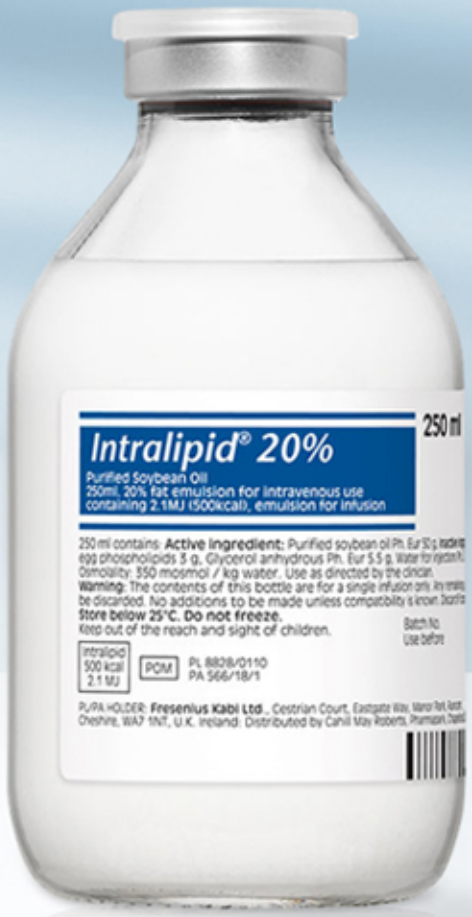


# Intralipid- A Magic Bullet?

Joseph Eldor

MedCrave
Step into the World of Research

# Intralipid-A Magic Bullet?

***Corresponding author:**

Joseph Eldor
Theoretical Medicine Institute, Jerusalem, Israel
Email: csen_international@csen.com

**Published By:**

**MedCrave Group LLC**

September 15, 2017

# Table of Contents

# Chapter I

## Intralipid-Iodine for Hysterosalpingography in Infertile Women

## Abstract

Tubal disease is the cause of female infertility in approximately 30% of women and 10-25% of these are due to proximal tubal obstruction. Selective salpingography represent an approach in the diagnosis and treatment of proximal tubal abnormalities. Intralipid (IL) is a synthetic product composed of 10 % soybean oil, 1,2 % egg yolk phospholipids, 2.25 % glycerin, and water. When indicated, IL is infused 7–10 days prior to embryo transfer (ET), Rates of ongoing pregnancy and live births were higher among women who underwent hysterosalpingography with oil contrast than among women who underwent this procedure with water contrast. It is the first time that intralipid-iodine is suggested to be used for hysterosalpingography in infertile women.

**Keywords:** Intralipid- Iodine; Hysterosalpingography; Infertility

Tubal disease is the cause of female infertility in approximately 30% of women [1] and 10-25% of these are due to proximal tubal obstruction [2]. Selective salpingography represent an approach in the diagnosis and treatment of proximal tubal abnormalities. The common indications for selective salpingography are to differentiate spasm from true obstruction [3]. In addition to that it allows clarifying findings from an equivocal hysterosalpingogram. Isthmic as well as intramural blockages were included. The tubal obstruction may due to amorphous materials occluding the tubal lumen, inflammatory changes and adhesions [4]. The use of selective salpingography and fallopian tube recanalization has revolutionized the diagnosis and treatment of infertility [3]. Diagnostic procedure has been used since 1980 [3]. It consists on opacification of the fallopian tube directly through a catheter placed in the tubal ostium. The objective is to differentiate spasm from true obstruction and to clear it with a catheter and guide wire system. Pregnancy rates among infertile women have been reported to increase after hysterosalpingography, but it is unclear whether the type of contrast medium used (oil-based or water-soluble contrast) influences this potential therapeutic effect.

Dreyer et al. [5] performed a multicenter, randomized trial in 27 hospitals in the Netherlands in which infertile women who were undergoing hysterosalpingography were randomly assigned to undergo this procedure with the use of oil-based or water-based contrast. Subsequently, couples received expectant management or the women underwent intrauterine insemination. The primary outcome was ongoing pregnancy within 6 months after randomization. Outcomes were analyzed according to the intention-to-treat principle. A total of 1119 women were randomly assigned to hysterosalpingography with oil contrast (557 women) or water contrast (562 women). A total of 220 of 554 women in the oil group (39.7%) and 161 of 554 women in the water group (29.1%) had an ongoing pregnancy (rate ratio, 1.37; 95% confidence interval [CI], 1.16 to 1.61; P<0.001), and 214 of 552 women in the oil group (38.8%) and 155 of 552 women in the water group (28.1%) had live births (rate ratio, 1.38; 95% CI, 1.17 to 1.64; P<0.001). Rates of adverse events were low and similar in the two groups.

Rates of ongoing pregnancy and live births were higher among women who underwent hysterosalpingography with oil contrast than among women who underwent this procedure with water contrast. Hysterosalpingography, which should be done in the follicular phase of the cycle, evaluates the contour of the uterine cavity, cervical canal, and tubal lumina. Other than being diagnostic, it can prove to be therapeutic. The instrument used to introduce the radio-opaque medium should be chosen to give the least discomfort and to cause no leakage of dye from the cervix. Water-soluble medium is usually used rather than an oil-based medium. Fluoroscopy with image intensification gives the best results. Insufficient dye injection will give an incomplete study. Too much dye injection, especially under pressure, might cause extravasation of the dye into the vascular system or conceal the fimbrial ends of the tubes [6]. To determine whether hysterosalpingography (HSG) increases the conception rate and to compare the therapeutic effectiveness of oil and water soluble contrast media, the histories of 744 women who attended the Yale Infertility

Clinic in 1965-69 were reviewed. Exclusion of women who had been infertile less than 1 year before coming to the clinic, in whom a HSG was done outside the clinic, and in whom there was no follow-up resulted in a study population of 460. The pregnancy rate for the study group (49%) was identical to that for the larger population. The study group was divided into women who became pregnant during their clinic attendance or within 1 year of terminating clinic care and women who did not become pregnant in this period. These 2 categories were then subdivided according to whether a HSG was done. To determine the relative effectiveness of different media, the conception rate following HSG in the Yale Clinic, where the iodized oil Ethiodol was used, was compared with that among 63 women who had HSG done by a private practitioner with the water soluble dye Salpix. 221 women were in the Ethiodol HSG group, 121 (55%) of whom conceived. Of the 239 Yale Clinic patients who did not have HSG, 103 (43%) became pregnant. Exclusion of couples with organic factors that could account for infertility resulted in a pregnancy rate of 58% for the HSG group and 47% for the non-HSG group. 25 (40%) of the 63 women in the Salpix group conceived, but exclusion of couples with organic factors lowered the pregnancy rate to 38% in this group. The average length of infertility was 0.5 years longer in the non-HSG group than in the Ethiodol HSG group, but shorter in the Salpix HSG group compared to the Ethiodol group. These results suggest that Ethiodol HSG may enhance fertility. Although the oil media has been criticized on the basis that it may cause granuloma formation or embolization, documented complications in the Yale series were rare. In contrast to earlier studies, unilateral nonpatency on x-ray was not found to affect eventual conception (58% pregnancy rate among women in the Ethiodol HSG group with normal tubes compared with 50% in those with filling of only 1 tube) [7].

Previous studies have suggested increased fertility rates following hysterosalpingography (HSG) using oil as compared with aqueous contrast medium. To compare the possible fertility-enhancing effects of two agents used for HSG, this prospective randomized study evaluated the subsequent fertility rates in 121 patients who underwent HSG, in which either oil or aqueous contrast medium was used. After random assignment to either agent, patients were observed for four menstrual cycles after HSG without resorting to any therapy other than clomiphene citrate where indicated. The pregnancy rates for the four cycles after HSG were compared with chi-square analysis in the total study and in the diagnostic subgroups. The subgroup with infertility of unknown cause had a significantly higher pregnancy rate after HSG with oil than after HSG with aqueous contrast medium. No significant difference was seen for any other subgroup or for the overall cohort [8].

In a prospective randomized study, the number of pregnancies after hysterosalpingography (HSG) was estimated in 398 patients who had been infertile for longer than 1 year. Iohexol was used in 101 patients, ioxaglate in 102 patients, diatrizoate meglumine in 97 patients, and ethiodized poppy-seed oil in 98 patients. Ten months after HSG, the patient, referring physician, and/or hospital department was consulted for information about pregnancies. Questionnaires were obtained from the patients who became pregnant during the waiting period of 3 months. No differences in demographic parameters, infertility status, or diagnosis made with HSG were detected among the four contrast media groups. Significantly more patients became pregnant after HSG in the ethiodized poppy-seed oil group than in the three water-soluble contrast media groups (P less than .01). When only intrauterine pregnancies resulting in full-term births were considered, significant differences in pregnancy rates between the oil-soluble and the water-soluble contrast media groups became more obvious. In the group that received ethiodized poppy-seed oil, almost one-third of the infertile women had normal pregnancies and childbirths after HSG [9].

Meta-analysis of four randomized clinical trials (RCTs) and six nonrandomized controlled studies evaluated pregnancy rates after the use of oil- or water-soluble contrast media during HSG. Four identified RCTs studied 800 patients and six nonrandomized studies comprised an additional 1,806 patients, all experiencing primary or secondary infertility. Pregnancy rates were significantly higher in the oil-soluble contrast media group compared with the water-soluble contrast media group in the RCTs. Inclusion of the six nonrandomized studies did not alter this conclusion. This apparent benefit was greatest for patients with unexplained infertility. Oil-soluble contrast media have a therapeutic effect compared with water-soluble media and this effect is greatest for patients who have been diagnosed as having unexplained infertility. New techniques for the evaluation of tubal patency support the hypothesis that tubal “plugs” may be involved in proximal tubal blockage (10). Ninety-three patients with unilateral or bilateral proximal tubal occlusion confirmed by hysterosalpingography or laparoscopy underwent FTR with use of water-soluble contrast material alone (n = 50) or also had an oil-based agent injected into each tube after recanalization (n = 43). Pregnancy rates and outcomes of the two groups were studied retrospectively.

With respect to differences between groups, only the body mass index proved to be a significant predictor (oil, 28.4; water, 24.7; P =.008). Mean age, duration of infertility, type of infertility, and initial diagnosis were comparable. There was a weak trend toward a higher pregnancy rate in the oil-based contrast material group, but it was not significant (P =.64). The average time to pregnancy was 4.4 months with use of oil-based contrast material, compared to 7.7 months with use of only water-soluble contrast material (P =.03). The use of an oil-based agent had little effect on the rate of conception, but time to conception was reduced by more than 3 months [11].

Hysterosalpingography is used commonly in the evaluation of infertility and in the diagnosis of anomalies of the uterus and fallopian tubes. There is continued debate over the safety and diagnostic or therapeutic efficacy of water-soluble versus oil-based contrast media. A 29-year-old woman with secondary infertility underwent hysterosalpingography with both water-soluble and oil-based contrast. The fallopian tubes appeared normal. Six months later, a plain abdominal radiograph obtained at the occasion of a minor motor vehicle accident revealed evidence of retained loculated pelvic contrast material. Subsequent laparoscopy identified adhesions and cul-de-sac implants strongly suspicious for endometriosis. Biopsy and pathologic study documented lipogranuloma.

Oil-based contrast media instilled into the pelvis at hysterosalpingography can persist for prolonged periods and create granulomatous lesions mimicking endometriosis. In view of the controversy whether oil-based contrast materials are superior to water-soluble media, the routine use of oil-based contrast media should be considered carefully [12].

Hysterosalpingography (HSG) has assumed a diagnostic and possibly therapeutic role in the evaluation of the infertile couple. The procedure is done using either an oil-based (OBCM) or a water-based (WBCM) contrast medium. Data from several retrospective studies suggest that higher pregnancy rates may be achieved when OBCM is used. Interpretation of these results, however, may be confounded by various methodologic flaws in study design and comparisons of heterogeneous populations. Letterie and Rose [13]sought to compare the therapeutic benefit of OBCM and WBCM in a prospective randomized study of infertile patients, controlling for pelvic anatomy by laparoscopic assessment. They used ethiodized oil (Ethiodol) or iothalamate meglumine (Conray 60) for tubal lavage at the time of laparoscopy only in patients with normal pelvic anatomy. Of the 225 patients who had diagnostic laparoscopy in the evaluation of infertility, 40 (18%) had normal pelvic anatomy and an otherwise unremarkable evaluation. Adequate follow-up was available on 29 patients randomized to receive either OBCM (n =15) or WBCM (n = 14). A significant difference in pregnancy rates was noted between OBCM (40%) and WBCM (14%) by chi-square analysis. No short- or long-term adverse reactions were noted. Results of this study suggest that in patients with normal pelvic anatomy as assessed laparoscopically, OBCM may offer a therapeutic benefit not evident with WBCM [13].

Moore et al. [14] evaluated the effect of different iodinated contrast agents on the fallopian tube and adnexal tissue in 15 rabbits. Ethiodized oil, an oil-soluble agent, was used in five rabbits. The following water-soluble agents were used: iothalamate meglumine 30% (n = 3), iothalamate meglumine 60% (n = 3), and ioxilan (n = 4). The agents were injected through catheters placed in the fallopian tubes. Fallopian tubes and peritoneal cavities were

histologically evaluated. The contralateral tube served as a control. Ioxilan and iothalamate meglumine 30% produced no pathologic response in the tube or peritoneal cavity. Iothalamate meglumine 60% was associated with mild inflammatory infiltrate, mucosal edema, giant cell reaction, and periovarian adhesions that were bilateral but more pronounced on the injected side. Use of ethiodized oil resulted in papillary fibrous adhesions on the ovarian surface, and fat granulomas were seen in the periovarian tissues. The safety of oil-based contrast agents for use in hysterosalpingography is therefore questioned. No significant differences were found among the water-soluble contrast agents [14].

Hysterosalpingography can be accomplished with either oil or water-soluble contrast medium. This randomized prospective study compared pregnancy rates in women who had hysterosalpingography with either water- or oil-soluble contrast material and were followed for six months. Fifteen of 60 (25%) patients who received water-soluble dye conceived compared with 14 of 46 (30%) patients in the oil-soluble group, a statistically insignificant difference. Furthermore, no difference in pregnancy rates within each subgroup of fertility diagnosis was detected. Intravasation was more common in patients administered oil-based contrast materials (six of 46 versus one of 60 patients, P = .02), although no serious consequences occurred. No difference in the amount of pain as assessed by pain scoring was experienced by patients in each group. The authors conclude that pregnancy rates are similar after hysterosalpingography with oil- and water-soluble contrast material, during at least the first six months after the procedure [15].

Aspects of the immunological relationship between mother and conceptus still remain a mystery, although the recent advances in molecular biology have enlightened some of the parameters that participate in feto-maternal cross-talk during implantation [16]. The atypical expression of major histocompatibility complex (MHC), the specific roles of some hormones and cytokines, as well as the temporal and spatial distributions of uterine natural killer (uNK) cells, represent substantive parameters of feto-maternal immunotolerance during implantation [17]. Although human maternal and fetal immunology is difficult to investigate, aberrant immune responses and an imbalanced cytokine network may be related to infertility, implantation failures after IVF, and recurrent pregnancy losses [18]. Patients with recurrent implantation failure (RIF) should be tested for inherited and acquired thrombophilias. Each patient should be individually assessed and counseled regarding management with low-molecular-weight heparin (LMWH). Empirical treatment with LMWH, aspirin, or corticosteroids is not effective for women with RIF who have negative thrombophilic tests [19]. If thrombophilic tests are normal, patients should be tested for immunological causes. The findings of a recent study suggest that increases in the percentage of CD56(dim) cells and NK cytotoxicity in peripheral blood may be important contributing factors for both RSA and IVF failure [20]. Human leukocyte antigen (HLA)-DQA1*0505 sharing or the maternal killer immunoglobulin-like receptor (KIR) repertoire is associated with recurrent spontaneous abortion (RSA) or repeated implantation failure (RIF) [21] and if abnormal, the patient might then benefit from intravenous immunoglobulin (IVIg) therapy [19]. IVIg has been successful in the treatment of recurrent miscarriage and recurrent implantation failure among women with elevated anti-phospholipid antibodies (APA) and/or NK cell activity [22]. When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with women treated with IVIg, no differences were seen [22]. Side-by-side comparison showed that synthetic pre-implantation factor (sPIF) is equally effective to inhibit NK cell toxicity at a lower dose than intravenous gamma immunoglobulin or intralipid treatment currently used [23]. sPIF is not yet available commercially, but intralipid infusions are available globally. Intralipid (IL) is a synthetic product composed of 10 % soybean oil, 1,2 % egg yolk phospholipids, 2.25 % glycerin, and water. When indicated, IL is infused 7–10 days prior to embryo transfer (ET), and one more time again after a positive pregnancy in women whose NKa is due to an autoimmune causes (antiphospholipid antibodies and/or antithyroid antibodies) (24). In cases of alloimmune implantation dysfunction (DQa and/HLA matching between the embryo recipient and the male partner), the same applies, but in this situation, the infusion is repeated at 2–4 week intervals until the 24th week of pregnancy [24]. IL costs about 10 times less than IVIg, is not a blood product, and is without significant side effects [24].

Intravenous lipid emulsions have been used experimentally since at least the 19th century. An early product marketed in 1957 under the name Lipomul was briefly used in the United States but was subsequently withdrawn due to side effects. Intralipid was invented by the Swedish physician and nutrition researcher Arvid Wretlind, and was approved for clinical use in Sweden in 1962 [25]. In the United States, the Food and Drug Administration initially declined to approve the product due to prior experience with another fat emulsion. It was approved in the United States in 1972.

## Conclusion

It is the first time that intralipid-iodine is suggested to be used for hysterosalpingography in infertile women.

## References


1. Juras J, Lipski M (2002) Imaging in tubal infertility. Rev Prat 52(16): 1768-1774.
2. Elstein M (2008) Tubal disease and fertility outcome. Reprod Biomed Online 16(2): 167-169.
3. Thurmond AS, Machan LS, Maubon AJ (2000) A Review of Selective Salpingography and Fallopian Tube Catheterization. RadioGraphics 20(6): 1759-1768
4. Papaioannou S, Afnan M, Girling AJ, Ola B, Olufowob O, et al. (2003) Diagnostic and therapeutic value of selective salpingography and tubal catheterization in an unselected infertile population. Fertil steril 79(3): 613-617.
5. Dreyer K, Van Rijswijk J, Mijatovic V, Goddijn M, Verhoeve HR, et al. (2017) Oil-Based or Water-Based Contrast for Hysterosalpingography in Infertile Women. N Engl J Med 376(21): 2043-2052.
6. Baramki TA (2005) Hysterosalpingography. Fertil Steril Jun 83(6): 1595-15606.
7. Mackey RA, Glass RH, Olson LE, Vaidya R (1971) Pregnancy following hysterosalpingography with oil and water soluble dye. Fertil Steril 22(8): 504-507.
8. Schwabe MG, Shapiro SS, Haning RV Jr (1983) Hysterosalpingography with oil contrast medium enhances fertility in patients with infertility of unknown etiology. Fertil Steril 40(5): 604-606.
9. Rasmussen F, Lindequist S, Larsen C, Justesen P (1991) Therapeutic effect of hysterosalpingography: oil- versus water-soluble contrast media--a randomized prospective study. Radiology 179(1): 75-78.
10. Watson A, Vandekerckhove P, Lilford R, Vail A, Brosens I, et al. (1994) A meta-analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result? Fertil Steril 61(3): 470-477.
11. Pinto AB, Hovsepian DM, Wattanakumtornkul S, Pilgram TK (2003) Pregnancy outcomes after fallopian tube recanalization: oil-based versus water-soluble contrast agents. J Vasc Interv Radiol 14(1): 69-74.
12. Grosskinsky CM, Clark RL, Wilson PA, Novotny DB (1994) Pelvic granulomata mimicking endometriosis following the administration of oil-based contrast media for hysterosalpingography. Obstet Gynecol 83(5 Pt 2): 890-892.
13. Letterie GS, Rose GS (1990) Pregnancy rates after the use of oil-based and water-based contrast media to evaluate tubal patency. South Med J 83(12): 1402-1403.
14. Moore DE, Segars JH Jr, Winfield AC, Page DL, Eisenberg AD, et al. (1990) Effects of contrast agents on the fallopian tube in a rabbit model. Radiology 176(3): 721-724.
15. Alper MM, Garner PR, Spence JE, Quarrington AM (1986) Pregnancy rates after hysterosalpingography with oil- and water-soluble contrast media. Obstet Gynecol 68(1): 6-9.
16. Gautam N Allahbadia (2015) Intralipid Infusion is the Current Favorite of Gynecologists for Immunotherapy. J Obstet Gynaecol India 65(4): 213-217.
17. Oreshkova T, Dimitrov R, Mourdjeva M (2012) A cross-talk of decidual stromal cells, trophoblast, and immune cells: a prerequisite for the success of pregnancy. Am J Reprod Immunol 68(5): 366-373.
18. Makrigiannakis A, Petsas G, Toth B, Relakis K, Jeschke U, et al. (2011) Recent advances in understanding immunology of reproductive failure. J Reprod Immunol 90(1): 96-104.
19. Fatemi H, Popovic-Todorovic B (2013) Implantation in assisted reproduction: a look at endometrial receptivity. Reprod Biomed Online 27(5): 530-538.
20. Karami N, Boroujerdnia MG, Nikbakht R, Khodadadi A (2012) Enhancement of peripheral blood CD56(dim) cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure. J Reprod Immunol 95(-2): 87-92.
21. Varla-Leftherioti M, Keramitsoglou T, Parapanissiou E, Kurpisz M, Kontopoulou-Antonopoulou V, et al. (2010) HLA-DQA1*0505 sharing and killer immunoglobulin-like receptors in sub fertilecouples: report from the 15th International Histocompatibility Workshop. Tissue Antigens 75(6): 668-672.
22. Coulam CB, Acacio B (2012) Does immunotherapy for treatment of reproductive failure enhance live births? Am J Reprod Immunol 67(4): 296-304.
23. Roussev RG, Donskoi BV, Stamatkin C, Ramu S, Chernyshov VP,et al. (2013) Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy. Reprod Biomed Online 26(1): 79-87.
24. CARE Fertility Forum Index. Intralipids-All you need to know
25. Hallberg D, Holm I, Obel AL, Schuberth O, Wretlind A, et al. (1967) Fat emulsions for complete intravenous nutrition .Postgrad med J 43(498): 307-316.

# Chapter 2

## Intralipid Treatment for Post Operative Delirium

## Abstract

Postoperative delirium (POD) is a common and serious adverse event in the elderly patient and is associated with significant morbidity and mortality. A new treatment for POD by intravenous Intralipid injection in the recovery room is first suggested in the medical literature.

**Keywords:** Postoperative delirium; Intralipid

## Post Operative Delirium

Delirium is defined by either the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM- 5) [1] or by the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD 10, Table 3) [2]. Delirium is an acute and fluctuating alteration of mental state of reduced awareness and disturbance of attention. POD (Post Operative Delirium) often starts in the recovery room and occurs up to 5 days after surgery [3-5]. One investigation [4] found that many patients with POD on the peripheral ward already had POD in the recovery room.

More than 230 million surgical procedures are performed each year worldwide, of which more than 80 million are in Europe [6-8]. In Europe, the in-hospital mortality rate up to a maximum of 60 days is 3% after elective surgery and nearly 10% after emergency surgery [7]. In addition to mortality, postoperative cognitive impairments such as POD and postoperative cognitive dysfunction (POCD) impose a huge burden on individuals and society [9]. The incidence of POD is dependent on perioperative and intraoperative risk factors [10]. Therefore, the incidence of POD varies within a broad range [11,12]. For example, a meta-analysis of 26 studies of POD reported an incidence of 4.0 to 53.3% in hip fracture patients and 3.6 to 28.3% in elective patients [13].

Delirium is one of the most common complications following hip fracture surgery in older people. This study identified pre- and peri-operative factors associated with the development of post-operative delirium following hip fracture surgery. Published and unpublished literature were searched to identify all evidence reporting variables on patient characteristics, on-admission, intra-operative and post- operative management assessing incident delirium in older people following hip fracture surgery. Pooled odds ratio (OR) and mean difference of those who experienced delirium compared to those who did not were calculated for each variable. Evidence was assessed using the Downs and Black appraisal tool and interpreted using the GRADE approach. A total of 6704 people (2090 people with post-operative delirium) from 32 studies were analysed. There was moderate evidence of nearly a two-times greater probability of post-operativedelirium for those aged 80 years and over (OR: 1.77; 95% CI: 1.09, 2.87), whether patients lived in a care institution pre-admission (OR: 2.65; 95% CI: 1.79, 3.92), and a six-time greater probability of developing post-operative delirium with a pre-admission diagnosis of dementia (OR: 6.07, 95% CI: 4.84, 7.62). There was no association with intra-operative variables and probability of delirium.

Clinicians treating people with a hip fracture should be vigilant towards post-operativedelirium if their patients are older, have pre-existing cognitive impairment and poorer overall general health. This is also the case for those who experience post-operative complications such as pneumonia or a urinary tract infection [14]. Post-operative cerebral dysfunction includes delirium, usually occurring early and reversible, and post-operative cognitive disorders, usually occurring later and prolonged. This is a frequent complication in patients older than 75 years old. The two neurological pictures are often inter-related. The pathophysiology of both entities is similar and related to post-operative neuro-inflammation; therefore onset may occur independently of any surgical complication. Post-operative cerebral dysfunction is a serious organic complication. Reduction of inflammation represents the most logical preventive measure but currently there are no studies that show this to be effective. Prevention therefore means combining several minor measures, elements that fit well into programs of enhanced post-operative recovery after surgery. Diminished pre-operative cognitive status being a major risk factor, pre-operative rehabilitation combining nutritional, physical and cognitive support can be helpful [15].

Postoperative delirium is a common and serious adverse event in the elderly patient and is associated with significant morbidity and mortality. It is of great importance to identify patients at risk for delirium, in order to focus preventive strategies. The aim of this article is to systematically review current available literature on pre-operative risk factors for delirium after vascular surgery. A systematic literature search was conducted using PubMed and EMBASE, using the MeSH terms and key words “delirium”, “surgery” and “risk factor”. Studies were retained for review after meeting strict inclusion criteria that included only prospective studies evaluating risk factors for delirium in patients who had elective vascular surgery. Diagnosis of delirium needed to be confirmed using the Diagnostic and Statistical Manual of Mental Disorders (DSM) or ICD-10. Fifteen articles were selected for inclusion, incidence of delirium across the studies ranged from 5% to 39%. Many factors have been associated with increased risk of delirium, including age, cognitive impairment, comorbidity, depression, smoking, alcohol, visual and hearing impairment, ASA-score, biochemical abnormalities, operative strategies and blood loss.

Delirium is a common complication after elective vascular surgery in elderly. The highest delirium incidence was observed after open aortic surgery as well as after surgery for critical limb ischemia. A picture starts to form of which predisposing factors lead to increased risk of delirium. The leading risk factors consistently identified in this systematic review were advanced age and cognitive impairment. Multi-disciplinary specialist-led interventions in the preoperative phase could decrease incidence and severity of delirium and should be focused on identified high-risk patients [16]. This study [17] investigates the relationship between cognitive dysfunction or delirium detected in the early post-surgical phase and the 1-year mortality among 514 hip fracture hospitalized older persons. Patients with early cognitive dysfunction or delirium experienced a 2-fold increased mortality risk. Early post-operative cognitive dysfunction and delirium are negative prognostic factors for mortality.

Premorbid cognitive impairment and dementia in older individuals negatively affect functional recovery after hip fracture. Additionally, post-operative delirium is an established risk factor for negative outcomes among hip fracture patients. While the majority of hip fracture patients experience minor post-surgical cognitive dysfunction, the prognostic value of this phenomenon is unknown. Therefore, we investigated the relationship between minor cognitive dysfunction or delirium detected in the early post-surgical phase and the 1-year mortality after index hip fracture. We enrolled 514 patients with hip fracture (77.4 % women), aged 65 years or older (mean age 83.1 ± 7.3 years), who underwent surgical hip fracture repair. Patients were assessed daily from the second to the fourth post-operative day and at 3, 6, and 12 months thereafter. All participants underwent comprehensive assessment, including detection of delirium by using the confusion assessment method and evaluation of cognitive function by using mini-mental state examination (MMSE; score range 0 to 30, with lower scores indicating poorer performance). In the absence of delirium, post-surgical cognitive dysfunction was defined as having low performance on MMSE. Vital status of 1 year after the index fracture and date of death were gathered from local registries.

The observed 1-year mortality rate was 14.8 %. Men were more likely to die than women within 1 year of the index fracture ($p < 0.01$). Compared to participants with better cognitive performance, those with MMSE < 24, as well as those with delirium in the post-operative phase, showed a significantly higher 1-year mortality rate (23.3 versus 17.9 and 8.1 %, respectively). Independent of age and sex, post-operative cognitive dysfunction as well as delirium was both associated with a 2-fold increased mortality risk. The presence of minor cognitive dysfunction in the early post-surgical phase is a negative prognostic factor for mortality among elderly hip fracture patients. The burden of minor cognitive dysfunction is likely superimposed on that of delirium in subgroups of frail patients [17]

Perioperative cerebral hypoperfusion/ischemia is a major inciting factor of postoperativedelirium, which is coupled with adverse outcome in elderly patients. Cerebral oximetry enables noninvasive assessment of the regional cerebral oxygen saturation (rSO2). This study aimed to investigate whether perioperative rSO2 variations were linked to delirium in elderly patients after spinal surgery. Postoperative delirium was assessed for 48 hours postsurgery in 109 patients aged over 60 years without a prior history of cerebrovascular or psychiatric diseases by the Confusion Assessment Method for the intensive care unit and the intensive care delirium screening checklist. The rSO2 values immediately before and throughout surgery were acquired. The preoperative cognitive functions, patient characteristics, and perioperative data were recorded. During the 48-h postoperative period, 9 patients (8%) exhibited delirium. The patients with delirium showed similar perioperative rSO2 values as those without, in terms of the median lowest rSO2 values (55% vs. 56%; P=0.876) and incidence (22%, both) and duration of decline of rSO2<80% of the baseline values. The serially assessed hemodynamic variables, hematocrit levels, and blood gas analysis variables were also similar between the groups, except for the number of hypotensive events per patient, which was higher in the patients with delirium than in those without (4, interquartile range [IQR] 3 to 6 vs. 2, IQR: 1to 3; P=0.014). The degree and duration of decrease of the perioperative rSO2 measurements were not associated with delirium in elderly patients after spinal surgery [18] .

Three-dimensional Arterial Spin Labeling (ASL) MRI was performed before surgery in a cohort of 146 prospectively enrolled subjects≥70 years old scheduled to undergo elective surgery. We investigated the prospective association between ASL-derived measures of cerebral blood flow (CBF) before surgery with postoperative delirium incidence and severity using whole-brain and globally normalized voxel-wise analysis. We also investigated the cross-sectional association of CBF with patients' baseline performance on specific neuropsychological tests, and with a composite general cognitive performance measure (GCP). Out of 146 subjects, 32 (22%) developed delirium. We found no significant association between global and voxel-wise CBF with delirium incidence or severity. We found the most significant positive associations between CBF of the posterior cingulate and precuneus and the Hopkins Verbal Learning Test - Revised total score, Visual Search and Attention Test (VSAT) score and the GCP composite. VSAT score was also strongly associated with right parietal lobe CBF. ASL can be employed in a large, well-characterized older cohort to examine associations between CBF and age-related cognitive performance. Although ASL CBF measures in regions previously associated with preclinical Alzheimer's Disease were correlated with cognition, they were not found to be indicators of baseline pathology that may increase risk for delirium [19].

Oxidative stress may be involved in occurrence of postoperative delirium (POD) and cognitive dysfunction (POCD). 8-iso-Prostaglandin F2α (8-iso-PGF2α), an isoprostane derived from arachidonic acid via lipid peroxidation, is considered a gold standard for measuring oxidative stress. The present study aimed to investigate the ability of postoperative plasma 8-iso-PGF2α levels to predict POD and POCD in elderly patients undergoing hip fracture surgery. Postoperative plasma 8-iso-PGF2α levels of 182 patients were measured by an enzyme-linked immunosorbent assay. We assessed the relationships between plasma 8-iso-PGF2α levels and the risk of POD and POCD using a multivariate analysis. Plasma 8-iso-PGF2α levels and age were identified as the independent predictors for POD and POCD. Based on areas under receiver operating characteristic curve, the predictive values of 8-iso-PGF2α were obviously higher than those of age for POD and POCD. In a combined logistic-regression model, 8-iso-PGF2α significantly enhanced the areas under curve

of age for prediction of POD and POCD. Postoperative plasma 8-iso-PGF2α levels may have the potential to predict POD and POCD in elder patients undergoing hip fracture surgery (Zheng YB e al. 2016) (20). Oxidative stress may be involved in occurrence of postoperative delirium (POD) and cognitive dysfunction (POCD). 8-iso-Prostaglandin F2α (8-iso-PGF2α), an isoprostane derived from arachidonic acid via lipid peroxidation, is considered a gold standardfor measuring oxidative stress. The present study aimed to investigate the ability of postoperative plasma 8-iso-PGF2α levels to predict POD and POCD in elderly patients undergoing hip fracture surgery.

Postoperative plasma 8-iso-PGF2α levels of 182 patients were measured by an enzyme-linked immunosorbent assay. We assessed the relationships between plasma 8-iso-PGF2α levels and the risk of POD and POCD using a multivariate analysis. Plasma 8-iso-PGF2α levels and age were identified as the independent predictors for POD and POCD. Based on areas under receiver operating characteristic curve, the predictive values of 8-iso-PGF2α were obviously higher than those of age for POD and POCD. In a combined logistic-regression model, 8-iso-PGF2α significantly enhanced the areas under curve of age for prediction of POD and POCD. Postoperative plasma 8-iso-PGF2α levels may have the potential to predict POD and POCD in elder patients undergoing hip fracture surgery [21].

Risk factors for delirium following cardiac surgery are incompletely understood. The aim of this study was to investigate whether intra-operative pathophysiological alterations and therapeutic interventions influence the risk of post-operative delirium. This retrospective cohort study was performed in a 12-bed cardiosurgical intensive care unit (ICU) of a university hospital and included patients consecutively admitted after cardiac surgery during a 2-month period. The diagnosis of delirium was made clinically using validated scores. Comparisons between patients with and without delirium were performed with non-parametric tests. Logistic regression was applied to identify independent risk factors. Results are given as number (percent) or median (range). Of the 194 consecutive post-cardiac surgery patients, 50 (26 %) developed delirium during their ICU stay. Univariate analysis revealed that significant differences between patients with and without delirium occurred in the following intra-operative variables: duration of cardiopulmonary bypass (184 [72-299] vs 113 (37-717) minutes, $p < 0.001$), lowest mean arterial pressure (50 [30-70] vs 55 [30-75] mmHg, $p = 0.004$), lowest haemoglobin level (85 [56-133] vs 98 [53-150] g/L, $p = 0.005$), lowest body temperature (34.5 [24.4-37.2] vs 35.1 [23.9-37.2] °C, $p = 0.035$), highest noradrenaline support (0.11 [0.00-0.69] vs 0.07 [0.00-0.42] µg/kg/minute, $p = 0.001$), and frequency of red blood cell transfusions (18 [36 %] vs 26 [18 %], $p = 0.018$) and platelet transfusions (23 [46 %] vs 24 [17 %], $p < 0.001$). Only platelet transfusions remained an independent risk factor in the multivariate analysis ($p < 0.001$). In patients undergoing cardiac surgery, various intra-operative events, such as transfusion of platelets, were risk factors for the development of a post-operative delirium in the ICU. Further research is needed to unravel the underlying mechanisms [22].

In this study, Bilge EÜ et al. [23] aimed to determine the risk factors and the incidence of delirium in patients who were followed postoperatively in our surgical intensive care unit for 24 h using the confusion assessment method (CAM). After obtaining approval from the ethics committee, 250 patients were included in the study. Patients who were operated under general anaesthesia or regional anaesthesia and followed in the surgical intensive care unit were evaluated by the Ramsay Sedation Scale on the first postoperative day. CAM was applied to the patients who had a Ramsey Sedation Score of ≤4. Patients' age, gender, American Society of Anesthesiologists (ASA) scores, preoperative risk factors, type of anaesthesia, operation time, intra-operative procedures, pain scores evaluated by the visual analogue scale (VAS) and postoperative analgesia methods were recorded.

The incidence of delirium was found to be 18.4%. The average age of patients who developed delirium was greater than the others (68.8±12.7 and 57.6±12, p=0.001, respectively). It was observed that a one-unit increase in the ASA score resulted in a 3.3-fold increase in the risk of delirium. The incidence of delirium in patients undergoing regional anaesthesia was 34.6%, whereas it was 16.5% in patients receiving general anaesthesia (p=0.024). The existence of preoperative diabetes mellitus (DM) and chronic obstructive pulmonary disease (COPD) was shown to improve the development of delirium ($p<0.05$). Delirium incidence was significantly higher in patients who were administered meperidine for postoperative analgesia (p=0.013). The VAS scores of patients who developed delirium were found to be significantly higher (p=0.006). As a result, we found that older age, high ASA score, preoperative DM and COPD are important risk factors for the development of delirium. Regional anaesthesia, high postoperative pain scores and meperidine use were observed to be associated with the development of delirium. In the postoperative period, addition of CAM, a simple measurement technique, to the daily follow-up forms can provide the early recognition of delirium, which is often under diagnosed. We think that identification and prevention of effective risk factors have the primary importance for postoperative delirium [23].

Delirium after cardiac surgery is a major problem. The exact mechanisms behind delirium are not understood. Potential pathways of delirium include neurotransmitter interference, global cognitive disorder, and neuro inflammation. Several predisposing and precipitating risk factors have been identified for postoperative delirium. The development of delirium following cardiac surgery is associated with worse outcomes in the perioperative period. Multiple interventions are being explored for the prevention and

treatment of delirium. Studies investigating the potential roles of biomarkers in delirium as well as pharmacological interventions to reduce the incidence and duration of delirium are necessary to mitigate this negative outcome [24]. Perhaps the most frequently described mechanism of brain injury in CABG surgery is based on the recognition that microemboli are generated by the surgeon manipulating the heart and aorta, through cardiotomy suctioning, and by the cardiopulmonary bypass circuit itself. Microemboli can be detected intraoperatively as high-intensity transient signals by transcranial Doppler sonography. They have the potential to lodge in cerebral microvasculature, impairing blood supply to the brain and thus cerebral oxygenation. Several phases during cardiac surgery have been associated with increased risk of embolic showers. Aortic cannulation and clamping (during application of cardiopulmonary bypass) increase the high-intensity transient signal rate, particularly if there is extensive atheroma in the ascending aorta [25]. It is not surprising, therefore, that most (81%) microemboli are generated at the point of aortic cross-clamp release [26]. Retaining the shed mediastinal blood with cardiotomy suckers provides an additional source of lipid emboli and other fragments [27].

## Conclusion

A new treatment for POD by intravenous Intralipid injection in the recovery room is first suggested in the medical literature.

## References


1. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (Dsm-51). In: (5th edn), Amer Psychiatric Pub, USA.
2. International Statistical Classification of Diseases and Related Health Problems 10th Revision.
3. Olin K, Eriksdotter-Jonhagen M, Jansson A, Herrington MK, Kristiansson M, et al. (2005) Postoperative delirium in elderly patients after major abdominal surgery. Br J Surg 92(12): 1559-1564.
4. Sharma PT, Sieber FE, Zakriya KJ, Pauldine RW, Gerold KB, et al. (2005) Recovery room delirium predicts postoperative delirium after hip-fracture repair. Anesth Analg 101(14): 1215-1220.
5. Radtke FM, Franck M, Schneider M, Luetz A, Seeling M,et al. (2008) Comparison of three scores to screen for delirium in the recovery room. Br J Anaesth 101(3): 338- 343.
6. Eurostat (2015) Your key to European statistics.
7. Pearse RM, Moreno RP, Bauer P, Pelosi P, Metnitz P, et al. (2012) Mortality after surgery in Europe: a 7 day cohort study. Lancet 380(9847): 1059-1065.
8. Weiser TG, Regenbogen SE, Thompson KD, Haynes AB, Lipsitz SR, et al. (2008) An estimation of the global volume of surgery: a modelling strategy based on available data. Lancet 372(9633): 139-144.
9. Saczynski JS, Marcantonio ER, Quach L, (2012) Cognitive trajectories after postoperative delirium. N Engl J Med 367: 30-39.
10. Smulter N, Lingehall HC, Gustafson Y, Olofsson B, Engström KG, et al. (2013) Delirium after cardiac surgery: incidence and risk factors. Interact Cardiovasc Thorac Surg 17(5): 790-796.
11. Dasgupta M, Dumbrell AC (2006) Preoperative risk assessment for delirium after noncardiac surgery: a systematic review. J Am Geriatr Soc 54(10): 1578-1589.
12. Dyer CB, Ashton CM, Teasdale TA (1995) Postoperative delirium. A review of 80 primary data-collection studies. Arch Intern Med 155(5): 461-465.
13. Bruce AJ, Ritchie CW, Blizard R, Lai R, Raven P, et al. (2007) The incidence of delirium associated with orthopedic surgery: a meta-analytic review. Int Psychogeriatr 19(2): 197-214.
14. Smith TO, Cooper A, Peryer G, Griffiths R, Fox C3, et al. (2017) Factors predicting incidence of post-operative delirium in older people following hip fracture surgery: a systematic review and meta-analysis. Int J Geriatr Psychiatry 32(4): 386-396.
15. Benhamou D, Brouquet A (2016) Postoperative cerebral dysfunction in the elderly: Diagnosis and prophylaxis. J Visc Surg 153(6S): S27-S32.
16. Raats JW, Steunenberg SL, De Lange DC, Van der Laan L (2016) Risk factors of post-operative delirium after elective vascular surgery in the elderly: A systematic review. Int J Surg 35: 1-6.
17. Ruggiero C, Bonamassa L, Pelini L, Prioletta I, Cianferotti L, et al. (2017) Early post-surgical cognitive dysfunction is a risk factor for mortality among hip fracture hospitalized older persons. Osteoporos Int 28(2): 667-675.
18. Soh S, Shim JK, Song JW, Kim KN, Noh HY, et al. (2016) Postoperative Delirium inElderly Patients Undergoing Major Spinal Surgery: Role of Cerebral Oximetry. J Neurosurg Anesthesiol 25.
19. Hshieh TT, Dai W, Cavallari M, Guttmann CR, Meier DS, et al. (2016) Cerebral blood flow MRI in the nondemented elderly is not predictive of post-operative delirium but is correlated with cognitive performance. J Cereb Blood Flow Metab 37(4): 1386-1397.
20. Zheng YB, Ruan GM, Fu JX, Su ZL, Cheng P, et al. (2016) Postoperative plasma 8-iso-prostaglandin F2α levels are associated with delirium and cognitive dysfunction in elderly patients after hip fracture surgery. Clin Chim Acta 455: 149-53.
21. Rudiger A, Begdeda H, Babic D, Krüger B, Seifert B, et al. (2016) Intra-operative events during cardiac surgery are risk factors for the development of delirium in the ICU. Crit Care 20: 264.
22. Bilge EÜ, Kaya M, Şenel GÖ, Ünver S (2015) The Incidence of Delirium at the Postoperative Intensive Care Unit in Adult Patients. Turk J Anaesthesiol Reanim 43(4): 232-239.
23. O Neal JB, Shaw AD (2016) Predicting, preventing, and identifying delirium after cardiac surgery. Perioper Med (Lond) 26;5: 7.
24. Mackensen GB, Ti LK, Phillips-Bute BG, Mathew JP, Newman MF, et al. (2003) Neurologic Outcome Research Group (NORG). Cerebral embolization during cardiac surgery: impact of aortic atheroma burden. Br J Anaesth 91(5): 656-661.
25. Reinsfelt B, Ricksten SE, Zetterberg H, Blennow K, Fredén-Lindqvist J, et al. (2012) Cerebrospinal fluid markers of brain injury, inflammation, and blood-brain barrier dysfunction in cardiac surgery. Ann Thorac Surg 94(2): 549-555
26. Brooker RF, Brown WR, Moody DM, Hammon JW Jr, Reboussin DM, et al. (1998) Cardiotomy suction: a major source of brain lipid emboli during cardiopulmonary bypass. Ann Thorac Surg 65(6): 1651-1655.

# Chapter 3

## Can Intralipid Infusion Open a Coronary Occlusion Causing Acute Myocardial Infarction?

## Abstract

CORONARY heart disease remains the leading cause of morbidity and mortality in Western countries. The best hope of salvaging viable myocardium after a coronary occlusion is by rapid reperfusion of the ischemic myocardium, either by thrombolysis or primary percutaneous coronary intervention. The use of intravenous intralipid infusion instead of thrombolysis or primary percutaneous coronary intervention is first suggested in the medical literature.

**Keywords:** Intralipid; Myocardial infarction; Coronary occlusion

## Thrombolysis or Primary Percutaneous Coronary Intervention or Intralipid Infusion?

CORONARY heart disease remains the leading cause of morbidity and mortality in Western countries. The best hope of salvaging viable myocardium after a coronary occlusion is by rapid reperfusion of the ischemic myocardium, either by thrombolysis or primary percutaneous coronary intervention. Although reperfusion restores blood flow, oxygen, and nutrients to the cardiac muscle, it also has the potential to induce reperfusion injury. Postconditioning of the heart with brief episodes of reperfusion/occlusion at the onset of reflow has been shown to limit infarct size. However, this approach is not practical for patients treated with thrombolytic agents and therefore a more generic pharmacologic postconditioning is still needed. The ideal pharmacologic candidates need to be safe and effective when administered during the first few minutes of reperfusion by inducing cellular protection or enhancing myocardial tolerance to ischemia/reperfusion injury. Several drugs have yielded encouraging results in animals and a few have been tested in humans; however, none of these modalities has been widely accepted. Lipids and in particular polyunsaturated fatty acids have received special cardiovascular research attention because polyunsaturated fatty acid-rich diets are associated with a decreased risk of coronary artery disease. Acute application of polyunsaturated fatty acids to cardiomyocytes has also been shown to shorten action potential duration and this could account for the antiarrhythmic mechanism of the polyunsaturated fatty acids. Intralipid (Sigma, St. Louis, MO) is a brand name for the first safe fat emulsion for human use; Intralipid 20% is an emulsion of soybean oil (20%), egg yolk phospholipids (1.2%), and glycerol (2.2%). Intralipid has been widely used in patients who need total parenteral nutrition and as a vehicle for different drugs such as propofol. It has been shown recently that postischemic administration of Intralipid protects the isolated rat heart against ischemia/reperfusion injury. However, the molecular mechanism in which Intralipid mediates cardioprotection is completely unknown [1].

Intralipid, a brand name for the first safe fat emulsion for human use, has been shown to be cardioprotective. However, the mechanism of this protection is not known. The authors investigated the molecular mechanism(s) of Intralipid-induced cardioprotection against ischemia/reperfusion injury, particularly the role of glycogen synthase kinase-3β (GSK-3β) and mitochondrial permeability transition pore in this protective action. In vivo rat hearts or isolated Langendorff-perfused mouse hearts were subjected to ischemia followed by reperfusion with Intralipid (1% in ex vivo and one bolus of 20% in in vivo) or vehicle. The hemodynamic function, infarct size, threshold for the opening of mitochondrial permeability transition pore, and phosphorylation levels of protein kinase B (Akt)/extracellular signal regulating kinase (ERK)/GSK-3β were measured. Administration of Intralipid at the onset of reperfusion resulted in approximately 70% reduction in infarct size in the in vivo rat model. Intralipid also significantly improved functional recovery of isolated Langendorff-perfused mouse hearts as the rate pressure product was increased from 2,999±863 mmHg*beats/min in the control group to 13,676±611 mmHg*beats/min (mean±SEM) and the infarct size was markedly smaller (18.3±2.4% vs. 54.8±2.9% in the control group, P <0.01). The Intralipid-induced cardioprotection was fully abolished by LY294002, a specific inhibitor of PI3K, but only partially by PD98059, a specific ERK inhibitor. Intralipid also increased the phosphorylation levels of Akt/ERK1/glycogen synthase kinase-3β by eightfold, threefold, and ninefold, respectively. The opening of mitochondrial permeability transition pore was inhibited by Intralipid because calcium retention capacity was higher in the Intralipid group (274.3±8.4 nM/mg vs. 168.6±9.6 nM/mg in the control group).

Postischemic treatment with Intralipid inhibits the opening of mitochondrial permeability transition pore and protects the heart through glycogen synthase kinase-3β via PI3K/Akt/ERK pathways [1]. It was recently shown that postischemic administration of intralipid protects the heart against ischemia-reperfusion injury. Here we compared the cardioprotective effects of intralipid with cyclosporine-A, a potent inhibitor of the mitochondrial permeability transition pore opening. In vivo rat hearts or isolated Langendorff-perfused mouse hearts were subjected to ischemia followed by reperfusion with intralipid (0.5%, 1% and 2% ex-vivo, and 20% in vivo), cyclosporine-A (0.2 μM, 0.8 μM, and 1.5 μM ex- vivo and 10 mg/kg in vivo), or vehicle. The hemodynamic function, infarct size, calcium retention capacity, mitochondrial superoxide production, and phosphorylation levels of protein kinase B (Akt)/glycogen synthase kinase-3β (GSK-3β) were measured. The values are mean ± SEM. Administration of intralipid at reperfusion significantly reduced myocardial infarct size compared with cyclosporine-A in vivo (infarct size/area at risk)%: 22.9±2.5% vs. 35.2 ±3.5%; P =0.030, n =7/group). Postischemic administration of intralipid at its optimal dose(1%) was more effective than cyclosporine-A (0.8 μM) in protecting the ex vivo heart against ischemia-reperfusion injury, as the rate pressure product at the end of reperfusion was significantly higher (mmHg · beats/min: 12,740±675 [n =7] vs. 9,203±10,781 [n =5], P =0.024), and the infarct size was markedly smaller (17.3 ± 2.9 [n =7] vs. 29.2 ± 2.7 [n =5], P =0.014). Intralipid was as efficient as cyclosporine-A in inhibiting the mitochondrial permeability transition pore opening (calcium retention capacity = 280±8.2 vs. 260.3±2.9 nmol/mg mitochondria protein in cyclosporine-A, P =0.454, n =6) and in reducing cardiac mitochondrial superoxide production. Unlike intralipid, which increased phosphorylation of Akt (6-fold) and GSK-3β (5-fold), cyclosporine-A had no effect on the activation of these prosurvival kinases.

Although intralipid inhibits the opening of the mitochondrial permeability transition pore as efficiently as cyclosporine-A, intralipid is more effective in reducing the infarct size

and improving the cardiac functional recovery [2]. It was recently demonstrated that the heart of late pregnant (LP) rodents is more prone to ischemia/reperfusion (I/R) injury compared to non-pregnant rodents. Lipids, particularly polyunsaturated fatty acids, have received special attention in the field of cardiovascular research. Here, we explored whether Intralipid (ITLD) protects the heart against I/R injury in LP rodents and investigated the mechanisms underlying this protection. In-vivo female LP rat hearts or ex-vivo isolated Langendorff-perfused LP mouse hearts were subjected to ischemia followed by reperfusion with PBS or ITLD (one bolus of 5mg/kg of 20% in in-vivo and 1% in ex-vivo). Myocardial infarct size, mitochondrial calcium retention capacity, genome-wide expression profiling, pharmacological inhibition and co-immunoprecipitation were performed. One bolus of ITLD at reperfusion significantly reduced the in-vivo myocardial infarct size in LP rats (23.3±2% vs. 55.5±3.4% in CTRL, $p<0.01$). Postischemic administration of ITLD also protected the LP hearts against I/R injury ex-vivo. ITLD significantly increased the threshold for the opening of the mitochondrial permeability transition pore in response to calcium overload (nmol-calcium/mg-mitochondrial protein: 290±17 vs. 167±10 in CTRL, $p<0.01$) and significantly increased phosphorylation of STAT3 (1.8±0.08 vs. 1±0.16 in CTRL, $p<0.05$) and GSK-3β (2.63±0.55 vs. 1±0.0.34 in CTRL, $p<0.05$). The ITLD-induced cardioprotection was fully abolished by Stattic, a specific inhibitor of STAT3. Transcriptome analysis revealed caveolin 2 (Cav2) was significantly upregulated by ITLD in hearts of LP rats under I/R injury. Co-immunoprecipitation experiments showed that Cav2 interacts with STAT3. ITLD protects the heart in late pregnancy against I/R injury by inhibiting the mPTP opening through Cav2/STAT3/GSK-3β pathway [3].

Recent studies have demonstrated that intralipid (ILP) conferred myocardial protection against ischemia-reperfusion (IR) injury through activation of reperfusion injury salvage kinase (RISK) pathway. As RISK signal has been shown to be impaired in hypertrophied myocardium, we investigated whether ILP-induced cardiac protection was maintained in hypertrophied rat hearts. Transverse aortic constriction was performed on male Sprague-Dawley rats to induce left ventricular hypertrophy, then sham-operated or hypertrophied rat hearts were isolated and perfused retrogradely by the Langendorff for 30 min (equilibration) followed by 40 min of ischemia and then 120 min of reperfusion. The isolated hearts received 15-min episode of 1% ILP separated by 15 min of washout or three episodes of 5-min ischemia followed by 5-min reperfusion before ischemia. The hemodynamics, infarct size, apoptosis, phosphorylated protein kinase B (p-Akt), phosphorylated extracellular regulated protein kinase 1/2 (ERK1/2), phosphorylated glycogen synthase kinase 3β(GSK3β), Bcl-2, phosphorylated Bad, and Bax were determined. We found that ILP significantly improved left ventricular hemodynamics and reduced infarct size and the number of TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling)-positive cells in the sham-operated rat hearts exposed to IR. However, such myocardial infarct-sparing effect of ILP was completely blocked by phosphatidylinositol-3-kinase inhibitor wortmannin, but only partially by mitogen-activated protein kinase kinase inhibitor PD98059 in sham-operated hearts. Intralipd upregulated the phosphorylation of Akt, extracellular regulated protein kinase 1/2 (ERK1/2), and their downstream target of GSK3β and antiapoptotic Bcl-2 expression in healthy rat hearts. Nonetheless, ILP failed to improve left ventricular hemodynamics and reduced infarct size and apoptosis and increase the phosphorylated Akt, ERK1/2, GSK3β, and antiapoptotic Bcl-2 in hypertrophied myocardium. In contrast, ischemic preconditioning increased the phosphorylation of Akt, ERK1/2 and GSK3β, improved heart pump function, and reduced myocardial necrosis in sham-operated hearts, a phenomenon partially attenuated by ventricular hypertrophy. Interestingly, GSK inhibitor SB216763 conferred cardioprotection against IR injury in sham-operated hearts, but failed to exert cardioprotection in hypertrophied myocardium. Our results indicated that ventricular hypertrophy abrogated ILP-induced cardioprotection against IR injury by alteration of RISK/GSK3β signal [4].

Elevated low-density lipoprotein cholesterol and triglycerides are major risk factors for coronary artery disease. However, fatty acids from triglycerides are a major energy source, low-density lipoprotein cholesterol is critical for cell membrane synthesis, and both are critical for cell survival. This study was designed to clarify the relationship between lipid profile, morbidity as assessed by Killip classification, and 30-day mortality in patients with acute myocardial infarction. Seven hundred twenty-four patients with acute myocardial infarction in the coronary care program of the Bureau of Health Promotion were analyzed. Low-density lipoprotein cholesterol and triglyceride levels were significantly lower in high-Killip (III+IV) patients compared with low-Killip (I+II) patients and in those who died compared with those who survived beyond 30 days (both $p<0.001$). After adjustment for risk factors, low-density lipoprotein cholesterol less than 62.5 mg/dL and triglycerides less than 110 mg/dL were identified as optimal threshold values for predicting 30-day mortality and were associated with hazard ratios of 1.65 (95% CI, 1.18-2.30) and 5.05 (95% CI, 1.75-14.54), and the actual mortality rates were 23% in low low-density lipoprotein, 6% in high low-density lipoprotein, 14% in low triglycerides, and 3% in high triglycerides groups, respectively. To test the synergistic effect, high-Killip patients with triglycerides less than 62.5 mg/dL and low-density lipoprotein cholesterol less than 110 mg/dL had a 10.9-fold higher adjusted risk of mortality than low-Killip patients with triglycerides greater than or equal to 62.5 mg/dL and low-density lipoprotein cholesterol greater than or equal to 110 mg/dL ($p<0.001$). The lipid paradox also improved acute myocardial infarction short-term outcomes prediction on original Killip and thrombolytic in myocardial infarction scores. Low low-density lipoprotein cholesterol, low triglycerides, and high Killip severity were associated with significantly higher 30-day in-hospital mortality in patients presenting with acute myocardial infarction. The

initial lipid profile of patients with acute myocardial infarction may therefore hold prognostic value [5].

## Emulsified isoflurane (EIso)

Volatile anesthetic postconditioning reduces myocardial infarct size against ischemia/reperfusion (I/R) injury. We tested the hypothesis that emulsified isoflurane (EIso) administrated after ischemia exerts cardioprotection in a rat model of myocardial I/R. Male SD rats underwent 30-min coronary occlusion followed by 3-h reperfusion except for sham rats. All vehicles were administrated intravenously at reperfusion onset for 30 min. In the first study, 56 rats were given saline (CON), 30% intralipid (IL) and 1, 2, 4, 8 or 16 mL/kg EIso for infarct size measurement. In a second study, 32 rats were randomized to four groups and administrated saline in sham (sham) and control (CON) groups, 30% intralipid in IL group and 2 mL/kg emulsified isoflurane in EIso group. Cardiomyocytic enzyme activity was determined. Myocardial mitochondria and cytosol were isolated to determine mitochondrial energy metabolism, cytochrome c release, mitochondrial membrane potential ($\Delta\Psi m$) and opening of the mitochondrial permeability transition pore (mPTP). Morphologic changes in mitochondria were observed by transmission electron microscopy. Compared with CON and IL, 2, 4 and 8 mL/kg EIso limited infarct size ($P < 0.01$). Serum levels of cardiac enzyme leakage were reduced in EIso-treated hearts compared with CON ($P < 0.01$ or $P < 0.05$). EIso preserved the ultrastructure of mitochondria, protected against mPTP opening, decreased cytochrome c release and preserved ATP production and $\Delta\Psi m$ . In conclusion, EIso is effective in reducing infarct size and in preserving mitochondrial function after ischemia and reperfusion injury [6]. The purpose of this study was to investigate whether adding emulsified isoflurane to St Thomas cardioplegia solution could enhance the cardiac protection after cardioplegic arrest in rats.

Thirty isolated heart preparations (male Sprague-Dawley rats) were randomly divided into 3 groups (n = 10/group) according to the different cardioplegia solutions being given: St Thomas solution mixed with emulsified isoflurane (containing 2.8% of isoflurane, group EI), St Thomas solution mixed with emulsified Intralipid (Huarui Pharmacy, Wuxi, Jiangsu, China) (group EL), and St Thomas solution alone (group St). In the 35-minute normothermic ischemia period, infusion of cardioplegia solution was repeated every 15 minutes. After the 35-minute ischemia period, the heart was perfused with Krebs-Henseleit buffer for another 2 hours. The functional parameters of the heart were monitored throughout the experiments. The coronary effluent was collected for measuring the activity of CK-MB 30 minutes after reperfusion, and the infarct size was assessed at the end of reperfusion. The infarct size in group EI (24% +/- 4%) was reduced when compared with that in group EL (31% +/- 8%, $p < 0.05$) and group St (43% +/- 9%, $p < 0.001$). The CK-MB activity in group EI was decreased significantly when compared with that in group EL and group St ($p < 0.05$). The functional recovery in group EI also was improved. Compared with standard St Thomas solution alone, adding 30% Intralipid alone also significantly reduced the infarct size and the CK-MB leakage and improved the recovery of the mechanical function. St Thomas cardioplegia solution supplemented with emulsified isoflurane enhanced its cardioprotection in an isolated heart ischemia reperfusion injury model in rats [7].

Pretreatment with volatile anesthetics has been demonstrated to exert cardioprotective effects. The purpose of this study was to examine the effect of emulsified isoflurane, a new formulation of isoflurane in lipid emulsion, administered intravenously in an ischemia and reperfusion model of myocardial injury. Thirty-two Sprague Dawley rats of both sexes were subjected to 30 min of myocardial ischemia followed by 180 min of reperfusion. Each was assigned to one of four pretreatment groups to receive an isovolumetric intravenous infusion of saline: control group, 30% intralipid group, 8% emulsified isoflurane 2 ml kg(-1) group, and sham group (each group, n =8). The vehicles were administered at a constant rate for 30 min and then discontinued 30 min before left anterior descending coronary artery occlusion. The cardioprotective effects were examined by determining hemodynamics, infarct size, enzyme activity, and cardiomyocytic apoptosis. Pretreatment with emulsified isoflurane 2 ml kg(-1) ($P = 0.000$) significantly reduced infarct size (22.6 +/- 2.2%) compared with control (34.8 +/- 2.3%) and 30% intralipid (31.1 +/- 2.9%). When compared with the control and intralipid groups, emulsified isoflurane increased Bcl-2 expression while decreasing Bax and Caspase-3 expression and enhancing Bcl-2/Bax ratios. The apoptotic index in the emulsified isoflurane treatment group showed a significant reduction compared with that in the control group ($P =0.000$) and the intralipid group ($P =0.001$). In addition, the serum levels of lactate dehydrogenase and creatine kinase were markedly reduced in the emulsified isoflurane treatment group compared with the control and intralipid groups (lactate dehydrogenase, $P =0.015$ vs. control; creatine kinase, $P =0.000$ vs. control and intralipid).

These data support a cardioprotective effect of intravenous emulsified isoflurane against myocardial ischemia and reperfusion injury, which are mediated, at least in part, by the inhibition of apoptosis and cell damage (8).

1. It has been shown that inhaled isoflurane limits the size of myocardial infarcts. The aim of the present study was to examine the effects of emulsified isoflurane on cardiac function and myocardial apoptosis in an ischaemia model of myocardial injury.

2. In the first study, 48 rats were randomly allocated to six groups (n =8 in each): control (saline); emulsified isoflurane (EIso) at 1, 2 or 4 mL/kg; 30% intralipid (vehicle for EIso); and sham operated. Rats received isovolumetric intravenous infusions for 30 min and then, 30 min after cessation of the infusion, 90 min coronary occlusion. Haemodynamics and myocardial infarct size were measured. In the second study, another 48 rats were randomized into six groups (n =8 in each). After

90 min ischaemia, rats were killed for histopathological study, immunohistochemical evaluation and apoptosis measurement. 3. Pretreatment with 2 and 4 mL/kg EIso significantly attenuated decreases in left ventricular systolic pressure and dP/dt(max), and increases in left ventricular end-diastolic pressure and -dP/dp(max), and alleviated myocardial injury compared with the control, intralipid and 1 mL/kg EIso groups (P <0.05). Infusion of 1 mL/kg EIso and intralipid had no effect on haemodynamics, infarct size or histological variables. 4. Expression of Bcl-2 was increased, whereas expression of Bax and caspase 3 was decreased, after preconditioning with 2 and 4 mL/kg EIso (P <0.05). The apoptotic index in the 2 and 4 mL/kg Eiso-treated groups was reduced compared with that in the control and intralipid groups (P <0.01). 5. In conclusion, EIso ameliorates cardiac dysfunction, attenuates myocardial damage and inhibits apoptosis after ischaemia, which may be attributed, in part, to diminished expression of apoptosis-related protein [9].

To evaluate the protective effects of 8% emulsified isoflurane after myocardial ischemia-reperfusion injury and its mechanism in rabbits: Twenty-four male adult New Zealand white rabbits were anesthetized with intravenous injection of 30 mg/kg pentobarbital followed by 5 mg x kg(-1) x h(-1) infusion. All rabbits were subjected to 30 minutes of left anterior descending coronary artery (LAD) occlusion and 3 hours of subsequent reperfusion. Before LAD occlusion, the rabbits were randomly allocated into three groups for preconditioning treatment (eight for each group). The control group (C group) received intravenously 0.9% NaCl for 30 minutes. The emulsified isoflurane group (EI group) received 8% emulsified isoflurane intravenously till 0.64% end-tidal concentration for 30 minutes that was followed by a 15-minute washout period.

The Intralipid group (IN group) received 30% Intralipid for 30 minutes. The infarcted area, plasma malondialdehyde (MDA) content, superoxide dismutase activity (SOD) and nitrite concentration after 3-hour myocardial perfusion were recorded simultaneously. For the myocardial ischemia-reperfusion injury animals, the infarcted size in the EI group was significantly reduced (91.9% +/- 8%) as compared with control group (39% +/- 6%, t=5.19, P<0.01). The plasma SOD activity and nitrite concentration in EI group were significantly higher than those in control group (t=2.82, t=8.46, P <0.05), but MDA content was lower in EI group than that in control group (t=2.56, P<0.05). The results indicate that emulsified isoflurane has a cardioprotection effect against ischemia-reperfusion injury. This beneficial effect of emulsified isoflurane is probably through NO release and consequently by increase in antioxidation of myocardium [10]. In this study, we examined the cardioprotective effects of parental emulsified isoflurane compared with inhaled isoflurane.

Thirty-two rabbits were subjected to 30 min of myocardial ischemia induced by temporary ligation of the left anterior descending coronary artery followed by 3 h of reperfusion. Before left anterior descending coronary artery occlusion, the rabbits were randomly allocated into one of four groups (eight for each group): group C, no ischemia preconditioning treatment; group IS, inhaled isoflurane 1.1% end-tidal; group EI, a continuous infusion of 8% emulsified isoflurane to an end-tidal concentration of 0.64%; and group IN, a continuous infusion of 30% Intralipid started 30 min. Treatments were started 30 min before ischemia followed by a 15 min washout period for isoflurane groups. Myocardial infarct volume, lactate dehydrogenase, and creatine kinase levels were measured and changes in mitochondrial ultrastructure assessed after 3 h myocardial reperfusion.

Myocardial infarct size 3 h after reperfusion was lower in groups IS and EI compared with groups C and IN (20% +/- 8%, 18% +/- 8%, 39% +/- 6%, and 34% +/- 9%, respectively, P < 0.01). There were no differences in myocardial infarct size between groups IS and EI or between groups C and IN. Plasma lactate dehydrogenase and creatine kinase levels were lower in group IS (456 +/- 58 U/L and 1725 +/- 230 U/L) and group EI (451 +/- 54 U/L and 1686 +/- 444 U/L) 3 h after myocardial reperfusion compared with groups C (676 +/- 82 U/L and 2373 +/- 529 U/L; P <0.01). Mitochondrial ultrastructure changes were less pronounced in groups IS and EI compared with group C. Our results indicate that, in rabbits, i.v. emulsified isoflurane provides similar myocardial protection against ischemia-reperfusion injury as inhaled isoflurane [11].

## The Intralipid Sink Effect

Papadopoulou A et al. [12] hypothesized that by substituting a dye surrogate in place of local anesthetic, they could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used. They selected 2 lipophilic dyes, acid blue 25 and Victoria blue, with log P values comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate “lipid rescue” treatment at dye concentrations equivalent to fatal, cardiotoxic, and neurotoxic local anesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75-kg adult. After mixing, the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colors compared against a graduated dye concentration scale.

Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the color intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log P value and the greatest amount of lipid emulsion. This study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may

reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable. Local anesthetic (LA) intoxication with cardiovascular arrest is a potential fatal complication of regional anesthesia. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. Aim of the study [13] was to compare four different rescue regimens using epinephrine and/or lipid emulsion and vasopressin to treat cardiac arrest caused by bupivacaine intoxication.

Twenty-eight piglets were randomized into four groups (4 × 7), anesthetized with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via central venous catheter at a rate of 1 $mg \cdot kg^{-1} \cdot min^{-1}$ until circulatory arrest. Bupivacaine infusion and sevoflurane were then stopped, chest compression was started, and the pigs were ventilated with 100% oxygen. After 1 min, epinephrine 10 $\mu g \cdot kg^{-1}$ (group 1), Intralipid(®) 20% 4 $ml \cdot kg^{-1}$ (group 2), epinephrine 10 $\mu g \cdot kg^{-1}$ + Intralipid(®) 4 $ml \cdot kg^{-1}$ (group)Or 2 IU vasopressin + Intralipid(®) 4 $ml \cdot kg^{-1}$ (group 4) were administered. Secondary epinephrine doses were given after 5 min if required.

Survival was 71%, 29%, 86%, and 57% in groups 1, 2, 3, and 4. Return of spontaneous circulation was regained only by initial administration of epinephrine alone or in combination with Intralipid(®). Piglets receiving the combination therapy survived without further epinephrine support. In contrast, in groups 2 and 4, return of spontaneous circulation was only achieved after secondary epinephrine rescue. In cardiac arrest caused by bupivacaine intoxication, first-line rescue with epinephrine and epinephrine + Intralipid(®) was more effective with regard to survival than Intralipid(®) alone and vasopressin + Intralipid(®) in this pig model [14].

Local anesthetic (LA) intoxication with severe hemodynamic compromise is a potential catastrophic event. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. However, there are no data about effectiveness of Intralipid for the treatment of severe cardiovascular compromise prior to cardiac arrest. Aim of this study was to compare effectiveness of epinephrine and Intralipid for the treatment of severe Hemodynamic compromise owing to bupivacaine intoxication, anesthetized Piglets were with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via a central venous catheter at a rate of 1 $mg \cdot kg^{-1} \cdot min^{-1}$ until invasively measured mean arterial pressure (MAP) dropped to 50% of the initial value. Bupivacaine infusion was then stopped, and epinephrine 3 $\mu g \cdot kg^{-1}$ (group 1), Intralipid(®) 20% 2 $ml \cdot kg^{-1}$ (group 2), or Intralipid 20% 4 $ml \cdot kg^{-1}$ (group 3) was immediately administered. Twenty-one piglets (3 × 7), were recorded. All animals in group 1 (100%) but only four of seven (57%) piglets in group 2 and group 3, respectively, survived. Normalization of hemodynamic parameters (HR, MAP) and ET($CO_2$) was fastest in group 1 with all piglets achieving HR and MAP values. hemodynamic compromise owing to bupivacaine intoxication in piglets, first-line rescue with epinephrine was more effective than Intralipid with regard to survival as well as normalization of hemodynamic parameters and ET($CO_2$) [15].

Intravenous lipid emulsion (ILE) has been proposed as a rescue therapy for severe local anesthetic drugs toxicity, but experience is limited with other lipophilic drugs. An 18-year-old healthy woman was admitted 8 h after the voluntary ingestion of sustained-release diltiazem (3600 mg), with severe hypotension refractory to fluid therapy, calcium salts, and high-dose norepinephrine (6.66 µg/ kg/ min). Hyperinsulinemic euglycemia therapy was initiated and shortly after was followed by a protocol of ILE (intralipid 20%, 1.5 ml/kg as bolus, followed by 0.25 ml/kg over 1h). The main finding attributed to ILE was an apparent rapid decrease in insulin resistance, despite a prolonged serum diltiazem elimination half-life. Diltiazem is a lipophilic cardiotoxic drug, which could be sequestered in an expanded plasma lipid phase. The mechanism of action of ILE is not known, including its role in insulin resistance and myocardial metabolism in calcium-channel blocker poisoning [16].

## Conclusion

The use of intravenous intralipid infusion instead of thrombolysis or primary percutaneous coronary intervention is first suggested in the medical literature.

## References


1. Rahman S, Li J, Bopassa JC, Umar S, Iorga A, et al. (2011) Phosphorylation of GSK-3β Mediates Intralipid-induced Cardioprotection against Ischemia/Reperfusion Injury. Anesthesiology 8(115): 242-253.
2. Li J, Iorga A, Sharma S, Youn JY, Partow-Navid R, et al. (2012) Intralipid, a clinically safe compound, protects the heart against ischemia-reperfusion injury more efficiently than cyclosporine-A. Anesthesiology. 117(4): 836-846.
3. Li J, Ruffenach G, Kararigas G, Cunningham CM, Motayagheni N, et al. (2017) Intralipid protects the heart in late pregnancy against ischemia/reperfusion injury via Caveolin2/STAT3/GSK-3β pathway. J Mol Cell Cardiol 102: 108-116.
4. Ma LL, Ge HW, Kong FJ, Qian LB, Hu BC, et al. (2014) Ventricular hypertrophy abrogates intralipid-induced cardioprotection by alteration of reperfusion injury salvage kinase/glycogen synthase kinase 3β signal. Shock 41(5): 435-442.
5. Cheng KH, Chu CS, Lin TH, Lee KT, Sheu SH, et al. (2015) Lipid paradox in acute myocardial infarction-the association with 30-day in-hospital mortality. Crit Care Med 43(6): 1255-1264.
6. Hu ZY, Peng XY, Liu F, Liu J (2014) Emulsified isoflurane protects rat heart in situ after regional ischemia and reperfusion. Fundam Clin Pharmacol 28(2): 190-198.
7. Huang H, Zhang W, Liu S, Yanfang C, Li T, et al. (2010) Cardioprotection afforded by St Thomas solution is enhanced by emulsified isoflurane in an isolated heart ischemia reperfusion injury model in rats. J Cardiothorac Vasc Anesth 24(1): 99-103.
8. Hu ZY, Luo NF, Liu J (2009) The protective effects of emulsified isoflurane on myocardial ischemia and reperfusion injury in rats. Can J Anaesth 56(2):115-25.
9. Hu ZY, Liu J (2009) Effects of emulsified isoflurane on haemodynamics and cardiomyocyte apoptosis in rats with myocardial ischaemia. Clin Exp Pharmacol Physiol 36(8): 776-783.
10. Rao Y, Wang YL, Chen YQ, Zhang WS, Liu J (2009) Protective effects of emulsified isoflurane after myocardial ischemia-reperfusion injury and its mechanism in rabbits. Chin J Traumatol 12(1): 18-21.
11. Rao Y, Wang YL, Zhang WS, Liu J (2008) Emulsified isoflurane produces cardiac protection after ischemia-reperfusion injury in rabbits. Anesth Analg 106(5): 1353-1359.
12. Papadopoulou JW, Willers TL, Samuels, DR Uncles (2012) The Use of Dye Surrogates to Illustrate Local Anesthetic Drug Sequestration by Lipid Emulsion: A Visual Demonstration of the Lipid Sink Effect. Regional Anesthesia & Pain Medicine 37(2): 183-187.
13. Grunbaum BM, Gilfix S, Gosselin, Blank DW, (2012) Analytical Interferences Resulting from Intravenous Lipid Emulsion. Clinical Toxicology (Phila) 50(9): 812-817.
14. Mauch J, Jurado OM, Spielmann N, Bettschart Wolfensberger R, Weiss M, et al. (2012)“Resuscitation Strategies from Bupivacaine-Induced Cardiac Arrest”. Pediatric Anesthesia 22(2): 124-129.
15. Mauch J, Jurado OM, Spielmann N, Bettschart Wolfensberger R, Weiss M, et al. (2011) Comparison of Epinephrine vs Lipid Rescue to Treat Severe Local Anesthetic Toxicity—An Experimental Study in Piglets. Pediatric Anesthesia 21(11): 1103-1108.
16. Montiel V1, Gougnard T, Hantson P (2011) “Diltiazem Poisoning Treated with Hyperinsulinemic Euglycemia Therapy and Intravenous Lipid Emulsion,” European Journal of Emergency Medicine 18(2): 121-123.

# Chapter 4

## Intralipid treatment for Alzheimer Disease

## Abstract

Currently, there is no cure for Alzheimer's. But drug and non-drug treatments may help with both cognitive and behavioral symptoms. Researchers are looking for new treatments to alter the course of the disease and improve the quality of life for people with dementia [1]. Intralipid treatment is first suggested here for the treatment of Alzheimer disease. It should be given intravenously on a monthly basis according to each patient's response. Clinical studies should be done in order to evaluate this new treatment modality.

**Keywords:** Alzheimer disease; Intralipid

## Dr. Aloysius "Alois" Alzheimer

Dr. Aloysius "Alois" Alzheimer (14 June 1864-19 December 1915) was a German psychiatrist and neuropathologist and a colleague of Emil Kraepelin. Alzheimer is credited with identifying the first published case of "presenile dementia", which Kraepelin would later identify as Alzheimer's disease [2].

In 1901, Dr. Alzheimer observed a patient at the Frankfurt Asylum named Auguste Deter. The 51-year-old patient had strange behavioral symptoms, including a loss of short-term memory; she became his obsession over the coming years. Auguste Deter was a victim of the politics of the time in the psychiatric community; the Frankfurt asylum was too expensive for her husband. Mr. Deter made several requests to have his wife moved to a less expensive facility, but Dr. Alzheimer intervened in these requests. Ms. Deter remained at the Frankfurt asylum, where Alzheimer had made a deal to receive her records and brain upon her death (3). On 8 April 1906, Ms. Deter died, and Dr. Alzheimer had her medical records and brain brought to Munich where he was working in Kraepelin's laboratory. With two Italian physicians, he used the staining techniques of Bielschowsky to identify amyloid plaques and neurofibrillary tangles. These brain anomalies would become identifiers of what later became known as Alzheimer's Disease [4].

### Alzheimer's disease

Some evidence indicates that disruption of the blood–brain barrier in Alzheimer's disease patients allows blood plasma containing amyloid beta (Aβ) to enter the brain where the Aβ adheres preferentially to the surface of astrocytes [5]. These findings have led to the hypotheses that [1] breakdown of the blood–brain barrier allows access of neuron- binding autoantibodies and soluble exogenous Aβ42 to brain neurons and [2] binding of these autoantibodies to neurons triggers and/or facilitates the internalization and accumulation of cell surface-bound Aβ42 in vulnerable neurons through their natural tendency to clear surface-bound autoantibodies via endocytosis. Eventually the astrocyte is overwhelmed, dies, ruptures, and disintegrates, leaving behind the insoluble Aβ42 plaque. Thus, in some patients, Alzheimer's disease may be caused (or more likely, aggravated) by a breakdown in the blood–brain barrier [6]. AD evolves with widespread loss of neurons and their synapses in such key brain areas as the cerebral cortex, entorhinal area, and hippocampus. At the gross level, this is evident as a general shrinkage of the brain away from the cranial vault and a corresponding dilation of the fluid-filled brain ventricles to fill the void. At the microscopic level, there are several different pathological changes that occur, but one consistent pathological hallmark is the early appearance of amyloid plaques. These plaques are abundant and widely scattered throughout AD-vulnerable brain regions. They contain a 42-amino acid protein fragment, known as amyloid 1-42 (A42),that is derived from the sequential enzymatic cleavage of the much larger amyloid precursor protein. Once produced, A42 has the ability to self-assemble into nondegradeable fibrils that can persist in AD brains long after the neurons in which they accumulated have died [7].

Alzheimer's disease (AD) was first described in 1906 by German psychiatrist Alois Alzheimer, who observed abnormal clumps and tangled bundles of protein in the brain of a patient who experienced memory loss, language difficulties, and abnormal behaviour (8). The risk of developing AD increases exponentially with age and is the leading cause of dementia and the most common neurodegenerative disease in the elderly; prevalence rates in 65-74 year olds are estimated to be 3%, rising to 19% for 75–85 year olds, and nearly 50% in those aged over 85 (9). AD is more common among older people but it is not a normal part of ageing. As the global population ages, the prevalence of AD is expected to rise from 36 million to 115 million sufferers by 2050 [9].

It is estimated that over 5% of the U.S. population over 65 and over 15% of the U.S. population over 85 are beset with some form of Alzheimer's disease (Cross, A. J., Eur J Pharmacol (1982) 82:77-80; Terry, R. D., et al., Ann Neurol (1983) 14:497506). It is believed that the principal cause for confinement of the elderly in long term care facilities is due to this disease, and approximately 65% of those dying in skilled nursing facilities suffer from it [10].

### Intravenous lipid emulsion

The idea that intravenous lipid emulsion could be used to affect the pharmacokinetics of a drug in circulation was first introduced fifty years ago. It was shown that rats infused lipid emulsion after an injection of the barbiturate thiopental emerged more rapidly from anaesthesia than rats infused the same volume of fat-free solution [11]. Other early studies were published on the effect of lipid emulsion on chlorpromazine availability in rabbits [12], and the effect of lipid emulsion on the elimination of phenytoin [13]. Although the studies show some effect of lipid emulsion, this did not kindle more widespread interest in the subject. The serendipitous discovery of the apparently shielding effect of a large intravenous dose of lipid emulsion against bupivacaine toxicity in rats triggered renewed interest in the field [14]. Additional experimental animal and isolated heart studies were performed [15,16], and although efficacy and safety had not been established by clinical trials, clinicians soon applied lipid therapy to seemingly hopeless cases of severe intoxication [17]. Intravenous lipid emulsion therapy for severe intoxication is a relatively young field. Although a few early studies on the pharmacokinetic effects of intravenous lipid emulsion exist [11-13,18], its use as a treatment for severe intoxication was proposed as late as 1998 [14]. Since this proposal, no randomized controlled human trials have been published. Thus, the evidence supporting this use of lipid emulsion consists only of animal studies and human case reports of varying quality [19].

Amyloid beta-peptide (Abeta) is a key molecule in Alzheimer disease (AD). Cerebral deposition of Abeta was earlier thought to initiate the pathological cascade of AD, including

the formation of senile plaques and neurofibrillary tangles, neuronal loss, and dementia. According to the classical amyloid hypothesis, the aggregation of Abeta into insoluble beta-sheet fibrils plays an important role in its neurotoxicity. However, this hypothesis is paradoxical: The concentrations of Abeta required for fibrillization and neurotoxicity are higher than its physiological concentrations. Cognitive decline in AD patients is not correlated with the levels of senile plaque formation or insoluble Abeta formation; instead it correlates with the levels of synapse loss and the levels of soluble Abeta. These observations suggest the existence of soluble toxic forms of Abeta in AD brains; these forms have recently been identified to be oligomeric assemblies of Abeta. At present, AD is believed to begin with synaptic dysfunction caused by soluble Abeta oligomers. This hypothesis termed the oligomer hypothesis, is based on the following observations: The levels of Abeta oligomers are high in AD brains. Exogenous Abeta oligomers at physiological concentrations cause synaptic and cognitive dysfunction in vivo and synapse loss and neuronal death in vitro. Furthermore, we observed that the E693delta mutation in the amyloid precursor protein found in AD patients causes disease by increasing the formation of Abeta oligomers without inducing the formation of Abeta fibrils or senile plaques. Currently, senile plaque formation is thought to occur in order to protect neurons from the toxicity of diffusible Abeta oligomers by sequestering them into deposits. Thus, soluble Abeta oligomers play a more important role in the etiology of AD insoluble Abeta fibrils [20].

The defining features of Alzheimer disease (AD) include conspicuous changes in both brain histology and behavior. The AD brain is characterized microscopically by the combined presence of 2 classes of abnormal structures, extracellular amyloid plaques and intraneuronal neurofibrillary tangles, both of which comprise highly insoluble, densely packed filaments. The soluble building blocks of these structures are amyloid-β(Aβ) peptides for plaques and tau for tangles. Amyloid-β peptides are proteolytic fragments of the transmembrane amyloid precursor protein, whereas tau is a brain-specific, axon-enriched microtubule-associated protein. The behavioral symptoms of AD correlate with the accumulation of plaques and tangles, and they are a direct consequence of the damage and destruction of synapses that mediate memory and cognition. Synapse loss can be caused by the failure of live neurons to maintain functional axons and dendrites or by neuron death. During the past dozen years, a steadily accumulating body of evidence has indicated that soluble forms of Aβ and tau work together, independently of their accumulation into plaques and tangles, to drive healthy neurons into the diseased state and that hallmark toxic properties of Aβ require tau. For instance, acute neuron death, delayed neuron death following ectopic cell cycle reentry, and synaptic dysfunction are triggered by soluble, extracellular Aβ species and depend on soluble, cytoplasmic tau. Therefore, Aβ is upstream of tau in AD pathogenesis and triggers the conversion of tau from a normal to a toxic state, but there is also evidence that toxic tau enhances Aβ toxicity via a feedback loop. Because soluble toxic aggregates of both Aβ and tau can self-propagate and spread throughout the brain by prionlike mechanisms, successful therapeutic intervention for AD would benefit from detecting these species before plaques, tangles, and cognitive impairment become evident and from interfering with the destructive biochemical pathways that they initiate [21].

The increasing prevalence of Alzheimer's disease (AD) and a lack of effective prevention or disease-modifying therapies are global challenges with devastating personal, social and economic consequences. The amyloid β (Aβ) hypothesis posits that cerebral β-amyloidosis is a critical early event in AD pathogenesis. However, failed clinical trials of Aβ-centric drug candidates have called this hypothesis into question. Whereas we acknowledge that the Aβ hypothesis is far from disproven, we here re-visit the links between Aβ, tau and neurodegeneration. We review the genetics, epidemiology and pathology of sporadic AD and give an updated account of what is currently known about the molecular pathogenesis of the disease [22]. The amyloid hypothesis has driven drug development strategies for Alzheimer's disease for over 20 years. We review why accumulation of amyloid-beta (Aβ) oligomers is generally considered causal for synaptic loss and neurodegeneration in AD. We elaborate on and update arguments for and against the amyloid hypothesis with new data and interpretations, and consider why the amyloid hypothesis may be failing therapeutically. We note several unresolved issues in the field including the presence of Aβ deposition in cognitively normal individuals, the weak correlation between plaque load and cognition, questions regarding the biochemical nature, presence and role of Aβ oligomeric assemblies in vivo, the bias of pre-clinical AD models toward the amyloid hypothesis and the poorly explained pathological heterogeneity and comorbidities associated with AD. We also illustrate how extensive data cited in support of the amyloid hypothesis, including genetic links to disease, can be interpreted independently of a role for Aβ in AD. We conclude it is essential to expand our view of pathogenesis beyond Aβ and tau pathology and suggest several future directions for AD research, which we argue will be critical to understanding AD pathogenesis [23].

The aggregation and deposition of amyloid-beta (Abeta) in the brain is thought to be an early event in the pathology of Alzheimer's disease (AD). Many studies have reported the association of Abeta with lipoproteins from plasma suggesting an involvement of lipoprotein particles in Abeta transport. Chylomicron-like lipid emulsions, resembling chylomicrons in composition, size and metabolism were prepared in the presence of [125I] Abeta1-40. Abeta was found to associate significantly with these lipid emulsions during their preparation. The chylomicron-like emulsions containing Abeta were then injected into a lateral ear vein of conscious rabbits and blood sampled at regular intervals up to 30 mins. It was observed that there was

no difference in the plasma clearance of [125I] Abeta and that of the 3H-cholesteryl ester, a marker of the emulsion particles, demonstrating that Abeta remains associated with these particles throughout both their lipolysis and tissue uptake. Our results show that Abeta can be metabolised in association with triglyceride rich lipoproteins (TRLs). In addition we report the presence of specific markers of TRLs of hepatic and intestinal origin in human CSF thus suggesting a potential means of cerebral Abeta delivery [24].

Docosahexaenoic acid (DHA), the main n-3 polyunsaturated fatty acid (PUFA) in membranes, is particularly abundant in brain cells. Decreased cerebral concentrations of DHA, resulting from dietary n-3 deficiency, are associated with impaired cognitive function. Because the cellular causes of this impairment are still unknown, we need in vitro models that mimic the variations in n-3/n-6 PUFA seen in vivo. We have compared the PUFA profiles of hamster astrocytes cultured in medium supplemented with long-chain PUFA [DHA and/or arachidonic acid (AA)] with those of brain tissue from hamsters fed an n-6/n-3 PUFA-balanced diet or one lacking n-3 PUFA. Astrocytes were obtained from the brain cortex of newborn hamsters and cultured in minimum essential medium + 5% fetal calf serum (FCS) supplemented with DHA and/or AA for 10 days. The astrocytes cultured in medium + FCS had low n-3 PUFA contents, comparable to those of brain tissue from hamsters fed an n-3-deficient diet. We have shown that astrocytes grown in medium supplemented with DHA and/or AA, plus alpha-tocopherol to prevent lipid peroxidation, incorporated large amounts of these long-chain PUFA, so that the n-6/n-3 PUFA compositions of the phosphatidylethanolamine and phosphatidylcholine, the two main classes of membrane phospholipids, were greatly altered. Astrocytes cultured in medium plus DHA had a more physiological n-3 status, grew better, and retained their astrocyte phenotype. Thus astrocytes in culture are likely to be physiologically relevant only when provided with adequate DHA. This reliable method of altering membrane phospholipid composition promises to be useful for studying the influence of n-6/n-3 imbalance on astrocyte function [25].

Rat neural stem cells/neural progenitors (NSC/NP) are generally grown in serum-free medium. In this study, NSC/NP were supplemented with the main long-chain polyunsaturated fatty acids (PUFAs) present in the brain, arachidonic acid (AA), or docosahexaenoic acid (DHA), and were monitored for their growth. Lipid and fatty acid contents of the cells were also determined. Under standard conditions, the cells were characterized by phospholipids displaying a highly saturated profile, and very low levels of PUFAs. When cultured in the presence of PUFAs, the cells easily incorporated them into the phospholipid fraction. We also compared the presence of three membrane proteins in the lipid raft fractions: GFR and connexin 43 contents in the rafts were increased by DHA supplementation, whereas Gbeta subunit content was not significantly modified. The restoration of DHA levels in the phospholipids could profoundly affect protein localization and, consequently, their functionalities [26].

Lipids are the fundamental structural components of biological membranes. For a long time considered as simple barriers segregating aqueous compartments, membranes are now viewed as dynamic interfaces providing a molecular environment favorable to the activity of membrane-associated proteins. Interestingly, variations in membrane lipid composition, whether quantitative or qualitative, play a crucial role in regulation of membrane protein functionalities. Indeed, a variety of alterations in brain lipid composition have been associated with the processes of normal and pathological aging. Although not establishing a direct cause-and-effect relationship between these complex modifications in cerebral membranes and the process of cognitive decline, evidence shows that alterations in membrane lipid composition affect important physicochemical properties notably impacting the lateral organization of membranes, and thus microdomains. It has been suggested that preservation of microdomain functionality may represent an effective strategy for preventing or decelerating neuronal dysfunction and cerebral vulnerability, processes that are both aggravated by aging. The working hypothesis developed in this review proposes that preservation of membrane organization, for example, through nutritional supplementation of docosahexaenoic acid, could prevent disturbances in and preserve effective cerebral function [27].

To date, no study has been performed to evaluate the antidotal effect of intravenous lipid emulsion on the poisoned patients' level of consciousness and routine metabolic profile tests in non-local anesthetic drug overdose. [28] aim was to evaluate the effect of intravenous intralipid administration as an antidote on the poisoned patients' Glasgow Coma Scale (GCS), hemodynamic parameters, arterial blood gas analysis, and routine metabolic profile tests (i.e., urea, glucose, sodium, and potassium) in the setting of non-local anesthetic drug overdose. In this randomized controlled trial, a total of 30 patients with non-local anesthetic drug intoxication were enrolled and randomly assigned into case (n=15) and control (n=15) groups. In the case group, all patients received 10 cc/kg intralipid 10% infusion. The patients in the control group just received the supportive care. Patients' demographic and clinical characteristics and results of their laboratory tests were evaluated at presentation and 6 hours after that.

Mean age was 23 +/- 5 and 28 +/- 11 years in cases and controls, respectively. There were no significant statistical differences between these two groups regarding age, gender, elapsed time between intubation and extubation, and need for intubation and/or mechanical ventilation ($p = 0.70$ and $p = 1.00$, respectively). Also, systolic blood pressure, pulse rate, mean rate pressure product, respiratory rate, results of arterial blood gas analyses, serum sodium, potassium, urea, and creatinine on presentation and six hours later were not statistically significantly different between the two study groups. However, a significant difference was

found between the two groups in terms of GCS difference ($p = 0.048$) and blood glucose six hours after presentation ($p = 0.04$). In the setting of non-local anesthetic drug overdose, intravenous intralipid infusion can increase GCS and interestingly, decrease the blood glucose. Lipid emulsions are widely used as carriers for hypnotics such as propofol, etomidate, and diazepam. It is assumed that the emulsions alone exert no effect on cellular functions nor influence the pharmacokinetics, pharmacodynamics, or anesthetic and analgetic potency of the hypnotics they carry. To elucidate possible interactions between lipid emulsions and cell membranes, in particular membrane-bound proteins, we investigated the effects of commercially available lipid emulsions on the cell membranes of cultured cortical neurons from the mouse by using the whole-cell configuration of the patch-clamp technique. Of nine lipid emulsions tested, three, i.e., Intralipid, Structolipid, and, to a much lesser extent, Abbolipid, activated membrane currents in the neuronal cells in a dilution-dependent manner. The emulsion-induced currents were not affected by picrotoxin or bicuculline but were inhibited by DL-AP5 and ketamine. The voltage dependence of the currents was influenced by the presence of Mg(2+) in a way that is typical for currents conducted by N-methyl-D-aspartate receptor channels. We conclude that Intralipid, Structolipid, and Abbolipid activate N-methyl-D-aspartate receptor channels in cortical neurons. Lipid emulsions are widely used as carriers for hypnotics such as propofol, etomidate, or diazepam. We tested nine commercially available lipid emulsions and demonstrate that three of them--Intralipid, Structolipid, and Abbolipid--activate NMDA receptor channels in the membranes of cortical neuronal cells [29].

Haywood SC et al. [30] tested the hypothesis that lipids could act as an alternative fuel source in the brain during insulin-induced hypoglycemia. Male Sprague-Dawley rats were subjected to hyperinsulinemic (5 mU.kg(-1).min(-1)) hypoglycemic (approximately 50 mg/dl) clamps. In protocol 1, intralipid (IL), a fat emulsion, was infused intravenously to prevent the fall in free fatty acid levels that occurs in response to hyperinsulinemic hypoglycemia. Intravenous lipid infusion did not alter the counterregulatory responses to hypoglycemia. To test whether IL could have central effects in mediating the counterregulatory response to hypoglycemia, in protocol 2 the brains of precannulated rats were intracerebroventricularly (icv) infused with IL or artificial cerebrospinal fluid (aCSF) as control. Unexpectedly, the epinephrine and glucagon response to hypoglycemia was significantly augmented with icv IL infusion. To determine whether central IL infusion could restore defective counterregulation, in protocol 3 rats were made recurrently hypoglycemic (RH) for 3 days and on the 4th day underwent hyperinsulinemic hypoglycemic clamps with icv IL or aCSF infusion. RH rats had the expected impaired epinephrine response to hypoglycemia, and icv IL infusion again significantly augmented the epinephrine response in RH rats to normal. With regard to our experimental model of hypoglycemic counterregulation, we conclude that 1) systemic lipid infusion did not alter the counterregulatory response to hypoglycemia, 2) the icv infusion of lipids markedly increased CSF FFA levels and paradoxically augmented the epinephrine and glucagon responses, and 3) the blunted sympathoadrenal response in recurrently hypoglycemic rats was completely normalized with the icv lipid infusion. It is concluded that, in the setting of insulin-induced hypoglycemia, increased brain lipids can enhance the sympathoadrenal response. Lipid infusion reverses systemic local anesthetic toxicity. The acceptable upper limit for lipid administration is unknown and has direct bearing on clinical management. We hypothesize that high volumes of lipid could have undesirable effects and sought to identify the dose required to kill 50% of the animals (LD(50)) of large volume lipid administration.

Intravenous lines and electrocardiogram electrodes were placed in anesthetized, male Sprague-Dawley rats. Twenty percent lipid emulsion (20, 40, 60, or 80 mL/kg) or saline (60 or 80 mL/kg), were administered over 30 mins; lipid dosing was assigned by the Dixon “up-and-down” method. Rats were recovered and observed for 48 hrs then euthanized for histologic analysis of major organs. Three additional rats were administered 60 mL/kg lipid emulsion and euthanized at 1, 4, and 24 hrs to identify progression of organ damage. The maximum likelihood estimate for LD(50) was 67.72 (SE, 10.69) mL/kg. Triglycerides were elevated immediately after infusion but returned to baseline by 48 hrs when laboratory abnormalities included elevated amylase, aspartate aminotransferase, and serum urea nitrogen for all lipid doses. Histologic diagnosis of myocardium, brain, pancreas, and kidneys was normal at all doses. Microscopic abnormalities in lung and liver were observed at 60 and 80 mL/kg; histopathology in the lung and liver was worse at 1 hr than at 4 and 24 hrs.

The LD (50) of rapid, high volume lipid infusion is an order of magnitude greater than doses typically used for lipid rescue in humans and supports the safety of lipid infusion at currently recommended doses for toxin-induced cardiac arrest. Lung and liver histopathology was observed at the highest infused volumes [31]. Intravenous lipid emulsion has been suggested as treatment for severe intoxications caused by lipophilic drugs, including tricyclic antidepressants. We investigated the effect of lipid infusion on plasma and tissue concentrations of amitriptyline and haemodynamic recovery, when lipid was given after amitriptyline distribution into well-perfused organs. Twenty anaesthetized pigs received amitriptyline intravenously 10 mg/kg for 15 min. Thirty minutes later, in random fashion, 20% Intralipid(®) (Lipid group) or Ringer’s acetate (Control group) was infused 1.5 ml/kg for 1 min. followed by 0.25 ml/kg/min. for 29 min. Arterial and venous plasma amitriptyline concentrations and haemodynamics were followed till 75 min. after amitriptyline infusion. Then, frontal brain and heart apex samples were taken for amitriptyline measurements. Arterial plasma total amitriptyline concentrations were higher in the Lipid than in the Control group ($p < 0.03$)

from 20 min. on after the start of the treatment infusions. Lipid emulsion reduced brain amitriptyline concentration by 25% ($p = 0.038$) and amitriptyline concentration ratios brain/arterial plasma ($p = 0.016$) and heart/arterial plasma ($p = 0.011$). There were no differences in ECG parameters and no severe cardiac arrhythmias occurred. Two pigs developed severe hypotension during the lipid infusion and were given adrenaline. In conclusion, lipid infusion, given not earlier than after an initial amitriptyline tissue distribution, was able to entrap amitriptyline back into plasma from brain and possibly from other highly perfused, lipid-rich tissues. In spite of the entrapment, there was no difference in haemodynamics between the groups [32].

Malathion is one of the most widely used organophosphate pesticides and herbicides. It has given rise to major clinical problems by its poisoning in all over the world. Malathion also a highly lipophilic agent, and tends to accumulate within lipid-rich tissue like a brain in the body, causing toxicity. Therefore, the study was aimed to investigate if there is a possible beneficial effect of using intralipid fat emulsion (IFE) on the neurotoxicity, and to detect it time-dependently at the beginning, 6th and 12th hours of M intoxication. Forty-eight rats were randomly divided into six groups including: control (C), Lipid (L) group (18.6 mL/kg oral IFE), Malathion (M) group (10 mg/kg oral M), M0L group (IFE treated after immediate from M), M6L group (IFE treated after 6 hours from M), M12L group (IFE treated after 12 hours from M). M group in comparison with all others group, there was an increase in the total oxidant status (TOS) level. M group in comparison with C, L, M0L groups, it was seen significantly decrease in the total antioxidant capacity (TAC) level. Interestingly, M group in comparison with M6L and M12L groups, there was no significant difference among these groups in terms of the TAC levels. Although there was no significant difference among C, L and M0L groups in terms of both TAC and TOS levels, but was significant difference C, L groups in comparison with M6L, M12L groups in terms of TAC levels. C group in comparison with L, M0L, M6L, M12L groups in terms of TOS levels, there was no significant difference. These findings have indicated that IFE seriously reduced TOS levels in all the groups depending on time. Also, M0L group in comparison with M6L and M12L groups, there was significantly increase of the TAC levels. There was no statistically significant difference between M6L and M12L groups. These biochemical results were confirmed with immunohistochemical results.

The study has had some certain evidence that IFE is a promising safe therapy for acutely intoxicated cases by organophosphate. It is much more effective if used at the beginning of organophosphate poisoning. As such, there is no need to avoid using IFE in clinical practice [33]. Chlorpyriphos is one of the most widely used organophosphate (OP) insecticide in agriculture with potential toxicity. Current post-exposure treatments consist of anti-cholinergic drugs and oxime compounds. We studied the effects of intralipid and caffeic acid phenethyl ester (CAPE) on chlorpyriphos toxicity to compose an alternative or supportive treatment for OP poisoning. Forty-nine rats were randomly divided into seven groups. Chlorpyriphos was administered for toxicity. Intralipid (IL) and CAPE administered immediately after chlorpyriphos. Serum acetylcholinesterase (AChE) level, total oxidant status (TOS), total antioxidant response (TAR), and histologic examination of cerebellum and brain tissue with Hematoxylin-Eosin and immunohistochemical dyes were examined.

Serum enzym levels showed that chlorpyriphos and CAPE inhibited AChE while IL alone had no effect, chlorpyriphos and CAPE intensifies the inhibition effect. Significant difference at AChE levels between the chlorpyriphos+IL and chlorpyriphos+CAPE verified that IL has a protective effect on AChE inhibition. TAR levels were significantly increased in all groups except chlorpyriphos group, TOS levels revealed that CAPE and IL decrease the amount of oxidative stress. Histologic examination revealed that neuronal degeneration was slightly decreased at chlorpyriphos+IL group, but CAPE had a significant effect on protection of neuronal degeneration. The results of this study gave us three key points. 1) AChE activity is important for diagnosis of OP intoxication but it has no value for determining the neuro-degeneration. 2) CAPE inhibits AChE activity and may increase the muscarinic-nicotinic hyperactivation. Therefore it should not be used for treatment of OP intoxication. 3) IL decreases the severity of neurodegeneration and symptoms of OP intoxication and it can be used as a supportive agent [34].

Intravenous lipid emulsion has been suggested as treatment for local anaesthetic toxicity, but the exact mechanism of action is still uncertain. Controlled studies on the effect of lipid emulsion on toxic doses of local anaesthetics have not been performed in man. In randomized, subject-blinded and two-phase cross-over fashion, eight healthy volunteers were given a 1.5 ml/kg bolus of 20% Intralipid (®) (200 mg/ml) or Ringer's acetate solution intravenously, followed by a rapid injection of lidocaine 1.0 mg/kg. Then, the same solution as in the bolus was infused at a rate of 0.25 ml/kg/min. for 30 min. Electroencephalography (EEG) was recorded, and 5 min. after lidocaine injection, the volunteers were asked to report subjective symptoms. Total and un-entrapped lidocaine plasma concentrations were measured from venous blood samples. EEG band power changes (delta, alpha and beta) after the lidocaine bolus were similar during lipid and during Ringer infusion. There were no differences between infusions in the subjective symptoms of central nervous system toxicity. Lidocaine was only minimally entrapped in the plasma by lipid emulsion, but the mean un-entrapped lidocaine area under concentration-time curve from 0 to 30 min. was clearly smaller during lipid than Ringer infusion (16.4 versus 21.3 mg × min/l, $p = 0.044$). Intravenous lipid emulsion did not influence subjective toxicity symptoms nor affect the EEG changes caused by lidocaine [35].

### Intralipid treatment for Alzheimer Disease

Lange DB et al. [36] described a case of severe central

nervous system toxicity after an overdose of lidocaine by local infiltration in a peritoneal dialysis patient and subsequent treatment of the toxicity with lipid emulsion. A 31-year-old male received an iatrogenic overdose of 1600 mg of lidocaine 2% by infiltration during an attempt to remove and replace a peritoneal dialysis catheter. Within 10 minutes after the last lidocaine injection, the patient exhibited features of local anesthetic toxicity, which included tachycardia, hypertension, shortness of breath, dizziness, and a choking sensation that progressed to hallucinations, dysarthria, and uncoordinated, weak limb movement. Within 10 minutes after administration of a single 1.5-mg/kg intravenous bolus of 1.5 mL/kg [corrected], the patient improved dramatically. After observation overnight in a monitored care setting, the patient was discharged home with no apparent neurologic sequelae.

Systemic toxicity due to regional anesthesia with local anesthetic agents such as lidocaine has been well described in the medical literature. The use of lipid emulsion as an antidote to the toxicity of local anesthetics and other lipophilic drugs has been suggested as a valuable intervention in both early, rapidly progressive toxicity, as well as toxicity that is refractory to standard treatment. Patients with advanced chronic kidney disease may be more susceptible to systemic effects of lidocaine due to decreased drug elimination. Central nervous system toxicity due to an overdose of lidocaine was quickly reversed by intravenous lipid emulsion in our patient [36].

The major component fatty acids in Intralipid are linoleic acid (44-62%), oleic acid (19-30%), palmitic acid (7-14%), a-linolenic acid (4-11%) and stearic acid (1.4-5.5%) (37). Oxidative stress is a hallmark of many degenerative disorders. The brain is particularly vulnerable to this phenomenon owing to high oxygen consumption, enrichment in polyunsaturated fatty acids (PUFAs) and high levels in redox metal ions [38]. Lipid peroxidation products (LPPs) have been found in brain, cerebrospinal fluid and plasma from patients with Alzheimer's disease (AD) [38]. Primary substrates for lipid peroxidation are PUFAs and include ω-6 fatty acids (for example, linoleic acid and arachidonic acid) as well as ω-3 fatty acids (for example, docosahexaenoic acid). Reactive oxygen species are responsible for starting the chain by the production of an unstable lipid radical that is converted to a lipid peroxyl radical, leading to the peroxidation of other fatty acids (propagation). This chain reaction stops (termination) when two radicals react to produce a non-radical species, or as a result of antioxidants (for example, vitamin C and vitamin E) and enzymes of the superoxide dismutase, catalase and peroxidase families [38]. Oxidized PUFAs are further degraded to toxic products, such as 4-hydroxy-2-nonenal (HNE), acrolein and other short-chain aldehydes. Importantly, amyloid-β has been shown to cause oxidative stress through its interaction with transition metal ions, such as Cu2+ and Zn2+, which are enriched in senile plaques [38]. Amyloid-β can reduce these metal ions, thus producing hydrogen peroxide. During this process, amyloid-β becomes oxidized, thereby leading to the crosslinking of some of its residues' side-chains and the formation of aggregate-prone adducts. Alternatively, hydrogen peroxide can be generated catalytically from Cu2+- or Zn2+-bound amyloid-β using other electron donors (for example, PUFAs and cholesterol), a process leading to the generation of toxic LPPs, such as oxysterol and HNE. Finally, amyloid-β itself can be crosslinked by HNE. Key challenges in the field are to understand the role of LPP accumulation in the progression of AD-associated manifestations.

The linoleic acid metabolism was examined in the brain cortex of 4 month-old and 24 month-old rats. After the injection of [1-14C]-linoleate into the lateral ventricle of the brain the animals were sacrificed at 1,3 and 6 hours from the injection. The linoleate (18:2) incorporation into lipids, the presence of fatty acid peroxidation products, as well as the 18:2 transformation into elongated and desaturated derivatives were determined. Both an age-related reduction in linoleate incorporation rate into glycerophospholipids and a decrease in fatty acid turnover were found. Furthermore, in glycerophospholipids from 24 month-old rat brain cortex a higher level of hydroperoxide derivative of linoleate was found as compared to 4 month-old animals, and this damaged fatty acid is eliminated more slowly in aged rats than in adults. Finally, unlike 4 month-old animals, a stimulation of the transformation rate of linoleate into desaturation (6,9,12-C18:3) and elongation (8,11,14,C20:3) products was found in 24 month-old rat brain cortex. On the contrary, as far as arachidonic acid (one of the most important end products of the mechanism of linoleate modification) is concerned, the differences between aged and control animals were small, making it quite difficult to attribute a physiological meaning to this phenomenon [39].

Alzheimer's disease and associated diseases constitute a major public health concern worldwide. Nutrition-based, preventive strategies could possibly be effective in delaying the occurrence of these diseases and lower their prevalence. Arachidonic acid is the second major polyunsaturated fatty acid (PUFA) and several studies support its involvement in Alzheimer's disease. The objective of this review is to examine how dietary arachidonic acid contributes to Alzheimer's disease mechanisms and therefore to its prevention. First, we explore the sources of neuronal arachidonic acid that could potentially originate from either the conversion of linoleic acid, or from dietary sources and transfer across the blood-brain-barrier. In a second part, a brief overview of the role of the two main agents of Alzheimer's disease, tau protein and Aβ peptide is given, followed by the examination of the relationship between arachidonic acid and the disease. Third, the putative mechanisms by which arachidonic acid could influence Alzheimer's disease occurrence and evolution are presented. The conclusion is devoted to what remains to be determined before integrating arachidonic acid in the design of preventive strategies against Alzheimer's disease

and other neurodegenerative diseases [40].

Insulin resistance and type 2diabetes are associated with an increased risk of neurodegenerative diseases. Brain-derived neurotrophic factor (BDNF) regulates neuronal differentiation and synaptic plasticity, and its decreased levels are supposed to play a role in the pathogenesis of Alzheimer's disease and other disorders. The aim of the current study was to estimate the effects of hyperinsulinemia and serum free fatty acids (FFA) elevation on circulating BDNF concentration in humans. We studied 18 healthy male subjects (mean age 25.6 ± 3.0 years; mean BMI 26.6 ± 4.8 kg/m(2)). Serum and plasma BDNF concentration was measured in the baseline state and in the 120 and 360 min of euglycemic hyperinsulinemic clamp with or without intralipid/heparin infusion. Furthermore, plasma BDNF was measured in 20 male subjects (mean age 22.7 ± 2.3 years; mean BMI 24.9 ± 1.5 kg/m(2)) 360 min after a high-fat meal.

Insulin sensitivity was reduced by ~40% after 6 h of intralipid/heparin infusion ($P < 0.001$). During both clamps, serum and plasma BDNF followed the same pattern. Hyperinsulinemia had no effect on circulating BDNF. Raising FFA had no effect on circulating BDNF in 120 min; however, it resulted in a significant decrease by 43% in serum and by 35% in plasma BDNF after 360 min ($P = 0.005$ and 0.006, respectively). High-fat meal also resulted in a decrease by 27.8% in plasma BDNF ($P = 0.04$). Our data show that raising FFA decreases circulating BDNF. This might indicate a potential link between FFA-induced insulin resistance and neurodegenerative disorders [41].

Rodriguez-Navas C et al. [42] analyzed the fatty acid profile of brains and plasma from male and female mice fed chow or a western-style high fat diet (WD) for 16 weeks to determine if males and females process fatty acids differently. Based on the differences in fatty acids observed in vivo, we performed in vitro experiments on N43 hypothalamic neuronal cells to begin to elucidate how the fatty acid milieu may impact brain inflammation. Using a comprehensive mass spectrometry fatty acid analysis, which includes a profile for 52 different fatty acid isomers, we assayed the plasma and brain fatty acid composition of age-matched male and female mice maintained on chow or a WD. Additionally, using the same techniques, we determined the fatty acid composition of N43 hypothalamic cells following exposure to palmitic and linoleic acid, alone or in combination.

The data demonstrate there is a sexual dimorphism in brain fatty acid content both following the consumption of the chow diet, as well as the WD, with males having an increased percentage of saturated fatty acids and reductions in ω6-polyunsaturated fatty acids when compared to females. Interestingly, we did not observe a sexual dimorphism in fatty acid content in the plasma of the same mice. Furthermore, exposure of N43 cells to the ω6-PUFA linoleic acid, which is higher in female brains when compared to males, reduces palmitic acid-induced inflammation. The data suggest male and female brains, and not plasma, differ in their fatty acid profile. This is the first time, to our knowledge, lipidomic analyses has been used to directly test the hypothesis there is a sexual dimorphism in brain and plasma fatty acid composition following consumption of the chow diet, as well as following exposure to the WD.

## Blood-Brain Barrier

Treatment strategies for Alzheimer's disease (AD) are still elusive. Thus, new strategies are needed to understand the pathogenesis of AD in order to provide suitable therapeutic measures. Available evidences suggest that in AD, passage across the blood-brain barrier (BBB) and transport exchanges for amyloid-β-peptide (ABP) between blood and the central nervous system (CNS) compartments play an important regulatory role for the deposition of brain ABP. New evidences suggest that BBB is altered in AD. Studies favoring transport theory clearly show that ABP putative receptors at the BBB control the level of soluble isoform of ABP in brain. This is achieved by regulating influx of circulating ABP into brain via specific receptor for advanced glycation end products (RAGE) and gp330/megalin-mediated transcytosis. On the other hand, the efflux of brain-derived ABP into the circulation across the vascular system via BBB is accomplished by low-density receptor-related protein-1 (LRP1). Furthermore, an increased BBB permeability in AD is also likely since structural damage of endothelial cells is quite frequent in AD brain. Thus, enhanced drug delivery in AD is needed to induce neuroprotection and therapeutic success. For this purpose, nanodrug delivery could be one of the available options that require active consideration for novel therapeutic strategies to treat AD cases. This review is focused on these aspects and provides new data showing that BBB plays an important role in AD-induced neurodegeneration and neurorepair [43].

The blood-brain barrier (BBB) is a tightly regulated barrier in the central nervous system. Though the BBB is thought to be intact during neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD), recent evidence argues otherwise. Dysfunction of the BBB may be involved in disease progression, eliciting of peripheral immune response, and, most importantly, altered drug efficacy. In this review, we will give a brief overview of the BBB, its components, and their functions. We will critically evaluate the current literature in AD and PD BBB pathology resulting from insult, neuroinflammation, and neurodegeneration. Specifically, we will discuss alterations in tight junction, transport and endothelial cell surface proteins, and vascular density changes, all of which result in altered permeability. Finally, we will discuss the implications of BBB dysfunction in current and future therapeutics. Developing a better appreciation of BBB dysfunction in AD and PD may not only provide novel strategies in treatment, but will prove an interesting milestone in understanding neurodegenerative disease etiology and progression [44].

It is not clear whether Alzheimer's Disease (AD) is primarily a neurodegenerative disorder or not. A body of evidence

suggests that vascular disorder in brains of individuals with AD contributes to the extremes of this disease. This raises a question whether Alzheimer's dementia is secondary to vascular dysfunction in the central nervous system (CNS) and, therefore, the neurodegeneration that follows is a consequence of inadequate cerebral blood flow, altered brain metabolism and failure in physiological functions of brain endothelium which represents a site at the blood-brain barrier (BBB). In this paper the evidence for a primary role of the CNS vascular system in pathogenesis of Alzheimer's dementia is reviewed to show how alterations in transport across the BBB contribute to development of cerebral beta-amyloidosis in AD. In addition, vascularly-based therapeutic strategies to limit the development of beta-amyloidosis and to remove amyloid and plaques from the CNS of AD individuals are discussed [35].

Protection of the brain is strengthened by active transport and ABC transporters. P-glycoprotein (P-gp) at the blood-brain barrier (BBB) functions as an active efflux pump by extruding a substrate from the brain, which is important for maintaining loco-regional homeostasis in the brain and protection against toxic compounds. Importantly, dysfunctional BBB P-gp transport is postulated as an important factor contributing to accumulation of aggregated protein in neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Furthermore, P-gp is a major factor in mediating resistance to brain entry of numerous exogenous compounds, including toxins that can be involved in PD pathogenesis. This review highlights the role of altered P-gp function in the pathogenesis and progression of neurodegenerative disease. Also the implications of alterations in P-gp function for the treatment of these diseases are discussed [46].

The blood-brain barrier (BBB) is a tightly regulated barrier in the central nervous system. Though the BBB is thought to be intact during neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD), recent evidence argues otherwise. Dysfunction of the BBB may be involved in disease progression, eliciting of peripheral immune response, and, most importantly, altered drug efficacy. In this review, we will give a brief overview of the BBB, its components, and their functions. We will critically evaluate the current literature in AD and PD BBB pathology resulting from insult, neuro inflammation, and neurodegeneration. Specifically, we will discuss alterations in tight junction, transport and endothelial cell surface proteins, and vascular density changes, all of which result in altered permeability. Finally, we will discuss the implications of BBB dysfunction in current and future therapeutics. Developing a better appreciation of BBB dysfunction in AD and PD may not only provide novel strategies in treatment, but will prove an interesting milestone in understanding neurodegenerative disease etiology and progression [47].

Although intravenous lipid emulsion (ILE) was first used to treat life-threatening local anesthetic (LA) toxicity, its use has expanded to include both non-local anesthetic (non-LA) poisoning and less severe manifestations of toxicity. A collaborative workgroup appraised the literature and provides evidence-based recommendations for the use of ILE in poisoning. Following a systematic review of the literature, data were summarized in four publications: LA and non-LA poisoning efficacy, adverse effects, and analytical interferences. Twenty-two toxins or toxin categories and three clinical situations were selected for voting. Voting statements were proposed using a predetermined format. A two-round modified Delphi method was used to reach consensus on the voting statements. Disagreement was quantified using RAND/UCLA Appropriateness Method.

For the management of cardiac arrest, we recommend using ILE with bupivacaine toxicity, while our recommendations are neutral regarding its use for all other toxins. For the management of life-threatening toxicity, (1) as first line therapy, we suggest not to use ILE with toxicity from amitriptyline, non-lipid soluble beta receptor antagonists, bupropion, calcium channel blockers, cocaine, diphenhydramine, lamotrigine, malathion but are neutral for other toxins, (2) as part of treatment modalities, we suggest using ILE in bupivacaine toxicity if other therapies fail, but are neutral for other toxins, (3) if other therapies fail, we recommend ILE for bupivacaine toxicity and we suggest using ILE for toxicity due to other LAs, amitriptyline, and bupropion, but our recommendations are neutral for all other toxins. In the treatment of non-life-threatening toxicity, recommendations are variable according to the balance of expected risks and benefits for each toxin. For LA-toxicity we suggest the use of Intralipid® 20% as it is the formulation the most often reported. There is no evidence to support a recommendation for the best formulation of ILE for non-LAs. The voting panel is neutral regarding ILE dosing and infusion duration due to insufficient data for non-LAs. All recommendations were based on very low quality of evidence.

Clinical recommendations regarding the use of ILE in poisoning were only possible in a small number of scenarios and were based mainly on very low quality of evidence, balance of expected risks and benefits, adverse effects, laboratory interferences as well as related costs and resources. The workgroup emphasizes that dose-finding and controlled studies reflecting human poisoning scenarios are required to advance knowledge of limitations, indications, adverse effects, effectiveness, and best regimen for ILE treatment [48]. Intralipid emulsion therapy is well-established for the treatment of local-anesthetic systemic toxicities. In recent years, its role has expanded as an important therapeutic agent in the reversal of other types of drug overdoses, including certain types of antipsychotics, antidepressants, antiarrhythmics, and calcium channel blockers. A literature review identified thirty-one case reports including forty-nine separate drug overdose cases involving ten separate drug classes which were successfully reversed with Intralipid. The present clinical case study describes an elderly unresponsive woman refractory to conventional treatments after ingesting a potentially lethal amount of 5.6 grams of diltiazem in a suicide attempt. After treatment

with Intralipid over a twenty-four hour period, the patient's hemodynamic and metabolic derangements were corrected and stabilized completely. Intralipid emulsion rescue therapy provides another potential strategy for the reversal of many drug toxicities, most likely by providing a lipid layer safety net for drug overdose by passive diffusion. Clinicians are urged to embrace an expanded role of Intralipid emulsion rescue therapy, not only for local anesthetic drug toxicities, but also for other lipophilic drug overdoses [49].

Caffeine is arguably the most widely used stimulant drug in the world. Here we describe a suicide attempt involving caffeine overdose whereby the patient's severe intoxication was successfully treated with the prompt infusion of Intralipid. A 19-year-old man was found in an agitated state at home by the volunteer emergency team about 1 h after the intentional ingestion of 40 g of caffeine (tablets). His consciousness decreased rapidly, followed quickly by seizures, and electrocardiographic monitoring showed ventricular fibrillation. Advanced life support maneuvers were started immediately, with the patient defibrillated 10 times and administered 5 mg epinephrine in total and 300 + 150 mg of amiodarone (as well as lidocaine and magnesium sulfate). The cardiac rhythm eventually evolved to asystole, necessitating the intravenous injection of epinephrine to achieve the return of spontaneous circulation. However, critical hemodynamic instability persisted, with the patient's cardiac rhythm alternating between refractory irregular narrow complex tachycardia and wide complex tachycardia associated with hypotension. In an attempt to restore stability we administered three successive doses of Intralipid (120 + 250 + 100 mg), which successfully prevented a severe cardiovascular collapse due to a supra-lethal plasma caffeine level (>120 mg/L after lipid emulsion). The patient survived without any neurologic complications and was transferred to a psychiatric ward a few days later. The case emphasizes the efficacy of intravenous lipid emulsion in the resuscitation of patients from non-local anesthetic systemic toxicity. Intralipid appears to act initially as a vehicle that carries the stimulant drug away from heart and brain to less well-perfused organs (scavenging mechanism) and then, with a sufficient drop in the caffeine concentration, possibly as a tonic to the depressed heart [50]. Thrombosis and immune dysfunction are two important complications that result from the administration of parenteral nutrition. Endothelial cells within the vasculature are crucial components necessary for maintenance of normal coagulation and immune function. We compared the effects of three commercial lipid emulsions (LEs; Intralipid®, ClinOleic® [or Clinolipid®], and Omegaven®) differing in the levels of omega-6 polyunsaturated fatty acids, omega-3 polyunsaturated fatty acids, omega-9 monounsaturated fatty acids, and saturated fatty acids upon endothelial cell fatty acid composition using Gas chromatography, endothelial cell integrity by assessing measurement of apoptosis and necrosis using flow cytometry, endothelial cell inflammatory activation by assessing the induction of ICAM-1 by lipopolysaccharide [LPS]), and transcription factor activation (phosphorylation of NF-κB) using western blot analysis.

Gas chromatographic analysis confirmed cellular uptake of the fatty acids within the LEs; furthermore, these fatty acid changes reflected the composition of the oils and egg phosphatides used in the manufacturing of these emulsions. However, the kinetics of fatty acid uptake and processing differed between LEs. Fish oil LE negatively impacted cell viability by doubling the percentage of apoptotic and necrotic cell populations quantified by flow cytometry using Annexin V/Fluorescein and propidium iodide. The soybean oil LE did not alter cell viability, while the olive oil-predominate emulsion improved cell viability. All LEs were capable of suppressing LPS-induced ICAM-1 expression; however, the fish oil LE was more potent than the other emulsions. Fish oil LE supplementation of cells also suppressed LPS-induced phosphorylation of NF-κB, while the soybean oil and olive predominant LE had no effect upon NF-κB phosphorylation. Lipid emulsions are readily incorporated and stored in the form of triacylglycerols. Soybean oil-based, olive oil-predominant and fish-oil based LEs differentially affected endothelial cell integrity. Importantly, these three LEs were capable of suppressing endothelial cell inflammatory response despite their fatty acid content [51].

Membrane currents conducted by the NMDA receptor channels were investigated in cultured cortical neurons and TsA cells transfected with NR1-1a/NR2A subunits of the NMDA receptor. The whole-cell recording technique was used. Current transients evoked by bath application of NMDA for 5 s were characterized by a fast peak and a slow decay to 46.1 +/-15.5% of the peak level at the end. When NMDA was applied in combination with various lipid emulsions (Intralipid, ClinOleic, Lipofundin or Abbolipid, the NMDA-induced currents were reduced, although this reduction did not affect the fast peak, it did affect the decay phase. The amount of reduction depended on the concentration of the lipids (in the case of Abbolipid diluted at 1:40, the current at the end of the 5-s drug application was approximately 2/3 of control). When Abbolipid was applied 40 s before NMDA, peak and late current were reduced to approximately 2/3. The effect of current reduction was the same at either of the two chosen membrane potentials (-80 and +40 mV) which indicates that the effect was not mediated by contamination of the emulsions with Mg (2+). The current reduction produced by Abbolipid was about the same in native neuronal cells and in TsA cells expressing the NR1-1a/NR2A subunits. The current-reducing effect of the lipid emulsions may add to the anesthetic, analgesic and neuroprotective effects seen with hypnotics administered by way of lipid carriers [52].

Little is known about the impact of circulating lipids on brain processes. Building on evidence that chronic fat consumption stimulates hypothalamic peptides in close

association with elevated triglycerides (TG), this study examined whether an acute rise in TG levels induced by fat emulsion can affect these hypothalamic systems. In normal weight rats, ip injection of Intralipid (20%, 5 ml) during the first 4 h after injection produced a robust increase in TG levels and nonesterified fatty acids, but had no impact on glucose, insulin, or leptin levels. This was accompanied by a marked increase in the expression of particular orexigenic peptides, galanin, orexins, and the opioid, enkephalin, which are known to be positively related to fat ingestion. This effect, similarly induced by 4 h of high fat diet consumption, was detected in the paraventricular nucleus (PVN) for galanin, in the perifornical hypothalamus (PFH) for orexins, and in the PVN, PFH, as well as the arcuate nucleus (ARC) for enkephalin. It was not seen, however, for neuropeptide Y and agouti-related protein localized in the ARC, which are unaffected or reduced by dietary fat. This site specificity was confirmed by c-Fos immunostaining, a marker of neuronal activity, which was increased by Intralipid in the PVN and PFH, but not in the ARC, and was detected in 20% of orexin-expressing neurons in the PFH. These findings suggest that circulating lipids, through different mechanisms, may stimulate hypothalamic neurons, which synthesize specific feeding stimulatory peptides that possibly contribute to hyperphagia during consumption of a fat-rich diet [53].

## Conclusion

Intralipid treatment is first suggested here for the treatment of Alzheimer disease. It should be given intravenously on a monthly basis according to each patient's response. Clinical studies should be done in order to evaluate this new treatment modality.

## References


1. http://www.alz.org/alzheimers_disease_treatments.asp
2. Berrios G E (1991) Alzheimer's Disease: A Conceptual History. International Journal of Geriatric Psychiatry 5: 355-365.
3. Engstrom, Eric J (2007-10-04) Researching Dementia in Imperial Germany: Alois Alzheimer and the Economies of Psychiatric Practice. Culture, Medicine and Psychiatry 31(3): 405-413.
4. https://en.wikipedia.org/wiki/Alois_Alzheimer
5. Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, et al. (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiology of Aging 28 (7): 977-986.
6. Nagele, Robert G (2006) Alzheimer's disease: new mechanisms for an old problem. *UMDNJ Research*. University of Medicine and Dentistry of New Jersey **7** (2).
7. http://www.umdnj.edu/research/publications/fall0 6/4.htm
8. Daviglus ML, Bell CC, Berrettini W, Bowen PE, Connolly ES Jr, et al. (2010) National Institutes of Health state-of-the-science conference statement: preventing Alzheimer disease and cognitive decline. NIH Consens State Sci Statements 27(4): 1-30.
9. World Health Organization (WHO) and Alzheimers Disease International (2012) Dementia: A Public Health Priority. World Health Organization, Geneva.
10. David M, Stern, Ann Marie Schmidt, Shi Du Yan, Berislav Zlokovic, et al. Method to increase cerebral blood flow in amyloid angiopathy, US 6677299 B2
11. Russell, RL, Westfall BA (1962) Alleviation of barbiturate depression. Anesth Analg 41(5): 582-585.
12. Krieglstein J, Meffert A, Niemeyer D (1974) Influence of emulsified fat on chlorpromazine availability in rabbit blood. Experientia 30(8): 924-926.
13. Straathof DJ, Driessen O, Meijer JW, Van Rees H, Vermeij P, et al. (1984) Influence of Intralipid infusion on the elimination of phenytoin. Arch Int Pharmacodyn Ther 267(2):180-186.
14. Weinber GL, Vade Boncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ, et al. (1998) Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anesthesiology 88(4): 1071-1075.
15. Weinberg G (2002) Current concepts in resuscitation of patients with local anesthetic cardiac toxicity. Reg Anesth Pain Med 27(96): 568-575.
16. Cave G, Harvey M, (2009a) Intravenous lipid emulsion as antidote beyond local anesthetic toxicity: a systematic review. Acad Emerg Med 16(9):815-824.
17. Rosenblatt MA, Abel M, Fischer GW, Itzkovich CJ, Eisenkraft JB, et al. (2006) Successful use of a 20% lipid emulsion to resuscitate a patient after a presumed bupivacaine-related cardiac arrest. Anesthesiology 105(1): 217-218.
18. Weinberg G, Di Gregorio G, Hiller D, Hewett A, Sirianni A, et al. (2009) Reversal of haloperidol-induced cardiac arrest by using lipid emulsion. Ann Intern Med 150(10): 737-738.
19. Minton NA, Goode AG, Henry JA (1987) The effect of a lipid suspension on amitriptyline disposition. Arch Toxicol 60(6): 467-469.
20. Cave G, Harvey M, Graudins A (2011) Intravenous lipid emulsion as antidote: A summary of published human experience. Emerg Med Australas 23(2): 123- 141.
21. Tomiyama T (2010) Involvement of beta-amyloid in the etiology of Alzheimer's disease. Brain Nerve 62(7): 691-699.
22. Bloom GS (2014) Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol 71(4): 505-508.
23. Zetterberg H, Mattsson N (2014) Understanding the cause of sporadic Alzheimer's disease. Expert Rev Neurother 14(6): 621-630.
24. Morris GP, Clark IA, Vissel B (2014) Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun 18;2: 135.
25. James AP, Pal S, Gennat HC, Vine DF, Mamo JC, et al. (2003 ) The incorporation and metabolism of amyloid-beta into chylomicron-like lipid emulsions. J Alzheimers Dis 5(3): 179-88.
26. Champeil-Potokar G, Denis I, Goustard-Langelier B, Alessandri JM, Guesnet P, et al. (2004) Astrocytes in culture requires docosahexaenoic acid to restore the n-3/n-6 polyunsaturated fatty acid balance in their membrane phospholipids. J Neurosci Res 75(1): 96-106.
27. Langelier B, Linard A, Bordat C, Lavialle M, Heberden C, et al. (2010) Long chain-polyunsaturated fatty acids modulate membrane phospholipid composition and protein localization in lipid rafts of neural stem cell cultures. J Cell Biochem 110(6): 1356-1364.
28. Colin J, Gregory-Pauron L, Lanhers MC, Claudepierre T, Corbier C, et al. (2016) Membrane raft domains and remodeling in aging brain. Biochimie 130: 178-187.
29. Taftachi F, Sanaei-Zadeh H, Sepehrian B, Zamani N (2012) Lipid emulsion improves Glasgow coma scale and decreases blood glucose level in the setting of acute non-local anesthetic drug poisoning--a randomized controlled trial. Eur Rev Med Pharmacol Sci 16 Suppl 1:38-42.
30. Weigt HU, Georgieff M, Beyer C, Föhr KJ (2002) Activation of neuronal N-methyl-D-aspartate receptor channels by lipid emulsions. Anesth Analg 94(2): 331-317.
31. Haywood SC, Bree AJ, Puente EC, Daphna-Iken D, Fisher SJ, et al. (2009) Central but not systemic lipid infusion augments the counterregulatory response to hypoglycemia. Am J Physiol Endocrinol Metab. 297(1): E50-E56.
32. Hiller DB, Di Gregorio G, Kelly K, Ripper R, Edelman L, et al. (2010) Safety of high volume lipid emulsion infusion: a first approximation of LD50 in rats. Reg Anesth Pain Med 35(2): 140-144.
33. Heinonen JA, Litonius E, Backman JT, Neuvonen PJ, Rosenberg PH, et al. (2013) Intravenous lipid emulsion entraps amitriptyline into plasma and can lower its brain concentration an experimental intoxication study in pigs. Basic Clin Pharmacol Toxicol 113(3): 193-200.
34. Basarslan SK, Alp H, Senol S, Evliyaoglu O, Ozkan U, et

al. (2014) Is intralipid fat emulsion a promising therapeutic strategy on neurotoxicity induced by malathion in rats? Eur Rev Med Pharmacol Sci 18(4): 471-476.

35. Ozkan U, Osun A, Basarslan K, Senol S, Kaplan I, et al. (2014) Effects of intralipid and caffeic acid phenethyl ester on neurotoxicity, oxidative stress, and acetylcholinesterase activity in acute chlorpyriphos intoxication. Int J Clin Exp Med 7(4): 837-46.

36. Heinonen JA, Litonius E, Salmi T, Haasio J, Tarkkila P, et al. (2015) Intravenous lipid emulsion given to volunteers does not affect symptoms of lidocaine brain toxicity. Basic Clin Pharmacol Toxicol 116(4): 378-83.

37. Lange DB, Schwartz D, DaRoza G, Gair R (2012) Use of intravenous lipid emulsion to reverse central nervous system toxicity of an iatrogenic local anesthetic overdose in a patient on peritoneal dialysis. Ann Pharmacother 46(12):e37. Erratum in Ann Pharmacother 47(1): 139.

38. http://ecatalog.baxter.com/ecatalog/loadResource.blob?bid=20000307

39. Gilbert Di Paolo, Tae-Wan Kim (2011) Linking lipids to Alzheimer's disease: cholesterol and beyond. Nature Reviews Neuroscience 12(5): 284-296.

40. Terracina L , Brunetti M , Avellini L ,De Medio GE , Trovarelli G , et al. (1992) Linoleic acid metabolism in brain cortex of aged rats. The Italian Journal of Biochemistry 41(4): 225-235.

41. Thomas MH, Pelleieux S, Vitale N, Olivier JL (2016) Dietary arachidonic acid as a risk factor for age-associated neurodegenerative diseases: Potential mechanisms. Biochimie 130: 168-177.

42. Karczewska-Kupczewska M, Kowalska I, Nikołajuk A, Adamska A, Zielińska M, et al. (2012) Circulating brain-derived neurotrophic factor concentration is downregulated by intralipid/heparin infusion or high-fat meal in young healthy male subjects. Diabetes Care 35(2): 358-362.

43. Rodriguez-Navas C, Morselli E, Clegg DJ (2016) Sexually dimorphic brain fatty acid composition in low and high fat diet-fed mice. Mol Metab 5(8): 680-689.

44. Sharma HS, Castellani RJ, Smith MA, Sharma A (2012) The blood-brain barrier in Alzheimer's disease: novel therapeutic targets and nanodrug delivery. Int Rev Neurobiol102: 47- 90.

45. Desai BS, Monahan AJ, Carvey PM, Hendey B (2007) Blood-brain barrier pathology in Alzheimer's and Parkinson's disease: implications for drug therapy. Cell Transplant 16(3): 285-299.

46. Zlokovic BV (2002) Vascular disorder in Alzheimer's disease: role inpathogenesis of dementia and therapeutic targets. Adv Drug Deliv Rev 54(12): 1553-1539.

47. Bartels AL (2011) Blood-brain barrier P-glycoprotein function in neurodegenerative disease. Curr Pharm Des 17(26): 2771-2777.

48. Desai BS, Monahan AJ, Carvey PM, Hendey B (2007) Blood-brain barrier pathology in Alzheimer's and Parkinson's disease: implications for drug therapy. Cell Transplant 16(3): 285-299.

49. Gosselin S, Hoegberg LC, Hoffman RS, Graudins A, Stork CM, et al. (2016) Evidence-based recommendations on the use of intravenous lipid emulsion therapy in poisoning. Clin Toxicol (Phila). 54(10): 899-923.

50. Muller SH, Diaz JH, Kaye AD (2016) Intralipid Emulsion Rescue Therapy: Emerging Therapeutic Indications in Medical Practice. J La State Med Soc 168(3): 101-103.

51. Muraro L, Longo L, Geraldini F, Bortot A, Paoli A, et al. (2016) Intralipid in acute caffeine intoxication: a case report. J Anesth 30(5): 895-859.

52. Harvey KA, Xu Z, Pavlina TM, Zaloga GP, Siddiqui RA, et al. (2015) Modulation of endothelial cell integrity and inflammatory activation by commercial lipid emulsions. Lipids Health Dis 18;14: 9.

53. Weigt H, Georgieff M, Beyer C, Georgieff EM, Kuhse J, et al. (2004) Lipid emulsions reduce NMDA-evoked currents. Neuropharmacology 47(3): 373-380.

54. Chang GQ, Karatayev O, Davydova Z, Leibowitz SF (2004) Circulating triglycerides impact on orexigenic peptides and neuronal activity in hypothalamus. Endocrinology145(8): 3904-3912.

# Chapter 5

## Intralipid Treatment for Newborns with Meconium Stained Amniotic Fluid (MSAF)

## Abstract

One in every seven pregnancies ends with meconium-stained amniotic fluid (MSAF). MSAF can be harmful to the newborn with short and long-term sequelae. A new treatment of Intravenous Intralipid is first suggested for MSAF.

**Keywords:** Meconium stained amniotic fluid (MSAF); Intralipid

## MAS and MSAF

One in every seven pregnancies ends with meconium-stained amniotic fluid (MSAF). MSAF can be harmful to the newborn with short and long-term sequelae. This study [1] was aimed to find out the incidence, predictors, onset and severity of respiratory distress among vigorous babies born through meconium stained amniotic fluid which may or may not be evident at birth. This study [1] included one hundred and forty-one vigorous babies born through meconium stained amniotic fluid, of which 36.9% (52) babies developed respiratory distress. Of the 52 babies who developed respiratory distress 19.23%(10 babies) developed meconium aspiration syndrome (MAS). In this study, it was observed factors like caesarean section and thick meconium increased risk of respiratory distress in the neonates born through meconium stained amniotic fluid who were vigorous. 98.07% (51 babies) developed respiratory distress at birth or within one hour of life. All the babies who developed MAS had mild or moderate form of MAS. None of the babies required assisted ventilation. Risk factors like thick meconium, caesarean section showed significant increase in the incidence of respiratory distress. Therefore intrapartum monitoring and timely intervention can prevent the complications of MAS [1].

In developing countries, meconium aspiration syndrome (MAS) is an important cause of morbidity and mortality among neonates. The concepts of pathophysiology and management of meconium stained amniotic fluid (MSAF) and meconium aspiration syndrome have undergone tremendous change in recent years. Routine intranatal and postnatal endotracheal suctioning of meconium in vigorous infants is no longer recommended. Recent studies have challenged its role even in non-vigorous infants. Supportive therapy like oxygen supplementation, mechanical ventilation and intravenous fluids are the cornerstone in the management of meconium aspiration syndrome. Availability of surfactant, inhaled nitric oxide, high frequency ventilators and extracorporeal membrane oxygenation has made it possible to salvage more infants with meconium aspiration syndrome [2].

## Endotracheal Suction

To evaluate the effect of 'No endotracheal suction' on occurrence of meconium aspiration syndrome (MAS) and/or all-cause mortality in non-vigorous neonates born through meconium stained amniotic fluid (MSAF). This pilot [3] randomized controlled trial enrolled term non-vigorous neonates (≥37 weeks) born through MSAF. Neonates randomized to 'No Endotracheal suction group' ('No ET' Group; n=88) did not undergo endotracheal suction before the definitive steps of resuscitation. Neonates randomized to 'Endotracheal suction group' ('ET' Group; n=87) underwent tracheal suction as part of the initial steps as per the current NRP recommendations. The primary outcome was occurrence of MAS and/or death. Secondary outcome variables were duration and severity of respiratory distress, need for respiratory support, development of hypoxic ischemic encephalopathy (HIE) and duration of oxygen therapy and hospitalization. Baseline characters including birth weight and gestational age were similar between the two groups. MAS was present in 23 (26.1%) vs. 28 (32.2%) neonates in 'No ET' and 'ET' groups respectively (OR 0.4 (0.12-1.4); p=0.14) with 4 (4.6%) and 9 (10.34%) deaths amongst these neonates with MAS in respective groups (OR 0.75 (0.62-1.2); p=0.38). Other parameters like severity and duration of respiratory distress, need for respiratory support, incidence of HIE, duration of oxygen therapy and duration of hospitalization were comparable. This study demonstrates that it is feasible to randomize non-vigorous infants born through meconium stained liquor to receive or not receive endotracheal suction [3].

### Antibiotics

To identify and assess the characteristics, risk and outcome of neonates treated with empiric antibiotics for suspected early onset sepsis (EOS). This is a retrospective study (4) conducted at a Malaysian government hospital. Records of neonatal patients admitted within 72 h of life and prescribed with empirical antibiotic therapy for suspected EOS were reviewed. Three hundred and twenty-three cases met the inclusion criteria and were divided into gestational age (premature < 36 weeks; term ≥ 37 weeks) and birth weight (low birth weight (LBW) < 2.5 kg; normal body weight (NBW) ≥ 2.5 kg) groups. Premature (n = 197) and LBW (n = 194) neonates required significantly longer hospital stay, a higher degree of ventilator support and more surfactant (p = 0.001). More than 90.0% of premature and LBW neonates were diagnosed with respiratory distress syndrome, congenital pneumonia and presumed sepsis. Term (n = 123) and NBW (n = 129) neonates had greater maternal risk factors, especially meconium- stained amniotic fluid (MSAF) and perinatal asphyxia. The incidence of demonstrated EOS was 3.1%. Crystalline penicillin plus gentamicin was the standard therapy for all groups and was started within 24 h of life, with a mean treatment duration of □4 days. The treatment success rate was 89.0%, and only LBW neonates showed a higher risk of overall treatment failure (OR=3.75; 95% CI: 1.22-11.53). Seventy-four percent of term and NBW neonates discharged well, while 42.0% of premature and LBW neonates required referral. Crystalline penicillin plus gentamicin prescribed within 24 h of life is effective in the prevention of EOS. However, low birth weight neonates have a higher risk of treatment failure [4].

### Oronasopharyngeal suction

Oronasopharyngeal suction (ONPS) is regularly performed in neonates at delivery in many hospitals across the country today [5]. Although ONPS is a technique that has essentially become habitual for most obstetricians, its theorized usefulness to help promote expeditious lung aeration after delivery by removal of amniotic fluid, meconium, mucus and blood that may otherwise be aspirated by the newborn, is currently not recommended. ONPS can cause vagal stimulation-induced bradycardia and thus hypercapnea, iatrogenic infection due to mucous membrane injury, and development of subsequent neonatal brain injury due to changes in cerebral blood flow regulation, particularly in

premature infants. Multiple studies that have been performed comparing routine use of ONPS to no intervention controls indicate that newborns receiving ONPS took a longer time to achieve normal oxygen saturations, caused apneic episodes, and caused disturbances in heart rate (mainly bradycardia) compared to the control groups. Although the ONPS groups revealed no significantly different APGAR scores at 1 and 5 minutes, the ONPS groups took longer than the control group to reach an arterial oxygen saturation greater than or equal to 92% in the first minutes of life. Currently, Neonatal Resuscitation Program guidelines discourage the use of or meconium-stained amniotic fluid and in the absence of obvious obstruction. Furthermore, this manuscript highlights various literature sources revealing that the routine use of ONPS at the time of delivery can cause more harm than good, if any good at all [5].

Evidence about IP-OP suction and selective tracheal intubation in meconium stained neonates is from developed countries. Little information is available about their role in developing countries with high incidence of meconium staining and MAS. This randomized trial [6] was planned to evaluate the effectiveness of IP-OP suction in meconium stained term neonates on prevention of MAS and reduction of its severity. Out of 540 meconium stained full term, cephalic presentation, singleton neonates without major congenital malformations born from June'08 to January'09, 31 were excluded and 509 randomized. In the intervention group IP-OP suction was done at the time of delivery of head using a 10 Fr suction catheter with a negative pressure of 100 mmHg. No IP-OP suction was performed in control group. All neonates with MSAF were assessed as vigorous or non-vigorous after birth and provided care as per NRP guidelines 2005.

Two hundred and fifty three neonates were randomized to IP-OP suction and 256 to no IP-OP suction. Eighty-two neonates (16%) developed MAS, the primary outcome parameter, with 40 infants in the intervention group (15.8%) and 42 (16.4%) in the non-intervention group (RR 0.86, 95% CI 0.60-1.54). Incidence of severe MAS was comparable (3.55% vs. 2.34%) (P value=0.40). Other variables like requirement of oxygen >48 h (9.8% vs. 10.5%) and mortality (2.7% vs. 1.7%) were also comparable. IP-OP suctioning did not reduce the incidence or severity of MAS even in a setting of high incidence of MAS in a developing country. The mortality in two groups was comparable [6].

## Gastric lavage

Neonates born with meconium stained amniotic fluid (MSAF) can develop feed intolerance during first few days of post -natal period. A randomized controlled trial was conducted with the objectives of to find out the incidence of feed intolerance in vigorous neonates with MSAF who received gastric lavage (GL) as compared to those in whom it was not performed. This was a randomized controlled trial on 500 neonates satisfying the inclusion criteria, 230 were allocated to GL and 270 to no lavage group through computer generated random numbers. No significant difference in the incidence of vomiting was found between GL and no lavage group (8.7 % vs 11.5 %, p = 0.305). Feed intolerance had no relationship with gestational age, gender, birth weight and mode of delivery. No neonates of GL group developed any complications related to the procedure. Thus, it may be concluded that gastric lavage is not required in neonates born with MSAF [7].

## Endotracheal suctioning

To assess whether endotracheal suctioning of nonvigorous infants born through meconium stained amniotic fluid (MSAF) reduces the risk and complications of meconium aspiration syndrome (MAS). Term, nonvigorous babies born through MSAF were randomized to endotracheal suction and no-suction groups (n=61 in each). Risk of MAS, complications of MAS and endotracheal suction, mortality, duration of neonatal intensive care unit stay, and neurodevelopmental outcome at 9 months were assessed.

Maternal age, consistency of meconium, mode of delivery, birth weight, sex, and Apgar scores were similar in the groups. In total, 39 (32%) neonates developed MAS and 18 (14.8%) of them died. There were no significant differences in MAS, its severity and complications, mortality, and neurodevelopmental outcome for the 2 groups. One infant had a complication of endotracheal suctioning, which was mild and transient. The current practice of routine endotracheal suctioning for nonvigorous neonates born through MSAF should be further evaluated [8].

## Prophylactic antibiotics

The objective of the study [9] was to evaluate the effect of administering prophylactic antibiotics on the development of neonatal sepsis in term neonates born through meconium-stained amniotic fluid (MSAF). Two hundred and fifty eligible neonates were randomized to study group (Antibiotic group-receiving first-line antibiotics for 3 days) and control group (No Antibiotic group). Both groups were evaluated clinically and by laboratory parameters (sepsis screen and blood cultures) for development of sepsis. All neonates were monitored for respiratory, neurological, and other systemic complications and received supportive treatment according to standard management protocol of the unit. One hundred and twenty one neonates were randomized to 'Antibiotic' group and 129 to 'No Antibiotic' group. The overall incidence of suspect sepsis was 9.6 % in the study population with no significant difference between 'No Antibiotic' and 'Antibiotic' groups (10.8 vs. 8.2 %, p = 0.48, odds ratio (OR) 0.74, 95 % confidence interval (CI) 0.32- 1.73). Incidence of culture-proven sepsis was also not significantly different between the two groups (5.42 vs. 4.13 %, p = 0.63, OR 0.75, 95 % CI 0.23- 2.43). The incidence of mortality, meconium aspiration syndrome, and other complications was comparable amongst the two groups. Routine antibiotic prophylaxis in neonates born through MSAF did not reduce the incidence of sepsis in this study population [9].

## Chorioamnionitis

Chorioamnionitis is more likely to occur when meconium-stained amniotic fluid (MSAF) is present. Meconium may

enhance the growth of bacteria in amniotic fluid by serving as a growth factor, inhibiting bacteriostatic properties of amniotic fluid. Many adverse neonatal outcomes related to MSAF result from meconium aspiration syndrome (MAS). MSAF is associated with both maternal and newborn infections. Antibiotics may be an effective option to reduce such morbidity. The objective of this review [10] is to assess the efficacy and side effects of prophylactic antibiotics for MSAF during labour in preventing maternal and neonatal infections. We (10) searched the Cochrane Pregnancy and Childbirth Group's Trials Register (30 September 2014). Randomised controlled trials (RCTs) comparing prophylactic antibiotics with placebo or no treatment during labour for women with MSAF.

Two review authors independently assessed trials for inclusion and risk of bias, extracted data and checked them for accuracy. We (10) included two studies with 362 pregnant women. Both studies compared ampicillin-sulbactam (N = 183) versus normal saline (N = 179) in pregnant women with MSAF. Prophylactic antibiotics appeared to have no statistically significant reduction in the incidence of neonatal sepsis (risk ratio (RR) 1.00, 95% CI 0.21 to 4.76), neonatal intensive care unit (NICU) admission (RR 0.83, 95% CI 0.39 to 1.78) and postpartum endometritis (RR 0.50, 95% CI 0.18 to 1.38). However, there was a significant decrease in the risk of chorioamnionitis (RR 0.36, 95% CI 0.21 to 0.62). No serious adverse effects were reported. Drug resistance, duration of mechanical ventilation and duration of admission to NICU/hospital were not reported. Most of the domains for risk of bias were at low risk of bias for one study and at unclear risk of bias for the other study. The quality of the evidence using GRADE was low for neonatal sepsis, postpartum endometritis, and neonatal mortality and morbidity prior to discharge (Neonatal intensive care admissions) and of moderate quality for chorioamnionitis. Current evidence indicates that compared to placebo, antibiotics for MSAF in labour may reduce chorioamnionitis. There was no evidence that antibiotics could reduce postpartum endometritis, neonatal sepsis and NICU admission. This systematic review identifies the need for more well-designed, adequately powered RCTs to assess the effect of prophylactic antibiotics in the incidence of maternal and neonatal complications (10). The role of gastric lavage in preventing retching, vomiting and secondary meconium aspiration syndrome in neonates with meconium-stained amniotic fluid is uncertain, and no there are no definitive guidelines. To evaluate the effect of gastric lavage in preventing retching, vomiting and secondary meconium aspiration syndrome in neonates with meconium-stained amniotic fluid. This was an open-label, parallel, randomized controlled trial conducted in the labour room, postnatal and neonatal wards of a tertiary-care teaching hospital. Vigorous neonates of □34 weeks gestation with meconium-stained amniotic fluid were randomised into two groups using block randomisation. Infants requiring oxygen, in respiratory distress or with major congenital malformations were excluded. Infants in the study group received elective gastric lavage in the labour room after initial stabilisation. No gastric lavage was done in the control group. The newborns were assessed for retching, vomiting and secondary meconium aspiration syndrome in the first 48 hrs of life or until discharge from the hospital, whichever was later. A total of 267 newborns were randomly assigned to the gastric lavage group and 269 to the no gastric lavage group. There were no statistical differences in overall feeding between the two groups (6•74% vs 10•78%). Feeding of two newborns in the no-lavage group had to be omitted for the initial few hours because of vomiting; this did not happen in any newborn in the lavage group. No newborn in either group developed secondary meconium aspiration syndrome. Gastric lavage in newborns with meconium-stained amniotic fluid does not prevent or reduce the occurrence of feeding problems or secondary meconium aspiration syndrome [11].

## Amnioinfusion

Amnioinfusion is thought to dilute meconium present in the amniotic fluid and so reduce the risk of meconium aspiration. To assess the effects of amnioinfusion for meconium-stained liquor on perinatal outcome. We [12] searched the Cochrane Pregnancy and Childbirth Group's Trials Register (1 December 2013). Randomised trials comparing amnioinfusion with no amnioinfusion for women in labour with moderate or thick meconium staining of the amniotic fluid. Three review authors independently assessed eligibility and trial quality, and extracted data. Fourteen studies of variable quality (4435 women) are included. Subgroup analysis was performed for studies from settings with limited facilities to monitor the baby's condition during labour and intervene effectively, and settings with standard peripartum surveillance. Settings with standard peripartum surveillance: there was considerable heterogeneity for several outcomes. There was no significant reduction in the primary outcomes meconium aspiration syndrome, perinatal death or severe morbidity, and maternal death or severe morbidity. There was a reduction in caesarean sections (CSs) for fetal distress but not overall. Meconium below the vocal cords diagnosed by laryngoscopy was reduced, as was neonatal ventilation or neonatal intensive care unit admission, but there was no significant reduction in perinatal deaths or other morbidity. Planned sensitivity analysis excluding trials with greater risk of bias resulted in an absence of benefits for any of the outcomes studied. Settings with limited peripartum surveillance: three studies were included. In the amnioinfusion group there was a reduction in CS for fetal distress and overall; meconium aspiration syndrome (three studies, 1144 women; risk ratio (RR) 0.17, 95% confidence interval (CI) 0.05 to 0.52); perinatal mortality (three studies, 1151 women; RR 0.24, 95% CI 0.11 to 0.53) and neonatal ventilation or neonatal intensive care unit admission. In one of the studies, meconium below the vocal cords was reduced and, in the other, neonatal encephalopathy was reduced.

Amnioinfusion is associated with substantive improvements in perinatal outcome only in settings where facilities for perinatal surveillance are limited. It is not clear whether the benefits are due to dilution of meconium or relief of oligohydramnios. In settings with standard peripartum

surveillance, some non-substantive outcomes were improved in the initial analysis, but sensitivity analysis excluding trials with greater risk of bias eliminated these differences. Amnioinfusion is either ineffective in this setting, or its effects are masked by ther strategies to optimise neonatal outcome. The trials reviewed are too small to address the possibility of rare but serious maternal adverse effects of amnioinfusion [12].

## Respiratory distress

This study [13] aimed to find out incidence, predictors, onset and severity of respiratory distress including meconium aspiration syndrome (MAS) among vigorous neonates born through meconium stained amniotic fluid (MSAF), which may or may not be evident at birth. Two hundred ninety vigorous neonates were studied. Data were collected on perinatal risk factors, clinical course and development of respiratory distress. Predictors of respiratory distress were identified by logistic regression and a score based on adjusted OR was assigned for each. Diagnostic performance of the score (0-24) was assessed on another 247 vigorous neonates using receiver operator characteristic analysis (ROC).

Respiratory distress developed in 97(33.4 %) infants, MAS in 75(25.9 %). The distress appeared within 12 h in 97.9 %, was severe in only 21.7 %. Of 10 risk factors significantly associated with respiratory distress, seven entered in regression analysis. Fetal distress (adj OR = 11.8; 95%CI = 6.2-22.5), prolonged labor(adj OR = 5.2; 95%CI = 2.5-10.7), and absent/poor cry(adj OR = 5.6; 95%CI = 2.4-13.3) were identified as independent predictors; each assigned a score of 12, 6 and 6, respectively. To predict respiratory distress, a cut-off score of 9 points had sensitivity-74.1 % (95%CI = 63.3 %-82.7 %), specificity-84.6 % (95 % CI = 77.9 %-89.6 %), positive predictive value- 71.6 % (95%CI = 60.8 %-80.4 %), negative predictive value-86.2 % (95 % CI = 79.6 %-90.9 %), likelihood ratio (LR) + ve 4.8(95%CI = 3.3-7.0) and LR-ve 0.3(95%CI = 0.2-0.4). Respiratory distress occurred in one third neonates, mostly had onset within 12 h of birth, and it was mild to moderate in majority. Fetal distress, prolonged labor, and absent/poor cry predicted respiratory distress and were validated. However, larger studies in different settings are required to confirm its utility [13].

## Feed intolerance

To compare reduction in incidence of feed intolerance in neonates born with meconiumstained amniotic fluid (MSAF) by use of gastric lavage to those who did not receive lavage. This Randomized controlled trial was conducted in all vigorous newborns delivered through MSAF, with birth weight ≥1800 g and gestation ≥34 wk. In the lavage group, gastric lavage with 10 ml/kg of normal saline was done. Twelve neonates in the lavage group (n = 124) developed feed intolerance compared to 16 neonates in control group (n = 120), (p = .309; OR 0.69; 95%CI 0.27-1.58). No difference in any other morbidity was noted. Gastric lavage in neonates with MSAF does not reduce feed intolerance, irrespective of thickness of MSAF and it confers no advantages [14].

## Intralipid

The optimal dosing regimens of lipid emulsion, epinephrine, or both are not yet determined in neonates in cases of local anaesthetic systemic toxicity (LAST). Newborn piglets received levobupivacaine until cardiovascular collapse occurred. Standard cardiopulmonary resuscitation was started and electrocardiogram (ECG) was monitored for ventricular tachycardia, fibrillation, or QRS prolongation. Piglets were then randomly allocated to four groups: control (saline), Intralipid (®) alone, epinephrine alone, or a combination of Intralipd plus epinephrine. Resuscitation continued for 30 min or until there was a return of spontaneous circulation (ROSC) accompanied by a mean arterial pressure at or superior to the baseline pressure and normal sinus rhythm for a period of 30 min. ROSC was achieved in only one of the control piglets compared with most of the treated piglets. Mortality was not significantly different between the three treatment groups, but was significantly lower in all the treatment groups compared with control. The number of ECG abnormalities was zero in the Intralipid only group, but 14 and 17, respectively, in the epinephrine and epinephrine plus lipid groups ($P<0.05$). Lipid emulsion with or without epinephrine, or epinephrine alone were equally effective in achieving a return to spontaneous circulation in this model of LAST. Epinephrine alone or in combination with lipid was associated with an increased number of ECG abnormalities compared with lipid emulsion alone [15].

This study [16] aimed to compare the effect of 2 lipid emulsions (LEs), a medium-chain triglyceride (MCT)/ω-3-polyunsaturated fatty acid (PUFA)-containing LE and a soybean-based LE, on the incidence of neonatal cholestasis, bronchopulmonary dysplasia (BPD), and lipid profile of preterm infants. Patients and In this prospective, observational study, 2 groups of preterm neonates, the very low birth weight (VLBW) (n = 129) and the low birth weight (LBW) groups (n = 153), which received parenteral LEs for at least 7 days, were included. Infants received either MCT/ω-3-PUFA-containing LE (SMOFlipid, subgroup I) or soybean-based LE (Intralipid, subgroup II) according to the attending neonatologist's preference and availability. Full biochemical assessment was performed on days of life 15, 30, and 45 and on discharge.

Of the VLBW infants, 7.4% and 13.3% of infants in subgroups I and II, respectively, developed cholestasis ($P = .39$; odds ratio [OR], 0.52; 95% confidence interval [CI], 0.15-1.76). The duration of LE administration was independently associated with cholestasis ($P < .001$; OR, 0.925; 95% CI, 0.888-0.963). The maximum amounts of lipids administered ranged between 1.6 and 3.6 g/kg/d in both VLBW subgroups. The VLBW subgroup I had lower incidence of BPD, lower alkaline phosphatase and phosphate, higher high-density lipoprotein (HDL), and lower cholesterol-to-HDL ratio on discharge than the VLBW subgroup II. The type of LE was independently associated with BPD and alkaline phosphatase. In the LBW group, the type of LE was not associated with clinical and biochemical parameters.

In VLBW infants, the MCT/ω-3-PUFA-containing LE administration is associated with decreased BPD and more favorable lipoprotein profile. Although a trend toward a lower incidence of cholestasis was observed, a preventive effect of MCT/ω-3-PUFA-containing LE on parenteral nutrition-associated cholestasis is not supported [16]. We [17] report a case of bupivacaine-induced cardiotoxicity in a neonate following caudal epidural block under general anesthesia for urologic surgery. Prompt recognition of the complication allowed early intervention with both standard resuscitative measures and administration of 20% Intralipid (®) , resulting in a good outcome [17]. To review the current state of the science regarding intravenous fat emulsions (IVFEs), with an emphasis on their safety profile.

Articles were identified via a search of the MEDLINE database, including publications from 1979 to December 2009, using a search string that included the terms parenteral nutrition, lipid emulsion, fat emulsion, IVFE, safety, adverse effect, neonate intralipid, and terms describing a range of specific adverse events (AEs) such as pancreatitis. We [18] selected articles that allowed us to compare the results of clinical trials involving delivery of medications via IVFEs with the historical use and effects of IVFEs in parenteral nutrition, with an emphasis on AEs. We [18] focused on 2 drugs in current use that are administered intravenously in lipid emulsions: propofol and clevidipine.

Clearance of the fat particles in IVFEs is mediated by the enzyme lipoprotein lipase. AEs are more likely if the rate or duration of IVFE administration exceeds the enzyme's clearance capacity. AEs are also more likely after administration of a 10% IVFE formulation than a 20% formulation, because the higher concentration of free phospholipid in the 10% formulation interferes with lipoprotein lipase activity. AEs can be reduced by administering IVFEs at a dosage < or = 2.5 g/kg/day and at a rate < or = 0.11 g/kg/h. The anesthetic agent propofol, which is formulated in a 10% IVFE, has been used clinically for 25 years. Typical AEs associated with propofol use include infection, high plasma triglyceride concentrations, and pancreatitis. Recent clinical trials involving clevidipine, which is formulated in a 20% IVFE, have demonstrated a low rate of lipid-related AEs. The results of this review demonstrate that IVFEs are well tolerated when administered in accordance with guideline recommendations [18].

These findings suggest that most intralipids errors occur during the administration phase. This complex process can generate high opportunities for error directly related to the use of IV pumps. Nursing staff members are prone to making dosing errors while accurately programming the infusion devices, especially during times of high workload. The evening hours around shift change appeared most vulnerable to such errors occurring. A further analysis to include error rates as a function of error opportunities is critical. The tracking and tallying of such opportunity for error can be accomplished using smart pump technology. A detailed analysis of the existing intralipid administration workflow process will guide the overall strategy of an error prevention plan. Understanding the nursing workload as a function of time of day and census is essential. These mission-critical tasks often require the hard work of a dedicated task force, the commitment of the hospital leadership, and cooperation from the health care providers [19].

In 1998 [20] it was first showed that intravenous Intralipid could prevent or improve resuscitation from cardiovascular collapse by severe bupivacaine overdose in rats. Since then published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haldoperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Amniotic fluid embolism (AFE) is a rare but potentially catastrophic obstetric emergency. Despite earlier recognition and aggressive treatment, morbidity and mortality rates remain high. An estimated 5% - 15% of all maternal deaths in Western countries are due to AFE. The pathophysiology of AFE is not completely understood. AFE most commonly occurs during labor, delivery, or the immediate postpartum period. However, it has been reported to occur up to 48 h postpartum. Pulmonary hypertension and right heart strain/failure may be the result of physical amniotic fluid debris in the pulmonary vasculature or, perhaps more likely, result from circulating pulmonary vasoconstrictive mediators. Therapy with Intralipid in male rats resulted in 100% survival and prevented Pulmonary arterial hypertension-induced right ventricular failure by preserving right ventricular pressure and right ventricular ejection fraction and preventing right ventricular hypertrophy and lung remodeling. In pre existing severe Pulmonary arterial hypertension, Intralipid attenu-ated most lung and right ventricular abnormalities. The beneficial effects of Intralipid in Pulmonary arterial hypertension seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and right ventricular, appear to be major contributors. In conclusion, Intralipid not only prevents the development of Pulmonary arterial hypertension and right ventricular failure but also rescues pre existing severe Pulmonary arterial hypertension. Intralipid treatment is a new treatment for AFE (amniotic fluid embolism) which was never suggested before [20]. Amniotic fluid embolism (AFE) is a rare and often fatal complication that occurs in the peripartum period. We [21] present a patient with an AFE who developed disseminated intravascular coagulation and cardiovascular collapse who may have benefitted from intravascular lipid emulsion rescue. This is the first published case in which lipid emulsion was a part of the successful treatment of AFE [21].

## Conclusion

A new treatment of Intravenous Intralipid is first suggested for MSAF.

## References


1. Kulkarni Poornima Prakash, Shilpa Dinesh BK (2017) Respiratory distress in vigorous babies born through meconium stained amniotic fluid: incidence, onset, risk factors and predictors at birth. Int J Contemp Pediatr 4(2): 390-393.
2. Chettri S, Bhat BV, Adhisivam B (2016) Current Concepts in the Management of Meconium Aspiration Syndrome. Indian J Pediatr 83(10): 1125-1130.
3. Nangia S, Sunder S, Biswas R, Saili A (2016) Endotracheal suction in term non vigorous meconium stained neonates-A pilot study. Resuscitation 105: 79-84.
4. Manan MM, Ibrahim NA, Aziz NA, Zulkifly HH, Al-Worafi YM, et al. (2016) Empirical use of antibiotic therapy in the prevention of early onset sepsis in neonates: a pilot study. Arch Med Sci 12(3): 603-613.
5. Evans MB, Po WD (2016) Clinical Question: Does Medical Evidence Support Routine Oronasopharyngeal Suction at Delivery? J Okla State Med Assoc 109(4-5): 140-142.
6. Nangia S, Pal MM, Saili A, Gupta U (2015) Effect of intrapartum oropharyngeal (IP-OP) suction on meconium aspiration syndrome (MAS) in developing country: A RCT. Resuscitation 97: 83-87.
7. Shah L, Shah GS, Singh RR, Pokharel H, Mishra OP, et al. (2015) Status of gastric lavage in neonates born with meconium stainedamniotic fluid: a randomized controlled trial. Ital J Pediatr 31;41: 85.
8. Chettri S, Adhisivam B, Bhat BV (2015) Endotracheal Suction for Nonvigorous Neonates Born through Meconium Stained Amniotic Fluid: A Randomized Controlled Trial. J Pediatr 166(5):1208-1213.
9. Goel A, Nangia S, Saili A, Garg A, Sharma S, et al. (2015) Role of prophylactic antibiotics in neonates born through meconium-stained amniotic fluid (MSAF) a randomized controlled trial. Eur J Pediatr 174(2): 237-243.
10. Siriwachirachai T, Sangkomkamhang US, Lumbiganon P, Laopaiboon M (2014) Antibiotics for meconium-stained amniotic fluid in labour for preventing maternal and neonatal infections. Cochrane Database Syst Rev 6(11):CD007772.
11. Sharma P, Nangia S, Tiwari S, Goel A, Singla B, et al. (2014) Gastric lavage for prevention of feeding problems in neonates with meconium-stained amniotic fluid: a randomised controlled trial. Paediatr Int Child Health 34(2): 115-119.
12. Hofmeyr GJ, Xu H, Eke AC (2014) Amnioinfusion for meconium-stained liquor in labour. Cochrane Database Syst Rev (1): CD000014.
13. Singh SN, Srivastava R, Singh A, Tahazzul M, Kumar M, et al. (2013) Respiratory distress including meconium aspiration syndrome invigorous neonates born through meconium stained amniotic fluid: incidence, onset, severity and predictors at birth. Indian J Pediatr 80(7): 538-543.
14. Ameta G, Upadhyay A, Gothwal S, Singh K, Dubey K, et al. (2013) Role of gastric lavage in vigorous neonates born with meconium stainedamniotic fluid. Indian J Pediatr 80(3): 195-198.
15. De Queiroz Siqueira M, Chassard D, Musard H, Heilporn A, Cejka JC, et al. (2014) Resuscitation with lipid, epinephrine, or both in levobupivacaine-induced cardiac toxicity in newborn piglets. Br J Anaesth 112(4): 729-734.
16. Skouroliakou M, Konstantinou D, Agakidis C, Delikou N, Koutri K, et al. (2012) Cholestasis, bronchopulmonary dysplasia, and lipid profile in preterm infants receiving MCT/ω-3-PUFA-containing or soybean-based lipid emulsions. Nutr Clin Pract 27(6): 817-824.
17. Lin EP, Aronson LA (2010) Successful resuscitation of bupivacaine-induced cardiotoxicity in a neonate. Paediatr Anaesth 20(10): 955-957.
18. Mirtallo JM, Dasta JF, Kleinschmidt KC, Varon J (2010) State of the art review: Intravenous fat emulsions: Current applications, safety profile, and clinical implications. Ann Pharmacother 44(4): 688-700.
19. Chuo J, Lambert G, Hicks RW (2007) Intralipid medication errors in the neonatal intensive care unit. Jt Comm J Qual Patient Saf 33(2): 104-111.
20. Joseph Eldor (2012) Vladimir Kotlovker. Intralipid for Amniotic Fluid Embolism (AFE)? OJAnes, 2(4). 127-133.
21. Windrik Lynch, Russell K McAllister, Jack F Lay Jr, William C Culp Jr (2017) Lipid Emulsion Rescue of Amniotic Fluid EmbolismInduced Cardiac Arrest: A Case Report. A&A Case Reports 8: 64-66.

# Chapter 6

## Warburg Effect and Intralipid Effect on the Mitochondria of Cancer Cells

## Abstract

Otto Warburg observed that cancers ferment glucose in the presence of oxygen, suggesting that defects in mitochondrial respiration may be the underlying cause of cancer. As stated by Otto Warburg nearly a century ago, cancer is a metabolic disease, a fermentation caused by malfunctioning mitochondria, resulting in increased anabolism and decreased catabolism. Treatment should, therefore, aim at restoring the energy yield. It was shown that a lipidic emulsion (LDE) composed of phospholipids and cholesterol esters which binds to low-density lipoprotein (LDL) receptors may concentrate in acute myeloid leukemia cells. LDE concentrates much more in malignant breast tumor tissue than in the normal tissue. Using Intralipid combined with the patient auto urotherapy will lead to an immune activation of the cancer antigens in urine creating antibodies and together with the intralipid attacking the cancer cells through its mitochondria as it is suggested by the Warburg effect

**Keywords:** Warburg effect; Mitochondria; Intralipid; Lipid Emulsion; Auto-urotherapy; Cancer

## Dr. Otto Heinrich Warburg

Otto Heinrich Warburg (1883-1970) was a member of an illustrious Jewish family, known for some five centuries. From humble beginnings, the family became prominent in the world for their contributions to all aspects of society. The son of a German mother and a Jewish (converted) father, Otto H. Warburg became a major contributor to medical science in the field of cancer research. Considered for Nobel Prize more than once, he finally received it in 1931 for his discovery of the nature and mode of action of the cellular respiratory enzyme. Warburg's personality was controversial: he was intolerant of opposing scientific views yet tolerant toward Nazi abuses. Accused of collaboration under the Nazi regime, Otto H. Warburg was nevertheless readmitted to the global scientific community after World War II. His contribution to cancer research remains influential to this day and has been superseded by discoveries that have built upon his work [1].

Decades ago, Otto Warburg observed that cancers ferment glucose in the presence of oxygen, suggesting that defects in mitochondrial respiration may be the underlying cause of cancer. We now know that the genetic events that drive aberrant cancer cell proliferation also alter biochemical metabolism, including promoting aerobic glycolysis, but do not typically impair mitochondrial function. Mitochondria supply energy; provide building blocks for new cells; and control redox homeostasis, oncogenic signaling, innate immunity, and apoptosis. Indeed, mitochondrial biogenesis and quality control are often upregulated in cancers. While some cancers have mutations in nuclear-encoded mitochondrial tricarboxylic acid (TCA) cycle enzymes that produce oncogenic metabolites, there is negative selection for pathogenic mitochondrial genome mutations. Eliminating mtDNA limits tumorigenesis, and rare human tumors with mutant mitochondrial genomes are relatively benign. Thus, mitochondria play a central and multifunctional role in malignant tumor progression, and targeting mitochondria provides therapeutic opportunities [2].

Cancer cells consume more glucose by glycolytic fermentation to lactate than by respiration, a characteristic known as the Warburg effect. In contrast with the 34 moles of ATP produced by respiration, fermentation produces two moles of ATP per mole of glucose consumed, which poses a puzzle on the function of the Warburg effect. Productions of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) per mole linearly vary with the fraction (x) of glucose consumed by fermentation that is frequently estimated around 0.9. Hence, calculation shows that, in respect to pure respiration, the predominant fermentative metabolism decreases around 10% the production of entropy per mole of glucose consumed in cancer cells. We hypothesize that increased fermentation could allow cancer cells to accomplish the Prigogine theorem of the trend to minimize the rate of production of entropy. According the theorem, open cellular systems near the steady state could evolve to minimize the rates of entropy production that may be reached by modified replicating cells producing entropy at low rate. Remarkably, at CO2 concentrations above 930 ppm, glucose respiration produces less entropy than fermentation, which suggests experimental tests to validate the hypothesis of minimization of the rate of entropy production through the Warburg effect [3].

## Mitochondria In Cancer Cells

Prominent features of cancer cells include metabolic imbalances and enhanced resistance to mitochondrial apoptosis. The fact that tumors rely heavily on glycolysis to meet their metabolic demands has been recognized since the beginning of the twentieth century, yet a complete elucidation of the so-called Warburg effect has not been achieved. Several mechanisms have been proposed to explain this phenomenon, including the up regulation of rate-limiting steps of glycolysis, the accumulation of mutations in the mitochondrial genome, the hypoxia-induced switch from mitochondrial respiration to glycolysis or the metabolic reprogramming resulting from the loss-of-function of enzymes like fumarate and succinate dehydrogenases. How aerobic glycolysis and apoptosis resistance are linked remains to be elucidated. On the one hand, these alterations may be acquired independently by cancer cells during multistep oncogenesis. On the other hand, the suppression of the intrinsic apoptotic program may be achieved through mechanisms that directly lead to the Warburg phenotype. Cancer-specific mitochondrial alterations and bioenergetics may be taken advantage for the development of two novel classes of antineoplastic agents. A first approach would target glycolysis and/or revert the Warburg phenomenon, whereas a second approach would aim at inducing apoptosis by targeting mitochondrial proteins and membranes. In both instances, encouraging pre-clinical results have been obtained [4]. Given the role of mitochondria in oxygen consumption, metabolism and cell death regulation, alterations in mitochondrial function or dysregulation of cell death pathways contribute to the genesis and progression of cancer. Cancer cells exhibit an array of metabolic transformations induced by mutations leading to gain-of-function of oncogenes and loss-of-function of tumor suppressor genes that include increased glucose consumption, reduced mitochondrial respiration, increased reactive oxygen species generation and cell death resistance, all of which ensure cancer progression. Cholesterol metabolism is disturbed in cancer cells and supports uncontrolled cell growth. In particular, the accumulation of cholesterol in mitochondria emerges as a molecular component that orchestrates some of these metabolic alterations in cancer cells by impairing mitochondrial function. As a consequence, mitochondrial cholesterol loading in cancer cells may contribute, in part, to the Warburg effect stimulating aerobic glycolysis to meet the energetic demand of proliferating cells, while protecting cancer cells against mitochondrial apoptosis due to changes in mitochondrial membrane dynamics. Further understanding the complexity in the metabolic alterations

of cancer cells, mediated largely through alterations in mitochondrial function, may pave the way to identify more efficient strategies for cancer treatment involving the use of small molecules targeting mitochondria, cholesterol homeostasis/trafficking and specific metabolic pathways [5].

It is a longstanding debate whether cancer is one disease or a set of very diverse diseases. The goal of this paper is to suggest strongly that most of (if not all) the hallmarks of cancer could be the consequence of the Warburg's effect. As a result of the metabolic impairment of the oxidative phosphorylation, there is a decrease in ATP concentration. To compensate the reduced energy yield, there is massive glucose uptake, anaerobic glycolysis, with an up-regulation of the Pentose Phosphate Pathway resulting in increased biosynthesis leading to increased cell division and local pressure. This increased pressure is responsible for the fractal shape of the tumor, the secretion of collagen by the fibroblasts and plays a critical role in metastatic spread. The massive extrusion of lactic acid contributes to the extracellular acidity and the activation of the immune system. The decreased oxidative phosphorylation leads to impairment in CO2 levels inside and outside the cell, with increased intracellular alkalosis and contribution of carbonic acid to extracellular acidosis-mediated by at least two cancer-associated carbonic anhydrase isoforms. The increased intracellular alkalosis is a strong mitogenic signal, which bypasses most inhibitory signals. Mitochondrial disappearance (such as seen in very aggressive tumors) is a consequence of mitochondrial swelling, itself a result of decreased ATP concentration. The transmembrane pumps, which extrude, from the mitochondria, ions, and water, are ATP-dependant. Therapy aiming at increasing both the number and the efficacy of mitochondria could be very useful [6].

A shift from respiration to fermentation is a common metabolic hallmark of cancer cells. As a result, glucose and glutamine become the prime fuels for driving the dysregulated growth of tumors. The simultaneous occurrence of "Press-Pulse" disturbances was considered the mechanism responsible for reduction of organic populations during prior evolutionary epochs. Press disturbances produce chronic stress, while pulse disturbances produce acute stress on populations. It was only when both disturbances coincide that population reduction occurred. This general concept can be applied to the management of cancer by creating chronic metabolic stresses on tumor cell energy metabolism (press disturbance) that are coupled to a series of acute metabolic stressors that restrict glucose and glutamine availability while also stimulating cancer-specific oxidative stress (pulse disturbances). The elevation of non-fermentable ketone bodies protect normal cells from energy stress while further enhancing energy stress in tumor cells that lack the metabolic flexibility to use ketones as an efficient energy source. Mitochondrial abnormalities and genetic mutations make tumor cells vulnerable metabolic stress. The press-pulse therapeutic strategy for cancer management is illustrated with calorie restricted ketogenic diets (KD- R) used together with drugs and procedures that create both chronic and intermittent acute stress on tumor cell energy metabolism, while protecting and enhancing the energy metabolism of normal cells. Optimization of dosing, timing, and scheduling of the press-pulse therapeutic strategy will facilitate the eradication of tumor cells with minimal patient toxicity. This therapeutic strategy can be used as a framework for the design of clinical trials for the non-toxic management of most cancers [7].

The potential role of the mitochondrial genome has recently attracted interest because of its high mutation frequency in tumors. Different aspects of mtDNA make it relevant for cancer's biology, such as it encodes a limited but essential number of genes for OXPHOS biogenesis, it is particularly susceptible to mutations, and its copy number can vary. Moreover, most ROS in mitochondria are produced by the electron transport chain. These characteristics place the mtDNA in the center of multiple signaling pathways, known as mitochondrial retrograde signaling, which modifies numerous key processes in cancer. Cybrid studies support that mtDNA mutations are relevant and exert their effect through a modification of OXPHOS function and ROS production. However, there is still much controversy regarding the clinical relevance of mtDNA mutations. New studies should focus more on OXPHOS dysfunction associated with a specific mutational signature rather than the presence of mutations in the mtDNA [8].

In the last years, metabolic reprogramming, fluctuations in bioenergetic fuels, and modulation of oxidative stress became new key hallmarks of tumor development. In cancer, elevated glucose uptake and high glycolytic rate, as a source of adenosine triphosphate, constitute a growth advantage for tumors. This represents the universally known Warburg effect, which gave rise to one major clinical application for detecting cancer cells using glucose analogs: the positron emission tomography scan imaging. Recent Advances: Glucose utilization and carbon sources in tumors are much more heterogeneous than initially thought. Indeed, new studies emerged and revealed a dual capacity of tumor cells for glycolytic and oxidative phosphorylation (OXPHOS) metabolism. OXPHOS metabolism, which relies predominantly on mitochondrial respiration, exhibits fine-tuned regulation of respiratory chain complexes and enhanced antioxidant response or detoxification capacity.

OXPHOS-dependent cancer cells use alternative oxidizable substrates, such as glutamine and fatty acids. The diversity of carbon substrates fueling neoplastic cells is indicative of metabolic heterogeneity, even within tumors sharing the same clinical diagnosis. Metabolic switch supports cancer cell stemness and their bioenergy-consuming functions, such as proliferation, survival, migration, and invasion. Moreover, reactive oxygen species-induced mitochondrial metabolism and nutrient availability are important for interaction with tumor microenvironment components.

Carcinoma-associated fibroblasts and immune cells participate in the metabolic interplay with neoplastic cells. They collectively adapt in a dynamic manner to the metabolic needs of cancer cells, thus participating in tumorigenesis and resistance to treatments.

Characterizing the reciprocal metabolic interplay between stromal, immune, and neoplastic cells will provide a better understanding of treatment resistance [9]. In differentiated normal cells, the conventional route of glucose metabolism involves glycolysis, followed by the citric acid cycle and electron transport chain to generate usable energy in the form of adenosine triphosphate (ATP). This occurs in the presence of oxygen. In hypoxic conditions, normal cells undergo anaerobic glycolysis to yield significantly less energy producing lactate as a product. As first highlighted in the 1920s by Otto Warburg, the metabolism exhibited by tumor cells involves an increased rate of aerobic glycolysis, known as the Warburg effect. In aerobic glycolysis, pyruvate molecules yielded from glycolysis are converted into fewer molecules of ATP even in the presence of oxygen. Evidence indicates that the reasons as to why tumor cells undergo aerobic glycolysis include: (1) the shift in priority to accumulate biomass rather than energy production, (2) the evasion of apoptosis as fewer reactive oxygen species are released by the mitochondria and (3) the production of lactate to further fuel growth of tumors [10].

As stated by Otto Warburg nearly a century ago, cancer is a metabolic disease, a fermentation caused by malfunctioning mitochondria, resulting in increased anabolism and decreased catabolism. Treatment should, therefore, aim at restoring the energy yield. To decrease anabolism, glucose uptake should be reduced (ketogenic diet). To increase catabolism, the oxidative phosphorylation should be restored. Treatment with a combination of α-lipoic acid and hydroxycitrate has been shown to be effective in multiple animal models. This treatment, in combination with conventional chemotherapy, has yielded extremely encouraging results in glioblastoma, brain metastasis and lung cancer. Randomized trials are necessary to confirm these preliminary data. The major limitation is the fact that the combination of α-lipoic acid and hydroxycitrate can only be effective if the mitochondria are still present and/or functional. That may not be the case in the most aggressive tumors. The increased intracellular alkalosis is a strong mitogenic signal, which bypasses most inhibitory signals. Concomitant correction of this alkalosis may be a very effective treatment in case of mitochondrial failure [11].

## Intralipid

Accidental intravascular or high-dose injection of local anesthetics (LA) can result in serious, potentially life-threatening complications. Indeed, adequate supportive measures and the administration of lipid emulsions are required in such complications. The study's objectives were threefold: (i) evaluate the myocardial toxicity of levobupivacaine when administered intravenously; (ii) investigate levobupivacaine toxicity on cardiomyocytes mitochondrial functions and cellular structure; (iii) assess the protective effects of a lipid emulsion in the presence or absence of myocardial ischemia. Domestic pigs randomized into two groups of 24 animals each, with either preserved coronary circulation or experimental myocardial ischemia. Six animals from each group received either: (i) single IV injection of saline, (ii) lipid emulsion (Intralipid(®) ), (iii) levobupivacaine, (iv) combination levobupivacaine-Intralipid(®) . Serially measured endpoints included: heart rate, duration of the monophasic action potentials (dMAP), mean arterial pressure, and peak of the time derivative of left ventricular pressure (LV dP/dtmax ). In addition, the following cardiomyocytes mitochondrial functions were measured: reactive oxygen species (ROS) production, oxidative phosphorylation, and calcium retention capacity (CRC) as well as the consequences of ROS production on lipids, proteins, and DNA. IV injection of levobupivacaine induced sinus bradycardia and reduced dMAP and LV dP/dtmax . At the mitochondrial level, oxygen consumption and CRC were decreased. In contrast, ROS production was increased leading to enhanced lipid peroxidation and structural alterations of proteins and DNA. Myocardial ischemia was associated with global worsening of all changes. Intralipid(®) quickly improved haemodynamics. However, beneficial effects of Intralipid(®) were less clear after myocardial ischemia [12].

We hypothesized that acute lipid-induced insulin resistance would be attenuated in high-oxidative muscle of lean trained (LT) endurance athletes due to their enhanced metabolic flexibility and mitochondrial capacity. Lean sedentary (LS), obese sedentary (OS), and LT participants completed two hyperinsulinemic euglycemic clamp studies with and without (glycerol control) the coinfusion of Intralipid. Metabolic flexibility was measured by indirect calorimetry as the oxidation of fatty acids and glucose during fasted and insulin-stimulated conditions, the latter with and without lipid oversupply. Muscle biopsies were obtained for mitochondrial and insulin-signaling studies. During hyperinsulinemia without lipid, glucose infusion rate (GIR) was lowest in OS due to lower rates of nonoxidative glucose disposal (NOGD), whereas state 4 respiration was increased in all groups. Lipid infusion reduced GIR similarly in all subjects and reduced state 4 respiration. However, in LT subjects, fat oxidation was higher with lipid oversupply, and although glucose oxidation was reduced, NOGD was better preserved compared with LS and OS subjects. Mitochondrial performance was positively associated with better NOGD and insulin sensitivity in both conditions. We conclude that enhanced mitochondrial performance with exercise is related to better metabolic flexibility and insulin sensitivity in response to lipid overload [13].

Intralipid® administration at reperfusion elicits protection against myocardial ischemia-reperfusion injury. However, the underlying mechanisms are not fully understood. Sprague-Dawley rat hearts were exposed to 15 min of

ischemia and 30 min of reperfusion in the absence or presence of Intralipid® 1% administered at the onset of reperfusion. In separate experiments, the reactive oxygen species (ROS) scavenger N-(2-mercaptopropionyl)-glycine was added either alone or with Intralipid®. Left ventricular work and activation of Akt, STAT3, and ERK1/2 were used to evaluate cardioprotection. ROS production was assessed by measuring the loss of aconitase activity and the release of hydrogen peroxide using Amplex Red. Electron transport chain complex activities and proton leak were measured by high-resolution respirometry in permeabilized cardiac fibers. Titration experiments using the fatty acid intermediates of Intralipid® palmitoyl-, oleoyl-and linoleoylcarnitine served to determine concentration-dependent inhibition of complex IV activity and mitochondrial ROS release. Intralipid® enhanced postischemic recovery and activated Akt and Erk1/2, effects that were abolished by the ROS scavenger N-(2-mercaptopropionyl) glycine. Palmitoylcarnitine and linoleoylcarnitine, but not oleoylcarnitine concentration-dependently inhibited complex IV. Only palmitoylcarnitine reached high tissue concentrations during early reperfusion and generated significant ROS by complex IV inhibition. Palmitoylcarnitine (1μM), administered at reperfusion, also fully mimicked Intralipid®-mediated protection in an N-(2-mercaptopropionyl)-glycine -dependent manner. Our data describe a new mechanism of postconditioning cardioprotection by the clinically available fat emulsion, Intralipid®. Protection is elicited by the fatty acid intermediate palmitoylcarnitine, and involves inhibition of complex IV, an increase in ROS production and activation of the RISK pathway [14]. We have recently shown that postischemic administration of intralipid protects the heart against ischemia-reperfusion injury. Here we compared the cardioprotective effects of intralipid with cyclosporine-A, a potent inhibitor of the mitochondrial permeability transition pore opening.

In vivo rat hearts or isolated Langendorff-perfused mouse hearts were subjected to ischemia followed by reperfusion with intralipid (0.5%, 1% and 2% ex-vivo, and 20% in vivo), cyclosporine-A (0.2 μM, 0.8 μM, and 1.5 μM ex- vivo and 10 mg/kg in vivo), or vehicle. The hemodynamic function, infarct size, calcium retention capacity, mitochondrial superoxide production, and phosphorylation levels of protein kinase B (Akt)/glycogen synthase kinase-3β (GSK-3β) were measured. The values are mean ± SEM. Administration of intralipid at reperfusion significantly reduced myocardial infarct size compared with cyclosporine-A in vivo (infarct size/area at risk)%: 22.9 ± 2.5% vs. 35.2 ± 3.5%; P = 0.030, n = 7/group). Postischemic administration of intralipid at its optimal dose (1%) was more effective than cyclosporine-A (0.8 μM) in protecting the ex vivo heart against ischemia-reperfusion injury, as the rate pressure product at the end of reperfusion was significantly higher (mmHg • beats/min: 12,740 ± 675 [n = 7] vs. 9,203 ± 10,781 [n = 5], P = 0.024), and the infarct size was markedly smaller (17.3 ± 2.9 [n = 7] vs. 29.2 ± 2.7 [n = 5], P = 0.014). Intralipid was as efficient as cyclosporine-A in inhibiting the mitochondrial permeability transition pore opening (calcium retention capacity = 280 ± 8.2 vs. 260.3 ± 2.9 nmol/mg mitochondria protein in cyclosporine-A, P = 0.454, n = 6) and in reducing cardiac mitochondrial superoxide production. Unlike intralipid, which increased phosphorylation of Akt (6-fold) and GSK-3β (5-fold), cyclosporine-A had no effect on the activation of these prosurvival kinases. Although intralipid inhibits the opening of the mitochondrial permeability transition pore as efficiently as cyclosporine-A, intralipid is more effective in reducing the infarct size and improving the cardiac functional recovery [15].

Free fatty acid (FFA)- and obesity-induced insulin resistance has been associated with disturbed mitochondrial function. Elevated plasma FFA can impair insulin-induced increase of adenosine triphosphate synthesis and down regulate the expression of genes important in the biogenesis of mitochondria in human skeletal muscle. Whether FAs have a direct effect on intrinsic mitochondrial capacity remains to be established. Therefore, we measured ex vivo mitochondrial respiratory capacity in human skeletal muscle after exposure to hyperinsulinemia and high levels of plasma FFA. Nine healthy lean men were studied during a 6-hour hyperinsulinemic (600 pmol/L) euglycemic clamp with concomitant infusion of Intralipid (Fresensius Kabi Nederland, Den Bosch, the Netherlands) (FFA clamped at 0.5 mmol/L) or saline. Mitochondrial respiratory capacity was measured by high-resolution respirometry in permeabilized muscle fibers using an Oxygraph (OROBOROS Instruments, Innsbruck, Austria). Each participant served as his own control. Peripheral glucose uptake (rate of disappearance) was significantly lower during infusion of the lipid emulsion compared with the control saline infusion (68 μmol/kg•min [saline] vs 40 μmol/kg•min [lipid], P = .008). However, adenosine diphosphate-stimulated and maximal carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone-stimulated uncoupled respiration rates were not different in permeabilized skeletal muscle fibers after exposure to high levels of FFA compared with the control condition. We conclude that short-term elevation of FFA within the physiological range induces insulin resistance but does not affect intrinsic mitochondrial capacity in skeletal muscle in humans [16].

Local anesthetics are used broadly to prevent or reverse acute pain and treat symptoms of chronic pain. Local anesthetic-induced cardiotoxic reaction has been considered the accidental event without currently effective therapeutic drugs except for recently reported intralipid infusion whose possible mechanism of action is not well known. Cardiolipin, an anionic phospholipid, plays a key role in determining mitochondrial respiratory reaction, fatty acid metabolism and cellular apoptosis. Mitochondrial energy metabolism dysfunction is suggested as associated with local anesthetic cardiotoxicity, from an in vitro study report that the local anesthetic cardiotoxicity may be due to the strong electrostatic interaction of local anesthetics and cardiolipin in the mitochondria membrane, although there is a lack for experimental evidence. Herein we hypothesized that local anesthetic-cardiolipin interactions

were the major determinant of local anesthetic-associated cardiotoxic reaction, established by means of theoretic and structural biological methods. The interaction between local anesthetic and mitochondrial cardiolipin may be the underlying mechanism for cardiotoxicity affecting its energy metabolism and electrostatic status [17]. We have previously shown that lack of thioredoxin-interacting protein (TXNIP) protects against diabetes and glucotoxicity-induced beta-cell apoptosis. Because the role of TXNIP in lipotoxicity is unknown, the goal of the present study was to determine whether TXNIP expression is regulated by fatty acids and whether TXNIP deficiency also protects beta-cells against lipoapoptosis. To determine the effects of fatty acids on beta-cell TXNIP expression, INS-1 cells and isolated islets were incubated with/without palmitate and rats underwent cyclic infusions of glucose and/or Intralipid prior to islet isolation and analysis by quantitative real-time RT-PCR and immunoblotting. Using primary wild-type and TXNIP-deficient islets, we then assessed the effects of palmitate on apoptosis (transferase-mediated dUTP nick-end labeling [TUNEL]), mitochondrial death pathway (cytochrome c release), and endoplasmic reticulum (ER) stress (binding protein [BiP], C/EBP homologous protein [CHOP]). Effects of TXNIP deficiency were also tested in the context of staurosporine (mitochondrial damage) or thapsigargin (ER stress). Glucose elicited a dramatic increase in islet TXNIP expression both in vitro and in vivo, whereas fatty acids had no such effect and, when combined with glucose, even abolished the glucose effect. We also found that TXNIP deficiency does not effectively protect against palmitate or thapsigargin-induced beta-cell apoptosis, but specifically prevents staurosporine- or glucose-induced toxicity. Our results demonstrate that unlike glucose, fatty acids do not induce beta-cell expression of proapoptotic TXNIP. They further reveal that TXNIP deficiency specifically inhibits the mitochondrial death pathway underlying beta-cell glucotoxicity, whereas it has very few protective effects against ER stress-mediated lipoapoptosis [18]. To investigate the mechanism of beta-cell dysfunction induced by glucolipotoxicity in high fat-fed obese rats:

Eighteen high-fat obese male Wistar rats were assigned into 3 groups and underwent 48-hour infusion through the jugular vein with normal saline (n=6), 20% intralipid + heparin (FFA group, n=6), or 25%glucose +20% intralipid + heparin (GS-FFA group, n=6). The plasma beta-hydroxybutyric acid (beta-HBA) was measured before and at the end of the infusion. After the infusion, the rats were sacrificed following an intravenous glucose tolerance test (IVGTT) to remove the tail of the pancreas for detection of apoptotic islet cells using TUNEL method. Immunohistochemical staining was performed to detect the expression of cytochrome c (cyt c), apoptosis-inducing factor (AIF), caspase-9 and caspase-3 in the islet cells. At the end of the infusion, all the rats exhibited increased plasma beta-HBA levels, which was the highest in the GS-FFA group ($P<0.05$). IVGTT performed after the infusion showed a significantly lower insulinogenic index in GS-FFA group than that in NS and FFA groups. Greater number of apoptotic islet cells was found in the GS-FFA group than in the FFA and NS groups ($P<0.05$), and the islets had significantly higher levels of cyt c, AIF, caspase-9 and caspase-3 in the former group than in the latter two groups ($P<0.05$). Hyperglycemia and high free fatty acid level synergistically impair insulin secretions to cause ketone overproduction in high fat-fed obese rats. The beta-cell dysfunction due to glucolipotoxicity is associated with increased beta-cell apoptosis and activation of mitochondrial apoptotic pathway [19].

To investigate pyruvate dehydrogenase (PDH)-E1alpha subunit phosphorylation and whether free fatty acids (FFAs) regulate PDH activity, seven subjects completed two trials: saline (control) and intralipid/heparin (intralipid). Each infusion trial consisted of a 4-h rest followed by a 3-h two-legged knee extensor exercise at moderate intensity. During the 4-h resting period, activity of PDH in the active form (PDHa) did not change in either trial, yet phosphorylation of PDH-E1alpha site 1 (PDH-P1) and site 2 (PDH-P2) was elevated in the intralipid compared with the control trial. PDHa activity increased during exercise similarly in the two trials. After 3 h of exercise, PDHa activity remained elevated in the intralipid trial but returned to resting levels in the control trial. Accordingly, in both trials PDH-P1 and PDH-P2 decreased during exercise, and the decrease was more marked during intralipid infusion. Phosphorylation had returned to resting levels at 3 h of exercise only in the control trial. Thus, an inverse association between PDH-E1alpha phosphorylation and PDHa activity exists. Short-term elevation in plasma FFA at rest increases PDH-E1alpha phosphorylation, but exercise overrules this effect of FFA on PDH-E1alpha phosphorylation leading to even greater dephosphorylation during exercise with intralipid infusion than with saline [20]. We have developed a stable isotope breath test to trace physiological remnant metabolism. Validity of the test depends on the injected lipid emulsion mimicking chylomicron remnant (CR) clearance and on subsequent metabolism of the emulsion cholesteryl ester (CE). Oxidation of CE fatty acids could involve both mitochondrial and peroxisomal pathways. In the present studies various agents were used to inhibit the binding of remnants, CE hydrolysis or mitochondrial fatty acid oxidation. Treatment of mice with suramin or lactoferrin markedly delayed the clearance and metabolism of remnants as shown by the significantly lower enrichment of 13CO(2) in the breath when compared with untreated mice. In hepatectomized rats injected with remnant-like emulsions, enrichment with 13CO(2) was virtually abolished. Treatment of mice with chloroquine or rats with methyl palmoxirate (an inhibitor of mitochondrial fatty acid oxidation) markedly impaired the recovery of label in the breath. Compared with mice fasted overnight, Intralipid by gavage decreased the breath enrichment with 13CO(2) consistent with competition between endogenous CR and the injected emulsion particles. These findings show that the breath test reliably measures the metabolism of CR and that CE fatty acid is metabolised by mitochondrial pathways [21]. Diminishing oxidative stress may protect the heart against ischaemia-reperfusion injury by preventing opening

of the mitochondrial permeability transition (MPT) pore. The general anaesthetic agent propofol, a free radical scavenger, has been investigated for its effect on the MPT and its cardioprotective action following global and cardioplegic ischaemic arrest. Isolated perfused Wistar rat hearts were subjected to either warm global ischaemia (Langendorff) or cold St. Thomas' cardioplegia (working heart mode) in the presence or absence of propofol. MPT pore opening was determined using [3H]-2-deoxyglucose-6-phosphate ([3H]-DOG-6P) entrapment. The respiratory function of isolated mitochondria was also determined for evidence of oxidative stress. Propofol (2 micrograms/ml) significantly improved the functional recovery of Langendorff hearts on reperfusion (left ventricular developed pressure from 28.4 +/- 6.2 to 53.3 +/- 7.3 mmHg and left ventricular end diastolic pressure from 52.9 +/- 4.3 to 37.5 +/- 3.9 mmHg). Recovery was also improved in propofol (4 micrograms/ml) treated working hearts following cold cardioplegic arrest. External cardiac work on reperfusion improved from 0.42 +/- 0.05 to 0.60 +/- 0.03 J/s, representing 45-64% of baseline values, when compared to controls ($P < 0.05$). Propofol inhibited MPT pore opening during reperfusion, [3H]-DOG-6P entrapment being 16.7 vs. 22.5 ratio units in controls ($P < 0.05$). Mitochondria isolated from non-ischaemic, propofol-treated hearts exhibited increased respiratory chain activity and were less sensitive to calcium-induced MPT pore opening. Propofol confers significant protection against global normothermic ischaemia and during cold cardioplegic arrest. This effect is associated with less opening of mitochondrial MPT pores, probably as a result of diminished oxidative stress. Propofol may be a useful adjunct to cardioplegic solutions in heart surgery [22].

We studied the variations arising in plasma and liver lipids after intravenous (i.v.), intraperitoneal (IP), and intragastric (IG) administration of a fat overdose on the order of 4 g.kg-1 body wt.day-1 in the form of Intralipid (ITL) 20% to 33 New Zealand rabbits for 15 days. The control group was submitted for surgery but did not receive an ITL supplement. The results show weight gain in all animals and normal liver enzyme values. There was an increase in plasma lipids in groups supplemented by the parenteral route (i.v. and IP), and fatty acids showed a similar distribution, in terms of percentages, to that for ITL. In liver tissue, there was an increase in the fractions related to ethanolamine and a decrease in phospholipids of choline and serine. In the i.v. group, neutral lipids predominated compared with other groups. The livers of all supplemented animals (i.v., IP, and IG) showed a higher content of stearic and linoleic acid and a reduction in oleic acid. Study with optical microscopy showed a microvacuolization affecting the three areas of the hepatic acini in the i.v. group, seen with electron microscopy as vacuoles lacking membranes and surrounded by mitochondria. In conclusion, there is an increase in hepatic steatosis in parenteral groups and a greater deposit of neutral lipids in the i.v. group, related to the administration route, without biochemical signs of liver dysfunction [23].

The metabolism of Intralipid (intravenously injected) was studied in rats fasted for 48 h. At all doses used, the Intralipid triacylglycerols disappeared rapidly from circulation and concomitantly the hepatic content of triacylglycerols and the level of circulating ketone bodies increased, indicating an active metabolism of Intralipid by the liver. To study this possibility further we used an ultrastructural approach. In rats given Intralipid we detected numerous lipid particles in the spaces of Disse, retained in the interdigitations of the hepatocyte. There were also lipid particles attached to the luminal surface of the endothelial cells. Small lipid particles were seen in close contact with endocytic vesicles internalized into hepatocytes but were present mainly in endothelial cells. Inside the endothelial cells, the endocytic vesicles were detected in contact with lysosomes. Inside hepatocytes, a process of sterification seemed to occur in the endoplasmic reticulum as deduced from the presence of small lipid droplets with ill-defined outlines. Large lipid droplets were seen in close contact with mitochondria, indicating a mitochondrial uptake and metabolism of fatty acids to synthesize and release ketone bodies. The possible role of lipoprotein lipase in the liver for the hepatic uptake of Intralipid particles is discussed [24].

Effects of high plasma free fatty acids (FFA) on the free radical formation of myocardial mitochondria, isolated from normal and ischemic dog hearts, were studied by electron spin resonance (ESR) spectrometry. Free radical concentrations in state 4 respiration were used for the evaluation of the function in the mitochondria in this study. High plasma FFA levels were induced either by intravenous injection of Intralipid and heparin, or by infusion of norepinephrine. Ischemic hearts were induced by inserting a Cournand's 7F catheter into the left coronary artery under fluoroscopic control. Exogenous high plasma FFA induced by Intralipid and heparin caused the decrease of free radicals in state 4 respiration in the mitochondria isolated from normal and ischemic dog hearts. Endogenous high FFA induced by continuous infusion of norepinephrine also caused the decrease of free radicals. On the other hand, nicotinic acid prevented the decrease of free radicals as well as the rise of plasma FFA by the norepinephrine infusion. These results suggest that high plasma FFA itself, whether it may be exogenous or endogenous, may impair the oxidative phosphorylation of the mitochondria isolated from normal and ischemic hearts [25]. Low-density lipoprotein (LDL) could be used as a carrier of chemotherapeutic agents to neoplastic cells that overexpress LDL receptors (rLDL), but LDL is difficult to obtain and handle. Recently, it was observed that a protein-free emulsion resembling the lipid portion of LDL (LDE) behave like native LDL when injected into the bloodstream. In this study, the evidence that LDE is taken up by rLDL was expanded by comparing LDL and LDE plasma decay curves in rabbits and by competition experiments with lymphocytes. To verify whether LDE could be removed from the plasma by neoplastic cells with increased rLDL, LDE labeled with 14Ccholesteryl ester was injected into 14 patients with acute myeloid leukemia (AML) and into 7 with acute lymphocytic leukemia (ALL). In

AML rLDL expression is increased but in ALL it is normal. LDE plasma fractional clearance rate (FCR, in h-1) was calculated from the remaining radioactivity measured in plasma samples collected during 24 h following injection. LDE FCR was 3-fold greater in AML than in ALL patients 0.192 +/- 0.210 (SD) and 0.066 +/- 0.033 h-1, respectively, $P < 0.035$. When LDE injection was repeated in 9 AML patients in hematological remission, LDE FCR diminished 66% compared to the pretreatment values (from 0.192 +/- 0.210 to 0.065 +/- 0.038 h-1, $P < 0.02$), so that it could be estimated that nearly 66% of the emulsion was taken up by AML cells and only 34% by the normal tissues. As expected, LDE FCR was unchanged in 4 patients with ALL in hematological remission (0.069 +/- 0.044 h-1). Gamma camera images obtained 6 h after the injection of 99mTc-label LDE into one patient with ALL showed biodistribution similar to that of LDL. In one AML patient LDE was comparatively more concentrated over the areas corresponding to the bone marrow infiltrated by AML cells. Our results indicate that LDE FCR is increased in a disease known to contain malignant cells that overexpress rLDL, suggesting that LDE is taken up by malignant cells with increased rLDL [26]. Previously, it was shown that a lipidic emulsion (LDE) composed of phospholipids and cholesterol esters which binds to low-density lipoprotein (LDL) receptors may concentrate in acute myeloid leukemia cells. In this study, we aimed to verify whether LDE also has the ability to concentrate in malignant ovarian cancer after being injected into the blood circulation of the patients. Three groups of women scheduled for surgery were included in the survey: 13 bearing malignant tumors, 9 with benign ovarian tumors, and 13 without ovarian tumor who were scheduled to undergo oophorectomy due to malignant disease of the uterine cervix or endometrium. On the day prior to surgery they were injected with LDE labeled with [(14)C]cholesteryl oleate. Specimens of tumors and normal ovaries excised during surgery were lipid extracted and analyzed for radioactivity counting. Results were expressed in radioactive count (cpm) per gram of tissue.

The mean of the uptakes of the emulsion radioactivity by the malignant tumors was roughly eightfold greater when compared with that of the contralateral normal ovaries (2261 +/- 1444 and 275 +/- 137 cpm/g, respectively, $P < 0.012$), benign tumors, and normal ovaries of the patients without ovarian tumors. LDE has the ability to concentrate in malignant ovarian tumor tissue. Therefore, it can be used as a vehicle to direct cytotoxic drugs against malignant ovarian tumors, thus diminishing the side effects of chemotherapy [27]. Over expression of low-density lipoprotein (LDL) receptors occurs in several cancer cell lines and offers a unique strategy for drug targeting by using LDL as vehicle. However, the native lipoprotein is difficult to obtain and handle. Previously, we showed that a lipidic emulsion (LDE) similar to the lipid structure of native LDL may bind to LDL receptors and be taken up by acute myelocytic leukemia cells. We also showed that LDE can also concentrate in ovarian cancer tissue. In this study, we tested whether LDE is taken up by breast carcinoma. LDE labeled with (99m)Tc was injected into 18 breast cancer patients, and nuclear medicine images of the tumor and metastatic sites were acquired. Subsequently, LDE labeled with [3H] cholesteryl oleate was intravenously injected into 14 breast cancer patients 24-30 h before total mastectomy procedure. Fragments of normal and of breast cancer tissue excised during surgery were lipid extracted with chloroform/methanol and their radioactivity was measured in a scintillation solution. (99m) Tc-LDE images of the primary tumor and of metastasis sites were obtained in all 18 breast cancer patients. As directly measured in the tumor and in the normal mammary tissue, the amount of the emulsion radioactive label in the tumor was 4.5 times greater than in the normal tissue (range 1.2- to 8.8-fold). LDE concentrates much more in malignant breast tumor tissue than in the normal tissue. Thus it has potential to carry drugs or radionuclides directed against mammary carcinoma cells for diagnostic or therapeutic purposes [28].

Cancer cells release various antigens, some of which appear in the urine. Oral autourotherapy is suggested as a new treatment modality for cancer patients. It will provide the intestinal lymphatic system with the many tumor antigens against which antibodies may be produced. These antibodies may be pierced through the blood stream and attack the tumor and its cells. Using Intralipid combined with the patient auto urotherapy will lead to an immune activation of the cancer antigens in urine creating antibodies and together with the intralipid attacking the cancer cells through its mitochondria as it is suggested by the Warburg effect [29].

## Conclusion

It is suggested that based on the Warburg effect the combination of Intralipid and auto urotherapy is a new modality in cancer treatment.

## References


1. Root-Bernstein RS (1993) Causality, complementarity, evolution, and emergent properties. In Iversen OH (Eds.), New Frontiers in Cancer Causation. Taylor & Francis, UK, p. 1-14
2. Burnet FM (1970) The concept of immunological surveillance. Prog Exp Tumor Res 13: 1-27
3. Hellstrom I, Hellstrom KE, Zeidman L, Bernstein ID, Brown JP, et al. (1979) Cell-mediated reactivity to antigens shared by Moloney virus induced lymphoma cells (LSTRA) and cells from certain 3-methylcholanthrene induced mouse sarcomas. Int J Cancer 23(4): 555-564.
4. Hellstrom I, Hellstrom KE, Shepard TH (1970) Cell-mediated immunity against antigens common to tumor colonic carcinomas and fetal gut epithelium. Int J Cancer 6(3): 346-351.
5. Hellstrom KE, Hellstrom I (1989) Immunological approach to tumor therapy: Monoclonal antibodies, tumor vaccines, and anti-idiotypes. In Quash GA, Rodwell JD & Marcel Dekker Covalently Modified Antigens and Antibodies in Diagnosis and Therapy, New York, p.1
6. Ehrlich P (1957) In Himmelweit S (Eds.), The Collected Papers of Paul Ehrlich (translated by P. Alexander), Pergamon Press, UK.
7. Watanabe T, Punkel CS, Takeyama H, Lloyd KO, Shiku H, et al. (1982) Human melanoma antigen AH is an autologous ganglioside related to GD2. J Exp Med 156(6): 1884-1889.
8. Rote NS, Gupta RK, Morton DL (1980) Tumor-associated antigens detected by autologous sera in urine of patients with solid neoplasms. J Surg Res 29(1): 18-22.
9. Gupta RK, Huth JF, Korn EL, Morton DL (1983) Prognostic significance of urinary antigen analysis by enzyme-linked immunosorbant assay in melanoma patients. Diag Immunol 1(4): 303-309.
10. DeVere White RW, Meyers FJ, Soares SE, Miller OG, Soriano TF (1992) Urinary prostate specific antigen levels: Role in monitoring the response of prostate cancer to therapy. J Urol 147(3 Pt 2): 947-951.
11. Huland E, Huland H, Meier T, Baricordi O, Fradet Y, Get al. (1991) Comparison of 15 monoclonal antibodies against tumor-associated antigens of transitional cell carcinoma of the human bladder. J Urol 146: 1631-1636.
12. Farinati F, Cardin F, Costa F, Nitti D, Di Mario F, et al. (1986) Gastric juice CEA levels: importance of age and gastric mucosal damage. Europ J Cancer Clin Oncol 22(4): 527-529.
13. Touitou Y, Proust J, Klinger E, Nakache JP, Huard D, et al. (1984) Cumulative effects of age and pathology on plasma carcinoembryonic antigen in an unselected elderly population. Europ J Cancer Clin Oncol 20(3): 369-374.
14. Lindholm L, Holmgren J, Svennerholm L, Fredman P, Nillson O, et al. (1983) A Monoclonal antibodies against gastro-intestinal tumor-associated antigens isolated as monosialogangliosides. Int Arch Allergy appl Immun 71: 178-181.
15. Mansson JE, Fredman P, Nilsson O, Lindholm L, Holmgren J, Svennerholm C, et al. (1985) Chemical structure of carcinoma ganglioside antigens defined by monoclonal antibody CA50 and some allied gangliosides of human pancreatic adenocarcinoma. Biochim Biophys Acta 834: 110-117.
16. Holmgren J, Lindholm L, Persson B, Lagergard T, Nilsson O, et al. (1984) Detection by monoclonal antibody of carbohydrate antigen CA50 in serum of patients with carcinoma. Br Med J (Clin Res Ed) 288(6429): 1479-1482.
17. Dienst C, Clodius T, Oldorp T, Uhlenbruch G, Diehl V (1987) CA 19-9, CA50 und CEA bei Pankreas und gastrointestinal Tumoren. Medizin Klin 82: 45-50.
18. Bruhn HD, Broers H, Euler H, Everding A, Feller AC, et al. (1986) CA im serum von Karzinom-Patienten. Deutsch Med Wochensch 34: 1267-1272.
19. Farinati F, Holmgren J, Di Mario F, Cardin F, Valliante F, et al. (1991) CA 50 determination in body fluids: Can we screen patients at risk for gastric cancer? Int J Cancer 47(1): 7-11.
20. Hoffmann JC, Dengler TJ, Knolle PA, Albert-Wolf M, Roux M, et al. (1993) A soluble form of the adhesion receptor CD58 (LFA-3) is present in human body fluids. Eur J Immunol 23: 3003-3010.
21. Tobi M, Darmon E, Rozen P, Harpaz N, Fink A, et al. (1995) Urinary organ specific neoantigen. A potentially diagnostic test for colorectal cancer. Dig Dis Sci 40: 1531-1537.
22. Boutwell RK (1983) Evidence that an elevated level of ornithine decarboxylase activity is an essential component of tumor promotion. Adv Polyamine Res;4:127-133
23. lipton A, Sheehan L, Harvey HA (1975) Urinary polyamine levels in patients with gastrointestinal malignancy. Cancer 36(6 Suppl): 2351-2354.
24. Loser C, Folsch UR, Paprotny C, Creutzfeldt W (1990) Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum and urine of 50 patients with colorectal cancer. Cancer 65(4): 958-966.

25. Matsumura Y, Tarin D (1992) Significance of CD44 gene products for cancer diagnosis and disease evaluation. Lancet 340(8827): 1053-1058.

26. Matsumura Y, Hanbury D, Smith JC, Tarin D (1994) Non-invasive detection of malignancy by identification of unusual CD44 gene activity in exfoliated cancer cells. Br Med J 308(6929): 619-624.

27. Matsumura Y, Sugiyama M, Matsumura S, Hayle AJ, Robinson P, et al. (1995) Unusual retention of introns in CD44 gene transcripts in bladder cancer provides new diagnostic and clinical oncological opportunities. J Pathol 177(1): 11-20.

28. Zbar B, Tanaka T (1971) Immunotherapy of cancer: regression of tumors after intralesional injection of living Mycobacterium bovis. Science 172(3980): 271-273.

29. Rosenberg SA, Packard BS, Aebersold PM, et al. (1988) Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med 319(25): 1676-1680.

30. Hellstrom KE, Hellstrom I (1969) Cellular immunity against tumor specific antigens. Adv Cancer Res 12: 167-223.

31. Penn I (1988) Tumors of the immunocompromised patient. Ann Rev Med 39: 63-73.

32. Vanky F, Klein E, Willems J, Böök K, Ivert T, et al. (1987) Lysis of Autologous tumor cells by blood lymphocytes activated in Autologous mixed lymphocyte tumor cell culture - no correlation with the postsurgical clinical course. Cancer Immunol Immunother 24(2): 180-183.

33. Vanky F, Klein E (1982) Specificity of auto-tumor cytotoxicity exerted by fresh, activated and propagated human T lymphocytes. Int J Cancer 29(45: 547-553.

34. Knuth A, Wolfel T, Klehmann E, Boon T, Meyer zum Buschenfelde KH, et al. (1989) Cytolytic T cell clones against an autologous human melanoma: Specificity study and definition of three antigens by immunoselection. Proc Natl Acad Sci USA 86(8): 2804-2808.

35. Lo Buglio AF, Neidhart JA (1974) A review of transfer factor immunotherapy in cancer. Cancer 34: 1563-1570.

36. Levin AS, Byers VS, Fudenberg HH, Wybran J, Hackett AJ, et al. (1975) Osteogenic sarcoma. Immunologic parameters before and during immunotherapy with tumor-specific transfer factor. J Clin Invest 55(3): 487-499.

37. Rosenberg SA, Schearz SL, Speiss PJ (1988) Combination immunotherapy for cancer: synergistic antitumor interactions of interleukin-2, alpha interferon and tumor-infiltrating lymphocytes. JNCI 80(17): 1393-1397.

38. Rosenberg SA, Aebersold P, Cornetta K, et al. (1990) Gene transfer into humans: immunotherapy of melanoma using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 323(9): 570-578.

39. Russell SJ, Eccles SA, Fleming CL, Johnson CA, Collins MKL (1991) Decreased tumorigenicity of a transplantable rat sarcoma following transfer and expression of an IL-2 cDNA. Int J Cancer 47(2): 244-252.

40. Hellstrom KE, Hellstrom I, Kant JA, Tamerius JD (1978) Regression and inhibition of sarcoma growth by interference with a radiosensitive T cell population. J Exp Med 148(3): 799-804.

41. Estin CD, Stevenson US, Hellstrom I, Hellstrom KE (1989) Cyclophosphamide potentiates the antitumor activity of v-p97NY. Cell Immunol 120(1): 126-131.

42. Nepom GT, Hellstrom KE (1987) Anti-idiotypic antibodies and the induction of specific tumor immunity. Cancer Metast Rev 6(4): 489-502.

43. Schwartz RH (1990) A cell culture model for T lymphocyte clonal anergy. Science 248(4961): 1349-1356.

44. Jenkins MK, Schwartz RH (1987) Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. J Exp Med 165(2): 302-319.

45. Mowat AM (1987) The regulation of immune responses to dietary protein antigens. Immunol Today 8(3): 93-98.

46. Weiner HL, Zhang ZJ, Khoury SJ, Miller A, Al-Sabbagh A, et al. (1991) Antigen-driven peripheral immune tolerance. Suppression of organ-specific autoimmune diseases by oral administration of autoantigens. Ann NY Acad Sci 636: 227-232.

47. Sercarz E, Krzych U (1991) The distinctive specificity of antigen-specific suppressor T cells. Immunol Today 12(4): 111-118.

48. Green DR, Flood PM, Gershon RK (1983) Immunoregulatory T-cell pathways. Annu Rev Immunol 1: 439-463.

49. Thurau SR, Caspi RR, Chan CC, Weiner HL, Nussenblatt RB, et al. (1991) Immunological suppression of experimental autoimmune uveitis. Fortschr Ophthalmol 88: 404-407.

50. Nussenblatt RB, Caspi RR, Mahdi R, Chan CC, Roberge F, et al. (1990) Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J immunol 144(5): 1689-1695.

51. Brandtzaeg P (1989) Overview of the mucosal immune system. Curr top Microbiol Immunol 146: 13-28.

52. MesteckyJ, McGhee JR (1989) Oral immunization: Past and present. Curr Top Microbiol Immunol 146: 3-12.

53. Stokes CR (1984) Induction and control of intestinal immune responses. In Newby TJ & Stokes CR (eds.) ,Local immune responses of the gut,CRC Press, Boca Raton, USA, pp.97-142.

54. Hanson DG, Vaz NM, Rawlings LA, Lynch JM (1979) Inhibition of specific immune responses by feeding protein antigens. II. Effects of prior passive and active immunization. J Immunol 122(6): 2261-2266.

55. Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, et al. (1987) Nature and properties of the human gastrointestinal immune system. In Miller K & Nicklin S (eds.), Immunology of the Gastrointestinal Tract, CRC Press, Boca Raton, USA, pp. 1-88.

56. Peng HJ, Turner MW, Strobel S (1990) The generation of a “tolerogen” after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. Clin Exp Immunol 81(3): 510-515.

57. Stokes CR, Swarbrick ET, Soothill JF (1983) Genetic differences in immune exclusion and partial tolerance to ingested antigens. Clin Exp Immunol 52(3): 678-684.

58. Swarbrick ET, Stokes CR, Soothill JF (1979) Absorption of antigens after oral immunization and the simultaneous induction of specific systemic tolerance. Gut 20(2): 121-125.

59. Miller A, Zhang AJ, Prabdu-Das M, Sobel A, Weiner HL, et al. (1992) Active suppression vs. clonal anergy following oral or IV administration of MBP in actively and passively induced EAE. Neurology 42(Suppl 3): 301.

60. Lamont AG, Gordon M, Ferguson A (1987) Oral tolerance in protein deprived mice. II. Evidence of normal ‘gut processing’ of ovalbumin, but suppressor cell deficiency, in deprived mice. Immunology 61(3): 339-343.

61. Hanson DG, Vaz NM, Maia LC, Hornbrook MM, Lynch JM, et al. (1977) Inhibition of specific immune responses by feeding protein antigens. Int Arch Allergy Appl Immunol 55: 526-532.

62. Matthews JB, Fivaz BH, Sewell HF (1981) Serum and salivary antibody responses and the development of oral tolerance after oral and intragastric antigen administration. Int Arch Allergy Appl Immunol 65(1): 107-113.

63. Higgins P, Weiner HL (1988) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J Immunol 140(2): 440-445.

64. Miller A, Lider O, Al-Sabbagh A, Weiner HL (1992) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. J Neuroimmunol 39(3): 243-250.

65. Jausion H (1935) Sur lauto-ouro-therapie. Journal D Urologie 39: 58-59.

66. Cimino T (1927) Premiers essais de vaccine-proteine-therapie des infections non gonococciques ni tuberculeuses des voies urinaires a l’aide des injections sous-cutanees de l’urine purulente du sujet, sterilisee par l’ebullition (ouro-therapie). Rivista Sanitaria 186:

67. Rabinowitch IM (1931) Auto-urine-therapy in gonarthritis. Vratchebnaia gazeta 35: 677-678.

68. Jausion H, Giard R, Martinaud G (1933) Lauto-ouro-therapie. La Presse Medicale 76:1467-1470.

69. Day HB (1936) Treatment of glomerulonephritis by antigen. Lancet 1228(5912): 1456-1459.

70. Sandweiss DJ, Saltzstein HC, Farbman AA (1938) The prevention or healing of experimental ulcer in Mann-Williamson dogs with the Anterior-Pituitary-Like hormone (Antuitrin-S). Am J Dig Dis 5(1): 24-30.

71. Sandweiss DJ, Saltzstein HC, Farbman AA (1939) The relation of sex hormones to peptic ulcer. Am J Dig Dis 6(1):6-12.

72. Seiffert L (1935) Die “Darn-Siphonblase”. Arch fur Klin Chir 183: 569.

73. Bricker EM (1929) Bladder substitution after pelvic evisceration. Surg Clin North Am 1950;30:1511 74. Hammer E. Cancer du colon sigmoide dix ans après implantation des ureteres d’une vessie exstrophiee. J Urol Nephrol 28: 260.

74. Miller-Schoop JW, Good RA (1975) Functional studies of Peyer`s patches: Evidence for their participation in intestinal immune responses. J Immunol 144(6): 1757-1760.

75. Barclay THC, Schapira DV (1983) Malignant tumors of the small intestine. Cancer 51(5): 878-881.

76. Loeffler M, Stein R, Wichmann HE, Potten CS, Kaur P, et al. (1986) Intestinal cell proliferation. I. A comprehensive model of steady-state proliferation in the crypt. Cell Tissue Kinet 19(6): 627-645.

77. Tapper D, Folkman J (1976) Lymphoid depletion in ileal loops: Mechanism and clinical implications. J Pediatr Surg 11(5): 871-880.

78. Wilson WEC, Kirkpatrick CH, Talmage DW (1965) Suppression of immunologic responsiveness in uremia. Ann Intern Med 62(1): 1-14.

79. Starkey RH, Cohen S, Orth DN (1975) Epidural growth factor: Identification of a new hormone in human urine. Science 189(4205): 800-802.

80. Urdaneta LF, Duffell D, Creevy CD, Aust JB (1966)

Late development of primary carcinoma of the colon following ureterosigmoidostomy: report of three cases and literature review. Ann Surg 164(3): 503-513.

81. Harguindey SS, Colbeck RC, Bransome ED JR (1975) Ureterosigmoidostomy and cancer: new observations (letter). Ann Intern Med 83(6): 833.

82. Rivard JY, Bedard A, Dionne L (1975) Colonic neoplasms following ureterosigmoidostomy. J Urol 113(6): 781-786.

83. Carswell JJ III, Skeel DA, Witherington R, Otken LB Jr (1976) Neoplasia at the site of ureterosigmoidostomy. J Urol 115(6): 750-752.

84. Lasser A, Acosta AE (1975) Colonic neoplasms complicating ureterosigmoidostomy. Cancer 35(4): 1218-1222.

85. Sooriyaarachchi GS, Johnson RO, Carbone PP (1977) Neoplasms of the large bowel following ureterosigmoidostomy. Arch Surg 112(10): 1174-1147.

86. Eraklis AJ, Folkman MJ (1978) Adenocarcinoma at the site of ureterosigmoidostomies for exstrophy of the bladder. J Pediatr Surg 13(4): 730-734.

87. Everson T (1964) Spontaneous regression of cancer. Ann NY Acad Sci 114: 721-735.

88. Stephenson H, Delmez J, Renden D, Kimpton R, Todd P, et al. (1971) Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. Surg Gynecol Obstet 133(4): 649-655.

89. Cole W (1974) Spontaneous regression of cancer: The metabolic triumph of the host? Ann NY Acad Sci 230: 111-141.

90. Burnet F (1967) Immunological aspects of malignant disease. Lancet 1(7501): 1171-1174.

91. Droller M (1980) Immunotherapy and genitourinary neoplasia. Urol Clin N Am 7(3): 831-846.

92. Enomoto K, Kawakita M, Kishimoto S, Katayama N, Miyake T, et al. (1980) Thrombopoiesis and megakaryocyte colony stimulating factor in the urine of patients with aplastic anemia. Br J Haematol 45(4): 551-556.

93. Kawakita M, Enomoto K, Katayama N, Kishimoto S, Miyake T, et al. (1981) Thrombopoiesis and megakaryocyte colony stimulating factors in the urine of patients with idiopathic thrombocytopenic purpura. Br J Haematol 48(4): 609-615.

94. Stanley ER, McNeill TA, Chan SH (1970) Antibody production to the factor in human urine stimulating colony formation in vitro by bone marrow cells. Br J Haematol 18(5): 585-590.

95. Galdston I (1939) The ideological basis of discovery. Bull Hist Med 7: 729-735.

# Chapter 7

## Combined Intralipid - Urotherapy for Patients with Cancer (Part 1)

## Abstract

Cancer cells release various antigens, some of which appear in the urine. Combined oral Intralipid and auto-urotherapy is suggested as a new treatment modality for cancer patients. It will provide the intestinal lymphatic system the many tumor antigens against which antibodies may be produced. These antibodies may be transpierced through the blood stream and attack the tumor and its cells. Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced.

**Keywords:** Cancer; Urotherapy; Intralipid

## The Philosophy Of Cancer

Microbes were known long before the germ theory of disease was invented. It was not the discovery of germs that revolutinized medicine, but the invention of a philosophy of medical explanation that permitted germs to be causative agents of disease [1]. Burnet and Thomas [2] postulated that specific cell mediated immunity may have evolved in vertebrates specially for defense against the "enemy within" rather than against infecting microorganisms and parasites. Most human cancers appear to lack truly tumor-specific antigens. The same neoplastic cell can express several different tumor antigens. For example, relatively cross-reacting tumor-specific transplantation antigens have been demonstrated in many chemically induced tumors [3]. Tumor-associated differentiation antigens are shared by neoplastic and embryonic cells [4]. The extent to which human patients react immunologically against their cancers has been a subject of much controversy [5]. Paul Ehrlich, in 1909, said:"I am convinced that during development and growth malignant cells arise extensively frequently but that in the majority of people they remain latent due to the protective action of the host. I am also convinced that this natural immunity is not due to the presence of antimicrobial bodies but is determined purely by cellular factors. These may be weakened in the older age groups in which cancer is more prevalent"[6].

### Tumor antigens in urine

Human melanoma cells express membrane antigens distinct from those of the normal ectodermal counterparts [7]. Urinary-tumor-associated antigen (U-TAA) is one such antigen. This high-molecular weight glycoprotein was first described when melanoma urine was found to react with autologous antibody [8]. The antigen has since been detected in the urine of 68% of melanoma patients. In addition, high levels of U-TAA are found to correlate positively with disease occurrence in surgically treated patients [9]. Prostatic specific antigen (PSA) has become an important laboratory test in the management of prostate cancer. PSA levels can be as readily obtained from voided urine as from serum samples [10].

Quantitative urinary immunocytology with monoclonal antibody (mab) 486p 3/12 proved to be valuable for diagnostic use in bladder-cancer patients` urine, especially in the followup of patients with superficial bladder carcinoma [11]. Quantitative urinary immunocytology is a general tool to test the diagnostic usefulness of mabs, assuming that normal and malignant cells differ in their quantitative expression of a given antigen. Selective criteria for selecting mabs for diagnostic approaches should ask not for tumor specificity, but for different quantitative expression of antigen in the tissues or cells in question. Gastric juice oncofetal antigen determination, due to direct shedding of antigens into the fluid around tumor tissues, appears to accurately indicate the presence and degree of gastric mucosal damage and to be to a slight extent influenced by unrelated factors [12]. Patients` age, for example, modifies CEA serum levels [13]. A monoclonal antibody (mab) against a human colorectal adenocarcinoma cell line has been raised [14], which reacts with sialosylfucosyllactoteraose [15] corresponding to the sialylated blood group antigen Lewis (a). The antigen defined by this antibody, CA50, is elevated in the serum of many patients with gastrointestinal tumors [16], with a sensitivity for gastric cancer ranging from 20 [17] to 65% [18]. CA50 (a tumor-associated gangliosidic antigen) levels have been determined by an RIA test in serum, gastric juice and urine of patients undergoing upper gastrointestinal tract endoscopy. Sensitivity and specificity were respectively 23% and 89% for CA50 determination in urines [19]. Soluble forms of membrane proteins such as cytokine receptors or cellular adhesion molecules (CD14, TNF receptor, CD25, IL-6 receptor, IFN-ç-receptor and CD54) have been detected in human body fluids. They may have important functions in immune regulation by blocking receptor/ligand interactions. The human adhesion receptor CD58 (LFA-3) is expressed on most cell types. A soluble form of CD58 (sCD58) was purified from human urine and partially purified from supernatant of the Hodgkin-derived cell line L428 [20]. Urinary organ-specific neoantigen from colorectal cancer patients has been used to make a monoclonal antibody, BAC 18.1 [21]. Organ-specific neoantigen originates in the colon and is excreted into the urine, so the BAC 18.1 binding levels in the urine may be a diagnostic aid for colorectal cancer. The polyamines spermidine, spermine and their diamine precursor putrescine are ubiquitous constituents of mammalian cells that are fundamentally involved in normal, malignant and induced proliferative states. The polyamines and ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine metabolism, were found to play an important role in tumor promotion [22]. The suggestion that polyamines play an important role in colorectal cancer was confirmed by studies that found elevated polyamine concentrations in blood or urine [23] of patients with colon carcinoma. Sensitivity of urinary polyamines for colon cancer were highest for total spermidine (92.1%), acetylated putrescine (84.5%), total putrescine (84.0%), N1-acetylspermidine (79.3%) and N8-acetylspermidine (78.6%), but in all these cases specificity was lower than 65% [24]. In patients with successful curative surgical treatment all preoperatively elevated urinary polyamine concentrations markedly decreased and returned to normal, whereas they were elevated and increased further in patients with proven relapse of the tumor and/or metastases in different organs [24]. The function of the CD44 gene is severely damaged, beginning with the very early pre-invasive stages of tumor development. This can be used as a means of tumor detection and diagnosis both on solid tissue specimens [25] and on exfoliated cells in

clinically obtained excreta and body fluids [26]. Urine cell lysates obtained from patients with bladder cancer can be discriminated from normal urine lysates [27] using Western blotting with a monoclonal antibody against the standard form of the CD44 protein.

## Immunotherapy

Zbar and Tanaka [28] first reported on animal immunotherapy based on the principle that tumor growth is inhibited at sites of delayed hypersensitivity reactions provoked by antigens unrelated to the tumor.They injected living Mycobacterium bovis (strain BCG) into established intradermal tumors and caused tumor regression and prevented the development of metastases. For optimum therapeutic effect contact between BCG and tumor cells was necessary. The ability of tumor immune lymphocytes to localize

Specifically to tumor offers a possibility for therapy which has been utilized over the past several years [29]. The rejection of murine tumors expressing tumor-specific transplantation antigens has been shown to be mediated primarily by immune cells [30]. Some 6 to 7% of transplant recipients may develop cancer as a consequence of iatrogenic immunosuppression [31]. Studies on the ability of patient lymphocytes to lyse tumor cells in short term (2-8 hr) isotope release assays have shown that lymphocytes from cancer patients can generally destroy only tumor cells from the same patient [32-34], unless the effector cells are not cytolytic T cells but, for example, Natural Killer cells or Lymphokine Activated Killer cells, in which case neoplastic cells representing many different types are sensitive. Immunotherapy is believed to be capable of eliminating only relatively small amounts of neoplastic cells and, therefore, the failure to induce a regression in patients with excessive tumor burden is not unexpected [35,36]. One approach of immunotherapy is to “xenogenize” tumor cells by virus infection. Another is to culture tumor infiltrating lymphocytes with interleukin-2 and reinoculate them into the host with cytokines [37]. The introduction of recombinant vectors expressing cytokine genes into tumor infiltrating lymphocyte cells [38] or into the tumor cells themselves [39] may enhance the migration of effector immune cells into the tumor with consequent immunomediated control. The considerable heterogeneity in the expression of tumor associated differentiation antigens by cells within the same tumor constitutes a problem for any immunotherapy, since it facilitates the escape of antigen-negative tumor variants. An alternative approach toward increasing the immune response to tumor-associated differentiation antigens is to treat the host to be immunized so as to abolish a “suppressor” response. Such treatment can be provided in the form of sublethal whole body x-irradiation [40], injection of a drug such as cyclophosphamide [41], or by the administration of certain anti-idiotypic antibodies [42]. Anergy is defined as a state of T lymphocyte unresponsiveness characterized by absence of proliferation, IL-2 production and diminished expression of IL-2R [43,44]. Most available data support suppression as a mechanism of oral tolerance [45,46]. Immunological suppression is classically demonstrated by the suppression of antigen-specific immune responses by T lymphocytes [47,48).

### Autoantigens

Oral administration of S-antigen (S-Ag), a retinal autoantigen that induces experimental autoimmune uveitis, prevented or markedly diminished the clinical appearance of S-Ag-induced disease as measured by ocular inflammation [49,50]. Gut associated lymphoid tissue has the capacity to generate potent immune responses on one hand, and to induce peripheral tolerance to external antigens on the other [51-53]. Both processes require antigen stimulation [53], involve cytokine production [51] and might occur at the same time - the first leading to potent local and systemic immune responses, while the latter leads to systemic antigen-specific nonresponsiveness [54]. The generation of acquired immune responses in the small intestine is believed to occur in Peyer`s patches [51,55]. Orally fed protein antigens are found in the blood within 1 hr of feeding [56]. Peripheral tolerance is not induced locally, but rather is induced systemically upon transfer of intact antigen, or its peptides, into the circulation [57,59].Oral tolerance may be induced by a single feeding of a protein antigen [60,61] or by several intermittent feedings [46,62]. In order to test whether feeding on autoantigen could suppress an experimental autoimmune disease, the Lewis rat model of experimental autoimmune encephalomyelitis was studied [63]. With increasing dosages of GP-MBP, the incidence and severity of disease was suppressed, as well as proliferative responses of lymph node cells to MBP. Antibody responses to MBP were decreased but not as dramatically as proliferative responses. Thus it appears that oral tolerance to MBP, as to other non-self antigens (45), preferentially suppress cellular immune responses. It appears that homologous MBP is a more potent oral tolerogen for experimental autoimmune encephalomyelitis than heterologous MBP [64].

Tumor cells may escape immune recognition in immunocompetent hosts by clonal evolution.Attention could be directed to activate the resident immune effectors to break the anergy or tolerance.

### Urotherapy

Subcutaneous urine injections was practiced in 1912 by Duncan [65] from New York under the name of auto-pyotherapy for urinary infections, and in 1919 by Wildbolz [65] from Bern for diagnostic purposes. Cimino [66] from Palermo reported in 1927 on the use of auto uro-therapy for urinary infections. Rabinowitch [67] in 1931 described this auto-urine therapy for gonarthritis. Jausion et al.

[68] used this kind of therapy in 1933 for desensitization and endocrinological problems. They treated with auto Urotherapy injections patients who suffered from migraine, pruritus, asthma, urticaria, eczema, psoriasis, etc. Day [69] in 1936 treated patients with acute and subacute glomerulonephritis by injection of an autogenous urinary extract. Sandweiss, Saltzstein and Farbman [70] reported in 1938 that an extract from urine of pregnant women has a prophylactic and therapeutic effect on experimental ulcers in dogs. Shortly thereafter the same group noted that an extract from urine of normal women has a similar beneficial effect [71]. In 1926 Seiffert first described the construction of ileal loop conduits for urinary diversion [72]. Bricker in the 1950s popularized the use of the ileal loop as a means of supravesical urinary diversion following exenteration for pelvic malignancy in adults [73]. Ureterosigmoidostomy as a means of urinary diversion was used widely from 1920 to 1955. It was this type of implant which Hammer first reported in 1929 associated with tumor [74]. Peyer`s patches are immunocompetent lymphoid organs which participate in intestinal immune responses [75]. Epithelial cells within the crypts of the small bowel are one of the fastest dividing cells in the body and yet they show one of the lowest rate of malignant transformation [76]. Stem cells in the mucosa of the small bowel can divide every 8 to 12 hours [77]. Tapper and Folkman [78] demonstrated that exposure of intestinal segments to urine causes marked lymphoid depletion in the segments. These studies give additional support to the idea that a lymphocyte suppressive factor exist in urine [79]. The continued presence of urine bathing the intestinal mucosa appears to locally inhibit regeneration of the Peyer`s patches. Starkey et al. [80] detected in human urine a material that is biologically and immunologically similar to epidermal growth factor that causes proliferation and keratinization of epidermal tissues. The increased susceptibility of the colon to cancer associated with the existence of an implanted ureter has been theorized to relate to 3 factore: 1. The role of the urine in the colon [81,82]. 2. The mechanical effect of the fecal stream on the stoma [83]. 3. The age of the anastomosis [84]. Adenocarcinoma of the colon mucosa is a recognized complication of ureterosigmoidostomy. The tumor, which develops adjacent to the junction of the ureter with the bowel, occurs 500 times as often as in the population at large and, in children so operated , 7,000 times as often as in all persons under age 25. The latency period is 5 to 50 years [81,85-87]. It is common knowledge that malignant tumors may disappear spontaneously although very infrequently [88-90]. Usually it is accepted that this could be due at least partly to an immunological reaction [91,92]. Renal adenocarcinoma is one of the cancer types in which such spontaneous regressions have been described most frequently [88,90]. Urinary extracts from patients with aplastic anemia [93] and idiopathic thrombocytopenic purpura [94] are capable of stimulating megakaryocyte colony growth in culture, and when injected into rats could also induce thrombocytosis in peripheral blood and megakaryocytosis in the spleens of these animals. Stanley et al. [95] demonstrated that rabbits immunized with human urine concentrates from leukemic patients developed antibody which neutralized the mouse bone marrow colony stimulating factor in human urine and human serum.

## Preconclusion

Henry Sigerist said, more than 50 years ago:"I personally have the feeling that the problem of cancer is not merely a biological and laboratory problem, but it belongs to a certain extent to the realm of philosophy. All experiments require certain philosophical preparation. And I have the feeling that in the case of cancer many experiments were undertaken without the necessary philosophical background, and therefore proved useless" [96].

## Conclusion No.1

Urotherapy is suggested as a new kind of immunotherapy for cancer patients. Unlike the clonal immunotherapy the urine of the cancer patients contain the many tumor antigens which constitute the tumor. Oral auto-urotherapy will provide the intestinal lymphatic system the tumor antigens against which they may produce antibodies due to non-self recognition. These antibodies may be transpierced through the blood stream and attack the tumor and its cells. Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced.

## References


1. Root-Bernstein RS (1993) Causality, complementarity, evolution, and emergent properties. In Iversen OH (Eds.), New Frontiers in Cancer Causation. Taylor & Francis, UK, p. 1-14

1. Burnet FM (1970) The concept of immunological surveillance. Prog Exp Tumor Res 13: 1-27.

2. Hellstrom I, Hellstrom KE, Zeidman L, Bernstein ID, Brown JP, et al. (1979) Cell-mediated reactivity to antigens shared by Moloney virus induced lymphoma cells (LSTRA) and cells from certain 3-methylcholanthrene induced mouse sarcomas. Int J Cancer 23(4): 555-564.

3. Hellstrom I, Hellstrom KE, Shepard TH (1970) Cell-mediated immunity against antigens common to tumor colonic carcinomas and fetal gut epithelium. Int J Cancer 6(3): 346-351.

2. Hellstrom KE, Hellstrom I (1989) Immunological approach to tumor therapy: Monoclonal antibodies, tumor vaccines, and anti-idiotypes. In Quash GA, Rodwell JD & Marcel Dekker Covalently Modified Antigens and Antibodies in Diagnosis and Therapy, New York, p.1

3. Ehrlich P (1957) In Himmelweit S (Eds.), The Collected Papers of Paul Ehrlich (translated by P. Alexander), Pergamon Press, UK.

4. Watanabe T, Punkel CS, Takeyama H, Lloyd KO, Shiku H,

et al. (1982) Human melanoma antigen AH is an autologous ganglioside related to GD2. J Exp Med 156(6): 1884-1889.

5. Rote NS, Gupta RK, Morton DL (1980) Tumor-associated antigens detected by autologous sera in urine of patients with solid neoplasms. J Surg Res 29(1): 18-22.

6. Gupta RK, Huth JF, Korn EL, Morton DL (1983) Prognostic significance of urinary antigen analysis by enzyme-linked immunosorbant assay in melanoma patients. Diag Immunol 1(4): 303-309.

7. DeVere White RW, Meyers FJ, Soares SE, Miller OG, Soriano TF (1992) Urinary prostate specific antigen levels: Role in monitoring the response of prostate cancer to therapy. J Urol 147(3 Pt 2): 947-951.

8. Huland E, Huland H, Meier T, Baricordi O, Fradet Y, Get al. (1991) Comparison of 15 monoclonal antibodies against tumor-associated antigens of transitional cell carcinoma of the human bladder. J Urol 146: 1631-1636.

9. Farinati F, Cardin F, Costa F, Nitti D, Di Mario F, et al. (1986) Gastric juice CEA levels: importance of age and gastric mucosal damage. Europ J Cancer Clin Oncol 22(4): 527-529.

10. Touitou Y, Proust J, Klinger E, Nakache JP, Huard D, et al. (1984) Cumulative effects of age and pathology on plasma carcinoembryonic antigen in an unselected elderly population. Europ J Cancer Clin Oncol 20(3): 369-374.

11. Lindholm L, Holmgren J, Svennerholm L, Fredman P, Nillson O, et al. (1983) A Monoclonal antibodies against gastro-intestinal tumor-associated antigens isolated as monosialogangliosides. Int Arch Allergy appl Immun 71: 178-181.

4. Mansson JE, Fredman P, Nilsson O, Lindholm L, Holmgren J, Svennerholm C, et al. (1985) Chemical structure of carcinoma ganglioside antigens defined by monoclonal antibody CA50 and some allied gangliosides of human pancreatic adenocarcinoma. Biochim Biophys Acta 834: 110-117.

12. Holmgren J, Lindholm L, Persson B, Lagergard T, Nilsson O, et al. (1984) Detection by monoclonal antibody of carbohydrate antigen CA50 in serum of patients with carcinoma. Br Med J (Clin Res Ed) 288(6429): 1479-1482.

13. Dienst C, Clodius T, Oldorp T, Uhlenbruch G, Diehl V (1987) CA 19-9, CA50 und CEA bei Pankreas und gastrointestinal Tumoren. Medizin Klin 82: 45-50.

5. Bruhn HD, Broers H, Euler H, Everding A, Feller AC, et al. (1986) CA im serum von Karzinom-Patienten. Deutsch Med Wochensch 34: 1267-1272.

14. Farinati F, Holmgren J, Di Mario F, Cardin F, Valliante F, et al. (1991) CA 50 determination in body fluids: Can we screen patients at risk for gastric cancer? Int J Cancer 47(1): 7-11.

15. Hoffmann JC, Dengler TJ, Knolle PA, Albert-Wolf M, Roux M, et al. (1993) A soluble form of the adhesion receptor CD58 (LFA-3) is present in human body fluids. Eur J Immunol 23: 3003-3010.

6. Tobi M, Darmon E, Rozen P, Harpaz N, Fink A, et al. (1995) Urinary organ specific neoantigen. A potentially diagnostic test for colorectal cancer. Dig Dis Sci 40: 1531-1537.

7. Boutwell RK (1983) Evidence that an elevated level of ornithine decarboxylase activity is an essential component of tumor promotion. Adv Polyamine Res;4:127-133

16. lipton A, Sheehan L, Harvey HA (1975) Urinary polyamine levels in patients with gastrointestinal malignancy. Cancer 36(6 Suppl): 2351-2354.

17. Loser C, Folsch UR, Paprotny C, Creutzfeldt W (1990) Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum and urine of 50 patients with colorectal cancer. Cancer 65(4): 958-966.

18. Matsumura Y, Tarin D (1992) Significance of CD44 gene products for cancer diagnosis and disease evaluation. Lancet 340(8827): 1053-1058.

19. Matsumura Y, Hanbury D, Smith JC, Tarin D (1994) Non-invasive detection of malignancy by identification of unusual CD44 gene activity in exfoliated cancer cells. Br Med J 308(6929): 619-624.

20. Matsumura Y, Sugiyama M, Matsumura S, Hayle AJ, Robinson P, et al. (1995) Unusual retention of introns in CD44 gene transcripts in bladder cancer provides new diagnostic and clinical oncological opportunities. J Pathol 177(1): 11-20.

21. Zbar B, Tanaka T (1971) Immunotherapy of cancer: regression of tumors after intralesional injection of living Mycobacterium bovis. Science 172(3980): 271-273.

22. Rosenberg SA, Packard BS, Aebersold PM, et al. (1988) Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med 319(25): 1676-1680.

23. Hellstrom KE, Hellstrom I (1969) Cellular immunity against tumor specific antigens. Adv Cancer Res 12: 167-223.

24. Penn I (1988) Tumors of the immunocompromised patient. Ann Rev Med 39: 63-73.

25. Vanky F, Klein E, Willems J, Böök K, Ivert T, et al. (1987) Lysis of Autologous tumor cells by blood lymphocytes activated in Autologous mixed lymphocyte tumor cell culture - no correlation with the postsurgical clinical course. Cancer Immunol Immunother 24(2): 180-183.

26. Vanky F, Klein E (1982) Specificity of auto-tumor cytotoxicity exerted by fresh, activated and propagated human T lymphocytes. Int J Cancer 29(45: 547-553.

27. Knuth A, Wolfel T, Klehmann E, Boon T, Meyer zum Buschenfelde KH, et al. (1989) Cytolytic T cell clones against an autologous human melanoma: Specificity study and definition of three antigens by immunoselection. Proc Natl Acad Sci USA 86(8): 2804-2808.

8. Lo Buglio AF, Neidhart JA (1974) A review of transfer factor immunotherapy in cancer. Cancer 34: 1563-1570.

28. Levin AS, Byers VS, Fudenberg HH, Wybran J, Hackett AJ, et al. (1975) Osteogenic sarcoma. Immunologic parameters before and during immunotherapy with tumor-specific transfer factor. J Clin Invest 55(3): 487-499.

29. Rosenberg SA, Schearz SL, Speiss PJ (1988) Combination immunotherapy for cancer: synergistic antitumor interactions of interleukin-2, alpha interferon and tumor-infiltrating lymphocytes. JNCI 80(17): 1393-1397.

30. Rosenberg SA, Aebersold P, Cornetta K, et al. (1990) Gene transfer into humans: immunotherapy of melanoma using

tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 323(9): 570-578.

31. Russell SJ, Eccles SA, Fleming CL, Johnson CA, Collins MKL (1991) Decreased tumorigenicity of a transplantable rat sarcoma following transfer and expression of an IL-2 cDNA. Int J Cancer 47(2): 244-252.

32. Hellstrom KE, Hellstrom I, Kant JA, Tamerius JD (1978) Regression and inhibition of sarcoma growth by interference with a radiosensitive T cell population. J Exp Med 148(3): 799-804.

33. Estin CD, Stevenson US, Hellstrom I, Hellstrom KE (1989) Cyclophosphamide potentiates the antitumor activity of v-p97NY. Cell Immunol 120(1): 126-131.

34. Nepom GT, Hellstrom KE (1987) Anti-idiotypic antibodies and the induction of specific tumor immunity. Cancer Metast Rev 6(4): 489-502.

35. Schwartz RH (1990) A cell culture model for T lymphocyte clonal anergy. Science 248(4961): 1349-1356.

36. Jenkins MK, Schwartz RH (1987) Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. J Exp Med 165(2): 302-319.

37. Mowat AM (1987) The regulation of immune responses to dietary protein antigens. Immunol Today 8(3): 93-98.

38. Weiner HL, Zhang ZJ, Khoury SJ, Miller A, Al-Sabbagh A, et al. (1991) Antigen-driven peripheral immune tolerance. Suppression of organ-specific autoimmune diseases by oral administration of autoantigens. Ann NY Acad Sci 636: 227-232.

39. Sercarz E, Krzych U (1991) The distinctive specificity of antigen-specific suppressor T cells. Immunol Today 12(4): 111-118.

40. Green DR, Flood PM, Gershon RK (1983) Immunoregulatory T-cell pathways. Annu Rev Immunol 1: 439-463.

41. Thurau SR, Caspi RR, Chan CC, Weiner HL, Nussenblatt RB, et al. (1991) Immunological suppression of experimental autoimmune uveitis. Fortschr Ophthalmol 88: 404-407.

42. Nussenblatt RB, Caspi RR, Mahdi R, Chan CC, Roberge F, et al. (1990) Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J immunol 144(5): 1689-1695.

43. Brandtzaeg P (1989) Overview of the mucosal immune system. Curr top Microbiol Immunol 146: 13-28.

44. MesteckyJ, McGhee JR (1989) Oral immunization: Past and present. Curr Top Microbiol Immunol 146: 3-12.

9. Stokes CR (1984) Induction and control of intestinal immune responses. In Newby TJ & Stokes CR (eds.) ,Local immune responses of the gut,CRC Press, Boca Raton, USA, pp.97-142.

45. Hanson DG, Vaz NM, Rawlings LA, Lynch JM (1979) Inhibition of specific immune responses by feeding protein antigens. II. Effects of prior passive and active immunization. J Immunol 122(6): 2261-2266.

10. Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, et al. (1987) Nature and properties of the human gastrointestinal immune system. In Miller K & Nicklin S (eds.), Immunology of the Gastrointestinal Tract, CRC Press, Boca Raton, USA, pp. 1-88.

46. Peng HJ, Turner MW, Strobel S (1990) The generation of a "tolerogen" after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. Clin Exp Immunol 81(3): 510-515.

47. Stokes CR, Swarbrick ET, Soothill JF (1983) Genetic differences in immune exclusion and partial tolerance to ingested antigens. Clin Exp Immunol 52(3): 678-684.

48. Swarbrick ET, Stokes CR, Soothill JF (1979) Absorption of antigens after oral immunization and the simultaneous induction of specific systemic tolerance. Gut 20(2): 121-125.

11. Miller A, Zhang AJ, Prabdu-Das M, Sobel A, Weiner HL, et al. (1992) Active suppression vs. clonal anergy following oral or IV administration of MBP in actively and passively induced EAE. Neurology 42(Suppl 3): 301.

49. Lamont AG, Gordon M, Ferguson A (1987) Oral tolerance in protein deprived mice. II. Evidence of normal 'gut processing' of ovalbumin, but suppressor cell deficiency, in deprived mice. Immunology 61(3): 339-343.

12. Hanson DG, Vaz NM, Maia LC, Hornbrook MM, Lynch JM, et al. (1977) Inhibition of specific immune responses by feeding protein antigens. Int Arch Allergy Appl Immunol 55: 526-532.

50. Matthews JB, Fivaz BH, Sewell HF (1981) Serum and salivary antibody responses and the development of oral tolerance after oral and intragastric antigen administration. Int Arch Allergy Appl Immunol 65(1): 107-113.

51. Higgins P, Weiner HL (1988) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J Immunol 140(2): 440-445.

52. Miller A, Lider O, Al-Sabbagh A, Weiner HL (1992) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. J Neuroimmunol 39(3): 243-250.

53. Jausion H (1935) Sur lauto-ouro-therapie. Journal D Urologie 39: 58-59.

54. Cimino T (1927) Premiers essais de vaccine-proteine-therapie des infections non gonococciques ni tuberculeuses des voies urinaires a l'aide des injections sous-cutanees de l'urine purulente du sujet, sterilisee par l'ebullition (ouro-therapie). Rivista Sanitaria 186:

55. Rabinowitch IM (1931) Auto-urine-therapy in gonarthritis. Vratchebnaia gazeta 35: 677-678.

13. Jausion H, Giard R, Martinaud G (1933) Lauto-ouro-therapie. La Presse Medicale 76:1467-1470.

56. Day HB (1936) Treatment of glomerulonephritis by antigen. Lancet 1228(5912): 1456-1459.

57. Sandweiss DJ, Saltzstein HC, Farbman AA (1938) The prevention or healing of experimental ulcer in Mann-Williamson dogs with the Anterior-Pituitary-Like hormone

(Antuitrin-S). Am J Dig Dis 5(1): 24-30.

58. Sandweiss DJ, Saltzstein HC, Farbman AA (1939) The relation of sex hormones to peptic ulcer. Am J Dig Dis 6(1):6-12.

14. Seiffert L (1935) Die “Darn-Siphonblase”. Arch fur Klin Chir 183: 569.

15. Bricker EM (1929) Bladder substitution after pelvic evisceration. Surg Clin North Am 1950;30:1511 74. Hammer E. Cancer du colon sigmoide dix ans après implantation des ureteres d’une vessie exstrophiee. J Urol Nephrol 28: 260.

59. Miller-Schoop JW, Good RA (1975) Functional studies of Peyer`s patches: Evidence for their participation in intestinal immune responses. J Immunol 144(6): 1757-1760.

60. Barclay THC, Schapira DV (1983) Malignant tumors of the small intestine. Cancer 51(5): 878-881.

61. Loeffler M, Stein R, Wichmann HE, Potten CS, Kaur P, et al. (1986) Intestinal cell proliferation. I. A comprehensive model of steady-state proliferation in the crypt. Cell Tissue Kinet 19(6): 627-645.

62. Tapper D, Folkman J (1976) Lymphoid depletion in ileal loops: Mechanism and clinical implications. J Pediatr Surg 11(5): 871-880.

63. Wilson WEC, Kirkpatrick CH, Talmage DW (1965) Suppression of immunologic responsiveness in uremia. Ann Intern Med 62(1): 1-14.

64. Starkey RH, Cohen S, Orth DN (1975) Epidural growth factor: Identification of a new hormone in human urine. Science 189(4205): 800-802.

65. Urdaneta LF, Duffell D, Creevy CD, Aust JB (1966) Late development of primary carcinoma of the colon following ureterosigmoidostomy: report of three cases and literature review. Ann Surg 164(3): 503-513.

66. Harguindey SS, Colbeck RC, Bransome ED JR (1975) Ureterosigmoidostomy and cancer: new observations (letter). Ann Intern Med 83(6): 833.

67. Rivard JY, Bedard A, Dionne L (1975) Colonic neoplasms following ureterosigmoidostomy. J Urol 113(6): 781-786.

68. Carswell JJ III, Skeel DA, Witherington R, Otken LB Jr (1976) Neoplasia at the site of ureterosigmoidostomy. J Urol 115(6): 750-752.

69. Lasser A, Acosta AE (1975) Colonic neoplasms complicating ureterosigmoidostomy. Cancer 35(4): 1218-1222.

70. Sooriyaarachchi GS, Johnson RO, Carbone PP (1977) Neoplasms of the large bowel following ureterosigmoidostomy. Arch Surg 112(10): 1174-1147.

71. Eraklis AJ, Folkman MJ (1978) Adenocarcinoma at the site of ureterosigmoidostomies for exstrophy of the bladder. J Pediatr Surg 13(4): 730-734.

16. Everson T (1964) Spontaneous regression of cancer. Ann NY Acad Sci 114: 721-735.

72. Stephenson H, Delmez J, Renden D, Kimpton R, Todd P, et al. (1971) Host immunity and spontaneous regression of cancer evaluated by computerized data reduction study. Surg Gynecol Obstet 133(4): 649-655.

73. Cole W (1974) Spontaneous regression of cancer: The metabolic triumph of the host? Ann NY Acad Sci 230: 111-141.

74. Burnet F (1967) Immunological aspects of malignant disease. Lancet 1(7501): 1171-1174.

75. Droller M (1980) Immunotherapy and genitourinary neoplasia. Urol Clin N Am 7(3): 831-846.

76. Enomoto K, Kawakita M, Kishimoto S, Katayama N, Miyake T, et al. (1980) Thrombopoiesis and megakaryocyte colony stimulating factor in the urine of patients with aplastic anemia. Br J Haematol 45(4): 551-556.

77. Kawakita M, Enomoto K, Katayama N, Kishimoto S, Miyake T, et al. (1981) Thrombopoiesis and megakaryocyte colony stimulating factors in the urine of patients with idiopathic thrombocytopenic purpura. Br J Haematol 48(4): 609-615.

78. Stanley ER, McNeill TA, Chan SH (1970) Antibody production to the factor in human urine stimulating colony formation in vitro by bone marrow cells. Br J Haematol 18(5): 585-590.

17. Galdston I (1939) The ideological basis of discovery. Bull Hist Med 7: 729-735.

## Combined Intralipid - Urotherapy for Patients with Cancer (Part 2)

Malignant tumors express antigens that may stimulate and serve as targets for antitumor immunity. Virally induced tumors usually contain integrated proviral genomes in their cellular genomes and often express viral genome-encoded proteins that may stimulate specific host immune responses. Antigens unique to individual tumors that stimulate specific rejection of transplanted tumors have been demonstrated only in experimental animals. Other tumor antigens that potentially can stimulate immune responses are shared by different tumors. These include products of mutated or rearranged oncogenes or tumor-suppressor genes. Tumors may also overexpress tissue differentiation antigens or embryonic antigens, which also have the potential to be recognized by the immune system. The recent identification of tumor antigens recognized by cytotoxic T cells opens up new possibilities for constructing chemically defined antigens for specific immunotherapy. Treatment of malignant tumors in humans by immunologic approaches, although theoretically attractive, has not yet succeeded on a large scale. Important progress in immunotherapy of cancer is emerging with several different treatment modalities [1]. Recent studies have identified new melanoma antigens that are recognised by CD4(+) T cells. Analysis of tumour-specific CD4(+) T-cell responses may lead to the development of optimal anti-cancer vaccines that can induce an orchestrated effort of tumour-specific CD4(+) and CD8(+) T cells in the fight against cancer [2]. T cells play an important role in in vivo rejection of human melanoma. Human melanoma antigens recognized by autologous T cells were identified. These antigens are classified as tissue (melanocyte)-specific proteins, cancer-testis antigens (proteins expressed in normal testis and various cancers), tumor-specific peptides derived from mutations in tumor cells, and others. A variety of mechanisms generating T cell epitopes on tumor cells were discovered. Various clinical observations, including tumor regression observed in adoptive transfer of gp100-reactive T cells suggest that these identified melanoma peptides may function as tumor rejection antigens. Immunodominant common epitopes that could expand melanoma-reactive cytotoxic T lymphocytes (CTLs) in vitro were found in the MART-1 and gp100 antigens. New immunization protocols--including immunization with peptides, recombinant viruses, plasmid DNAs, and dendritic cells pulsed with peptides as well as adoptive transfer of in vitro-generated CTLs by stimulation with antigenic peptides--were developed (phase I clinical trials have been performed in the Surgery Branch of the National Cancer Institute, Bethesda, MD, U.S.A.). Immunization with the gp100(209(210M)) peptide that was modified to have high HLA-A2 binding affinity, along with incomplete Freund's adjuvant and interleukin (IL)-2, resulted in a 42% response rate in patients with melanoma. These immunotherapies need further improvement due to the mechanisms of tumor escape from T cell responses [3]. Most major advances in human cancer immunology and immunotherapy have come from studies in melanoma. We are beginning to understand the immune repertoire of T cells and antibodies that are active against melanoma, with recent glimpses of the CD4 (+) T cell repertoire. The view of what the immune system can see is extending to mutations and parts of the genome that are normally invisible [4]. Pancreatic cancer is the fifth leading cause of cancer deaths in the United States with little or no impact from conventional treatment options. Significant advances in understanding basic immunology have renewed interest in using immunotherapy to treat pancreatic cancer. Cancer immunotherapy, including humanized MAbs, cytokines, and potent vaccine strategies, has been successful in animal models and is being evaluated in clinical trials. Gene therapy is also being explored using methods to inactivate oncogenes, replace defective tumor suppressor genes, confer enhanced chemosensitivity to tumor cells, and increase immunogenicity of tumor cells. Angiogenesis, an essential step in the growth and metastasis of pancreatic cancer, has been targeted by many antiangiogenic agents. Several clinical trials have been initiated to evaluate the role of these innovative strategies in patients with pancreatic cancer with increasingly sophisticated correlative studies to learn more about the mechanisms of tumor rejection with these agents. The rapid translation of basic science discoveries to clinical trials should result in the development of new effective treatments for patients with pancreatic cancer [5].

The immune repertoire contains T cells and B cells that can recognize autologous cancer cells. This repertoire is directed against self, and in some cases altered self (mutations). Priming immune responses against self antigens can be difficult. Strategies are presented using altered self to elicit immunity against self in poorly immunogenic tumor models. Mechanisms underlying immunity to self antigens on cancer cells show that the immune system can use diverse strategies for cancer immunity, in both the immunization and the effector phases. CD4+ T cells are typically, but not always, required for immunization. The effector phase of tumor immunity can involve cytotoxic T cells, macrophages with activating Fc receptors, and/or killer domain molecules. This diversity in the effector phase is observed even when immunizing with conserved paralogs. A consequence of tumor immunity is potentially autoimmunity, which may be undesirable. Autoimmunity uses similar mechanisms as tumor immunity, but tumor immunity and autoimmunity can uncouple. These studies open up strategies for active immunization against cancer [6].

## Cancer Antigens

The spectrum of human antigens allows a monitoring of various pathological processes such as autoimmune disorders and tumorigenesis. Serological analysis of cDNA expression libraries (SEREX) is now used to search for new cancer-associated antigens, which are potential diagnostic markers or targets for immunotherapy of cancer [7]. The immune response can effectively hamper the progression of preclinical stages of tumor growth. Medicine in the postgenomic era offers an increasing possibility of detecting

healthy individuals at risk of developing cancer who could benefit from tumor-preventive vaccines. The identification of novel tumor antigens that fulfill two conditions will be crucial for the development of cancer immunoprevention. First, an ideal antigen should have a crucial pathogenetic role in tumor growth to avoid the selection of antigen-loss variants. Second, the antigen should be recognizable by the immune system even in MHC-loss variants and should therefore be recognized both by antibodies and T cells. Identifying such antigens will also provide new targets for cancer immunotherapy [8]. Cancer/testis (CT) antigens are a category of tumor antigens with normal expression restricted to male germ cells in the testis but not in adult somatic tissues. In some cases, CT antigens are also expressed in ovary and in trophoblast. In malignancy, this gene regulation is disrupted, resulting in CT antigen expression in a proportion of tumors of various types. Since their initial identification by T-cell epitope cloning, the list of CT antigens has been greatly expanded through serological expression cloning (SEREX) and differential mRNA expression analysis, and approximately 20 CT antigens or antigen families have been identified to date. Characteristics commonly shared by CT antigens, aside from the highly tissue-restricted expression profile, include existence as multigene families, frequent mapping to chromosome X, heterogeneous protein expression in cancer, likely correlation with tumor progression, induction of expression by hypomethylation and/or histone acetylation, and immunogenicity in cancer patients. Spontaneous humoral and cell-mediated immune responses have been demonstrated against several CT antigens, including NY-ESO-1, MAGE-A, and SSX antigens. Since CT antigens are immunogenic and highly restricted to tumors, their discovery has led directly to the development of antigen-specific cancer vaccines, and clinical trials with MAGE-A and NY-ESO-1 are in progress [9]. Our understanding of how immune responses are generated and regulated drives the design of possible immunotherapies for cancer patients. Cancer vaccines that are able to induce tumor-specific immune responses in cancer patients are not always followed by tumor rejection. Two possible reasons that might explain this dichotomy of cancer immunology. First, the immune response generated, although detectable, may not be quantitatively sufficient to reject the tumor. Second, the tumor microenvironment may modulate tumor cell susceptibility to the systemic immune response induced by the immunization [10]. Cytolytic T lymphocytes (CTL) play a major role in the recognition and destruction of tumor cells by the immune system. Some of these antigens, including those encoded by the MAGE genes, are absent on all normal cells, and therefore constitute ideal targets for cancer vaccines aimed at increasing the activity of anti-tumor lymphocytes. Such vaccines are currently tested in clinical trials with melanoma patients. These antigens consist of small peptides that are presented by HLA molecules and that result from the degradation of intracellular proteins. This degradation is performed by an intracellular proteolytic complex called the proteasome. Dendritic cells, which in the lymph node are responsible for antigen presentation to the lymphocytes in order to initiate the immune response, are inefficient to produce some peptides because they contain a different proteasome called “immunoproteasome” [11].

One of the most significant advances in the field of modern tumor immunology is the identification of genes encoding tumor-rejection antigens that are recognized by human leukocyte antigen (HLA) class I-restricted and tumor-specific cytotoxic T lymphocytes (CTLs). Several peptides encoded by these genes are now under clinical trial as cancer vaccines, and major tumor regression has been observed in some melanoma patients. These results indicate that identification of the peptides capable of inducing CTLs may provide a new modality of cancer therapy. Itoh et al. [12] investigated tumor-rejection antigens from epithelial cancers, and reported 7 genes encoding tumor-rejection antigens and peptides available for specific immunotherapy of HLA-A26 or -A24 patients with epithelial cancers. Furthermore, they identified more than 10 genes encoding tumor-rejection antigens and peptides available for specific immunotherapy of HLA-A2 patients with epithelial cancers. Therefore these new antigens and peptides could be applicable to the treatment of numerous epithelial cancer patients. Cytotoxic T-cell responses to shared tumor antigens have been characterized for several tumor types, and the MHC-associated peptides that comprise these antigens have been defined at a molecular level. These provide new tools to determine whether immune responses can be generated with these tumor antigens, and there are data to suggest that such immune responses can be generated. However, it is also clear that tumor cells can evade immune responses directed against some shared antigens, by downregulating expression of MHC or of the antigenic protein(s), as well as by more active methods such as secretion of immunosuppressive cytokines. Awareness of these mechanisms of immune escape will help to direct development of the next generation of tumor vaccines. Targeting unique antigens and modulating the cytokine environment likely will be critical to comprehensive vaccine systems in the future [13]. The adoptive transfer of tumor-infiltrating lymphocytes along with interleukin 2 into autologous patients resulted in the objective regression of tumor in about 30% of patients with melanoma, indicating that these T cells play a role in tumor rejection. To understand the molecular basis of the T cell-cancer cell interaction Wang [14] and others started to search for tumor antigens expressed on cancer cells recognized by T cells. This led to the identification of several major histocompatibility complex (MHC) class I restricted tumor antigens. These tumor antigens have been classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. Because CD4+ T cells play a central role in orchestrating the host immune response against cancer, infectious diseases, and autoimmune diseases, a novel genetic approach has recently been developed to identify these MHC class II restricted tumor antigens. The identification of both MHC class I and II restricted tumor antigens provides new opportunities for the development of

therapeutic strategies against cancer. In order to enhance cell mediated cytotoxicity, bispecific antibodies (BsAbs), molecules combining two or more antibodies with different antigenic specificities, have been developed as new agents for immunotherapy. Kudo et al. [15] recent studies revealed that simultaneous administration of two kinds of BsAbs (anti-tumor x anti-CD3 plus anti-tumor x anti-CD28) together with lymphokine activated killer cells with a T cell phenotype (T-LAK cells) inhibited growth of human xenotransplanted tumors in severe combined immunodeficient (SCID) mice, while single BsAb was without effect. Three kinds of BsAbs (anti-tumor x anti-CD3, anti-tumor x anti-CD28, anti-tumor x anti-CD2) showed the highest cytotoxicity against tumor cells when given simultaneously with T-LAK cells or peripheral blood mononuclear cells in vitro and in vivo. BsAbs can be preserved for immediate application, while cytotoxic T lymphocytes (CTLs) must be made-to-order, and are time-consuming to prepare. Tumor associated antigens, such as MAGE antigens, SART antigens, MUC1 antigen, c-erbB 2 antigen or cancer/testis antigens can be served to target antigens for BsAb production. By conjugation with antibodies to effector cells (anti-CD3, anti-CD28, anti-CD16, anti-CD64, anti-CD89 or anti-CD2), many kinds of BsAbs can be produced to cover most types of cancers from different organs. Therefore this strategy might be ubiquitously applicable to most malignancies.

Melanogenesis-related proteins play important roles in melanin synthesis and antigenicity of melanomas. Identification of highly expressed melanoma-associated antigens (MAA) that are immunogenic in humans will provide potential targets for cancer vaccines. Melanogenesis-related proteins have been shown to be MAA. Autoantibody responses to these MAA have been shown to react with melanoma cells and melanocytes, and suggested to play a role in controlling melanoma progression. To assess antibody responses to potential melanoma/melanocyte autoantigens, the open-reading frame sequences of tyrosinase, tyrosinase-related protein (TRP)-1, TRP-2, and melanoma-associated glycoprotein antigen family (gp100/pmel17) genes were cloned and expressed as recombinant proteins in E. coli [16]. Purified recombinant antigens were employed to detect antibodies in sera of melanoma patients and normal healthy donors. By affinity enzyme-linked immunosorbent assay and western blotting, all recombinant antigens were shown to be antigenic. The main subclass of antibody response to these antigens was IgG. Most importantly this study demonstrated anti-TRP-2 and anti-gp100/pmel17 IgG responses in melanoma patients. Only one of 23 normal donors had an antibody response to the antigens tested. MAA-specific IgG antibodies in sera were assessed in melanoma patients (n = 23) pre- and post-polyvalent melanoma cell vaccine treatment. Polyvalent melanoma cell vaccine treatment enhanced anti-MAA antibody responses; however, only anti-TRP-2 and anti-gp100/pmel17 antibody response was enhanced. These studies suggest that four melanogenesis-related proteins are autoimmunogenic and can be used as potential targets for active-specific immunotherapy.

The adoptive transfer of cytotoxic T lymphocytes (CTLs) derived from tumor-infiltrating lymphocytes (TIL) along with interleukin 2 (IL-2) into autologous patients with cancer resulted in the objective regression of tumor, indicating that these CTLs recognized cancer rejection antigens on tumor cells. To understand the molecular basis of T cell-mediated antitumor immunity, several groups started to search for such tumor antigens in melanoma as well as in other types of cancers. A number of tumor antigens were isolated by the use of cDNA expression systems and biochemical approaches. These tumor antigens could be classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. However, the majority of tumor antigens identified to date are nonmutated, self proteins. This raises important questions regarding the mechanism of antitumor activity and autoimmune disease. The identification of human tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer [17].

## Cancer Vaccines

Multiple novel immunotherapy strategies have reached the stage of testing in clinical trials that were accelerated by recent advances in the characterization of tumor antigens and by a more precise knowledge of the regulation of cell-mediated immune responses. The key steps in the generation of an immune response to cancer cells include loading of tumor antigens onto antigen-presenting cells in vitro or in vivo, presenting antigen in the appropriate immune stimulatory environment, activating cytotoxic lymphocytes, and blocking autoregulatory control mechanisms. This knowledge has opened the door to antigen-specific immunization for cancer using tumor-derived proteins or RNA, or synthetically generated peptide epitopes, RNA, or DNA. The critical step of antigen presentation has been facilitated by the coadministration of powerful immunologic adjuvants, the provision of costimulatory molecules and immune stimulatory cytokines, and the ability to culture dendritic cells. Advances in the understanding of the nature of tumor antigens and their optimal presentation, and in the regulatory mechanisms that govern the immune system, have provided multiple novel immunotherapy intervention strategies that are being tested in clinical trials [18].

The critical role of antigen-specific T cells in cancer immunotherapy has been amply demonstrated in many model systems. Though success of clinical trials still remains far behind expectation, the continuous improvement in our understanding of the biology of the immune response will provide the basis of optimized cancer vaccines and allow for new modalities of cancer treatment. The future will mainly be concerned with allogeneic bone marrow cell transplantation after non-myeloablative conditioning, because this approach could provide a major breakthrough in cancer immunotherapy [19]. Concerning active vaccination protocols the following aspects will be addressed: i) the targets of immunotherapeutic approaches; ii) the response elements needed for raising a therapeutically successful immune reaction; iii) ways to achieve an optimal

confrontation of the immune system with the tumor and iv) supportive regimen of immunomodulation. Many questions remain to be answered in the field of allogeneic bone marrow transplantation after non-myeloablative conditioning to optimize the therapeutic setting for this likely very powerful tool of cancer therapy.

Active immunotherapy using dendritic cells (DCs) to deliver tumor antigens has generated considerable excitement among oncologists worldwide. Although most tumor antigens used in immunotherapeutic approaches are tumor-associated, often, little is known about the underlying biology of the target. Antigen expression is a prerequisite for tumor formation or maintenance by the use of 'obligate' tumor antigens. The prototype for this class of antigens is the p53 tumor antigen, which is mutated in > 50% of human malignancies. The direct involvement of p53 in the malignant transformation of tumors makes it an attractive target for immunotherapy. p53-Reactive antibodies have been found in patients with various types of cancer, demonstrating that the human immune system can recognize and respond to tumor-associated p53. Extensive preclinical experimentation has now validated the translation of p53-expressing DCs into a clinical setting. Clinical trials are ongoing to evaluate the safety and antitumor responses elicited by DCs transduced with adenoviral-p53 in cancer patients [20].

Tumor vaccination strategies have been increased over the past years. This increase began with the identification of tumor antigens recognized by the immune system. Better understanding of the immune system and increasing knowledge about the antigen presentation process and the role of dendritic cells have opened new therapeutic possibilities. DNA vaccines, already successfully used against viral antigens and covering a broad repertoire of epitopes, might also be of advantage in tumor immunotherapy. Design and selection of vectors are of considerable importance for the vaccination. There are three major types of DNA-based recombinant cancer vaccines: DNA from tumor antigens can be used 1) to modify dendritic cells, 2) as 'naked' DNA-vaccine or 3) to construct recombinant viral vaccines [21].It is now clear that many human tumor antigens can be recognised by the immune system. These tumor antigens can be classified into several groups including cancer-testis, differentiation, tissue specific, over-expressed, and viral-associated antigens. In many cases, there is a known molecular basis of carcinogenesis which provides the explanation for the differentiated expression of these antigens in tumors compared with normal cells. Improved understanding of the biology of the immune response, particularly of immune recognition and activation of T-cells, allow better design of vaccines. Pre-clinical comparative studies allow evaluation of optimal vaccine strategies which can then be delivered to the clinic. Currently, a range of cancer vaccines are being tested including those using tumor cells, proteins, peptides, viral vectors, DNA or dendritic cells. Ultimately, this research should give rise to an entirely new modality of cancer treatments [22].

The identification of antigens on tumor cells has led to significant contributions to the field of immunotherapy. One of the most active areas under investigation in cancer immunotherapy is the development of vaccines against melanoma antigens. Induction of immunity against tumor antigens can follow multiple routes using different mechanisms. Crucial to the development of active immunization and other immunotherapies is the discovery and understanding of the molecular identity of antigens and the mechanisms involved in tumor immunity, as well as escape from immunity [23]. Antigenic differences between normal and malignant cells form the basis of clinical immunotherapy protocols. Because the antigenic phenotype varies widely among different cells within the same tumor mass, immunization with a vaccine that stimulates immunity to a broad array of tumor antigens expressed by the entire population of malignant cells is likely to be more efficacious than immunization with a vaccine for a single antigen. One strategy is to prepare a vaccine by transfer of DNA from the patient's tumor into a highly immunogenic cell line. Weak tumor antigens, characteristic of malignant cells, become strongly antigenic if they are expressed by immunogenic cells. In animal models of melanoma and breast cancer, immunization with a DNA-based vaccine is sufficient to deter tumor growth and to prolong the lives of tumor-bearing mice [24]. Berd [25] has devised a novel approach to active immunotherapy based on modification of autologous cancer cells with the hapten, dinitrophenyl (DNP). The treatment program consists of multiple intradermal injections of DNP-modified autologous tumor cells mixed with BCG. Administration of DNP-vaccine to patients with metastatic melanoma induces a unique reaction - the development of inflammation in metastatic masses. Histologically, this consists of infiltration of T lymphocytes, most of which are CD8+. These T cells usually produce gamma interferon in situ. Moreover, they represent expansion of T cell clones with novel T cell receptor structures. Occasionally, administration of DNP-vaccine results in partial or complete regression of measurable metastases. The most common site of regression has been small lung metastases. Administration of DNP-vaccine to patients in the post-surgical adjuvant setting produces a more striking clinical effect. Berd et al. have treated 214 patients with clinically evident stage III melanoma who had undergone lymphadenectomy. With a median follow-up time of 4.4 years (1.8-10.4 years) the 5-year overall survival (OS) rate is 47% (one nodal site = 51%, two nodal sites = 33%). These results appear to be comparable to those obtained with high dose interferon. More recent studies suggest that this therapeutic approach is also applicable to ovarian cancer. There appear to be no insurmountable impediments to applying this approach to much larger numbers of patients or to developing it as a standard cancer treatment. Certain anti-idiotypic antibodies that bind to the antigen-combining sites of antibodies can effectively mimic the three-dimensional structures and functions of the external antigens and can be used as surrogate antigens for active specific immunotherapy. Extensive studies in animal models have demonstrated the efficacy of these vaccines for triggering the immune

system to induce specific and protective immunity against bacterial, viral and parasitic infections as well as tumors. Several monoclonal anti-idiotype antibodies that mimic distinct human tumor-associated antigens have been developed and characterized. Encouraging results have been obtained in recent clinical trials using these anti-idiotype antibodies as vaccines [26]. Immunization with anti-idiotype (Id) antibodies represents a novel new approach to active immunotherapy. Extensive studies in animal tumor models have demonstrated the efficacy of anti-Id vaccines in preventing tumor growth and curing mice with established tumor. Bhattacharya-Chatterjee et al. [27] have developed and characterized several murine monoclonal anti-Id antibodies (Ab2) which mimic distinct human tumor-associated antigens (TAA) and can be used as surrogate antigens for triggering active anti-tumor immunity in cancer patients. Immunization with dendritic cells loaded with tumor antigens could represent a powerful method of inducing antitumor immunity. Studies from several laboratories have shown that immunization with dendritic cells pulsed with specific antigens prime cytotoxic T-cells and engender tumor immunity. The majority of cancer patients who lack an identified tumor antigen and/or cannot provide sufficient tumor tissue for antigen preparation are excluded from treatment with cancer vaccines based on using either specific tumor antigens or mixtures of tumor-derived antigens in the form of peptides or proteins isolated from tumor cells. Vaccination with the mRNA content of tumor cells would extend the scope of vaccination to this group of patients as well because RNA can be amplified from very few cancer cells [28].

The adoptive transfer of tumor-infiltrating lymphocytes (TIL) along with interleukin (IL)-2 into autologous patients with cancer resulted in the objective regression of tumor, indicating that T cells play an important role in tumor regression. In the last few years, efforts have been made towards understanding the molecular basis of T-cell-mediated antitumor immunity and elucidating the molecular nature of tumor antigens recognized by T cells. Tumor antigens identified thus far could be classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens and tumor-specific unique antigens. CD4+ T cells play a central role in orchestrating the host immune response against cancer, infectious diseases and autoimmune diseases. The identification of tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer [29]. Human tumors express a number of protein antigens that can be recognized by T cells, thus providing potential targets for cancer immunotherapy. Dendritic cells (DCs) are rare leukocytes that are uniquely potent in their ability to present antigens to T cells, and this property has prompted their recent application to therapeutic cancer vaccines. Isolated DCs loaded with tumor antigen ex vivo and administered as a cellular vaccine have been found to induce protective and therapeutic anti-tumor immunity in experimental animals. In pilot clinical trials of DC vaccination for patients with non-Hodgkin's lymphoma and melanoma, induction of anti-tumor immune responses and tumor regressions have been observed. Additional trials of DC vaccination for a variety of human cancers are under way, and methods for targeting tumor antigens to DCs in vivo are also being explored. Exploitation of the antigen-presenting properties of DCs thus offers promise for the development of effective cancer immunotherapies [30]. Recently, cancer immunotherapy has emerged as a therapeutic option for the management of cancer patients. This is based on the fact that our immune system, once activated, is capable of developing specific immunity against neoplastic but not normal cells. Increasing evidence suggests that cell-mediated immunity, particularly T-cell-mediated immunity, is important for the control of tumor cells. Several experimental vaccine strategies have been developed to enhance cell-mediated immunity against tumors. Some of these tumor vaccines have generated promising results in murine tumor systems. In addition, several phase I/II clinical trials using these vaccine strategies have shown extremely encouraging results in patients [31]. Animal studies have shown that vaccination with genetically modified tumor cells or with dendritic cells (DC) pulsed with tumor antigens are potent strategies to elicit protective immunity in tumor-bearing animals, more potent than "conventional" strategies that have been tested in clinical settings with limited success. While both vaccination strategies are forms of cell therapy requiring complex and costly ex vivo manipulations of the patient's cells, current protocols using dendritic cells are considerably simpler and would be more widely available. Vaccination with defined tumor antigens presented by DC has obvious appeal. However, in view of the expected emergence of antigen-loss variants as well as natural immunovariation, effective vaccine formulations must contain mixtures of commonly, if not universally, expressed tumor antigens. When, or even if, such common tumor antigens will be identified cannot be, predicted, however. Thus, for the foreseeable future, vaccination with total-tumor-derived material as source of tumor antigens may be preferable to using defined tumor antigens. Vaccination with undefined tumor-derived antigens will be limited, however, by the availability of sufficient tumor tissue for antigen preparation. Because the mRNA content of single cells can be amplified, tumor mRNA, or corresponding cDNA libraries, offer an unlimited source of tumor antigens. DC transfected with tumor RNA were shown to engender potent antitumor immunity in animal studies. Thus, immunotherapy using autologous DC loaded with unfractionated tumor-derived antigens in the form of RNA emerges as a potentially powerful and broadly useful vaccination strategy for cancer patients [32].

## Cancer Immunotherapy

Adoptive immunotherapy--the isolation of antigen-specific cells, their ex vivo expansion and activation, and subsequent autologous administration--is a promising approach to inducing antitumor immune responses. The molecular identification of tumor antigens and the ability to monitor the persistence and transport of transferred cells has provided new insights into the mechanisms of tumor immunotherapy. Recent studies have shown the effectiveness of cell-

transfer therapies for the treatment of patients with selected metastatic cancers. These studies provide a blueprint for the wider application of adoptive-cell-

transfer therapy, and emphasize the requirement for in vivo persistence of the cells for therapeutic efficacy [33]. There is clear evidence that certain forms of immunotherapy can be successful against certain cancers. However, it would appear that cancerous cells of various origin are exceptionally adept at subverting the immune response. Consequently, it is probable that the most efficacious therapy will be one in which multiple responses of the immune system are activated. There is currently an embarrassment of riches with regard to multiple vaccine strategies in the clinic, although no single method seems to hold the solution [34]. Despite advances in chemotherapy and surgical techniques, patients with cancer often develop local recurrence or metastatic spread. Recent advances in molecular biology, coupled with new insights in tumor immunology, have led to the design of novel antitumor vaccines. Poxviruses are a large family of DNA viruses that provide an effective and safe vector system for vaccine development. The poxvirus strategy has been successfully documented in animal models, and has been used to express both tumor-associated antigens and immune stimulatory molecules [35].

Prostate cancer is the most common malignancy in American men. Metastatic prostate cancer is incurable, with the currently best treatment, androgen ablation, being only palliative. Therefore, there is a need to develop new, more effective therapies against this disease. Multiple immunotherapeutic strategies are being explored for the treatment of prostate cancer, with the hope that such treatment will be more effective and have fewer side effects than current treatment options. Several immunotherapy strategies have been shown to be effective against prostate tumors in animal models, and many of these strategies are beginning to be tested in clinical trials for their efficacy against human prostate cancer. It is likely that effective treatment of prostate cancer will require the use of both immunotherapeutic and traditional approaches in multimodality treatments. In addition, for immunotherapy to be effective against prostate cancer, ways to overcome immune evasion and immunosuppression by the tumor cells will need to be developed [36]. Despite the identification of tumor antigens and their subsequent generation in subunit form for use as cancer vaccines, whole tumor cells remain a potent vehicle for generating anti-tumor immunity. This is because tumor cells express an array of target antigens for the immune system to react against, avoiding problems associated with major histocompatibility complex (MHC)-restricted epitope identification for individual patients. Furthermore, whole cells are relatively simple to propagate and are potentially efficient at contributing to the process of T cell priming. However, whole cells can also possess properties that allow for immune evasion, and so the question remains of how to enhance the immune response against tumor cells so that they are rejected. Scenarios where whole tumor cells may be utilised in immunotherapy include autologous tumor cell vaccines generated from resected primary tumor, allogeneic (MHC-disparate) cross-reactive tumor cell line vaccines, and immunotherapy of tumors in situ. Since tumor cells are considered poorly immunogenic, mainly because they express self-antigens in a non-stimulatory context, the environment of the tumor cells may have to be modified to become stimulatory by using immunological adjuvants. Recent studies have re-evaluated the relative roles of direct and cross-priming in generating anti-tumor immunity and have highlighted the need to circumvent immune evasion [37]. The Wilms tumor gene WT1 is expressed in leukemias and various kinds of solid tumors, including lung and breast cancer, and exerts an oncogenic function in these malignancies, suggesting that WT1 protein is a novel, over expressed tumor antigen. The WT1 protein, in fact, is an attractive tumor rejection antigen in animal models. Stimulation in vitro of peripheral blood mononuclear cells with HLA-A*2402--and HLA-A*0201--restricted 9-mer WT1 peptides elicits WT1-specific cytotoxic T-lymphocytes (CTLs), and the CTLs kill endogenously WT1-expressing leukemia or solid tumor cells. Furthermore, WT1 immunoglobulin M (IgM) and IgG antibodies are detected in patients with hematopoietic malignancies such as acute myeloid leukemia, chronic myeloid leukemia, and myelodysplastic syndromes, indicating that WT1 protein overexpressed by leukemia cells is indeed immunogenic. Taken together, these results demonstrate that WT1 protein is a promising tumor antigen for cancer immunotherapy against leukemias and various kinds of solid tumors, including lung and breast cancer [38].

In the last few years, a great deal of efforts have been directed towards understanding the molecular basis of T cell-mediated anti-tumor immunity and elucidating the molecular nature of tumor antigens recognized by T cells. Identification of a number of major histocompatibility complex (MHC) class I-restricted melanoma antigens has led to clinical trials aimed at developing effective cancer vaccines. These studies showed some evidence of therapeutic effect on the treatment of cancer, but the exclusive use of CD8+ T cells may not be effective in eradicating tumor. This rekindles interest in the role of CD4+ T cells in antitumor immunity, which play a central role in orchestrating the host immune response against cancer. Thus, Wang et al. [39] have attempted to identify MHC class II-restricted tumor antigens recognized by tumor-specific CD4+ T cells. The identification of tumor rejection antigens provides new opportunities for the development of therapeutic strategies against cancer. Interleukin (IL)-2 and IL-15 are two cytokine growth factors that regulate lymphocyte function and homeostasis. Early clinical interest in the use of IL-2 in the immunotherapy of renal cell carcinoma and malignant melanoma demonstrated the first efficacy for cytokine monotherapy in the treatment of neoplastic disease. Advances in our understanding of the cellular and molecular biology of IL-2 and its receptor complex have provided rationale to better utilize IL-2 to expand and activate immune effectors in patients with cancer. Exciting new developments in monoclonal antibodies recognizing tumor targets and

tumor vaccines have provided new avenues to combine with IL-2 therapy in cancer patients. IL-15, initially thought to mediate similar biological effects as IL-2, has been shown to have unique properties in basic and pre-clinical studies that may be of benefit in the immunotherapy of cancer [40]. Several recent developments have hallmarked progress in tumor immunology and immunotherapy. The use of interleukin-2 (IL-2) in cancer patients demonstrated that an immunological manipulation was capable of mediating the regression of established growing cancers in humans. The identification of the genes encoding cancer antigens and the development of means for effectively immunizing patients against these antigens has opened important new avenues of exploration for the development of effective active and cell-transfer immunotherapies for patients with cancer [41]. A wide range of strategies in cancer immunotherapy has been developed in the last decade, some of which are currently being used in clinical settings. The development of these immunotherapeutical strategies has been facilitated by the generation of relevant transgenic animal models. Since the different strategies in experimental immunotherapy of cancer each aim to activate different immune system components, a variety of transgenic animals have been generated either expressing tumor associated, HLA, oncogenic or immune effector cell molecule proteins [42].

Immunotherapy is in its infancy for many diseases, whether they are neoplastic or autoimmune. The major issues for cancer immunotherapy today involve the definition of molecular targets and the generation of effector mechanisms to attack the targets of interest. Soft tissue sarcomas provide a unique opportunity to examine the immune response against defined antigens. Many types of sarcomas contain tumor-specific chromosomal translocations encoding fusion proteins, which are attractive targets for immunotherapy. Our understanding of the immune system is also coming into clearer focus with the discovery of dendritic cells as powerful natural adjuvants and the teasing out of mechanisms leading to immunity versus tolerance as examples. It is hoped that the intersection of cellular immunology and sarcoma molecular biology will lead to new modalities of therapy for this group of patients with this heterogeneous group of diseases [43]. Studies of the administration of interleukin-2 to patients with metastatic melanoma or kidney cancer have shown that immunological manipulations can mediate the durable regression of metastatic cancer. The molecular identification of cancer antigens has opened new possibilities for the development of effective immunotherapies for patients with cancer. Clinical studies using immunization with peptides derived from cancer antigens have shown that high levels of lymphocytes with anti-tumor activity can be raised in cancer-bearing patients. Highly avid anti-tumor lymphocytes can be isolated from immunized patients and grown in vitro for use in cell-transfer therapies. Current studies are aimed at understanding the mechanisms that enable the cancer to escape from immune attack.

The idea that there might be an immune response to cancer has been around for many years. Immunotherapy has a long history, but is only rarely considered as the treatment of choice. Immunotherapy has encountered a number of intrinsic difficulties in cancer, such as the antigenic resemblance between the tumor and normal cells, the rapid kinetic proliferation of tumor cells and their reduced immunogenicity. There are various types of immunotherapy. Aspecific immunotherapy augments the body's immune response without targeting specific tumoral antigens. In adoptive immunotherapy, cells are administered with antitumoral reactivity to mediate neoplasm regression. Specific active immunotherapy is based on the principle that neoplasm cells contain immunogenic sites against which an antitumoral immune response can be induced in an attempt to stimulate the immune system to target specific tumoral antigens. Vaccines against cancer cells are based on a more precise identification of the tumoral antigen components. Passive immunotherapy was limited by the difficulty of obtaining high titering and specificity in early attempts using polyclonal antisera; monoclonal antibodies are currently used alone or in association with radioactive substances and cytotoxic agents. Enormous progress has been made this century in the use of immunotherapy for cancer treatment. It seems likely that the next century will see its increased afficacy, making it one of the possible therapeutic options [45].

Despite major advances in our understanding of adaptive immunity and dendritic cells, consistent and durable responses to cancer vaccines remain elusive and active immunotherapy is still not an established treatment modality. The key to developing an effective anti-tumor response is understanding why, initially, the immune system is unable to detect transformed cells and is subsequently tolerant of tumor growth and metastasis. Ineffective antigen presentation limits the adaptive immune response; however, we are now learning that the host's innate immune system may first fail to recognize the tumor as posing a danger. Recent descriptions of stress-induced ligands on tumor cells recognized by innate effector cells, new subsets of T cells that regulate tumor tolerance and the development of spontaneous tumors in mice that lack immune effector molecules, beckon a reflection on our current perspectives on the interaction of transformed cells with the immune system and offer new hope of stimulating therapeutic immunity to cancer [46]. Immunotherapy approaches to fight cancer are based on the principle of mounting an immune response against a self-antigen expressed by the tumor cells. In order to reduce potential autoimmunity side-effects, the antigens used should be as tumor-specific as possible. A complementary approach to experimental tumor antigen discovery is to screen the human genome in silico, particularly the databases of "Expressed Sequence Tags" (ESTs), in search of tumor-specific and tumor-associated antigens. The public databases currently provide a massive amount of ESTs from several hundreds of cDNA tissue libraries, including tumoral tissues from various types. Vinals et al. [47] described a novel method of EST database

screening that allows new potential tumor-associated genes to be efficiently selected. The resulting list of candidates is enriched in known genes, described as being expressed in tumor cells.

The concept of immunotherapy of cancer is more than a century old, but only recently have molecularly defined therapeutic approaches been developed. The identification of tumor antigens can now be accelerated by methods allowing the amplification of gene products selectively or preferentially transcribed in the tumor. However, determining the potential immunogenicity of such gene products remains a demanding task, since major histocompatibility complex (MHC) restriction of T cells implies that for any newly defined antigen, immunogenicity will have to be defined for any individual MHC haplotype. Tumor-derived peptides eluted from MHC molecules of tumor tissue are also a promising source of antigen. Tumor antigens are mostly of weak immunogenicity, because the vast majority are tumor-associated differentiation antigens already 'seen' by the patient's immune system. Effective therapeutic vaccination will thus require adjuvant support, possibly by new approaches to immunomodulation such as bispecific antibodies or antibody-cytokine fusion proteins. Tumor-specific antigens, which could be a more potent target for immunotherapy, mostly arise by point mutations and have the disadvantage of being not only tumor-specific, but also individual-specific. Therapeutic vaccination will probably focus on defined antigens offered as protein, peptide or nucleic acid. Irrespective of the form in which the antigen is applied, emphasis will be given to the activation of dendritic cells as professional antigen presenters. Dendritic cells may be loaded in vitro with antigen, or, alternatively, initiation of an immune response may be approached in vivo by vaccination with RNA or DNA, given as such or packed into attenuated bacteria. The importance of activation of T helper cells has only recently been taken into account in cancer vaccination. Activation of cytotoxic T cells is facilitated by the provision of T helper cell-derived cytokines. T helper cell-dependent recruitment of elements of non-adaptive defence, such as leucocytes, natural killer cells and monocytes, is of particular importance when the tumor has lost MHC class I expression. Barriers to successful therapeutic vaccination include: (i) the escape mechanisms developed by tumor cells in response to immune attack; (ii) tolerance or anergy of the evoked immune response; (iii) the theoretical possibility of provoking an autoimmune reaction by vaccination against tumor-associated antigens; and (iv) the advanced age of many patients, implying reduced responsiveness of the senescent immune system [48]. Generating an antitumor immune response in tumor-bearing host has been impaired by several characteristics of both patient and tumor cells. Amongst those requirements is the necessity of generating immune effectors that are specific to tumor cells. The last two decades have seen the description of many so called tumor "specific" antigens. Indeed, strictly specific tumor antigens are scarce. Most antigens are tumor-associated antigens, also shared by normal tissues. Telomerase and its activity have recently been recognized as a major marker of tumoral growth in more than 80% of cancers. Some telomerase subunits might be ideal, if not universal, targets to an antitumor immune response in patients with cancer. Many other major parameters remain to be understood and to be mastered [49].

The survival of patients with cancer has improved steadily but incrementally over the last century, with the advent of effective anticancer treatments such as chemotherapy and radiotherapy. However, the majority of patients with metastatic disease will not be cured by these measures and will eventually die of their disease. New and more effective methods of treating these patients are required urgently. The immune system is a potent force for rejecting transplanted organs or microbial pathogens, but effective spontaneous immunologically induced cancer remissions are very rare. In recent years, much has been discovered about the mechanisms by which the immune system recognizes and responds to cancers. The specific antigens involved have now been defined in many cases. Improved adjuvants are available. Means by which cancer cells overcome immunological attack can be exploited and overcome. Most importantly, the immunological control mechanisms responsible for initiating and maintaining an effective immune response are now much better understood. It is now possible to manipulate immunological effector cells or antigen-presenting cells ex vivo in order to induce an effective antitumour response. At the same time, it is possible to recruit other aspects of the immune system, both specific (e.g. antibody responses) and innate (natural killer cells and granulocytes) [50].

Immunotherapy of cancer is entering into a new phase of active investigation both at the pre-clinical and clinical level. This is due to the exciting developments in basic immunology and tumor biology that have allowed a tremendous increase in our understanding of mechanisms of interactions between the immune system and tumor cells. Clinical approaches are diverse but can now be based on strong scientific rationales. The analysis of the available clinical results suggests that, despite some disappointments, there is room for optimism that both active immunotherapy (vaccination) and adoptive immunotherapy may soon become part of the therapeutic arsenal to combat cancer in a more efficient way [51]. Advancements in the understanding of cellular immunity within the last decade, along with the characterization of tumor antigens, have led to immunotherapeutic approaches for cancer therapy. This mode of treatment is expected to provide more tumor-specific activity, thereby being less toxic to normal cells than standard modalities. Clinical trials are underway throughout the world to determine whether immunotherapy is a practical and viable alternative to conventional cancer therapies. Unlike radiotherapy and chemotherapy, wherein tumor regression is the standard for determining efficacy of the regimens, immunotherapy has to be evaluated by the examination of several immunological parameters within patients [52]. The identification of tumor-associated antigens recognized by cellular or humoral effectors of the

immune system has opened new perspectives for cancer therapy. Different groups of cancer-associated antigens have been described as targets for cytotoxic T lymphocytes (CTLs) in vitro and in vivo: 1) cancer-testis (CT) antigens, which are expressed in different tumors and normal testis; 2) melanocyte differentiation antigens; 3) point mutations of normal genes; 4) antigens that are overexpressed in malignant tissues; and 5) viral antigens. Clinical studies with peptides derived from these antigens have been initiated to induce specific CTL responses in vivo. Immunological and clinical parameters for the assessment of peptide-specific reactions have been defined, i.e., delayed-type hypersensitivity (DTH), CTL, autoimmmune, and tumor regression responses. Preliminary results demonstrate that tumor-associated peptides alone elicit specific DTH and CTL responses leading to tumor regression after intradermal injection. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was proven effective in enhancing peptide-specific immune reactions by amplification of dermal peptide-presenting dendritic cells. Long-lasting complete tumor regressions have been observed after induction of peptide-specific CTLs. However, in single cases with disease progression after an initial tumor response, either a loss of the respective tumor antigen targeted by CTLs or of the presenting major histocompatibility complex (MHC) class I allele was detected as a mechanism of immune escape under immunization. Based on these observations, cytokines to enhance antigen and MHC class I expression in vivo are being evaluated to prevent immunoselection. Recently, a strategy utilizing spontaneous antibody responses to tumor-associated antigens (SEREX) has led to the identification of a new CT antigen, NY-ESO-1, which is regarded as one of the most immunogenic antigens known today inducing spontaneous immune responses in 50% of patients with NY-ESO-1-expressing cancers. Clinical studies involving antigenic constructs that induce both antibody and CTL responses will show whether these are more effective for immunotherapy of cancer [53].

Tumors express proteins not commonly found in normal cells, or over-express certain proteins. These may in some cases serve as target antigens for immunological attack. It is therefore essential to improve our understanding of the nature of these target epitopes and the cells which recognize them, in order to develop immunotherapy as a realistic treatment for cancer [54]. Advances in molecular biology have enabled specific antigens present on colorectal cells to be characterized, against which immune responses may be generated. This, in combination with our inability to significantly alter survival from this condition, has resurrected an interest in immunotherapy as a potential treatment option. A number of approaches currently constitute immunotherapeutic options for colorectal cancer. A number of treatment modalities are already in phase III studies, although clearly not all will fulfill their initial promise. Surgeons need to be aware of the advances in this rapidly expanding field, and keep an open mind as to their efficacy [55]. The goal of harnessing the immune system to recognize tumor as “nonself” is not new. Now, thanks to new knowledge and new techniques, however, modalities that seek to activate the host immune system are becoming increasingly feasible as treatments for advanced malignancies [56]. The major impact of recent scientific advances, such as the discovery of genes and gene products, has been to facilitate development of immunotherapies based on the specific stimulation of immune reactions against characterized tumor antigens [57]. Over the last decade, there has been a considerable increase in understanding of immune responses against cancers, the antigenic structures on tumor cells recognised by the immune system, and the development of more effective vaccines. There is, however, very limited understanding of why the immune system most often fails to control tumor growth and progression. In some patients, it is difficult to demonstrate immune responses to their tumors, and it may be assumed that this reflects poor recognition of tumor antigens, induction of anergy in lymphocytes, or suppression of immune responses by tumor-derived factors. In other patients, tumor progression appears to occur despite the presence of antibody or cell-mediated responses. This may indicate selection of tumor cells that have lost tumor antigens or HLA antigens by immune responses against the tumor. Tumor cells may also become resistant to mediators of apoptosis, such as Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand used by lymphocytes to kill tumor cells. It is suggested that development of effective immunotherapy will need to include strategies that take into account these limitations of immune responses and classification of tumors according to the treatment approach most likely to succeed [58].

Heat shock proteins (Hsps), ubiquitous in nature, act as chaperones for peptides and other proteins. They have been implicated in loading immunogenic peptides onto major histocompatibility complex molecules for presentation to T cells. When isolated from tumor cells, Hsps are complexed with a wide array of peptides, some of which serve as tumor-specific antigens. Animal studies have demonstrated that heat shock protein--peptide complexes (HSPPCs) from tumor cells can act as vaccines to prevent or treat tumors. Potent and specific tumor antigens have long been the holy grail in cancer immunotherapy; HSPPCs from tumor cells could become a safe and reliable source of tumor-specific antigens for clinical application [59]. Immunotherapy of mice with preexisting cancers with heat shock protein preparations derived from autologous cancer resulted in retarded progression of the primary cancer, a reduced metastatic load, and prolongation of life-span. Treatment with heat shock protein preparations derived from cancers other than the autologous cancer did not provide significant protection. Spontaneous cancers (lung cancer and melanoma), chemically induced cancers (fibrosarcoma and colon carcinoma), and an ultraviolet radiation-induced spindle cell carcinoma were tested, and the results support the efficacy of autologous cancer-derived heat shock protein-peptide complexes in immunotherapy of cancers without the need to identify specific tumor antigenic epitopes [60].

### Dendritic cells

The identification of tumor specific antigens has provided important advance in tumor immunology. It is now established that specific cytotoxic T lymphocytes (CTL) and natural killer cells infiltrate tumor tissues and are effector cells able to control tumor growth. However, such a natural antitumor immunity has limited effects in cancer patients. Failure of host defenses against tumor is consecutive to several mechanisms which are becoming targets to design new immunotherapeutic approaches. CTL are critical components of the immune response to human tumors and induction of strong CTL responses is the goal of most current vaccine strategies. Effectiveness of cytokine therapy, cancer vaccines and injection of cells improving cellular immunity have been established in tumor grafted murine models. Clinical trials are underway. Today, interest is particularly focused on cell therapy: injected cells are either “ready to use” effector cells (lymphocytes) or antigen presenting cells able to induce a protective immune reaction in vivo (dendritic cells). The challenge ahead lie in the careful optimization of the most promising strategies in clinical situation [61]. The response of hepatocellular carcinoma (HCC) to therapy is often disappointing and new modalities of treatment are clearly needed. Active immunotherapy based on the injection of autologous dendritic cells (DC) co-cultured ex vivo with tumor antigens has been used in pilot studies in various malignancies such as melanoma and lymphoma with encouraging results. In the study of Ladhams et al. [62], the preparation and exposure of patient DC to autologous HCC antigens and re-injection in an attempt to elicit antitumor immune responses were described. Therapy was given to two patients, one with hepatitis C and one with hepatitis B, who had large, multiple HCC and for whom no other therapy was available. No significant side-effects were observed. The clinical course was unchanged in one patient, who died a few months later. The other patient, whose initial prognosis was considered poor, is still alive and well more than 3 years later with evidence of slowing of tumor growth based on organ imaging.

The characterization of tumor-associated antigens recognized by human T lymphocytes in a major histocompatibility complex (MHC)-restricted fashion has opened new possibilities for immunotherapeutic approaches to the treatment of human cancers. Dendritic cells (DC) are professional antigen presenting cells that are well suited to activate T cells toward various antigens, such as tumor-associated antigens, due to their potent costimulatory activity. The availability of large numbers of DC, generated either from hematopoietic progenitor cells or monocytes in vitro or isolated from peripheral blood, has profoundly changed pre-clinical research as well as the clinical evaluation of these cells. Accordingly, appropriately pulsed or transfected DC may be used for vaccination in the field of infectious diseases or tumor immunotherapy to induce antigen-specific T cell responses. These observations led to pilot clinical trials of DC vaccination for patients with cancer in order to investigate the feasibility, safety, as well as the immunologic and clinical effects of this approach. Initial clinical studies of human DC vaccines are generating encouraging preliminary results demonstrating induction of tumor-specific immune responses and tumor regression. Nevertheless, much work is still needed to address several variables that are critical for optimizing this approach and to determine the role of DC-based vaccines in tumor immunotherapy [63].

Dendritic cells are among the most efficient antigen-presenting cells of our immune system and they play a crucial role in immunity reactions such as the activation of T and B cells and the induction or maintenance of tolerance. New culture methods allow us to generate dendritic cells in sufficient numbers for further studies and for the preparation of antigen-loaded dendritic cells for clinical application in cancer patients. In animal studies immunization with antigen-loaded dendritic cells offered protection from growth of injected tumor cells. In experimental clinical studies in cancer patients with e.g. metastatic renal carcinoma, melanoma and B cell lymphoma some lasting remissions were observed after administration of antigen-loaded dendritic cells. Side effects were minor. Unanswered questions on tumor vaccines with antigen-loaded dendritic cells concern specific matters, such as optimal culture methods and antigen loading, and general matters, such as dose, frequency, duration and route of administration. Also, no method is currently available by which the in vivo immune response can be measured accurately [64]. Research over the last two years has explored a number of possible approaches to applying dendritic cell immunotherapy to the treatment of human cancers. The chosen strategy in clinical situations will vary for individual patients and will depend on the type of tumor, availability of tissue samples and potential source of dendritic cells. The isolation of fractionated tumor peptide from individual patients’ tumors for use with autologous stem cell-derived dendritic cells may provide, in at least some cases, an effective and practical approach to cancer immunotherapy. This approach will provide a ‘closed’ system of tumor immunotherapy with all components (dendritic cells, antigen and cytotoxic T lymphocytes) being provided by the patient and applied in a tailor-made fashion to individual patients as an adjuvant to current anti-tumor therapies [65].

## Cancer Antibodies

The specificity of antibodies has been harnessed to target cancer cells and the first therapeutic antibodies for use in oncology are now finding application in the clinic. Studies are currently under way to develop new and improved antibodies. Recent developments have been made in the identification of novel targets, including the use of genomic and proteomic technologies. Several methods are also being developed to enhance antibody efficacy [65]. Bi-specific antibodies (BsAbs) combine immune cell activation with tumor cell recognition as a result of which tumor cells are killed by pre-defined effector cells [67]. Antibody-based

therapy of human cancers has led to several remarkable outcomes, particularly in the therapy of breast cancer and lymphoma. Many solid tumors have proven less responsive, due in part to difficulties in the tumor-selective delivery of antibodies and potential cytolytic effectors. However, antibodies that directly perturb signaling mechanisms in cells derived from epithelial malignancies have shown benefit; examples include antibodies directed against the extracellular domains of HER2/neu and epidermal growth factor receptor. A long-term goal of immunotherapy has been to induce anti-tumor inflammatory responses that can directly cause tumor regression or induce adaptive responses against tumor-related antigens [68]. Specific targeting of tumor cells may be achieved by using monoclonal antibodies to tumor antigens. Edrecolomab is a mouse-derived monoclonal IGg2A antibody directed against the human tumor-associated CO17-1A (or Ep-CAM) antigen, and is the first monoclonal antibody approved for cancer therapy. Encouraging results of several clinical trials were recently reported using edrecolomab for adjuvant therapy after surgery of Duke's C colorectal cancer. Side effects and toxicity profiles compare favorably to conventional regimens of radio- or chemotherapy. Future challenges lie in further improvement of these novel therapeutics, hopefully generating benefit for a larger number of cancer patients [69]. Gangliosides on tumor cells have been suggested as potential target antigens for specific immunotherapy in various types of cancer including small cell lung cancer (SCLC). Brezicka et al. [70]) have compared the expression of three gangliosides that have been described as tumor-associated antigens, FucGM1, GM2 and GD3 in SCLC tissue specimens collected at autopsy, using a double-layer immunofluorescence staining method and specific monoclonal antibodies (Mabs) directed against these ganliosides. They found expression of FucGM1, GD3 and GM2 in 70% (n=20), 60% (n=15) and 40% of the tumor cells in all lesions from the same patient (five of eight cases). These results indicate that FucGM1 is a relevant ganglioside antigen in SCLC, and suggest that specific immunotherapy involving more than one ganglioside antigen in SCLC should at least include FucGM1 and GD3.

There is now a considerable body of information documenting the autoimmune consequences of antibodies induced by growing malignancies, or by passively administered and actively induced antibodies, in cancer patients against antigens shared by normal and malignant tissues. This provides a rich source of information addressing the consequences of autoantibodies against a range of antigens. Antibodies against cell-surface or intracellular antigens in the central nervous system (CNS) or on epithelial surfaces of normal tissues do not generally result in autoimmunity, but the same types and titers of antibodies against cell surface antigens in the subepidermal skin, peripheral nerves, blood, or vascular sites such as the spleen and bone marrow readily induce autoimmunity. The blood brain barrier of the CNS and apical antigen expression and the basement membrane in epithelial tissues, may protect these sites from antibody induced damage. Cancer cells, however, are protected by neither unidirectional antigen expression nor basement membranes. Vaccine induced antibodies against a variety of cancer cell surface antigens have been associated with prevention of tumor recurrence in preclinical models and in vaccinated cancer patients, in the absence of demonstrable autoimmunity. This forms the basis for a series of ongoing Phase III trials with single or polyvalent antigen cancer vaccines designed for optimal antibody induction [71].

Immunotherapy of cancer is still mainly an experimental treatment. Some monoclonal antibodies have been approved for adjuvant therapy of cancer in patients, but active immunization strategies have not yet matured to this stage. The fact that vaccination against viral diseases is effective has primed high expectations for successful vaccination against cancer as well. Indeed, in some animal models, therapeutic results could be obtained against short-term established tumors, which paved the way for clinical trials. However, the first results with active immunization in cancer patients were disappointing and this led to a careful examination of current protocols and the search for more effective approaches. Evaluation of the available data suggests that cancer patients may not be comparable in their immune response to cancer vaccines with healthy persons. Furthermore, the tumor seems to be able to develop several immune-escape mechanisms, which either inactivate the specific immune cells or prevent activation of potential effector mechanisms against the tumor [72].

## Genetic immunotherapy

The establishment of cancer in a host involves at least two major events: the escape of tumor cells from normal growth control and their escape from immunological recognition. Because of this nature of their development, cancer cells seem to be predominatly poorly immunogenic. In contrast to the previous idea that cancer cells express no recognizable antigens, recent progress in the identification and characterization of tumor antigens, as well as the expansion of knowledge on the cellular and molecular mechanisms of antigen recognition by the immune system, have raised the possibility of using immunotherapy to treat certain tumors. Information on these mechanisms has been obtained in three crucial areas: 1) the role of cytokines in the regulation of the immune response, 2) the molecular characterization of tumor antigens in both mouse and human tumors, and 3) the molecular mechanisms of T cell activation and antigen presentation. Such information has provided new insight into tumor immunology and immunotherapy. Furthermore, recombinant DNA technology allows for modification of the genome of mammalian cells for therapeutic purposes in several diseases. Several novel strategies have been developed to derive genetically modified tumor cells and use them as cellular vaccines to induce antitumor immunity in animal tumor models. This combined modality of genetically modified tumor cells and immunotherapy has been termed immunogene therapy of tumors. Crucial to this approach

has been the ability to transfer into normal or neoplastic cells genes known to increase the immunogenicity of cells, which subsequently can be used to augment immune reactions in tumor-bearing mice or cancer patients. While there has been success in inducing antitumor immunity in some tumor models, there are difficulties and limitations in the application of these gene-modified tumor cells for the treatment of preexisting tumors [73].

Genetic immunization refers to treatment strategies where gene transfer methods are used to generate immune responses against cancer. Our growing knowledge of the mechanisms regulating the initiation and maintenance of cytotoxic immune responses has provided the rationale for the design of several genetic immunization strategies. Tumor cells have been gene-modified to express immune stimulatory genes and are then administered as tumor vaccines, in an attempt to overcome tumor cell ignorance by the immune system. With the description of well-characterized tumor antigens, multiple strategies have been proposed mainly aimed at optimal tumor antigen presentation by antigen-presenting cells (APC). Among APC, the dendritic cells have been recognized as the most powerful cells in this class, and have become the target for introducing tumor antigen genes to initiate antitumor immune responses. The detailed knowledge of how the immune system can be activated to specifically recognize tumor antigens, and the mechanisms involved in the control of this immune response, provide the basis for modern genetic immunization strategies for cancer treatment [74]. T lymphocytes play a crucial role in the host's immune response to cancer. Although there is ample evidence for the presence of tumor-associated antigens on a variety of tumors, they are seemingly unable to elicit an adequate antitumor immune response. Modern cancer immunotherapies are therefore designed to induce or enhance T cell reactivity against tumor antigens. Vaccines consisting of tumor cells transduced with cytokine genes in order to enhance their immunogenicity have been intensely investigated in the past decade and are currently being tested in clinical trials. With the development of novel gene transfer technologies it has now become possible to transfer cytokine genes directly into tumors in vivo. The identification of genes encoding tumor-associated antigens and their peptide products which are recognized by cytotoxic T lymphocytes in the context of major histocompatibility complex class I molecules has allowed development of DNA-based vaccines against defined tumor antigens. Recombinant viral vectors expressing model tumor antigens have shown promising results in experimental models. This has led to clinical trials with replication-defective adenoviruses encoding melanoma-associated antigens for the treatment of patients with melanoma. An attractive alternative concept is the use of plasmid DNA, which can elicit both humoral and cellular immune responses following injection into muscle or skin. New insights into the molecular biology of antigen processing and presentation have revealed the importance of dendritic cells for the induction of primary antigen-specific T cell responses. Considerable clinical interest has arisen to employ dendritic cells as a vehicle to induce tumor antigen-specific immunity. Advances in culture techniques have allowed the generation of large numbers of immunostimulatory dendritic cells in vitro from precursor populations derived from blood or bone marrow. Experimental immunotherapies which now transfer genes encoding tumor-associated antigens or cytokines directly into professional antigen-presenting cells such as dendritic cells are under evaluation in pre-clinical studies at many centers. Gene therapy strategies, such as in vivo cytokine gene transfer directly into tumors as well as the introduction of genes encoding tumor-associated antigens into antigen-presenting cells hold considerable promise for the treatment of patients with cancer [75].

## Adjuvant immunotherapy

In the course of a century, tumor immunology has revealed a picture of a very complex immune system involving the recognition and eradication of malignancies. Many tumors evade the immune system, and understanding of tumor escape mechanisms is the key to a successful immunotherapy for cancer. A wide array of tumor immunotherapy modalities have been developed, many of which have reached the phase of clinical trials, with some satisfactory results [76]. Although surgery remains the mainstay for the treatment of most solid tumors, investigators are seeking complementary therapies to eradicate microscopic disease, which causes tumor relapse even after an apparently complete surgical excision. Although adjuvant chemotherapy has achieved some significant results, the control of minimal residual disease is still a challenge for clinicians. Among novel therapeutic approaches, immunotherapy holds promise. This anticancer strategy aims at triggering a highly specific endogenous killing machine against tumor cells. Recent progress in tumor immunology has improved our understanding of host-immune system interactions. In particular, new technologies have fostered the identification of potentially immunogenic tumor antigens that can be used as suitable targets for immune effector cells. After observing immunotherapy-mediated clinical responses in patients with metastatic disease, investigators have started evaluating this anticancer modality in the adjuvant setting [77].

## Conclusion No. 2

Urotherapy is suggested as a kind of immunotherapy for cancer patients. Unlike the clonal immunotherapy the urine of the cancer patients contain the many tumor antigens which constitute the tumor. Oral auto-urotherapy will provide the intestinal lymphatic system the tumor antigens against which they may produce antibodies due to non-self recognition. These antibodies may be transpierced through the blood stream and attack the tumor and its cells [78]. Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced.

## References


1. Shu S, Plautz GE, Krauss JC, Chang AE (1997) Tumor immunology. JAMA 278(22): 1972-1981.
2. Cerundolo V (1999) T cells work together to fight cancer. Curr Biol 9(18): R695-R697.
3. Kawakami Y (2000) New cancer therapy by immunomanipulation: development of immunotherapy for human melanoma as a model system. Cornea 19(3 Suppl): S2-S6.
4. Houghton AN, Gold JS, Blachere NE (2001) Immunity against cancer: lessons learned from melanoma. Curr Opin Immunol 13(2): 134-140.
5. Lieberman SM, Horig H, Kaufman HL (2001) Innovative treatments for pancreatic cancer. Surg Clin North Am 81(3): 715-739.
6. Turk MJ, Wolchok JD, Guevara-Patino JA, Goldberg SM, Houghton AN, et al. (2002) Multiple pathways to tumor immunity and concomitant autoimmunity. Immunol Rev 188: 122-135.
7. Koroleva EP, Lagarkova MA, Khlgatian SV, Shebzukhov IuV, Meshcheriakov AA, et al. (2004) Serological study of a repertoire of human cancer antigens and autoantigens. Mol Biol (Mosk). 38(2): 233-238.
8. Lollini PL, Forni G (2003) Cancer immunoprevention: tracking down persistent tumor antigens. Trends Immunol 24(2): 62-66.
9. Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT, et al. (2002) Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev 188: 22-32.
10. Perez-Diez A, Marincola FM (2002) Immunotherapy against antigenic tumors: a game with a lot of players. Cell Mol Life Sci 59(2): 230-240.
11. Van den Eynde B (2001) Identification of cancer antigens of relevance for specific cancer immunotherapy. Bull Mem Acad R Med Belg 156(10-12): 548-555.
12. Itoh K, Yamana H, Shichijo S, Yamada A (2000) Human tumor-rejection antigens and peptides from genes to clinical research. Nippon Geka Gakkai Zasshi 101(9): 612-627.
13. Slingluff CL (1999) Targeting unique tumor antigens and modulating the cytokine environment may improve immunotherapy for tumors with immune escape mechanisms. Cancer Immunol Immunother 48(7): 371-373.
14. Wang RF (1999) Human tumor antigens: implications for cancer vaccine development. J Mol Med 77(9): 640-655.
15. Kudo T, Suzuki M, Katayose Y, Shinoda M, Sakurai N, et al. (1999) Specific targeting immunotherapy of cancer with bispecific antibodies. Tohoku J Exp Med 188(4): 275-288.
16. Huang SK, Okamoto T, Morton DL, Hoon DS (1998) Antibody responses to melanoma/melanocyte autoantigens in melanoma patients. J Invest Dermatol 111(4): 662-627.
17. Wang RF (1997) Tumor antigens discovery: perspectives for cancer therapy. Mol Med 3(11): 716-731.
18. Ribas A, Butterfield LH, Glaspy JA, Economou JS (2003) Current developments in cancer vaccines and cellular immunotherapy. J Clin Oncol 21(12):2415-2432.
19. Zoller M (2003) Immunotherapy of cancer by active vaccination: does allogeneic bone marrow transplantation after non-myeloablative conditioning provide a new option? Technol Cancer Res Treat. 2(3): 237-260.
20. Chada S, Mhashilkar A, Roth JA, Gabrilovich D (2003) Development of vaccines against self-antigens: the p53 paradigm. Curr Opin Drug Discov Devel 6(2): 169-173.
21. Pecher G (2002) DNA-based tumor vaccines. Onkologie 25(6): 528-532.
22. Dermime S, Armstrong A, Hawkins RE, Stern PL (2002) Cancer vaccines and immunotherapy. Br Med Bull 62: 149-62.
23. Perales MA, Wolchok JD (2002) Melanoma vaccines. Cancer Invest 20(7-8): 1012-1026.
24. Cohen EP (2001) DNA-based vaccines for the treatment of cancer an experimental model. Trends Mol Med 7(4): 175-179.
25. Berd D (2001) Autologous, hapten-modified vaccine as a treatment for human cancers. Vaccine 19(17-19):2565-2570.
26. Bhattacharya-Chatterjee M, Chatterjee SK, Foon KA (2001) The anti-idiotype vaccines for immunotherapy. Curr Opin Mol Ther 3(1): 63-69.
27. Bhattacharya-Chatterjee M, Chatterjee SK, Foon KA (2000) Anti-idiotype vaccine against cancer. Immunol Lett 74(1): 51-58.
28. Mitchell DA, Nair SK (2000) RNA transfected dendritic cells as cancer vaccines. Curr Opin Mol Ther 2(2): 176-181.
29. Wang RF, Rosenberg SA (1999) Human tumor antigens for cancer vaccine development. Immunol Rev 170: 85-100.
30. Timmerman JM, Levy R (1999) Dendritic cell vaccines for cancer immunotherapy. Annu Rev Med 50: 507-529.
31. Chen CH, Wu TC (1998) Experimental vaccine strategies for cancer immunotherapy. J Biomed Sci. 5(4): 231-252.
32. Gilboa E, Nair SK, Lyerly HK (1998) Immunotherapy of cancer with dendritic-cell-based vaccines. Cancer Immunol Immunother 46(2): 82-87.
33. Dudley ME, Rosenberg SA (2003) Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer 3(9): 666-675.
34. Whelan M, Whelan J, Russell N, Dalgleish A (2003) Cancer immunotherapy: an embarrassment of riches? Drug Discov Today 8(6): 253-258.
35. Kwak H, Horig H, Kaufman HL (2003) Poxviruses as vectors for cancer immunotherapy. Curr Opin Drug Discov Devel 6(2): 161-168.
36. Markiewicz MA, Kast WM (2003) Advances in immunotherapy for prostate cancer. Adv Cancer Res 87: 159-194.
37. Ward S, Casey D, Labarthe MC, Whelan M, Dalgleish A, et al. (2002) Immunotherapeutic potential of whole tumour cells. Cancer Immunol Immunother 51(7): 351-357.
38. Sugiyama H (2002) Cancer immunotherapy targeting WT1 protein. Int J Hematol 76(2):127-132.
39. Wang RF, Zeng G, Johnston SF, Voo K, Ying H, et al. (2002) T cell-mediated immune responses in melanoma: implications for immunotherapy. Crit Rev Oncol Hematol 43(1): 1-11.

40. Fehniger TA, Cooper MA, Caligiuri MA (2002) Interleukin-2 and interleukin-15: immunotherapy for cancer. Cytokine Growth Factor Rev 13(2): 169-183.

41. Rosenberg SA (2001) Progress in the development of immunotherapy for the treatment of patients with cancer. J Intern Med 250(6): 462-475.

42. McLaughlin PM, Kroesen BJ, Harmsen MC, de Leij LF (2001) Cancer immunotherapy: insights from transgenic animal models. Crit Rev Oncol Hematol 40(1): 53-76.

43. Maki RG (2001) Soft tissue sarcoma as a model disease to examine cancer immunotherapy. Curr Opin Oncol 13(4): 270-274.

44. Rosenberg SA (2001) Progress in human tumour immunology and immunotherapy. Nature. 411(6835): 380-384.

45. Bertolaccini L, Olivero G (2001) Cancer immunotherapy. A future therapeutical choice? Minerva Chir. 56(2): 183-191.

46.

47. Smyth MJ, Godfrey DI, Trapani JA (2001) A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2(4): 293-299.

48. Vinals C, Gaulis S, Coche T (2001) Using in silico transcriptomics to search for tumor-associated antigens for immunotherapy. Vaccine 19(17-19): 2607-2614.

49. Matzku S, Zoller M (2001) Specific immunotherapy of cancer in elderly patients. Drugs 18(9): 639-664.

50. Rousseau R, Soria JC (2000) Telomerase, a universal target in immunotherapy strategies against tumor? Bull Cancer 87(12): 895-901.

51. Davis ID (2000) An overview of cancer immunotherapy. Immunol Cell Biol 78(3): 179-195.

52. Bremers AJ, Parmiani G (2000) Immunology and immunotherapy of human cancer: present concepts and clinical developments. Crit Rev Oncol Hematol 34(1): 1-25.

53. Shankar G, Salgaller ML (2000) Immune monitoring of cancer patients undergoing experimental immunotherapy. Curr Opin Mol Ther 2(1): 66-73.

54. Knuth A, Jager D, Jager E (2000) Cancer immunotherapy in clinical oncology. Cancer Chemother Pharmacol 46(Suppl): S46-S51.

55. Pawelec G, Engel A, Adibzadeh M (1999) Prerequisites for the immunotherapy of cancer. Cancer Immunol Immunother 48(4): 214-217.

56. Maxwell-Armstrong CA, Durrant LG, Scholefield JH (1999) Immunotherapy for colorectal cancer. Am J Surg 177(4): 344-348.

57. Lum LG (1999) T cell-based immunotherapy for cancer: a virtual reality? CA Cancer J Clin 49(2): 74-100.

58. Rosenberg SA (1999) A new era of cancer immunotherapy: converting theory to performance. CA Cancer J Clin 49(2): 70-73, 65.

59. Hersey P (1999) Impediments to successful immunotherapy. Pharmacol Ther 81(2): 111-119.

60. Przepiorka D, Srivastava PK (1998) Heat shock protein--peptide complexes as immunotherapy for human cancer. Mol Med Today 4(11): 478-484.

61. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK, et al. (1997) Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 278(5335): 117-120.

62. Catros-Quemener V, Bouet F, Genetet N (2003) Antitumor immunity and cellular cancer therapies. Med Sci (Paris) 19(1): 43-53.

63. Ladhams A, Schmidt C, Sing G, Butterworth L, Fielding G, et al. (2002) Treatment of non-resectable hepatocellular carcinoma with autologous tumor-pulsed dendritic cells. J Gastroenterol Hepatol 17(8): 889-896.

64. Meidenbauer N, Andreesen R, Mackensen A (2001) Dendritic cells for specific cancer immunotherapy. Biol Chem 382(4): 507-520.

65. Punt CJ, de Vries IJ, Mulders PF, Adema GJ, Figdor CG, et al. (1999) Immunology in medical practice. XXV. Use of dendritic cells in the immunotherapy of cancer. Ned Tijdschr Geneeskd 143(48): 2408-2414.

66. Hermans IF, Moroni-Rawson P, Ronchese F, Ritchie DS (1998) The emerging role of the dendritic cell in novel cancer therapies. N Z Med J 111(1063): 111-113.

67. Trikha M, Yan L, Nakada MT (2002) Monoclonal antibodies as therapeutics in oncology. Curr Opin Biotechnol 13(6): 609-614.

68. Withoff S, Helfrich W, De Leij LF, Molema G (2001) Bi-specific antibody therapy for the treatment of cancer. Curr Opin Mol Ther 3(1): 53-62.

69. Weiner LM, Adams GP (2000) New approaches to antibody therapy. Oncogene 19(53): 6144-6151.

70. Abicht A, Lochmuller H (2000) Technology evaluation: edrecolomab, Centocor Inc. Curr Opin Mol Ther. 2(5): 593-600.

71. Brezicka T, Bergman B, Olling S, Fredman P (2000) Reactivity of monoclonal antibodies with ganglioside antigens in human small cell lung cancer tissues. Lung Cancer 28(1): 29-36.

72. Livingston PO, Ragupathi G, Musselli C (2000) Autoimmune and antitumor consequences of antibodies against antigens shared by normal and malignant tissues. J Clin Immunol 20(2): 85-93.

73. Velders MP, Schreiber H, Kast WM (1998) Active immunization against cancer cells: impediments and advances. Semin Oncol 25(6): 697-706.

74. Yoshizawa H, Kagamu H, Gejyo F (2001) Cancer immunogene therapy. Arch Immunol Ther Exp (Warsz) 49(5): 337-343.

75. Ribas A, Butterfield LH, Economou JS (2000) Genetic immunotherapy for cancer. Oncologist 5(2): 87-98.

76. Tuting T, Storkus WJ, Lotze MT (1997) Gene-based strategies for the immunotherapy of cancer. J Mol Med 75(7): 478-491.

77. Bremers AJ, Kuppen PJ, Parmiani G (2000) Tumour immunotherapy: the adjuvant treatment of the 21st century? Eur J Surg Oncol 26(4): 418-424.

78. Mocellin S, Rossi CR, Lise M, Marincola FM (2002) Adjuvant immunotherapy for solid tumors: from promise to clinical application. Cancer Immunol Immunother 51(11-12): 583-595.

79. Eldor J (1997) Urotherapy for patients with cancer. Med Hypotheses 48(4): 309-315.

## Combined Intralipid - Urotherapy for Patients with Cancer (Part 3)

### Intralipid Effects on the Immune System

Intravenous lipid emulsions have been used experimentally since at least the 19th century. An early product marketed in 1957 under the name Lipomul was briefly used in the United States but was subsequently withdrawn due to side effects. Intralipid was invented by the Swedish physician and nutrition researcher Arvid Wretlind [1], and was approved for clinical use in Sweden in 1962. In the United States, the Food and Drug Administration initially declined to approve the product due to prior experience with another fat emulsion. It was approved in the United States in 1972. Recurrent embryo implantation failure (RIF) is a disorder with potentially devastating physiological and psychological manifestations for those affected. Although its prevalence is not uncommon, many of the mechanisms involved still require elucidation. Both organ-specific and systemic autoimmunity are associated with an increased prevalence of recurrent miscarriage and reproductive failure, rendering the role of the maternal immunological system in fertility a key concept. It is believed by some that central to this theme is the maternal cytokine profile, with particularly T-helper (Th) cells. Immune modulating therapies have therefore been mooted as potential therapeutic strategies. Recent reports of high pregnancy rates achievable in women with RIF have added fuel to the debate regarding the effectiveness of intralipid in modulating the immune system. We would like to assess if there is sufficient current evidence of acceptable quality to permit an assumption that intralipid therapy is an effective treatment for women undergoing repeated assisted reproduction cycles. We have concluded that appropriately controlled, large-scale, confirmatory studies are necessary to prove the efficacy of intralipid before it can be recommended for routine use [2]. In vitro investigations have revealed the ability of intralipids to suppress natural killer (NK) cytotoxicity. Evidence from both animal and human studies suggests that intralipid administered intravenously may enhance implantation and maintenance of pregnancy when the patient has an abnormal NK cell level or function. The aim of this study was to establish the duration and efficacy of Intralipids suppressive effect on NK cell functional activity. Fifty patients with abnormal NK activity results (NKa) received intralipid 20% i.v. (9 mg/mL total blood volume -corresponds to 2 mL of intralipid 20% diluted in 250 mL saline; or 18 mg/mL - corresponds to 4 mL of intralipid 20% diluted in 250 mL saline) infusions and their NKa were tested periodically. The determination of NK cell function was performed by flow cytometry using K562 cells as targets. Fifty women with abnormal NKa-testing received intralipid infusions. 39 (78%) showed NKa suppression within the normal range the first week after infusion, 11 (22%), showed suppression, but still above the normal threshold. They received second infusion 2-3 weeks later. In 10, the Nka activity was normalized the following week. Four patients had three intralipid infusions in 2-week periods in between and after the third infusion, and all showed NKa normal activity. In 47 patients the suppressive effect of the Intralipid after the normalization of NKa lasted between 6 and 9 weeks, in two patients this benefit lasted 5 weeks, and in one patient the effect was 4 weeks. Intralipid is effective in suppressing in vivo abnormal NK-cell functional activity. The results suggest that Intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive failure [3].

Novel anti-inflammatory effects of insulin have recently been described, and insulin therapy to maintain euglycemia suppresses the plasma levels of free fatty acids (FFA) and increases the survival of critically ill patients. We aimed to explore the effect of short-term high levels of plasma FFA on the inflammatory response to a low dose of endotoxin. Fourteen healthy male volunteers underwent the following two trials in a randomized crossover design: 1) continuous infusion of 20% Intralipid [0.7 ml.kg(-1).h(-1) (1.54 g/kg)] for 11 h, and 2) infusion of isotonic saline for 11 h (control). In each trial, heparin was given to activate lipoprotein lipase, and an intravenous bolus of endotoxin (0.1 ng/kg) was given after 6 h of Intralipid/saline infusion. Blood samples and muscle and fat biopsies were obtained before the Intralipid/saline infusion and before as well as after infusion of an endotoxin bolus. Plasma levels of FFA, triglycerides, and glycerol were markedly increased during the Intralipid infusion. Endotoxin exposure induced an increase in plasma levels of TNF-alpha, IL-6, and neutrophils and further stimulated gene expression of TNF-alpha and IL-6 in both skeletal muscle and adipose tissue. The systemic inflammatory response to endotoxin was significantly pronounced during Intralipid infusion. Short-term hyperlipidemia enhances the inflammatory response to endotoxin, and skeletal muscle and adipose tissue are capable of producing essential inflammatory mediators after endotoxin stimulation [4].

Because of its oxidative modification during the acute-phase response to an aggression, low density lipoprotein (LDL) can be regarded as a source of lipid mediators that can act both to promote and inhibit inflammation. This can be exemplified by the production of anti-inflammatory oxidized fatty acids and proinflammatory lysophosphatidylcholine (LPC) during LDL oxidation. We have shown previously that oxidized LDL (oxLDL) plays an active role at the interface between innate and adaptive immunity by delivering instructive molecules such as LPC, which promotes mature dendritic cell (DC) generation from differentiating monocytes. It is shown in this study that LPC affects the signalling pathway of peroxisome proliferator-activated receptors (PPARs). LPC-induced DC maturation is associated with complete inhibition of PPARgamma activity and up-regulation of the activity of an uncharacterized nuclear receptor that bind peroxisome proliferator response element. Oxidized fatty acids generated during LDL oxidation are natural ligands for PPARgamma and inhibit oxLDL- and LPC-induced maturation. Inhibition experiments with synthetic PPARgamma ligands suggested a PPARgamma-dependent and independent effect of LPC on DC maturation. Therefore, the relative amount of oxidized

fatty acids and LPC influences the immunological functions of oxLDL on DC, in part by regulating the PPAR pathway. By sensing the biochemical composition of lipoprotein particles, the innate immune system may thus identify various endogenous signals that influence the immune response during the acute-phase reaction. The therapeutic emulsion intralipid also blocks LPC action on PPAR activity and DC maturation. Intralipid may thus be an alternative therapeutic strategy for some chronic inflammatory diseases [5].

During the acute phase response, the interplay between high density lipoproteins and low density lipoproteins (LDL) favors transient generation of oxidized LDL with proinflammatory activities. We hypothesized that oxidative modification of LDL is an endogenous signal for the immune system, and we have shown that oxidized LDL promotes mature dendritic cell transition from monocyte, therefore linking the nonspecific acute phase response to adaptive immunity. Lysophosphatidylcholine (LPC) is a major lipid component of oxidized LDL with reported proinflammatory activities. We now report that LPC acts through G protein-coupled receptors on differentiating monocytes to generate mature dendritic cells with the ability to stimulate IL-2 and IFN-gamma production by allogeneic T lymphocytes. LPC is most effective in lipoprotein-deprived serum and can be inhibited by an excess of native LDLs reflecting normal plasma conditions. Therefore, by controlling the balance between native and oxidized lipoproteins and the resulting production of LPC, the acute phase reactants may provide a context of Ag presentation that is transiently favorable to immune activation. Intralipid, a therapeutic lipid emulsion for parenteral nutrition with unexplained immunomodulatory properties, also blocked LPC activity. This opens perspectives for the understanding and treatment of acute and chronic inflammatory diseases [6].

The lipid component of total parenteral nutrition (TPN) has reportedly been associated with trophic effects on the intestinal mucosa and suppressive effects on the immune system. We have challenged these hypotheses using a 7-day TPN rodent model comparing the effects of isocaloric, isonitrogenous lipid-based (TPN-lipid, 50% of calories as long-chain triacylglycerol) and carbohydrate-based TPN (TPN-CH, 100% of calories as carbohydrates) on mucosal morphology and immune function. Enterally fed animals were included to establish a baseline for immunologic read-outs. The study was performed in healthy, metabolically stable animals to avoid interference by septic or trauma-related stress factors. Both TPN regimens resulted in a significantly smaller weight gain (TPN-lipid, 29.8 +/- 4.0 g; TPN-CH, 30.3 +/- 4.4 g) compared with enterally fed reference animals (49.2 +/- 3.2 g; p = .007), with no difference in nitrogen balance between the TPN groups. Mucosal sucrase activity was significantly lower in both TPN groups (TPN-lipid, 8.8 +/- 1.0 x 10(-7) katal per gram (kat/g) of protein; CH: 11.9 +/- 1.6 x 10(-7) kat/g of protein) compared with enteral feeding (17.4 +/- 0.9 x 10(-7) kat/g of protein; ANOVA: p = .0007). Morphometric analysis of the small intestine revealed no differences between the two TPN groups although a significantly depressed villus height in the TPN-lipid group could be observed in comparison to enterally fed reference rats (TPN-lipid, 0.47 +/- 0.02; TPN-CH, 0.50 +/-0.01; enteral, 0.56 +/- 0.02 mm; ANOVA: p = .0298). Light and electron microscopy revealed a normal surface architecture in all three groups of rats. Cellular immune reactivity was evaluated using a novel specific immunization protocol: animals were immunized against OVA 4 weeks before TPN. OVA-induced lymph proliferative responses and phenotypic data from draining popliteal and mesenteric lymph nodes were evaluated after the different regimens. Results did not differ among the three groups.

In healthy rodents, short-term lipid-based and carbohydrate-based TPN regimens lead to limited mucosal atrophy with preserved surface architecture compared with enteral feeding. However, peripheral and mesenteric cellular immune responsiveness after both TPN regimens remained comparable to enterally fed reference animals. Therefore, mesenteric and systemic cellular immune reactivity does not appear to be impaired by lipid-based or carbohydrate-based TPN [7]. To detect possible interactions between lipid-based total parenteral nutrition (TPN) substrates and mononuclear phagocytes, ultra structural in vitro and in vivo studies were carried out on material from pigs. Mononuclear phagocytes isolated from peripheral blood, phagocytosed lipid after incubation with 1 mg/ml Intralipid for 24 h. Similarly, lipid was taken up by intravascular macrophages in the lungs and liver after central venous administration of TPN containing 2.3 g/kg body weight/day of Intralipid for 5-7 weeks. Lipid accumulation was almost exclusively found intravascularly in the lungs and liver, and not in macrophages obtained from bronchoalveolar lavage fluid. A morphometric study of the lung capillaries showed that the macrophages in TPN animals had increased in size and number, and occupied a larger portion of the capillary lumina. The macrophages appeared to be activated, but the endothelial lining was well preserved. Free intravascular lipid droplets had a diameter both in vitro and in vivo of about 0.5 micron, indicating good stability of the emulsion. We suggest that the lipid uptake stimulates the macrophages and thereby plays a role in the lung tissue inflammation seen in response to long-term lipid-based TPN in pigs [8].

Intravenous lipid emulsions depress lymphocyte proliferative responses and granulocyte function at concentrations found in the blood circulation during their administration. The effects of Intralipid, a widely used intravenous lipid emulsion, were measured on immunoglobulin production in vitro by pokeweed mitogen-activated lymphocytes as a test of B-cell function. Intralipid decreased IgG, IgM, and IgA production at soybean oil triglyceride concentrations of 2.5-20 mg/ml occurring in the blood circulation during Intralipid infusion. The effects on IgM and IgA production were highest and that on IgG production lowest. Hydrocortisone-sensitive and concanavalin A-inducible suppressor cells were more sensitive to Intralipid than other cell populations. In vivo Ig production may not be equally disturbed, inasmuch as Intralipid concentrations in the lymph nodes and the spleen may be lower than in the blood circulation. However, care should be taken to prevent Intralipid concentrations from

becoming high enough to depress immune responses [9]. We studied the effect of Intralipid (IL) in monocyte cultures based on the ability of the cultures to phagocytose and kill Candida albicans and produce the oxidative burst. The IL was taken up by monocytes in cultures, and these cells phagocytosed more Candida organisms than did the control cells [85 +/- 2.2% in the IL treated (1%) compared to 68 +/- 2.3% after 1 h in the control]. The percentage of killing of Candida albicans, which had been taken up by the IL-treated monocytes measured after 2 h in culture (48.3 +/- 6.0%), was no different when compared to control (47.0 +/- 5.8%). Following ingestion of IL, there was an increase in basal H2O2 production, however, the presence of the IL in the cells had no effect on the expected increase in H2O2 production following stimulation with either phorbol myristate acetate (PMA) or zymosan particles. Compared to untreated cells, a significant increase in the number of monocytes with positive nitroblue tetrazolium staining was observed in monocytes that had ingested IL (when they were stimulated with either PMA or Candida microorganisms). Similar results were obtained in monocyte-derived macrophages (i.e., monocytes in monolayer cultures for 10 days). These findings suggest that the essential monocyte functions of phagocytosis, microbicidal activity, and ability to elicit an oxidative burst are not directly altered by the conventional use of IL in clinical practice [10].

In many surgical departments it has been common practice to give patients with weight loss pre-operative parenteral nutrition before major surgery. The purpose of the present study was to elucidate the value of intravenous pre-operative nutrition in relation to the immune system. The study comprised 10 patients undergoing total gastrectomy. All patients had a weight loss of 15% of body weight or more within 6 months or 10% within 3 months. Before operation they all received parenteral nutrition for 1 week. They all had 1.5 g of protein per kg per day and energy corresponding to the basal metabolic rate + 50% as Vamin, Intralipid, and carbohydrate solutions. Before and after this treatment blood samples were taken to estimate neutrophil function (the rate of oxygen consumption and superoxide liberation, phagocytosis and intracellular lysis of Candida albicans, the concentration and consumption rate of ATP during phagocytosis, and chemotaxis) and immune globulins (IgG, IgM, & IgA). Cellular immunity (CMI) was estimated by intradermal application of seven different antigens. We found a significant increase in response to the intradermal antigens ($p < 0.01$) but no difference in any of the parameters expressing leukocyte function or immune globulins [11].

Eight healthy subjects were given Intralipid, a soybean oil emulsion, 20% intravenously for 2 h. During the infusion a significant increase in the nitroblue tetrazolium-reduction of blood monocytes was noted. Preincubation of monocytes in vitro with Intralipid (20 to 100 mg/ml) for 30 min was found to increase the ability of the cells to migrate chemotactically and to phagocytize yeast particles. On the contrary, when neutrophilic granulocytes were preincubated with Intralipid in the same concentrations for 30 min. their nitroblue-tetrazolium-reduction, chemotactic and spontaneous locomotion, as well as their ingestion of yeast particles was depressed [12]. Platinum (Pt) drugs are the most potent and commonly used anti- cancer chemotherapeutics. Nanoformulation of Pt drugs has the potential to improve the delivery to tumors and reduce toxic side effects. A major challenge for translating nanodrugs to clinical settings is their rapid clearance by the reticuloendothelial system (RES), hence increasing toxicities on off-target organs and reducing efficacy. We are reporting that an FDA approved parenteral nutrition source, Intralipid 20%, can help this problem. A dichloro (1, 2-diaminocyclohexane) platinum (II)-loaded and hyaluronic acid polymer-coated nanoparticle (DACHPt/HANP) is used in this study. A single dose of Intralipid (2 g/kg, clinical dosage) is administrated [intravenously (i.v.), clinical route] one hour before i.v. injection of DACHPt/HANP. This treatment can significantly reduce the toxicities of DACHPt/HANP in liver, spleen, and, interestingly, kidney. Intralipid can decrease Pt accumulation in the liver, spleen, and kidney by 20.4%, 42.5%, and 31.2% at 24-hr post nanodrug administration, respectively. The bioavailability of DACHPt/HANP increases by 18.7% and 9.4% during the first 5 and 24 hr, respectively [13].

## Conclusion No. 3

The use of oral intralipid together with auto urotherapy in patients with cancer is first suggested in the medical literature. Intralipid can increase the response to the cancer antigens in the intestinal lymphatic system against which antibodies may be produced. These antibodies may be transpierced through the blood stream and attack the tumor and its cells.

## References


1. http://pmj.bmj.com/content/43/498/307.full.pdf
2. Shreeve N, Sadek K (2012) Intralipid therapy for recurrent implantation failure: new hope or false dawn? J Reprod Immunol 93(1): 38-40.
3. Roussev RG, Acacio B, Ng SC, Coulam CB (2008) Duration of intralipid's suppressive effect on NK cell's functional activity. Am J Reprod Immunol 60(3): 258-263.
4. Krogh-Madsen R, Plomgaard P, Akerstrom T, Møller K, Schmitz O, et al. (2008) Effect of short-term intralipid infusion on the immune response during low-dose endotoxemia in humans. Am J Physiol Endocrinol Metab 294(2): E371-E379.
5. Coutant F, Agaugué S, Perrin-Cocon L, André P, Lotteau V, et al. (2004) Sensing environmental lipids by dendritic cell modulates its function. J Immunol 172(1): 54-60.
6. Coutant F, Perrin-Cocon L, Agaugué S, Delair T, André P, et al. (2002) Mature dendritic cell generation promoted by lysophosphatidylcholine. J Immunol 169(4): 1688-1695.
7. Gross T, Babst R, Juretic A, Herzog B, Stehle P, et al. (2000) Intralipid-based short-term total parenteral nutrition does not impair small intestinal mucosa-related cellular immune reactivity in the healthy rat. JPEN J Parenter Enteral Nutr 24(6): 337-344.
8. Aksnes J, Børsum K, Rollag H, Hovig T (1996) Intravascular lung macrophages play an essential role in lipid entrapment and the inflammatory tissue reaction seen after long-term lipid-based parenteral nutrition in pigs. An ultrastructural study. APMIS 104(6): 429-436.
9. Salo M (1990) Inhibition of immunoglobulin synthesis in vitro by intravenous lipid emulsion (Intralipid). JPEN J Parenter Enteral Nutr 14(5): 459-462.
10. Padeh S, Shor R, Passwell JH (1987) Effect of intralipid on the phagocytic and microbicidal capacity of human monocytes in culture. J Pediatr Gastroenterol Nutr 6(4): 575-580.
11. Rasmussen A, Segel E, Trier Aagaard M, Hessov I (1985) The effect of preoperative nutrition on the immune system. Clin Nutr 4(3): 175-178.
12. Wiernik A, Jarstrand C, Julander I (1983) The effect of intralipid on mononuclear and polymorphonuclear phagocytes. Am J Clin Nutr 37(2): 256-261.
13. Liu L, Ye Q, Lu M, Lo YC, Hsu YH, et al. (2015) A new approach to reduce toxicities and to improve bioavailabilities of platinum-containing anti-cancer nanodrugs. Sci Rep 5: 10881.

# Chapter 8

## Combined Spinal Epidural Catheters for Epidural Cooling, Cerebrospinal Fluid Aspiration and Spinal Intralipid Infusion for Treatment of Spinal and Brain Injuries, Diseases and Protection

## Abstract

A new proposal for spinal cord and brain treatment and protection due to injuries and diseases is made herein. It is composed of two 20G nylon catheters with 6 lateral holes arranged circumferentially within 3 cm from the tip and a closed end. One catheter is inserted into the epidural space and the other catheter is inserted into the spinal space in two different lumbar interspaces using an 18G Tuohy needle 90 mm. The epidural catheter is used for cooled saline injection and infusion. The spinal catheter is used for Intralipid spinal injections and CSF aspiration. The proposal is based on the current studies on spinal cord cooling and CSF aspiration as well as on the Intralipid resuscitation properties and lipid brain protection. A study is needed to evaluate the clinical value of this combined approach.

**Keywords:** Spinal catheter; Epidural catheter; Epidural cooling; Spinal intralipid; Spinal injury; Brain injury; Spinal disease; Brain Disease; Spinal protection; Brain protection

## Introduction

Following a traumatic injury to the central nervous system, a cascade of physiological events leads to neuronal loss including, for example, an inflammatory immune response and excitotoxicity. Despite advances in spinal cord protection, paraplegia continues to be a serious complication of descending and thoraco-abdominal aortic operations. Damage to the nervous system may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease or disorder including, but not limited to, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), diabetic neuropathy, senile dementia, stroke and ischemia. During the past 9 years, considerable new evidence has accumulated supporting the use of prophylactic hypothermia for traumatic brain injury (TBI). In 1998 it was first showed that intravenous intralipid could prevent or improve resuscitation from cardiovascular collapse by severe bupivacaine overdose in rats. Since then published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haloperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. A new multimodal proposal for spinal cord and brain treatment and protection due to injuries and diseases is made herein.

## CSF Role in Ischemic Injury

In ischemic injury, CSF has a toxic effect of facilitating cerebral edema. While intracellularly excessive water content is directly toxic to the CNS cells, the cerebral edema can also block cerebral blood flow and collateral circulation to damaged nerve tissue, causing "no reflow" phenomenon or "hypoperfusion". This failure of circulation results in continuing damage to CNS tissue after the interruption of blood flow is reversed leading to ireversible damage. Restoration of blood flow to the affected area of the CNS after a period of complete ischemia as short as six minutes does not result in blood reflow to the affected CNS tissue. After the CNS tissue is injured by an initiating insult, such as ischemia, trauma, the CSF infiltrates the CNS tissue through water channels on the injured cell membrane to cause edema. Swelling of the tissue makes the Virchow-Robin space (also known as the perivascular space or extracellular space) smaller and may even cause it to collapse, thereby compressing the small blood vessels and resulting in an obstruction of the blood flow, such as a "hypoperfusion" or even "no-reflow" phenomenon, which prolongs the original ischemic duration, blocks collateral circulation and induces a feedback loop. As the duration of blood flow interruption increases, the edema spreads throughout the CNS tissue causing additional damage in an ischemic cascade.

In the adult human, the average intra-cranial volume is about 1700 ml. The volume of the brain is approximately 1400 ml; CSF volume ranges from about 52 to 160 ml (mean 140 ml), and blood volume is about 150 ml. Thus the CSF occupies about 10 percent of the intra-cranial and intra-spinal volume. The choroid plexuses are the main sites of CSF formation. The average rate of CSF formation is about 21 to 22 ml/hr, or approximately 500 ml/day. The CSF as a whole is renewed four or five times daily. CSF formation is related to intracranial pressure. When the intracranial pressure is below about 70 mmH2O, CSF is not absorbed, and production increasrs. CSF is a very dilute aqueous solution with a low colloidal osmotic pressure.

The CSF has a mechanical function. It serves as a kind of water jacket for the spinal cord and brain, protecting them from trauma and acute changes in venous blood pressure. The CSF provides buoyancy and shock absorption, so that brain and spinal cord float in a CSF pool. CSF does not appear to be necessary to brain or spinal cord metabolism. However, during ischemic episodes, CSF has a toxic effect by facilitating cerebral edema and resulting in no-reflow phenomenon after disruption of blood flow to CNS tissue. In order to prevent cerebral edema, and the irreversible effects that occur after the CNS injuries, the CSF is withdrawn from the affected area of the CNS. It is preferred to completely remove all CSF. However, it is very difficult, almost impossible, mechanically to remove CSF completely from the subarachnoid spaces because the CNS (i.e. brain and spinal cord) contour is very complex with many sulci, gyri and pools. CSF pressure control has been used for protecting spinal cord during aortic surgery. It is said that controlling the pressure of CSF, in particular, maintaining a pressure lower than the central venous pressure could be advantageous in protecting the spinal cord from injury during aortic surgery. However, such pressure control does not achieve the neuroprotective effect in the case of more general ischemia. Removing CSF from the spinal cord's subarachnoid space is relatively easier (compared with the brain) because of spinal cord's simpler contour. However, simple withdrawal of CSF even under controlled conditions in thoraco-aortic surgery, is not predictably effective protecting CSF tissue. After the CSF has been withdrawn. The injured CNS tissue is washed with washing solutions. The first washing solution is an emulsion. It should contain oil, an osmotic agent, water and at least one emulsifier. Typically, the emulsion contains up to about 31% - 80% oil. Generally a water in oil emulsion is preferred. However, oil in water emulsions have also been effective. Intralipid solutions (10%, 20% and 30%), used clinically for parenteral nutrition, such as those manufactured and distributed by Baxter, Fresenius Kabi, Pharmacia & Upjohn etc. may also be effective. The first washing solution acts as a hook to pull out edematous CSF fluids away from the CNS tissue into the oil. The oil can be any non-toxic, organic liquid. Hydrocarbon oils and silicone oils are effective. Any hydrocarbon oils from plant, animal sources and mineral oil such as soybean oil, cod liver oil, vitamin E oil, canola oil, corn oil, and mixtures of these oils in any concentration ratio may be used [1].

## Traumatic Injury to the Central Nervous System

Following a traumatic injury to the central nervous system, a cascade of physiological events leads to neuronal loss

including, for example, an inflammatory immune response and excitotoxicity resulting from the initial impact disrupting the glutamate, acetylcholine, cholinergic, GABAA, and NMDA receptor systems. In addition, the traumatic CNS injury is frequently followed by brain and/or spinal cord edema that enhances the cascade of injury and leads to further secondary cell death and increased patient mortality. Methods are needed for the in vivo treatment of traumatic CNS injuries that are successful at providing subsequent trophic support to remaining central nervous system tissue, and thus enhancing functional repair and recovery, under the complex physiological cascade of events which follow the initial insult [2].

The nervous system comprises the central (CNS) and the peripheral nervous system (PNS). The CNS is composed of the brain, spinal cord and visual system; the PNS consists of all of the other neural elements, namely the nerves and ganglia outside of the brain and spinal cord. Damage to the nervous system may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease or disorder including, but not limited to, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), diabetic neuropathy, senile dementia, stroke and ischemia. Maintenance of CNS integrity is a complex "balancing act" in which compromises are struck with the immune system. In most tissues, the immune system plays an essential part in protection, repair, and healing. In the CNS, because of its unique immune privilege, immunological reactions are relatively limited. A growing body of evidence indicates that the failure of the mammalian CNS to achieve functional recovery after injury reflects an ineffective dialog between the damaged tissue and the immune system [3]. The predominant mechanism in most cases of traumatic brain injury (TBI) is diffuse axonal injury [4]. While axonal injury is common in all TBI regardless of severity, a shearing of the axons occurs in human diffuse axonal injury (DAI) leading to progressive changes that ultimately may result in the loss of connections between nerve cells. The slow progression of events in DAI continues for up to several weeks after injury creating a window of opportunity for therapeutic intervention. There are approximately 500,000 new cases of TBI in the U.S. each year [5], and the incidence requiring hospitalization is estimated to be approximately 200-225/100,000 population. Currently, it is estimated that brain injuries account for 12% of all hospital admissions in the United States [6]. When compared to spinal cord injury, which accounts for less than 1% of hospital admissions, it is clear that TBI is a medical care problem which has a significant impact financially within the United States. Approximately 30,000 - 44,000 people will survive a severe TBI with GCS score TBI (GCS#10). Yet with new medical management techniques, less than 10% will remain in a persistent vegetative state. A GCS score of eight or less generally reflects a state of unconsciousness in which the patient demonstrates no eye opening, does not follow simple commands to move muscles, and has vocalizations which are limited to sounds. Such signs are indicative of severe brain injury [7]. Approximately 52,000 to 56,000 people die each year from TBI [8], resulting in direct costs approximated at more than $50 billion annually [9]. The costs of severe TBI to the individual and family are extremely high [10]. Acute medical and rehabilitation bills are often around $100,000 with some considerably higher. The Model Systems Database for Traumatic Brain Injury demonstrates there is a correlation between the average Disability Rating Score and the combined acute care and rehabilitation charges [11]. Those with a severe TBI (GCS score of 6 - 8) have average combined charges of $110,842, and those with a very severe TBI (GCS score 3 - 5) have average combined charges of $154,256 [12]. About one-half of all TBIs are transportation related and these patients have some of the highest combined charges for acute care and rehabilitations. This may be related to the mechanism of TBI in high speed motor vehicle crashes, specifically the presence of diffuse axonal injury (DAI) being most prevalent in the midbrain and brain stem areas. Clearly, brain injuries of this severity that occur with high speed acceleration-deceleration injuries, have the highest costs to society. TBI clearly causes more mortality, morbidity and probably more economic loss than HIV infection in the United States. Motor vehicle crashes of all types are responsible for approximately 40% - 50% of the TBI admissions recorded in the Model TBI Systems Database. The predominant mechanism of injury is considered to be diffuse axonal injury (DAI). Approximately 30% - 40% of the fatal head injuries involve diffuse axonal injury by pathological examination [13]. However, based on betaamyloid precursor protein immunostaining, axonal injury may be present in all cases of fatal head injury. In cases of persistent vegetative states, recently found that all cases had evidence of DAI in magnetic resonance imaging (MRI). Diffuse axonal injury occurs even in the absence of a blow to the head and is more prevalent than previously realized. Even in mild head injury, diffuse axonal injury is present in almost 1/3 of the cases. The defining characteristic of DAI is the morphologic change to the axons which occurs over the course of several days to weeks and the fact that multiple regions of the brain are injured. While a component of DAI is present in blunt or penetrating trauma injury, it is at the periphery of the injury zone and is much less significant than the predominant mechanism of injury. DAI is the major mechanism of injury in high speed acceleration-deceleration injuries associated with motor vehicle crashes. While all four mechanisms of TBI (DAI, blunt trauma, penetrating trauma, axonia) may be involved in such an injury, it is the predominant mechanism of injury under this condition [14].

## Inadvertent Intralipid Spinal-Epidural Injection

A term female infant was admitted to the intensive care unit with the diagnosis of tetralogy of Fallot with critical pulmonary stenosis. On the seventh day of life a long saphenous line was inserted that remained without complications until seven days later when the infant appeared septic. A lumbar puncture demonstrated the presence of intra-lipid in the cerebrospinal fluid that was interpreted as due to migration of the saphenous catheter. The child had an uneventful recovery [15].

A patient had accidently received 300 ml of intralipid total parenteral nutrition solution via his epidural catheter. A 21-yr-old man with ulcerative colitis underwent a total colectomy. Postoperative pain control was begun with intravenous morphine using a patient-controlled analgesia device. The patient was unable to obtain satisfactory pain relief. A lumbar epidural catheter was placed, and he received excellent analgesia after 100 microgram of fentanyl was injected into the epidural space. A continuous epidural infusion of preservative-free morphine at 1.0 mg/h was then begun using a standard intravenous infusion set connected to the epidural catheter tubing. The tubing and intravenous infusion pump was labeled with bright green "EPIDURAL CATHETER" tags. He remained nearly pain-free for the next 24 h. It was then discovered that an intravenous infusion of intralipid total parenteral nutrition solution had been "piggy-backed" onto the intravenous tubing leading to the epidural catheter. The label covering one of the side ports on the infusion set tubing had apparently been lost. Approximately 300 ml of intralipid solution had been infused at a rate of 60 ml/h. The epidural catheter was removed from the patient. The catheter insertion site appeared normal and was not painful to palpation. Daily visits failed to reveal any neurological deficits caused by this unusual infusion. The epidural site remained normal. There is no known reports of a similar accidental epidural intralipid infusion [16].

## Epidural Saline Infusion

A 30-year-old female with a 4-month history of postlumbar puncture headache (PLPHA) resulting from an accidental dural puncture during an attempted epidural anesthetic for cesarean section. Epidural blood patches were attempted at 4 days and 3 months post-lumbar puncture, but were unsuccessful. At 4 months post-lumbar puncture, a 24-h epidural saline infusion relieved the PLPHA for 48 h, but the headache returned. Finally, a second epidural saline infusion was done, followed by an epidural blood patch, which permanently cured the PLPHA. Follow-up to 4 months showed no return of the PLPHA [17].

Concerns have been expressed about the potential danger of an autologous epidural blood patch for the treatment of post-dural puncture headache. The immediate resolution of the headache with a blood patch is attributable to thecal compression raising the CSF pressure. An epidural injection of saline would, in theory, produce the same mass effect, and restore normal CSF dynamics. As saline is a relatively inert and sterile solution, epidural saline bolus or infusion appears to be an attractive alternative. Regimens that have been advocated include: 1) 1.0 - 1.5 liter of epidural Hartman solution over 24 h, starting on the first day after dural puncture; 2) up to 35 ml•h–1 of epidural saline or Hartman solution for 24 - 48 h, or after development of the headache; 3) a single 30 ml bolus of epidural saline after development of headache; and 4) 10 - 120 ml of saline injected as a bolus via the caudal epidural space. Advocates of an epidural saline bolus or infusion maintain that the lumbar injection of saline raises epidural and intrathecal pressure. Reduction in the leak would allow the dura to repair. However, observations of the pressures produced in the subarachnoid and epidural space show that, despite a large rise in epidural pressure, the consequent rise in subarachnoid pressure maintains the differential pressure across the dura. The pressure rise is also not sustained and is dissipated within 10 min. The saline may induce an inflammatory reaction within the epidural space, promoting closure of the dural perforation. Histological studies have not demonstrated an inflammatory response following epidural Dextran 40 administration, however, in contrast to an autologous blood patch. There is no reason to suppose that epidural saline is more likely to accelerate dural healing through a proinflammatory action than Dextran 40. Thus, there are no studies that are able to demonstrate either a sustained rise in CSF pressure or accelerated closure of the dural perforation after the administration of epidural saline. Whilst there are many case reports describing the success of epidural saline, comparative trials with epidural blood patches have not demonstrated the long-term efficacy of epidural saline placement. It is difficult to conclude from the evidence therefore, that epidural saline administration will restore normal CSF dynamics. The administration of large volumes of epidural saline may result in intraocular haemorrhages through a precipitous rise in intracranial pressure [18].

## Paraplegia-A Serious Complication of Descending and Thoraco-Abdominal Aortic Operations

Despite advances in spinal cord protection, paraplegia continues to be a serious complication of descending and thoraco-abdominal aortic operations. A novel, self-contained catheter designed to cool the spinal cord topically after being threaded into the spinal column. A cooling catheter for this purpose was specifically designed and produced. The catheter has two lumina, one for ingress and one for egress of fluid. The system is self-contained, so that the fluid does not communicate in any way with the spinal fluid. A console device circulates cold fluid through the catheter. The catheter was tested in 5 adult sheep, with direct monitoring of core body temperature and spinal cord temperature in both active cooling and passive re-warming cycles. In testing in 4 sheep (five attempted implants, with one failure), the catheter worked without problem, producing effective cooling of the spinal cord, from a mean temperature of 36.8˚C (core temperature) to 30.5˚C (spinal temperature) ($p < 0.0001$). In no case did post-mortem examination or histology reveal any evidence of damage to the spinal cord from hypothermia. Temperature rose toward body temperature after cessation of active cooling. Effective topical cooling of the spinal cord can be achieved via a specially designed, self-contained cooling catheter placed into the intra-thecal space. This catheter holds promise for spinal cord protection in aortic surgery. Also, this catheter may be useful as well in mitigating injury to the spinal cord in cases of traumatic spinal column injury [19]. Paraplegia or paraparesis after otherwise successful thoracic or thoraco-abdominal aortic reconstruction is a

devastating complication for both patient and physician. Various strategies have been developed to minimize the incidence of neurological complications after aortic surgery. The incidence of spinal cord ischemia and subsequent neurological complications has been correlated with 1) the duration and severity of ischemia, 2) failure to establish a spinal cord blood supply, and 3) reperfusion injury. Preoperative identification of the arteria radicularis magna, the artery of Adamkiewicz, facilitates identification of critical intercostal vessels for reimplantation, resulting in reestablishing spinal cord blood flow. Techniques for monitoring spinal cord function using evoked potentials have been developed, and surgical techniques have evolved to reduce the duration of ischemia. Furthermore, sequentially sacrificing all the intercostal arteries while maintaining collateral circulation to the cord has produced good outcomes. The severity of ischemia can be minimized by using cerebrospinal fluid drainage, hypothermia, distal bypass, managing the blood pressure, and adjunctive pharmacological therapy. Reperfusion injury can be reduced with the use of antioxidant therapy. Recent advances in endovascular stent grafting have reduced the incidence of postoperative spinal complications, especially among high-risk patients [20].

## Hypothermia for Prevention of Paraplegia Associated with Thoracic Aortic Surgery

The most dreaded complication associated with thoracic aortic surgery remains paraplegia caused by spinal cord ischemia. The existence of this problem has been known since the early days of aortic surgery and much research, both clinical and experimental, has been devoted to the understanding and prevention of spinal cord injury. Intraoperative and peri-operative adjuncts have included both pharmacological and surgical approaches. Varying degrees of success have been reported for each new approach, with some remedies coming and going in and out of fashion as continued research either supports or refutes their effectiveness. Rather than finding any one therapy that completely removes the threat of peri-operative spinal cord injury, incremental improvements in surgical and anesthetic regimens have greatly reduced the incidence of this complication to an acceptable level. Among the earliest adjuncts used in the battle against paraplegia was hypothermia. In studies dating back 50 years, Pontius and colleagues demonstrated that systemic hypothermia successfully allowed prolongation of the spinal cord ischemic interval in laboratory dogs [1]. For many years, the combination of moderate hypothermia, coupled with rapid surgical techniques, remained the mainstay of protection in aortic surgery. As more complex and extensive operations were attempted, profound hypothermic and circulatory arrest approaches were introduced, but for less comprehensive repairs this was thought to be overkill and was selectively used.

Hypothermic techniques, even when applied systemically in moderate or mild amounts, can adversely affect the conduct of the surgery. Prolonged operating times due to cooling and warming, the need for heaters and coolers in the circuits, and increased bleeding tendencies have all given pause to surgeons unfamiliar with these problems. Because of these issues, investigations were turned to eliminating the need for systemic hypothermia and limiting the cooling efforts to those tissues most directly affected, namely the spinal cord. Locally applied cooling blankets and appliances were found not to be effective due to the rapid rewarming by surrounding tissues and interference with the sterile fields of the procedure. Administration of iced saline solutions directly into the isolated aorta was shown to effect cooling of the spinal cord but, as one might expect, this was abandoned due to its complexity. A more direct approach began to be explored experimentally; by installing a perfusion and drainage catheter system into the epidural or intrathecal spaces, localized sustained regional cooling could be generated. The necessity to perform a laminectomy to install this cooling apparatus as well as the direct installation of iced fluids into the epidural space made it unsuitable for aortic surgery, but alternative localized approaches continue to be explored [21].

## Epidural Cooling Catheter to Protect the Spinal Cord from Ischemia during Aortic Surgery

Using swine, Mori et al. [22] investigated whether epidural placement of a cooling catheter rather than infusing iced saline solution could protect the spinal cord from ischemia during aortic surgery. Fourteen domestic pigs were divided into two groups of 7 each. Each underwent epidural catheter placement preceding 30 minutes of aortic cross-clamping distal to the origin of the left subclavian artery. In group 1, cold water was circulated continuously through the lumen of the catheter connected to an external unit. In group 2, animals received catheter placement without cooling. Spinal cord somatosensory evoked potentials were recorded. Neurologic status involving hind limbs was graded sequentially after surgery. At aortic crossclamping, spinal temperature in group 1 (31.7˚C ± 0.6˚C) was significantly lower than in group 2 (37.8˚C ± 0.4˚C; $p < 0.0001$). No significant elevation of intrathecal pressure accompanied cooling with the catheter (group 1, 8.1 ± 1.7 mm Hg; group 2, 8.0 ± 1.5 mm Hg). Mean duration of total loss of potentials was significantly shorter in group 1 (7.4 ± 3.8 minutes) than group 2 (19.7 ± 7.3 minutes; $p = 0.0002$). Pigs in group 1 exhibited better hind limb function recovery (mean Tarlov score, 4.7 ± 0.5) than group 2 (0.6 ± 0.8; $p = 0.0017$). Group 1 showed normal histologic characteristics, whereas group 2 showed loss of motor neurons in the ventral horns. Epidural cooling catheter without iced saline infusion can cool the spinal cord without elevating intrathecal pressure, protecting the cord against ischemia [23].

Cambria et al. [23] summarized their experience with epidural cooling (EC) to achieve regional spinal cord hypothermia and thereby decrease the risk of spinal cord ischemic injury during the course of descending thoracic aneurysm (TA) and thoraco-abdominal aneurysm (TAA) repair.

During the interval July 1993 to Dec. 1995, 70 patients underwent TA (n = 9, 13%) or TAA (n = 61) (type I, 24 [34%], type II, 11 [15%], type III, 26 [37%]) repair using the EC technique. The latter was accomplished by continuous infusion of normal saline (4 degrees C) into a T11-12 epidural catheter; an intrathecal catheter was placed at the L3-4 level for monitoring of cerebrospinal fluid temperature (CSFT) and pressure (CSFP). All operations (one exception, atrio-femoral bypass) were performed with the clamp-and-sew technique, and 50% of patients had preservation of intercostal vessels at proximal or distal anastomoses (30%) or by separate inclusion button (20%). Neurologic outcome was compared with a published predictive model for the incidence of neurologic deficits after TAA repair and with a matched (Type IV excluded) consecutive, control group (n = 55) who underwent TAA repair in the period 1990 to 1993 before use of EC.

EC was successful in all patients, with a 1442 ± 718 ml mean (range, 200 to 3500 ml) volume of infusate; CSFT was reduced to a mean of 24 degrees ± 3 degrees C during aortic cross-clamping with maintenance of core temperature of 34 ± 0.8 degrees C. Mean CSFP increased from baseline values of 13 ± 8 mm Hg to 31 ± 6 mm Hg during cross-clamp. Seven patients (10%) died within 60 days of surgery, but all survived long enough for evaluation of neurologic deficits. The EC group and control group were well-matched with respect to mean age, incidence of acute presentations/aortic dissection/aneurysm rupture, TAA type distribution, and aortic cross-clamp times. Two lower extremity neurologic deficits (2.9%) were observed in the EC patients and 13 (23%) in the control group ($p < 0.0001$). Observed and predicted deficits in the EC patients were 2.9% and 20.0% ($p = 0.001$), and for the control group 23% and 17.8% ($p = 0.48$). In considering EC and control patients (n = 115), variables associated with postoperative neurologic deficit were prolonged (>60 min) visceral aortic cross-clamp time (relative risk, 4.4; 95% CI, 1.2 to 16.5; $p = 0.02$) and lack of epidural cooling (relative risk, 9.8; 95% CI, 2 to 48; $p = 0.005$). EC is a safe and effective technique to increase the ischemic tolerance of the spinal cord during TA or TAA repair. When used in conjunction with a clamp-and-sew technique and a strategy of selective intercostal reanastomosis, EC has significantly reduced the incidence of neurologic deficits after TAA repair. Despite advances in spinal cord protection, paraplegia continues to be a serious complication of descending and thoraco-abdominal aortic operations. Moomiaie et al. [24] devised and tested a novel, self-contained catheter designed to cool the spinal cord topically after being threaded into the spinal column. A cooling catheter for this purpose was specifically designed and produced. The catheter has two lumina, one for ingress and one for egress of fluid. The system is self-contained, so that the fluid does not communicate in any way with the spinal fluid. A console device circulates cold fluid through the catheter. The catheter was tested in 5 adult sheep, with direct monitoring of core body temperature and spinal cord temperature in both active cooling and passive re-warming cycles. In testing in 4 sheep (five attempted implants, with one failure), the catheter worked without problem, producing effective cooling of the spinal cord, from a mean temperature of 36.8 degrees C (core temperature) to 30.5 degrees C (spinal temperature) ($p < 0.0001$). In no case did post-mortem examination or histology reveal any evidence of damage to the spinal cord from hypothermia. Temperature rose toward body temperature after cessation of active cooling. Effective topical cooling of the spinal cord can be achieved via a specially designed, self-contained cooling catheter placed into the intra-thecal space. This catheter holds promise for spinal cord protection in aortic surgery. Also, this catheter may be useful as well in mitigating injury to the spinal cord in cases of traumatic spinal column injury.

Salzano et al. [25] tested in pigs the hypothesis that regional deep hypothermia of the spinal cord achieved by cerebrospinal fluid cooling will protect against ischemic injury during thoracic aortic cross-clamping. Eight control animals underwent aortic cross-clamping at the distal aortic arch and just above the diaphragm for 30 minutes. Eight experimental animals had placement of two subarachnoid perfusion catheters through laminectomies at T4 and the lower lumbar region. The subarachnoid space was perfused with normal saline solution at 6 degrees C delivered by gravity infusion, with infusion rates adjusted to maintain cord temperatures at less than 20 degrees C. After 30 minutes of aortic cross-clamping, the infusion was stopped and the cord allowed to warm to body temperature. Hind limb neurologic function was graded by Tarlov's scale. All of the animals in the control group had complete hind limb paraplegia (Tarlov grade 0) postoperatively. Seven of the 8 animals in the experimental group had preservation of hind limb motor function (Tarlov grade 2), and 1 animal had complete hind limb paraplegia (Tarlov grade 0) ($p = 0.002$, Fisher's exact test). It was concluded that regional deep hypothermia of the spinal cord in pigs does provide some protection from ischemic injury during thoracic aortic cross-clamping. Clinically this may be a useful adjunct for prevention of paraplegia during thoracic aortic operations.

It is reported that hypothermia has some protective effect against ischemia of the spinal cord during thoraco abdominal aneurysm repair. However, it has not been elucidated clinically whether regional spinal cord hypothermia by epidural perfusion cooling is effective and safe. Tabayashi et al. [26] assessed the effect and safety of perfusion cooling of the epidural space during most or all of descending thoracic or thoracoabdominal aneurysm repair. From January 1998 to December 2007, a total of 102 patients with a mean age of 61 years underwent replacement of most or all of the descending thoracic aorta or thoraco-abdominal aorta with the aid of mild hypothermia via epidural perfusion cooling and cerebrospinal fluid (CSF) drainage. Risk factors for spinal cord injury and hospital death were analyzed using univariate and multivariate analyses. The actuarial survival rate was calculated by the Kaplan-Meier method. The mean lowest CSF temperature was 23.3 degrees C during epidural perfusion cooling. The mean temperature difference between the nasopharynx and CSF was 8.4

degrees C. The incidence of spinal cord injury was 3.9% (4/102), and that of hospital death was 5.9% (6/102). There was no significant risk factor associated with spinal cord injury. Type

III aneurysm and postoperative cerebrovascular accident, respiratory failure, liver failure, and infection were predictors of hospital death. The actuarial survival rates at 3 and 5 years were 82.1% and 75.9%, respectively. Epidural perfusion cooling is a safe method to employ in clinical situations.

## The Effect of Intrathecal Tetracaine on the Neurological Sequelae of Spinal Cord Ischemia and Reperfusion with Aortic Occlusion

Spinal cord ischemia and resultant paraplegia are devastating sequelae in up to 40% of patients undergoing repair of thoraco-abdominal aneurysms. Breckwoldt et al. [27] investigated the effect of intrathecal tetracaine on the neurological sequelae of spinal cord ischemia and reperfusion with aortic occlusion. Cocaine-derived anesthetics (lidocaine and its analogues) have been shown to decrease neuronal cell metabolism and also have specific neuronal membrane stabilizing effects. New Zealand white rabbits were anesthetized and spinal cord ischemia was then induced by infrarenal aortic occlusion. Animals were divided into six treatment groups. Tetracaine (groups 2 and 4) or normal saline solution (group 5) was administered intrathecally before aortic cross-clamping. Groups 1 and 3 functioned as controls. Group 6 animals received intravenous thiopental. Rabbits were classified as either neurologically normal or injured (paralyzed or paretic). Among controls, 25 minutes of aortic occlusion produced varied neurological sequelae (group 1, 3/6 injured, 50%) whereas 30 minutes resulted in more consistent injury (group 3, 5/6 injured, 83%). All rabbits that received intrathecal saline solution were paralyzed (group 5, 4/4 injured, 100%). Animals treated with intrathecal tetracaine and aortic occlusion of 30 minutes (group 4) showed significantly better preservation of neurological function (6/7 normal, 86%) than controls and saline-treated animals (groups 3 and 5). All animals treated with intrathecal tetracaine and aortic occlusion for 25 minutes (group 2) showed no signs of injury (5/5 normal, 100%), but this was not significant versus controls (group 1). Intravenous thiopental (group 6, 5/5 injured, 100%) had no beneficial effect. Intrathecal tetracaine significantly and dramatically abrogated the neurological injury secondary to spinal cord ischemia and reperfusion after aortic occlusion at 30 minutes in the rabbit model.

## The Use of Prophylactic Hypothermia for Traumatic Brain Injury

During the past 9 years, considerable new evidence has accumulated supporting the use of prophylactic hypothermia for traumatic brain injury (TBI). Studies can be divided into 2 broad categories: studies with protocols for cooling for a short, predetermined period (e.g., 24 - 48 h), and those that cool for longer periods and/or terminate based on the normalization of intracranial pressure (ICP). There have been no systematic reviews of hypothermia for TBI that include this recent new evidence. This analysis followed the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions and the QUOROM (quality of reporting of meta-analyses) statement. Fox et al. [28] developed a comprehensive search strategy to identify all randomized controlled trials (RCTs) comparing therapeutic hypothermia with standard management in TBI patients. They searched Embase, MEDLINE, Web of Science, the Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews, ProceedingsFirst and PapersFirst. Additional relevant articles were identified by hand-searching conference proceedings and bibliographies. All stages of study identification and selection, quality assessment and analysis were conducted according to prospectively defined criteria. Study quality was determined by assessment of each study for the use of allocation concealment and outcome assessment blinding. Studies were divided into 2 a priori-defined subgroups for analysis based on cooling strategy: short term (≤48 h), and long term or goal-directed (>48 h and/or continued until normalization of ICP). Outcomes included mortality and good neurologic outcome (defined as Glasgow Outcome Scale score of 4 or 5). Pooling of primary outcomes was completed using relative risk (RR) and reported with 95% confidence intervals (CIs). Of 1709 articles, 12 studies with 1327 participants were selected for quantitative analysis. Eight of these studies cooled according to a long-term or goal-directed strategy, and 4 used a short-term strategy. Summary results demonstrated lower mortality (RR 0.73, 95% CI 0.62 - 0.85) and more common good neurologic outcome (RR 1.52, 95% CI 1.28 - 1.80). When only short-term cooling studies were analyzed, neither mortality (RR 0.98, 95% CI 0.75 - 1.30) nor neurologic outcome (RR 1.31, 95% CI 0.94 - 1.83) were improved. In 8 studies of long-term or goal-directed cooling, mortality was reduced (RR 0.62, 95% CI 0.51 - 0.76) and good neurologic outcome was more common (RR 1.68, 95% CI 1.44 - 1.96). The best available evidence to date supports the use of early prophylactic mild-to-moderate hypothermia in patients with severe TBI (Glasgow Coma Scale score ≤ 8) to decrease mortality and improve rates of good neurologic recovery. This treatment should be commenced as soon as possible after injury (e.g., in the emergency department after computed tomography) regardless of initial ICP, or before ICP is measured. Most studies report using a temperature of 32 degrees - 34 degrees C. The maximal benefit occurred with a long-term or goaldirected cooling protocol, in which cooling was continued for at least 72 hours and/or until stable normalization of intracranial pressure for at least 24 hours was achieved.

Peterson et al. [29] conducted an updated meta-analysis of the effects of hypothermia therapy on mortality, favorable neurologic outcome, and associated adverse effects in adults with traumatic brain injury (TBI) for use

by Brain Trauma Foundation (BTF)/American Association of Neurological Surgeons (AANS) task force to develop evidence-based treatment guidelines. The data sources relied on hand searches of four previous goodquality systematic reviews, which all conducted electronic searches of primarily MEDLINE (OVID), EMBASE, and Cochrane Library. An independent, supplemental electronic search of MEDLINE was undertaken as well (last searched June 2007). Only English-language publications of randomized controlled trials of therapeutic hypothermia in adults with TBI were selected for analysis. Two reviewers independently abstracted data on trial design, patient population, hypothermia and cointervention protocols, patient outcomes, and aspects of methodological quality. Pooled relative risks (RR) and associated 95% confidence intervals (CIs) were calculated for each outcome using random-effects models. In the current study, only 13 trials met eligibility criteria, with a total of 1339 randomized patients. Sensitivity analyses revealed that outcomes were influenced by variations in methodological quality. Consequently, main analyses were conducted based on eight trials that demonstrated the lowest potential for bias (n = 781). Reductions in risk of mortality were greatest (RR 0.51; 95% CI 0.33, 0.79) and favorable neurologic outcomes much more common (RR 1.91; 95% CI 1.28, 2.85) when hypothermia was maintained for more than 48 h. However, this evidence comes with the suggestion that the potential benefits of hypothermia may likely be offset by a significant increase in risk of pneumonia (RR 2.37; 95% CI 1.37, 4.10). In sum, the present study's updated metaanalysis supports previous findings that hypothermic therapy constitutes a beneficial treatment of TBI in specific circumstances. Accordingly, the BTF/AANS guidelines task force has issued a Level III recommendation for optional and cautious use of hypothermia for adults with TBI.

## Lipid Neuroprotection

The harmony and function of the complex brain circuits and synapses are sustained mainly by excitatory and inhibitory neurotransmission, neurotrophins, gene regulation, and factors, many of which are incompletely understood. A common feature of brain circuit components, such as dendrites, synaptic membranes, and other membranes of the nervous system, is that they are richly endowed in docosahexaenoic acid (DHA), the main member of the omega-3 essential fatty acid family. DHA is avidly retained and concentrated in the nervous system and known to play a role in neuroprotection, memory, and vision. Only recently has it become apparent why the surprisingly rapid increases in free (unesterified) DHA pool size take place at the onset of seizures or brain injury. This phenomenon began to be clarified by the discovery of neuroprotectin D1 (NPD1), the first-uncovered bioactive docosanoid formed from free DHA through 15-lipoxygenase-1 (15-LOX-1). NPD1 synthesis includes, as agonists, oxidative stress and neurotrophins. The evolving concept is that DHA-derived docosanoids set in motion endogenous signaling to sustain homeostatic synaptic and circuit integrity. NPD1 is anti-inflammatory, displays inflammatory resolving activities, and induces cell survival, which is in contrast to the pro-inflammatory actions of the many of omega-6 fatty acid family members. Bazan et al. [30] highlighted studies relevant to the ability of DHA to sustain neuronal function and protect synapses and circuits in the context of DHA signalolipidomics. DHA signalolipidomics comprises the integration of the cellular/tissue mechanism of DHA uptake, its distribution among cellular compartments, the organization and function of membrane domains containing DHA phospholipids, and the precise cellular and molecular events revealed by the uncovering of signaling pathways regulated by docosanoids endowed with prohomeostatic and cell survival bioactivity. Therefore, this approach offers emerging targets for prevention, pharmaceutical intervention, and clinical translation involving DHAmediated signaling. The significance of the selective enrichment in omega-3 essential fatty acids in photoreceptors and synaptic membranes of the nervous system has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neural degeneration, Palacios-Pelaez et al. [31] discovered a docosanoid synthesized from unesterified docosahexaenoic acid (DHA) by a 15-lipoxygenase (15-LOX), which they called neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z, 11E,13E,15E,19Z hexaenoic acid). This lipid mediator is a docosanoid because it is derived from the 22 carbon (22C) precursor DHA, unlike eicosanoids, which are derived from the 20 carbon (20C) arachidonic acid (AA) family member of essential fatty acids. They discovered that NPD1 is promptly made in response to oxidative stress, as a response to brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, in oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-beta (Abeta) peptides. They thus envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by imbalances in normal neural function. They provide here, in three sections, recent experimental examples that highlight the specificity and potency of NPD1 spanning beneficial bioactivity during initiation and early progression of neurodegeneration: 1) during retinal signal phototransduction, 2) during brain ischemia-reperfusion, and 3) in Alzheimer's disease (AD) and stressed human brain cell models of AD. From this experimental evidence, they conclude that DHA-derived NPD1 regulation targets upstream events of brain cell apoptosis, as well as neuro-inflammatory signaling, promoting and maintaining cellular homeostasis, and restoring neural and retinal cell integrity.

Deficiency in docosahexaenoic acid (DHA) is associated with impaired visual and neurological development, cognitive decline, macular degeneration, and other neurodegenerative diseases. DHA is concentrated in phospholipids of the brain and retina, with photoreceptor cells having the highest DHA content of all cell membranes. The discovery that neuroprotectin D1 (NPD1; 10R, 17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid) is a bioactive

mediator of DHA sheds light on the biological importance of this fatty acid. In oxidative stress-challenged human retinal pigment epithelial (RPE) cells, human brain cells, or brain ischemia-reperfusion, NPD1 synthesis is enhanced as a response for sustaining homeostasis. Thus, neurotrophins, Abeta peptide (Abeta42), calcium ionophore A23187, interleukin-1beta (IL-1beta), or DHA supply enhances NPD1 synthesis. NPD1, in turn, upregulates the antiapoptotic proteins of the Bcl-2 family and decreases the expression of proapoptotic Bcl-2 family members. In human neural cells, DHA attenuates Abeta42 secretion, resulting in concomitant formation of NPD1. NPD1 repressed Abeta42- triggered activation of proinflammatory genes and upregulated the antiapoptotic genes encoding Bcl-2, Bcl-xl, and Bfl-1(A1) in human brain cells in culture. Overall, NPD1 signaling regulates brain and retinal cell survival via the induction of antiapoptotic and neuroprotective gene-expression programs that suppress Abeta42-induced neurotoxicity and other forms of cell injury. These in turn support homeostasis during brain and retinal aging, counteract inflammatory signaling, and downregulate events that support the initiation and progression of neurodegenerative disease [32].

Maciá-Botejara et al. [33] studied the changes occurring in brain lipid composition after the administration of total parenteral nutrition (TPN) by comparing two lipid emulsions, one with long-chain triacylglycerols (LCT) and the other with long-chain and medium-chain triacylglycerols (MCT/LCT 50%/50%). They used 21 young New Zealand rabbits divided into three groups of seven animals each. Two groups were subjected to TPN for 7 d, with each group receiving using one of two different lipid emulsions: Intralipid 20% (group LCT) and Lipofundin MCT/LCT 20% (group MCT/LCT). The third control group received an oral diet and underwent the same surgical procedure with the administration of intravenous saline solution. The energy administered in the TPN formulas was non-protein 100 kcal•kg−1•d−1, with 40% corresponding to fats. There were modest increases in plasma cholesterol and triacylglycerols. In the brain tissue, there was a decrease of phosphatidylcholine in animals with TPN, which was greater in group LCT. There were no significant differences in the overall percentage distribution of brain fatty acids among the groups. The lipid emulsions administered in TPN, especially those prepared exclusively with LCT, cause changes in the brain lipid polar fractions of young rabbits.

## Lipid Resuscitation Therapy

On 1998 it was first showed that intravenous intralipid could prevent or improve resuscitation from cardiovascular collapse by severe bupivacaine overdose in rats. Since then published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haloperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Lipid resuscitation therapy using intravenous lipid emulsion (IVLE) for drug overdoses has gained widespread use. However, there is little information regarding its adverse effects. Grunbaum et al. [34] performed lipemic interference studies on typical automated platforms to investigate the potential of lipid resuscitation therapy to interfere with the reliability and turnaround time of analytes that would be of interest in acute intoxications. They also tested methods to minimize interferences. Serum pools were supplemented with increasing concentrations of Intralipid-20%() (0% - 30%). Analyses were performed on Beckman-Coulter DXC800 and DXI and Roche Modular-P. Analytes demonstrating significant interference were re-measured after centrifugation (14,000 × g for 10 minutes). Triglyceride and glycerol-blanked triglyceride concentrations were similar in IVLE-free samples. However, with addition of IVLE, concentrations were markedly different (139 vs. 76 mmol/L). There was no appreciable interference on the troponin-I, sodium, potassium, chloride, calcium, bicarbonate or urea assays. Albumin and magnesium assays demonstrated significant interference. Amylase, lipase, phosphate, creatinine, total protein, ALT, CK and bilirubin became unmeasurable in IVLEsupplemented samples. Whereas glucose measurement by potentiometry was free of interference, colorimetric methodology was error prone. Centrifugation removed > 90% of glycerol-blanked triglyceride (max = 5.8 mmol/ L), dramatically reducing lipid interferences. IVLE results in appreciable analytical interferences at concentrations demonstrated in lipid resuscitation therapy. Of particular concern is the marked interference on glucose and magnesium, which may result in unsuccessful and potentially harmful interventions. Major implications for patient care include reporting of incorrect results and delays in the reporting of time-sensitive results. Whenever possible, blood samples should be collected prior to initiating lipid therapy. Interferences can be minimized by brief centrifugation at relatively low speeds on equipment readily available in most core labs. Li et al. [35] have recently shown that post-ischemic administration of intralipid protects the heart against ischemia-reperfusion injury. They compared the cardioprotective effects of intralipid with cyclosporine-A, a potent inhibitor of the mitochondrial permeability transition pore opening.

In vivo rat hearts or isolated Langendorff-perfused mouse hearts were subjected to ischemia followed by reperfusion with intralipid (0.5%, 1% and 2% ex vivo, and 20% in vivo), cyclosporine-A (0.2 μM, 0.8 μM, and 1.5 μM ex vivo and 10 mg/kg in vivo), or vehicle. The hemodynamic function, infarct size, calcium retention capacity, mitochondrial superoxide production, and phosphorylation levels of protein kinase B (Akt)/glycolgen synthase kinase-3β (GSK-3β) were measured. The values are mean ± SEM. Administration of intralipid at reperfusion significantly reduced myocardial infarct size compared with cyclosporine-A in vivo (infarct size/area at risk)%: 22.9% ± 2.5% vs. 35.2% ± 3.5%; p = 0.030, n = 7/group). Post-ischemic administration of intralipid at its optimal dose (1%) was more effective than cyclosporine-A

(0.8 μM) in protecting the ex vivo heart against ischemia-reperfusion injury, as the rate pressure product at the end of reperfusion was significantly higher (mmHg•beats/min: 12,740 ± 675 [n = 7] vs. 9203 ± 10,781 [n = 5], p = 0.024), and the infarct size was markedly smaller (17.3 ±2.9 [n = 7] vs. 29.2 ± 2.7 [n = 5], p = 0.014). Intralipid was as efficient as cyclosporine-A in inhibiting the mitochondrial permeability transition pore opening (calcium retention capacity = 280 ± 8.2 vs. 260.3 ± 2.9 nmol/mg mitochondria protein in cyclosporine-A, p = 0.454, n = 6) and in reducing cardiac mitochondrial superoxide production. Unlike intralipid, which increased phosphorylation of Akt (6-fold) and GSK-3β (5-fold), cyclosporine-A had no effect on the activation of these prosurvival kinases. Although intralipid inhibits the opening of the mitochondrial permeability transition pore as efficiently as cyclosporine-A, intralipid is more effective in reducing the infarct size and improving the cardiac functional recovery.

## Pretreatment or Resuscitation with a Lipid Infusion

Intralipid 10% (10% i.v fat emulsion) (a 10% intravenous fat emulsion) is a sterile, non-pyrogenic fat emulsion prepared for intravenous administration as a source of calories and essential fatty acids. It is made up of 10% soybean oil, 1.2% egg yolk Phospholipids, 2.25% glycerin, and water for injection. In addition, sodium hydroxide has been added to adjust the ph so that the final product ph is 8. Ph range is 6 to 8.9. The soybean oil is a refined natural product consisting of a mixture of neutral triglycerides of predominantly unsaturated fatty acids. the major component fatty acids are linoleic (44% - 62%), oleic (19% - 30%), palmitic (7% - 14%), linolenic (4% - 11%) and stearic (1.4% - 5.5%). Purified egg phosphatides are a mixture of naturally occurring phospholipids which are isolated from the egg yolk. intralipid□ 10% (10% i.v fat emulsion) (a 10% intravenous fat emulsion) has an osmolality of approximately 300 mosmol/ kg water (which represents 260 mos-mol/ liter of emulsion) and contains emulsified fat particles of approximately 0.5 micron size. the total caloric value, including fat, phospholipid and glycerin, is 1.1 kcal per ml of intralipid 10% (10% i.v fat emulsion). the phospholipids present contribute 47 milligrams or approximately 1.5 mmol of phosphorus per 100 ml, of the emulsion. Weinberg et al. [36] first showed in 1998 that an infusion of a soybean oil emulsion normally used as a total parenteral nutrition solution could prevent (by pretreatment) or improve resuscitation from cardiovascular collapse caused by severe bupivacaine overdose in the intact, anesthetized rat. Subsequent studies from the same laboratory confirmed these findings in isolated rat heart [37] and anesthetized dog [38].

Under the latter experimental model, return of spontaneous circulation after a bupivacaine challenge occurred in all animals receiving lipid, but in none of the saline controls [38]. This study was accompanied by an editorial asking whether lipid might be the long-sought “silver bullet” for local anesthetic systemic toxicity (LAST). Since then, the effectiveness of lipid emulsion infusion in reversing LAST has been confirmed in other laboratories and by systematic analysis [39] and in the clinical setting as well. Lipid infusion is useful in reversing cardiac toxicity of local anesthetics, and recent reports indicate it may be useful in resuscitation from toxicity induced by a variety of other drugs. While the mechanism behind the utility of lipid rescue remains to be fully elucidated, the predominant effect appears to be creation of a “lipid sink”.

French D et al. [40] tried to determine whether the extraction of drugs by lipid, and hence the clinical efficacy of lipid rescue in toxicological emergencies can be predicted by specific drug properties. Each drug investigated was added individually to human drug-free serum. Intralipid□ was added to this drugcontaining serum, shaken and then incubated at 37˚C. The lipid was removed by ultracentrifugation and the concentration of drug remaining in the serum was measured by high-pressure liquid chromatography. In this in vitro model, the ability of lipid emulsion to bind a drug was largely dependent upon the drug’s lipid partition constant. Additionally, using a multiple linear regression model, the prediction of binding could be improved by combining the lipid partition constant with the volume of distribution together accounting for approximately 88% of the variation in the decrease in serum drug concentration with the administration of lipid emulsion.

The lipid partition constant and volume of distribution can likely be used to predict the efficacy of lipid infusion in reversing the cardiac toxicity induced by anesthetics or other medications. Local anaesthetics may induce cardiac arrest, usually because of rapid absorption from the site of injection or because of an intended intravascular injection. Early central nervous system symptoms usually precede seizures. Cardiac arrhythmias follow the CNS signs. These arrhythmias often resolve with the i.v. bolus injection of 100 to 150 mL of a lipid emulsion (20% Intralipid(□)). Although long acting local anaesthetics (bupivacaine, ropivacaine, levobupivacaine) are predominantly involved in this cardiac toxicity, lidocaine may also induce cardiac arrhythmias and clinician must be aware of this risk. In case of cardiac arrest, resuscitation manoeuvres are of major importance. They need to be performed immediately and the efficacy of the lipid rescue requires a correct coronary flow to be efficacious. Finally, prevention is the key of a safe injection. It is important to control the dose, to inject slowly, without any excessive pressure and to verify that no blood reflux occurs [41].

## Intralipid Reversing other Drugs Toxicity

These publications, along with other animal studies, opened the door to more widespread use of lipid emulsion for emergency treatment of toxicities caused by a range of lipophilic drugs. Notably, published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haloperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin,

dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Tricyclic antidepressant (TCA) toxicity results predominantly from myocardial sodium-channel blockade. Subsequent ventricular dysrhythmias, myocardial depression, and hypotension cause cardiovascular collapse. Animal studies have demonstrated the effectiveness of intravenous lipid-emulsion in treating TCA cardiotoxicity. Blaber MS et al. [42] report a case of dothiepin (tricyclic antidepressant) overdose causing refractory cardiovascular collapse, which seemed to be successfully reversed with lipid-emulsion therapy (Intralipid□; Fresenius, Cheshire, UK). Lipid emulsions are a potentially novel therapy for reversing cardiotoxicity seen in TCA overdose. Research is required into the role of lipid emulsion in the management of poisoning by oral lipophilic agents.

## The Lipid Sink Effect

Papadopoulou A et al. [43] hypothesized that by substituting a dye surrogate in place of local anesthetic, they could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used. They selected 2 lipophilic dyes, acid blue 25 and Victoria blue, with log P values comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate “lipid rescue” treatment at dye concentrations equivalent to fatal, cardiotoxic, and neurotoxic local anesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75-kg adult. After mixing, the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colors compared against a graduated dye concentration scale. Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the color intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log P value and the greatest amount of lipid emulsion. This study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable. Local anesthetic (LA) intoxication with cardiovascular arrest is a potential fatal complication of regional anesthesia. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. Aim of the study [34] was to compare four different rescue regimens using epinephrine and/or lipid emulsion and vasopressin to treat cardiac arrest caused by bupivacaine intoxication. Twenty-eight piglets were randomized into four groups (4 × 7), anesthetized with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via central venous catheter at a rate of 1 mg•kg−1•min−1 until circulatory arrest. Bupivacaine infusion and sevoflurane were then stopped, chest compression was started, and the pigs were ventilated with 100% oxygen. After 1 min, epinephrine 10 µg•kg−1 (group 1), Intralipid(□) 20% 4 ml•kg−1 (group 2), epinephrine 10 µg•kg−1 + Intralipid(□) 4 ml•kg−1 (group 3) or 2 IU vasopressin + Intralipid(□) 4 ml•kg−1(group 4) were administered. Secondary epinephrine doses were given after 5 min if required. Survival was 71%, 29%, 86%, and 57% in groups 1, 2, 3, and 4. Return of spontaneous circulation was regained only by initial administration of epinephrine alone or in combination with Intralipid(□). Piglets receiving the combination therapy survived without further epinephrine support. In contrast, in groups 2 and 4, return of spontaneous circulation was only achieved after secondary epinephrine rescue. In cardiac arrest caused by bupivacaine intoxication, first-line rescue with epinephrine and epinephrine + Intralipid(□) was more effective with regard to survival than Intralipid(□) alone and vasopressin + Intralipid(□) in this pig model [44].

Local anesthetic (LA) intoxication with severe hemodynamic compromise is a potential catastrophic event. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. However, there are no data about effectiveness of Intralipid for the treatment of severe cardiovascular compromise prior to cardiac arrest. Aim of this study was to compare effectiveness of epinephrine and Intralipid for the treatment of severe Hemodynamic compromise owing to bupivacaine intoxication, anesthetized Piglets were with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via a central venous catheter at a rate of 1 mg•kg−1•min−1 until invasively measured mean arterial pressure (MAP) dropped to 50% of the initial value. Bupivacaine infusion was then stopped, and epinephrine 3 µg•kg−1 (group 1), Intralipid(□) 20% 2 ml•kg−1 (group 2), or Intralipid 20% 4 ml•kg−1 (group 3) was immediately administered. Twenty-one piglets (3 × 7), were recorded. All animals in group 1 (100%) but only four of seven (57%) piglets in group 2 and group 3, respectively, survived. Normalization of hemodynamic parameters (HR, MAP) and ET(CO2) was fastest in group 1 with all piglets achieving HR and MAP values. hemodynamic compromise owing to bupivacaine intoxication in piglets, first-line rescue with epinephrine was more effective than Intralipid with regard to survival as well as normalization of hemodynamic parameters and ET(CO2) [45].

Intravenous lipid emulsion (ILE) has been proposed as a rescue therapy for severe local anesthetic drugs toxicity, but experience is limited with other lipophilic drugs. An 18-year-old healthy woman was admitted 8 h after the voluntary ingestion of sustained-release diltiazem (3600 mg), with severe hypotension refractory to fluid therapy, calcium salts, and high-dose norepinephrine (6.66 µg/kg/ min). Hyperinsulinemic euglycemia therapy was initiated and shortly after was followed by a protocol of ILE (intralipid 20%, 1.5 ml/kg as bolus, followed by 0.25 ml/kg over 1h). The main finding attributed to ILE was an apparent rapid decrease in insulin resistance, despite a prolonged serum diltiazem elimination half-life. Diltiazem is

a lipophilic cardiotoxic drug, which could be sequestered in an expanded plasma lipid phase. The mechanism of action of ILE is not known, including its role in insulin resistance and myocardial metabolism in calcium-channel blocker poisoning [46].

## Guidelines for the Management of Local Anaesthetic Toxicity

There is increasing evidence for the use of Intralipid in the management of acute local anaesthetic toxicity. This is supported by the recent Association of Anaesthetists of Great Britain and Ireland (AAGBI) guidelines for the management of local anaesthetic toxicity. Acute hospitals in England and Wales were surveyed to determine the proportion that currently stocked Intralipid, the locations of stocks within the hospital, guidelines related to its use and previous use in the last 12 months. The majority of hospitals surveyed stocked Intralipid in multiple locations, although not in all areas using high volumes of local anaesthetics. Guidelines were typically in place, although these were often local rather than those from the AAGBI. Use in the last 12 months was uncommon, but typically information was not available on indications for its use. More systematic data collection is required on the safety and efficacy of Intralipid in the management of acute drug toxicity [47]. Intralipid therapy has been used successfully as “rescue therapy” in several cases of overdose. West PL et al. [48] present a case of iatrogenic lipid emulsion overdose because of a dosing error:

A 71-year-old female overdosed on 27 tablets of 5 mg amlodipine. Although initially stable in the Emergency Department, she became hypotensive, oliguric, and respiratory failure developed despite medical therapy. The primary treating team felt that meaningful recovery was unlikely to occur without rapid improvement in clinical status, and 12.5 h after presentation, intralipid rescue therapy was initiated. A protocol for intralipid specifying a maximum infusion of 400 mL of 20% lipid emulsion was faxed, but the infusion was continued until 2 L of lipid emulsion was infused. There were no detectable adverse hemodynamic effects of the intralipid infusion. After this time, laboratory values were difficult to obtain. Three hours after the infusion, a metabolic panel was obtained from ultracentrifuged blood showing hyponatremia. A white blood cell (WBC) was obtained from a complete blood count (CBC) performed 22 h after the infusion, hemoglobin and hematocrit could not be obtained from this blood. A platelet count was obtained by smear estimate. Hematocrits were obtained from centrifuged blood and appeared elevated. No oxygenation could be obtained on blood gas. The patient’s family chose to withdraw care on hospital day 2 and no further laboratory draws were obtained. Amlodipine was 1500 ng/mL (ref. 3 - 11 ng/mL).”

## Lipid Emulsion Overdose

Lipid emulsion overdose caused no detectable acute adverse hemodynamic effects. The following laboratory values were unobtainable immediately after infusion: white blood cell count, hemoglobin, hematocrit, platelet count, and a metabolic panel of serum electrolytes. Ultracentrifugation of blood allowed for detection of a metabolic panel 3 h after the infusion. Centrifuged hematocrits appeared to be higher than expected. Lipid infusion reverses systemic local anesthetic toxicity. The acceptable upper limit for lipid administration is unknown and has direct bearing on clinical management. Hiller DB et al. [49] hypothesize that high volumes of lipid could have undesirable effects and sought to identify the dose required to kill 50% of the animals (LD(50)) of large volume lipid administration. Intravenous lines and electrocardiogram electrodes were placed in anesthetized, male Sprague-Dawley rats. Twenty percent lipid emulsion (20, 40, 60, or 80 mL/kg) or saline (60 or 80 mL/kg), were administered over 30 mins; lipid dosing was assigned by the Dixon “up-anddown” method. Rats were recovered and observed for 48 hrs then euthanized for histologic analysis of major organs. Three additional rats were administered 60 mL/kg lipid emulsion and euthanized at 1, 4, and 24 hrs to identify progression of organ damage. The maximum likelihood estimate for LD(50) was 67.72 (SE, 10.69) mL/kg. Triglycerides were elevated immediately after infusion but returned to baseline by 48 hrs when laboratory abnormalities included elevated amylase, aspartate aminotransferase, and serum urea nitrogen for all lipid doses. Histologic diagnosis of myocardium, brain, pancreas, and kidneys was normal at all doses. Microscopic abnormalities in lung and liver were observed at 60 and 80 mL/kg; histopathology in the lung and liver was worse at 1 hr than at 4 and 24 hrs. The LD (50) of rapid, high volume lipid infusion is an order of magnitude greater than doses typically used for lipid rescue in humans and supports the safety of lipid infusion at currently recommended doses for toxin-induced cardiac arrest. Lung and liver histopathology was observed at the highest infused volumes.

## Intralipid Rescue: 1966-2009

Cave G and Harvey M [50] evaluate the efficacy of lipid emulsion as antidotal therapy outside the accepted setting of local anesthetic toxicity. Literature was accessed through PubMed, OVID (1966-February 2009), and EMBASE (1947-February 2009) using the search terms “intravenous” AND [“fat emulsion” OR “lipid emulsion” OR “Intralipid”] AND [“toxicity” OR “resuscitation” OR “rescue” OR “arrest” OR “antidote”]. Additional author and conference publication searches were undertaken. Publications describing the use of lipid emulsion as antidotal treatment in animals or humans were included. Fourteen animal studies, one human study, and four case reports were identified. In animal models, intravenous lipid emulsion (ILE) has resulted in amelioration of toxicity associated with cyclic antidepressants, verapamil, propranolol, and thiopentone. Administration in human cases has resulted in successful resuscitation from combined bupropion/lamotrigine-induced cardiac arrest, reversal of sertraline/quetiapine-induced coma, and amelioration of verapamiland beta blocker-induced shock. Management of overdose with

highly lipophilic cardiotoxic medications should proceed in accord with established antidotal guidelines and early poisons center consultation. Data from animal experiments and human cases are limited, but suggestive that ILE may be helpful in potentially lethal cardiotoxicity or developed cardiac arrest attributable to such agents. Use of lipid emulsion as antidote remains a nascent field warranting further preclinical study and systematic reporting of human cases of use. Previous investigators have demonstrated amelioration of lipid-soluble drug toxidromes with infusion of lipid emulsions. Clomipramine is a lipid-soluble tricyclic antidepressant with significant cardiovascular depressant activity in human overdose. Harvey M and Cave G [51] compare resuscitation with Intralipid versus sodium bicarbonate in a rabbit model of clomipramine toxicity. Thirty sedated and mechanically ventilated New Zealand White rabbits were infused with clomipramine at 320 mg/kg per hour. At target mean arterial pressure of 50% initial mean arterial pressure, animals were rescued with 0.9% NaCl 12 mL/kg, 8.4% sodium bicarbonate 3 mL/kg, or 20% Intralipid 12 mL/kg. Pulse rate, mean arterial pressure, and QRS duration were sampled at 2.5-minute intervals to 15 minutes. In the second phase of the experiment, 8 sedated and mechanically ventilated rabbits were infused with clomipramine at 240 mg/kg per hour to a mean arterial pressure of 25 mm Hg. Animals received either 2 mL/kg 8.4% sodium bicarbonate or 8 mL/kg 20% Intralipid as rescue therapy. External cardiac compression and intravenous adrenaline were administered in the event of cardiovascular collapse. Mean difference in mean arterial pressure between Intralipidand saline solution-treated groups was 21.1 mm Hg (95% confidence interval [CI] 13.5 to 28.7 mm Hg) and 19.5 mm Hg (95% CI 10.5 to 28.9 mm Hg) at 5 and 15 minutes, respectively. Mean difference in mean arterial pressure between Intralipidand bicarbonatetreated groups was 19.4 mm Hg (95% CI 18.8 to 27.0 mm Hg) and 11.5 mm Hg (95% CI 2.5 to 20.5 mm Hg) at 5 and 15 minutes. The rate of change in mean arterial pressure was greatest in the Intralipid-treated group at 3 minutes (6.2 mm Hg/min [95% CI 3.8 to 8.6 mm Hg/min]). In the second phase of the experiment spontaneous circulation was maintained in all Intralipid-treated rabbits (n = 4). All animals in the bicarbonate-treated group developed pulseless electrical activity and proved refractory to resuscitation at 10 minutes (n = 4, p = 0.023). In this rabbit model, Intralipid infusion resulted in more rapid and complete reversal of clomipramine-induced hypotension compared with sodium bicarbonate. Additionally, Intralipid infusion prevented cardiovascular collapse in a model of severe clomipramine toxicity.

## Intralipid Prevents and Rescues Fatal Pulmonary Arterial Hypertension and Right Ventricular Failure and Enhances the Inflammatory Response to Endotoxin

Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling leading to right ventricular (RV) hypertrophy and failure. Intralipid (ILP), a source of parenteral nutrition for patients, contains γ-linolenic acid and soy-derived phytoestrogens that are protective for lungs and heart. Umar S. et al. [52] investigated the therapeutic potential of ILP in preventing and rescuing monocrotaline-induced PAH and RV dysfunction. PAH was induced in male rats with monocrotaline (60 mg/kg). Rats then received daily ILP (1 mL of 20% ILP per day IP) from day 1 to day 30 for prevention protocol or from day 21 to day 30 for rescue protocol. Other monocrotaline-injected rats were left untreated to develop severe PAH by day 21 or RV failure by approximately day 30. Saline or ILP-treated rats served as controls. Significant increase in RV pressure and decrease in RV ejection fraction in the RV failure group resulted in high mortality. Therapy with ILP resulted in 100% survival and prevented PAH-induced RV failure by preserving RV pressure and RV ejection fraction and preventing RV hypertrophy and lung remodeling. In preexisting severe PAH, ILP attenuated most lung and RV abnormalities. The beneficial effects of ILP in PAH seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and RV, appear to be major contributors. In conclusion, ILP not only prevents the development of PAH and RV failure but also rescues preexisting severe PAH [52].

Novel anti-inflammatory effects of insulin have recently been described, and insulin therapy to maintain euglycemia suppresses the plasma levels of free fatty acids (FFA) and increases the survival of critically ill patients. Krogh-Madsen et al. [53] aimed to explore the effect of short-term high levels of plasma FFA on the inflammatory response to a low dose of endotoxin. Fourteen healthy male volunteers underwent the following two trials in a randomized crossover design: 1) continuous infusion of 20% Intralipid [0.7 ml•kg−1•h−1 (1.54 g/kg)] for 11 h, and 2) infusion of isotonic saline for 11 h (control). In each trial, heparin was given to activate lipoprotein lipase, and an intravenous bolus of endotoxin (0.1 ng/kg) was given after 6 h of Intralipid/saline infusion. Blood samples and muscle and fat biopsies were obtained before the Intralipid/saline infusion and before as well as after infusion of an endotoxin bolus. Plasma levels of FFA, triglycerides, and glycerol were markedly increased during the Intralipid infusion. Endotoxin exposure induced an increase in plasma levels of TNFalpha, IL-6, and neutrophils and further stimulated gene expression of TNF-alpha and IL-6 in both skeletal muscle and adipose tissue. The systemic inflammatory response to endotoxin was significantly pronounced during Intralipid infusion. Short-term hyperlipidemia enhances the inflammatory response to endotoxin, and skeletal muscle and adipose tissue are capable of producing essential inflammatory mediators after endotoxin stimulation.

## Is Intralipid Approved by the FDA for Local Anesthesia-Induced Cardiovascular Collapse or Resuscitation?

As with any FDA-labeled medication, the individual Intralipid products carry with them a set of contraindications

to use and adverse effects. However, the extent to which these apply in the setting of Local Anesthesiainduced cardiovascular collapse or resuscitation, for which Intralipid is not FDA-approved, remains to be defined. The general contraindication to Intralipid use is the presence of disorders of fat metabolism. Other contraindications not published in the product's package insert include egg allergy and acute myocardial infarction. The use of Intralipid is cautioned in patients with anemia, severe liver disease, coagulopathies, pulmonary disease, and in patients at risk for fat embolism. The most common adverse effects from general Intralipid use are those related to contamination of the administration site and irritation of the veins likely due to other solutions coinfused with Intralipid. Early or immediate adverse effects of Intralipid include allergic reactions, headache, somnolence, dizziness, diaphoresis, dyspnea, nausea/vomiting, hyperthermia, and hypercoagulability. More delayed adverse effects of Intralipid include thrombocytopenia, jaundice, overloading syndrome, increased liver function tests, leucopenia, hepatomegaly, and splenomegaly; pancreatitis has rarely been associated with Intralipid use. Those adverse effects that may be doseor rate-related include pulmonary embolus or fat embolus, and pulmonary vasoconstriction may result from bolus administration of Intralipid. There were no adverse effects reported with Intralipid use in the four human case reports after Local Anesthesia -induced cardiovascular collapse, although further investigation is warranted [54].

## Conclusions

A new multimodal proposal for spinal cord and brain treatment and protection due to injuries and diseases has been described:

1. The epidural catheter is used for cooled saline injection and infusion.
2. The spinal catheter is used for Intralipid spinal injections and CSF aspiration.
3. The proposal is based on the current studies on spinal cord cooling and CSF aspiration as well as on the Intralipid resuscitation properties and lipid brain protection.
4. A study is needed to evaluate the clinical value of this multimodal spinal and brain treatment and disease prevention.

## References


1. Wang YM (2004) Compositions and Treatment Method for Brain and Spinal Cord Injuries.Patent No. US 6683066 B2.
2. Hoffman SW, Kellermann AL, Stein DG, Wright DW, Lowery-North DW, et al. (2007) Dosage Regimen for the Treatment of a Traumatic Brain Injury with Progesterone. Patent No. EP 1868614 A2.
3. Eisenbach-Schwartz M, Yoles E, Hauben E (2008) Use of Poly-Glu, Tyr for Neuroprotection Therapy of the Cns or Pns. Patent No. EP 1406650 B1.
4. Whyte J, Rosenthal M (1993) Rehabilitation of the Patient with Traumatic Brain Injury. In: J DeLisa (Ed.) Rehabilitation Medicine: Principles and Practice. (2nd edn), Lippincott, Philadelphia, pp. 825-860.
5. Frankowski RF, Annegers JF, Whitman S (1985) The Descriptive Epidemiology of Head Trauma in the United States. In: Becker DP & Povlishock JT (Eds.), Central Nervous System Research Status Report, NINCDS, Bethesda, p. 33-43.
6. Sandel ME, Finch M (1993) The Case for Comprehensive Residency Training in Traumatic Brain Injury: A Commentary. Am J Phys Med Rehabil 72(5): 325-326.
7. Jennett B, Bond M (1975) Assessment of Outcome in Severe Brain Damage. The Lancet 305(7905): 480-484.
8. Kraus JF, McArthur DL (1996) Epidemiologic Aspects of Brain Injury. Neurologic Clinics 14(2): 435-450.
9. Max W, Mackenzie EJ, Rice DP (1991) Head Injuries: Costs and Consequences. Journal of Head Trauma Rehabilitation 6(2): 76-91
10. McMordie WR, Barker S (1988) The Financial Trauma of Head Injury. Brain Inj 2(4): 357-364.
11. Bullock MR, Lyeth BG, Muizelaar JP (1999) Current Status of Neuroprotection Trials for Traumatic Brain Injury: Lessons from Animal Models and Clinical Studies. Neurosurgery 45(2): 207-217.
12. Lehmkuhl LD, Hall KM, Mann N, Gordon M (1993) Factors That Influence Costs and Length of Stay of Persons with Traumatic Brain Injury in Acute Care and Inpatient Rehabilitation. Journal of Head Trauma Rehabilitation 8(2): 88-100.
13. Bennett M, Obrien DP, Phillips JP, Farrell MA (1995) Clinicopathologic Observations in 100 Consecutive Patients with Fatal Head Injury Admitted to a Neurosurgical Unit. Ir Med J 88(2): 60-62.
14. Meythaler JM, Peduzzi J (2004) Method of Treating Traumatic Brain and Spinal Cord Injuries and Other Neurogenic Conditions Using Non-Steroidal Anti-Inflammatory Drugs and Naturally Occurring Conotoxins. Patent No. EP 1210100 A4.
15. Odaibo F, Fajardo CA, Cronin C (1992) Recovery of Intralipid from Lumbar Puncture after Migration of Saphenous Vein Catheter. Arch Dis Child 67(10): 1201-1203.
16. Bickler P, Spears R, McKay W (1990) Intralipid Solution Mistakenly Infused into Epidural Space. Anesthesia & Analgesia 71(6): 712-713.
17. Stevens RA, Jorgensen N (1988) Successful Treatment of Dural Puncture Headache with Epidural Saline Infusion after Failure of Epidural Blood Patch. Case Report. Acta Anaesthesiologica Scandinavica 32(5): 429-431.
18. Turnbull DK, Shepherd DB (2003) Post-Dural Puncture Headache: Pathogenesis, Prevention and Treatment. Br J Anaesth 91(5): 718-729.
19. Moomiaie RM, Ransden J, Stein J, Strugar J, Zhu QB, et al. (2007) Cooling Catheter for Spinal Cord Preservation in Thoracic Aortic Surgery. J Cardiovasc Surg (Torino) 48(1): 103-108.
20. Okita Y (2011) Fighting Spinal Cord Complication during Surgery for Thoracoabdominal Aortic Disease. Gen Thorac Cardiovasc Surg 59(2): 79-90.
21. Galla JD (2005) Regional Spinal Cord Cooling Using a Countercurrent Closed-Lumen Epidural Catheter. Invited Commentary. The Annals of Thoracic Surgery 80(5): 1833-1834.
22. Mori A, Ueda T, Hachiya T, Kabei N, Okano H, et al. (2005) An Epidural Cooling Catheter Protects the Spinal Cord Against Ischemic Injury in Pigs. The Annals of Thoracic Surgery 80(5): 1829-1833.
23. Cambria RP, Davison JK, Zannetti S, Italien LG, Brewster DC, et al. (1997) Clinical Experience with Epidural Cooling for Spinal Cord Protection during Thoracic and Thoracoabdominal Aneurysm Repair. J J Vasc Surg 25(2) 234-241; Discussion 241-243.
24. Moomiaie RM, Ransden J, Stein J, Strugar J, Zhu QB, et al. (2007) Cooling Catheter for Spinal Cord Preservation in Thoracic Aortic Surgery. J Cardiovasc Surg (Torino) 48(1): 103-108.
25. Salzano RP Jr, Ellison LH, Altonji PF, Richter J, Deckers PJ, et al. (1994) Regional Deep Hypothermia of the Spinal Cord Protects against Ischemic Injury during Thoracic Aortic Cross-Clamping," Ann Thorac Surg 57(1): 65-70; Discussion 71.
26. Tabayashi K1, Saiki Y, Kokubo H, Takahashi G, Akasaka J, et al. (2010) Protection from Postischemic Spinal Cord Injury by Perfusion Cooling of the Epidural Space during Most or All of a Descending Thoracic or Thoracoabdominal Aneurysm Repair. Gen Thorac Cardiovasc Surg 58(5): 228-234.
27. Breckwoldt WL, Genco CM, Connolly RJ, Cleveland RJ, Diehl JT, et al. (1991) Spinal Cord Protection during Aortic Occlusion: Efficacy of Intrathecal Tetracaine. Ann Thorac Surg 51(6): 959-963.
28. Fox JL, Vu EN, Doyle-Waters M, Brubacher JR, Abu-Laban R, et al. (2010) Prophylactic Hypothermia for Traumatic Brain Injury: A Quantitative Systematic Review. CJEM 12(4): 355-364.
29. Peterson K, Carson S, Carney N (2008) Hypothermia Treatment for Traumatic Brain Injury: A Systematic Review and Meta-Analysis. J Neurotrauma 25(1): 62-71.
30. Bazan NG, Musto AE, Knott EJ ()2011 Endogenous Signaling by Omega-3 Docosahexaenoic Acid-Derived Mediators Sustains Homeostatic Synaptic and Circuitry Integrity. Mol Neurobiol 44(2): 216-222.
31. Palacios-Pelaez R, Lukiw WJ, Bazan NG (2010) Omega-3 Essential Fatty Acids Modulate Initiation and Progression of Neurodegenerative Disease. Mol Neurobiol 41(2-3): 367-74.

32. Zhang C, Bazan NG (2010) Lipid-Mediated Cell Signaling Protects against Injury and Neurodegeneration. J Nutr 140(4): 858-63.

33. Maciá-Botejara E Morán-Penco JM, Espín-Jaime MT, Botello-Martínez F, Salas-Martínez J, et al. (2013) Brain Lipid Composition in Rabbits after Total Parenteral Nutrition with Two Different Lipid Emulsions. Nutrition29(1): 313-317.

34. Grunbaum AM, Gilfix BM, Gosselin S, Blank DW (2012) Analytical Interferences Resulting from Intravenous Lipid Emulsion. Clin Toxicol (Phila) 50(9): 812-817.

35. Li J Iorga A, Sharma S, Youn JY, Partow-Navid R, et al. (2012) Intralipid, a Clinically Safe Compound, Protects the Heart against Ischemia-Reperfusion Injury More Efficiently than Cyclosporine-A. Anesthesiology 117(4): 836-846.

36. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ, et al. (1998) Pretreatment or Resuscitation with a Lipid Infusion Shifts the Dose-Response to Bupivacaine-Induced Asystole in Rats. Anesthesiology 88(4): 1071-1075.

37. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, et al. (2006) Lipid Infusion Accelerates Removal of Bupivacaine and Recovery from Bupivacaine Toxicity in the Isolated Rat Heart. Reg Anesth Pain Med 31(4): 296-303.

38. Weinberg G, Ripper R, Feinstein DL, Hoffman W () Lipid Emulsion Infusion Rescues Dogs from Bupivacaine-Induced Cardiac Toxicity. Regional Anesthesia and Pain Medicine, Reg Anesth Pain Med 28(3): 198-202.

39. Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, et al. (2010) Lipid Emulsions in the Treatment of Acute Poisoning: A Systematic Review of Human and Animal Studies. Clin Toxicol (Phila) 48(1): 1-27.

40. French D, Smollin C, Ruan W, Wong A, Drasner K, et al. (2011) Partition Constant and Volume of Distribution as Predictors of Clinical Efficacy of Lipid Rescue for Toxicological Emergencies. Clin Toxicol (Phila) 49(9): 801-819.

41. JX Mazoit (2013) Cardiac Arrest and Local Anaesthetics. Presse Medicate 42(3): 280-286.

42. Blaber MS1, Khan JN, Brebner JA, McColm R (2012) Lipid Rescue for Tricyclic Antidepressant Cardiotoxicity. J Emerg Med 43(3): 465-467.

43. Papadopoulou A, Willers JW, Samuels TL, Uncles DR, et al. (2012) The Use of Dye Surrogates to Illustrate Local Anesthetic Drug Sequestration by Lipid Emulsion: A Visual Demonstration of the Lipid Sink Effect. Reg Anesth Pain Med 37(2): 183-187.

44. Mauch J, Jurado OM, Spielmann N, Bettschart-Wolfensberger R, Weiss M (2012) Resuscitation Strategies from Bupivacaine-Induced Cardiac Arrest," Pediatric Anesthesia, Paediatr Anaesth 22(2): 124-129.

45. Mauch J, Martin Jurado O, Spielmann N, Bettschart-Wolfensberger R, Weiss M, et al. (2011) Comparison of Epinephrine vs Lipid Rescue to Treat Severe Local Anesthetic Toxicity-An Experimental Study in Piglets. Paediatr Anaesth 21(11): 1103-1138.

46. Montiel V, Gougnard T, Hantson P (2011) Diltiazem Poisoning Treated with Hyperinsulinemic Euglycemia Therapy and Intravenous Lipid Emulsion. Eur J Emerg Med 18(2): 121-123.

47. Hamann P, Dargan PI, Parbat N, Ovaska H, Wood DM, et al. (2010) Availability of and Use of Intralipid (Lipid Rescue Therapy, Lipid Emulsion) in England and Wales. Emergency Medicine Journal 27(8): 590-592.

48. West PL, McKeown NJ, Hendrickson RG (2010) Iatrogenic Lipid Emulsion Overdose in a Case of Amlodipine Poisoning. Clinical Toxicology (Phila) 48(4): 393-396.

49. Hiller DB, Di Gregorio G, Kelly K, Ripper R, Edelman L, et al. (2010) Safety of High Volume Lipid Emulsion Infusion: A First Approximation of LD50 in Rats. Reg Anesth Pain Med 35(2): 140-144.

50. Cave G, Harvey M (2009) Intravenous Lipid Emulsion as Antidote beyond Local Anesthetic Toxicity: A Systematic Review. Acad Emerg Med 16(9): 815-824.

51. Harvey M, Cave G (2007) Intralipid Outperforms Sodium Bicarbonate in a Rabbit Model of Clomipramine Toxicity. Annals of Emergency Medicine 49(2): 178-185.

52. Umar S, Nadadur RD, Li J, Maltese F, Partownavid P, et al. (2011) Intralipid Prevents and Rescues Fatal Pulmonary Arterial Hypertension and Right Ventricular Failure in Rats. Hypertension 58(3): 512-518.

53. Krogh-Madsen R, Plomgaard P, Akerstrom T, Møller K, Schmitz O, et al. (2008) Effect of ShortTerm Intralipid Infusion on the Immune Response during Low-Dose Endotoxemia in Humans. Am J Physiol Endocrinol Metab 294(2): E371-E379.

54. Felice KL, Schumann H (2008) Intravenous Lipid Emulsion for Local Anesthetic Toxicity: A Review of the Literature. J Med Toxicol 4(3): 184-191.

# Chapter 9

## Intra lipid treatment: Is it only the tip of an iceberg? A new suggestion: Bone cement implantation syndrome (BCIS)

## Abstract

On 1998 it was first showed that intravenous intra lipid could prevent or improve resuscitation from cardiovascular collapse by severe bupivacaine overdose in rats. Since then published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haldoperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Intralipid treatment is a new treatment for Bone cement implantation syndrome (BCIS) which was never suggested before. Animal studies should be done in order to evaluate this new treatment modality.

**Keywords:** Intralipid; Bupivacaine toxicity; Bone cement implantation syndrome (BCIS)

## Pre treatment or resuscitation with a lipid infusion

Intralipid® 10% (10% i.v fat emulsion) (a 10% intravenous fat emulsion) is a sterile, non-myogenic fat emulsion prepared for intravenous administration as a source of calories and essential fatty acids. it is made up of 10% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerine, and water for injection. in addition, sodium hydroxide has been added to adjust the ph so that the final product ph is 8. Ph range is 6 to 8.9. The soybean oil is a refined natural product consisting of a mixture of neutral triglycerides of predominantly unsaturated fatty acids. The major component fatty acids are linoleic (44-62%), oleic (19-30%), palmitic (7-14%), Purified egg phosphatides are a mixture of naturally occurring phospholipids which are isolated from the egg yolk. Intralipid® 10% (10% i.v fat emulsion) (a 10% intravenous fat emulsion) has an osmolality of approximately 300 mos mol/kg water (which represents 260 mosmol/liter of emulsion) and contains emulsified fat particles of The total caloric value, including fat, phospholipid and glycerine, is 1.1 kcal per ml of intra lipid 10% (10% i.v fat emulsion). The phospholipids present contribute 47 milligrams or approximately 1.5 mmol of phosphorus per 100 ml, of the emulsion. Weinberg et al., [1] first showed in 1998 that an infusion of a soybean oil emulsion normally used as a total parenteral nutrition solution could prevent (by pre-treatment) or improve resuscitation from cardiovascular collapse caused by severe bupivacaine overdose in the intact, anesthetized rat. Subsequent studies from the same laboratory confirmed these findings in isolated rat heart [2] and anesthetized dog [3]. Under the latter experimental model, return of spontaneous circulation after a bupivacaine challenge occurred in all animals receiving lipid, but in none of the saline controls [3]. This study was accompanied by an editorial asking whether lipid might be the long-sought "silver bullet" for local aesthetic systemic toxicity (LAST). Since then, the effectiveness of lipid emulsion infusion in reversing LAST has been confirmed in other laboratories and by systematic analysis [4] in the clinical setting as well. Lipid infusion is useful in reversing cardiac toxicity of local anaesthetics, and recent reports indicate it may be useful in resuscitation from toxicity induced by a variety of other drugs. While the mechanism behind the utility of lipid rescue remains to be fully elucidated, the predominant effect appears to be creation of a "lipid sink". French D et al., [5] tried to determine whether the extraction of drugs by lipid, and hence the clinical efficacy of lipid rescue in toxicological emergencies can be predicted by specific drug properties each drug investigated was added individually to human drug-free serum. Intralipid® was added to this drug-containing serum, shaken and then incubated at 37°C. The lipid was removed by ultracentrifugation and the concentration of drug remaining in the serum was in this in vitro model, the ability of lipid emulsion to bind a drug was largely dependent upon the drug's lipid partition constant. Additionally, using a multiple linear regression model, the prediction of binding could be improved by combining the lipid partition constant with the volume of distribution together accounting for approximately 88% of the variation in the decrease in serum drug concentration with the administration of lipid emulsion. The lipid partition constant and volume of distribution can likely be used to predict the efficacy of lipid infusion in reversing the cardiac toxicity induced by aesthetics or other medications.

Local anaesthetics may induce cardiac arrest, usually because of rapid absorption from the site of injection or because of an intended intravascular injection. Early central nervous system symptoms usually precede seizures. Cardiac arrhythmias follow the CNS signs. These arrhythmias often resolve with the i.v. bolus injection of 100 to 150mL of a lipid emulsion (20% Intralipid (®)). Although long acting local anaesthetics (bupivacaine, ropivacaine, levobupivacaine) are predominantly involved in this cardiac toxicity, lidocaine may also induce cardiac arrhythmias and clinician must be aware of this risk. In case of cardiac resuscitation manoeuvres are of major importance. They need to be performed immediately and tharrest, e efficacy of the lipid rescue requires a correct coronary flow to be efficacious. Finally, prevention is the key of a safe injection. It is important to control the dose, to inject slowly, without any excessive pressure and to verify that no blood reflux occurs [6].

## Reversing other drugs toxicity

These publications, along with other animal studies, opened the door to more widespread use of lipid emulsion for emergency treatment of toxicities caused by a range of lipophilic drugs. Notably, published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haldoperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Tricyclic antidepressant (TCA) toxicity results predominantly from myocardial sodium-channel blockade. Subsequent ventricular dysrhythmias, myocardial depression, and hypotension cause cardiovascular collapse. Animal studies have demonstrated the effectiveness of intravenous lipid-emulsion in treating TCA cardio toxicity. Blaber MS et al., [7] report a case of dothiepin (Tricyclic antidepressant) overdose causing refractory cardiovascular collapse, which seemed to be successfully reversed with lipid-emulsion therapy (Intralipid®; Fresenius, Cheshire, UK). Lipid emulsions are a potentially novel therapy for reversing cardio toxicity seen in TCA overdose. Research is required into the role of lipid emulsion in the management of poisoning by oral lipophilic agents.

## The lipid sink effect

Papadopoulou A et al., [8] hypothesized that by substituting a dye surrogate in place of local aesthetic, they could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used. They selected 2 lipophilic dyes, acid blue 25 and Victoria blue, with log P values

comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate "lipid rescue" treatment at dye concentrations equivalent to fatal, cardio toxic, and neurotoxin local aesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75-kg adult. After mixing, the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colors compared against a graduated dye concentration scale. Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the colour intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log P value and the greatest amount of lipid emulsion. This study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable.

Local aesthetic (LA) intoxication with cardiovascular arrest is a potential fatal complication of regional anesthesia. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. Aim of the study [9] was to compare four different rescue regimens using epinephrine and/or lipid emulsion and vasopressin to treat cardiac arrest caused by bupivacaine intoxication. Twenty-eight piglets were randomized into four groups (4 × 7), anesthetized with Sevofluran, incubated, and ventilated. Bupivacaine was infused with a syringe driver via central venous catheter at a rate of 1 mg •kg (-1) •min (-1) until circulatory arrest. Bupivacaine infusion and Sevofluran were then stopped, chest compression was started, and the pigs were ventilated with 100% oxygen. After 1 min, epinephri-1) (group1),Intralipid(®)e10µg •kg(20%4 ml •kg(-1) (group 2), epinephrine-1)+Intralipid(®)10µg•kg (4ml•kg (-1) (group 3) or 2 IU vasopressin + Intralipid(®) 4 ml •kg(-1) (group 4) were administered. Secondary epinephrine doses were given after 5 min if required.

Survival was 71%, 29%, 86%, and 57% in groups 1, 2, 3, and 4. Return of spontaneous circulation was regained only by initial administration of epinephrine alone or in combination with Intralipid (®). Piglets receiving the combination therapy survived without further epinephrine support. In contrast, in groups 2 and 4, return of spontaneous circulation was only achieved after secondary epinephrine rescue. In cardiac arrest caused by bupivacaine intoxication, first line rescue with epinephrine and epinephrine + Intralipid (®) was more effective with regard to survival than Intralipid (®) alone and vasopressin + Intralipid (®) in this pig model (9). Local aesthetic (LA) intoxication with severe hemodynamic compromise is a potential catastrophic event. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. However, there are no data about effectiveness of Intralipid for the treatment of severe cardiovascular compromise prior to cardiac arrest. Aim of this study was to compare effectiveness of epinephrine and Intralipid for the treatment of severe hemodynamic. Piglets were compromise owing to bupivacaine intoxication, anesthetized with Sevofluran, incubated, and ventilated. Bupivacaine was infused with a syringe driver via a central venous catheter at a rate of 1 mg •kg (-1) •min (-1) until invasively measured mean arterial pressure (MAP) dropped to 50% of the initial value. Bupivacaine infusion was then stopped, and epinephrine (-1) (group3 1), µg •Intra lipid(®) 20% 2 ml •kg (-1) (group 2), or Intralipid 20% 4 ml• kg(-1) (group 3) was immediately administered. Twenty-one piglets (3 × 7), were recorded. All animals in group 1 (100%) but only four of seven (57%) piglets in group 2 and group 3, respectively, survived. Normalization of hemodynamic parameters (HR, MAP) and ET (CO2) was fastest in group 1 with all piglets achieving HR and MAP values. Hemodynamic compromise owing to bupivacaine intoxication in piglets, first-line rescue with epinephrine was more effective than Intralipid with regard to survival as well as normalization of hemodynamic parameters and ET(CO2) [10].

Intravenous lipid emulsion (ILE) has been proposed as a rescue therapy for severe local aesthetic drugs toxicity, but experience is limited with other lipophilic drugs. An 18-year-old healthy woman was admitted 8 h after the voluntary ingestion of sustained-release diltiazem (3600 mg), with severe hypotension refractory to fluid therapy, calcium salts, and high-dose norepinephrine (6.66 µg/kg/min). Hyper insulinemic euglycemia therapy was in was followed by a protocol of ILE (Intralipid 20%, 1.5 ml/kg as bolus, followed by 0.25 ml/kg over 1h). The main finding attributed to ILE was an apparent rapid decrease in insulin resistance, despite a prolonged serum diltiazem elimination half-life. Diltiazem is a lipophilic cardio toxic drug, which could be sequestered in an expanded plasma lipid phase. The mechanism of action of ILE is not known, including its role in insulin resistance and myocardial metabolism in calcium-channel blocker poisoning [11].

## Guidelines for the management of local anaesthetic toxicity

There is increasing evidence for the use of Intralipid in the management of acute local anaesthetic toxicity. This is supported by the recent Association of Anaesthetists of Great Britain and Ireland (AAGBI) guidelines for the management of local anaesthetic toxicity. Acute hospitals in England and Wales were surveyed to determine the proportion that currently stocked Intralipid, the locations of stocks within the hospital, guidelines related to its use and previous use in the last 12 months. The majority of hospitals surveyed stocked Intralipid in multiple locations, although not in all areas using high volumes of local anaesthetics. Guidelines were typically in place, although these were often local rather than those from the AAGBI. Use in the last 12 months was uncommon, but typically information was not available on indications for its use. More systematic data collection is required on the safety and efficacy of Intralipid in the management of acute drug toxicity [12].

Intralipid therapy has been used successfully as "rescue therapy" in several cases of overdose. West PL et al., [13] present a case of iatrogenic lipid emulsion overdose because of a dosing error: "A 71-year-old female overdosed on 27 tablets of 5 mg amlodipine. Although initially stable in the Emergency Department, she became hypertensive, oligarch, and respiratory failure developed despite medical therapy. The primary treating team felt that meaningful recovery was unlikely to occur without rapid improvement in clinical status, and 12.5 h after presentation, intra lipid rescue therapy was initiated. A protocol for intra lipid specifying a maximum infusion of 400 mL of 20% lipid emulsion was faxed, but the infusion was continued until 2 L of lipid emulsion was infused. There were no detectable adverse hemodynamic effects of the intra lipid infusion. After this time, laboratory values were difficult to obtain. Three hours after the infusion, a metabolic panel was obtained from ultracentrifuge blood showing hyponatraemia. A white blood cell (WBC) was obtained from a complete blood count (CBC) performed 22 h after the infusion, haemoglobin and haematocrit could not be obtained from this blood. A platelet count was obtained by smear estimate. Haematocrit were obtained from centrifuged blood and appeared elevated. No oxygenation could be obtained on blood gas. The patient's family chose to withdraw care on hospital day 2 and no further laboratory draws were obtained. Amlodipine was 1,500ng/mL (ref. 3-11ng/mL)."

## Lipid emulsion overdose

Lipid emulsion overdose caused no detectable acute adverse hemodynamic effects. The following laboratory values were unobtainable immediately after infusion: white blood cell count, haemoglobin, haematocrit, platelet count, and a metabolic panel of serum electrolytes. Ultracentrifugation of blood allowed for detection of a metabolic panel 3 h after the infusion. Centrifuged haematocrit appeared to be higher than expected. Lipid infusion reverses systemic local aesthetic toxicity. The acceptable upper limit for lipid administration is unknown and has direct bearing on clinical management. Hiller DB et al., [14] hypothesize that high volumes of lipid could have undesirable effects and sought to identify the dose required to kill 50% of the animals (LD (50)) of large volume lipid administration. Intravenous lines and electrocardiogram electrodes were placed in anesthetized, male Sprague-Dawley rats. Twenty percent lipid emulsion (20, 40, 60, or 80 mL/kg) or saline (60 or 80 mL/kg), were administered over 30 mins; lipid dosing was assigned by the Dixon "up-and-down" method. Rats were recovered and observed for 48 hrs then euthanized for Histologic analysis of major organs. Three additional rats were administered 60 mL/kg lipid emulsion and euthanized at 1, 4, and 24 hrs to identify progression of organ damage. The maximum likelihood estimate for LD (50) was 67.72 (SE, 10.69) mL/kg. Triglycerides were elevated immediately after infusion but returned to baseline by 48 hrs when laboratory abnormalities included elevated amylase, aspartate aminotransferase, and serum urea nitrogen for all lipid doses. Histologic diagnosis of myocardium, brain, pancreas, and kidneys was normal at all doses. Microscopic abnormalities in lung and liver were observed at 60 and 80 mL/kg; histopathology in the lung and liver was worse at 1 hr than at 4 and 24 hrs. The LD (50) of rapid, high volume lipid infusion is an order of magnitude greater than doses typically used for lipid rescue in humans and supports the safety of lipid infusion at currently recommended doses for toxin-induced cardiac arrest. Lung and liver histopathology was observed at the highest infused volumes. Cave G and Harvey M [15] evaluate the efficacy of lipid emulsion as antidote therapy outside the accepted setting of local aesthetic toxicity.

## Intralipid rescue: 1966-2009

Literature was accessed through PubMed, OVID (1966-February 2009), and EMBASE (1947-February 2009) using the search terms "intravenous" AND ["fat emulsion" OR "lipid emulsion" OR "Intralipid"] AND ["toxicity" OR "resuscitation" OR "rescue" OR "arrest" OR "antidote"]. Additional author and conference publication searches were undertaken. Publications describing the use of lipid emulsion as antidote treatment in animals or humans were included. Fourteen animal studies, one human study, and four case reports were identified. In animal models, intravenous lipid emulsion (ILE) has resulted in amelioration of toxicity associated with cyclic antidepressants, verapamil, propranolol, and thiopentone. Administration in human cases has resulted in successful resuscitation from combined bupropion/lamotrigine-induced cardiac arrest, reversal of sertraline/quetiapine-induced coma, and amelioration of verapamil- and beta blocker-induced shock. Management of overdose with highly lipophilic cardio toxic medications should proceed in accord with established antidote guidelines and early poisons centre consultation. Data from animal experiments and human cases are limited, but suggestive that ILE may be helpful in potentially lethal cardio toxicity or developed cardiac arrest attributable to such agents. Use of lipid emulsion as antidote remains a nascent field warranting further preclinical study and systematic reporting of human cases of use. Previous investigators have demonstrated amelioration of lipid-soluble drug toxidromes with infusion of lipid emulsions. Clomipramine is a lipid-soluble tricyclic antidepressant with significant cardiovascular depressant activity in human overdose. Harvey M and Cave G [16] compare resuscitation with Intralipid versus sodium bicarbonate in a rabbit model of clomipramine toxicity.

Thirty sedated and mechanically ventilated New Zealand White rabbits were infused with clomipramine at 320 mg/kg per hour. At target mean arterial pressure of 50% initial mean arterial pressure, animals were rescued with 0.9% NaCl 12 mL/kg, 8.4% sodium bicarbonate 3 mL/kg, or 20% Intralipid 12 mL/kg. Pulse rate, mean arterial pressure, and QRS duration were sampled at 2.5-minute intervals to 15 minutes. In the second phase of the experiment, 8 sedated and mechanically ventilated rabbits were infused with clomipramine at 240 mg/kg per hour to a mean arterial

pressure of 25 mm Hg. Animals received either 2 mL/kg 8.4% sodium bicarbonate or 8 mL/kg 20% Intralipid as rescue therapy. External cardiac compression and intravenous adrenaline were administered in the event of cardiovascular collapse. Mean difference in mean arterial pressure between Intralipid- and saline solution-treated groups was 21.1 mm Hg (95% confidence interval [CI] 13.5 to 28.7 mm Hg) and 19.5 mm Hg (95% CI 10.5 to 28.9 mm Hg) at 5 and 15 minutes, respectively. Mean difference in mean arterial pressure between Intralipid- and bicarbonate-treated groups was 19.4 mm Hg (95% CI 18.8 to 27.0 mm Hg) and 11.5 mm Hg (95% CI 2.5 to 20.5 mm Hg) at 5 and 15 minutes. The rate of change in mean arterial pressure was greatest in the Intralipid-treated group at 3 minutes (6.2 mm Hg/ min [95% CI 3.8 to 8.6 mm Hg/min] In the second phase of the experiment spontaneous circulation was maintained in all Intralipid-treated rabbits (n=4). All animals in the bicarbonate-treated group developed pulse less electrical activity and proved refractory to resuscitation at 10 minutes (n=4, P=.023). In this rabbit model, Intralipid infusion resulted in more rapid and complete reversal of clomipramineinduced hypotension compared with sodium bicarbonate. Additionally, Intralipid infusion prevented cardiovascular collapse in a model of severe clomipramine toxicity.

## Bone cement implantation syndrome (BCIS)

Bone cement implantation syndrome (BCIS) is characterised by hypoxia, hypotension and loss of consciousness occurring early after bone cementation. The haemodynamic perturbations during BCIS have not been extensively studied, particularly not in patients with femoral neck fracture. Kotyra M et al., [17] evaluated the effects of cemented hemiarthroplasty, in these patients, on pulmonary haemodynamics, right ventricular performance, intrapulmonary shunting and physiological dead space. Fifteen patients undergoing cemented hemiarthroplasty because of femoral neck fracture were included. Surgery was performed under total intravenous anaesthesia in the lateral position. All patients were catheterised with a radial and pulmonary artery catheter, for continuous measurements of mean arterial pressure (MAP), pulmonary arterial pressure (PAP), cardiac output, mixed venous oxygen saturation, right ventricular end-diastolic volume (RVEDV) and right ventricular ejection fraction (RVEF).

Haemodynamic measurements and blood gas analyses were performed after induction of anaesthesia, during surgical stimulation before and immediately after bone cementation and prosthesis insertion, 10 and 20 min after insertion and during skin closure. After bone cementation and prosthesis insertion, MAP (-10%), cardiac index (-10%) and stroke volume index (-10%) decreased, while PAPs (10-15%) and the pulmonary vascular resistance index (45%) increased. RVEF decreased by 10- 20%, while the RVEDV index increased by 10%. Pulmonary haemodynamic and RV variables changed progressively with time, while intra-pulmonary shunting and physiological dead space increased immediately after prosthesis insertion and then returned to baseline. Cemented hemiarthroplasty in patients with femoral neck fracture causes a pronounced pulmonary vasoconstriction and an impairment of RV function accompanied by pulmonary ventilation/perfusion abnormalities.

Acute hypotension, hypoxemia, cardiac arrhythmias, cardiac arrest, (or a combination of these), and sudden death are well-recognized complications of the cemented hip arthroplasty procedure. Collectively, these are known as the bone cement implantation syndrome (BCIS). The endogenous cannabinoids, anandamide (ANA) and 2-arachidonylglycerol (2-AG), Are reported to be strong vasodilators and play a role in the hypotension associated with hemorrhagic and septic shock. In the present study [18], a potential role for the endogenous cannabinoids in influencing hemodynamic variables in BCIS was investigated. Thirty-five patients (35 hips) entered a prospective, randomized clinical trial. The patients were divided into two groups. Group 1 comprised 16 patients who had the component inserted using a conventional cementing technique, whereas group 2 consisted of 19 patients who had the femoral component inserted without cement. Blood samples were taken at six consecutive time points: before anesthesia, after reaming the femur, 2 min after insertion of stems with or without cement into the femur, and 10 min, 20, and 30 min after stem insertion. In group 1 (with cement), the mean levels of ANA and 2-AG significantly increased after stem insertion. In a comparison of each group after stem insertion, mea ANA and 2-AG levels in group 1 also significantly differed from those in group 2. By contrast, in group 2 (without cement) neither ANA nor 2-AG levels exhibited a significant increase or change at any point in time. In conclusion, it has been shown for the first time that endogenous cannabinoids are candidates for lipid mediators of BCIS [18].

Fallon KM et al., [19] described a case report of a cardiac arrest during a cemented hip arthroplasty procedure. Hemodynamic instability during methylmethacrylate use in arthroplasty surgery can be explained by fat embolization rather than the inherent toxicity of the monomer. A 78-yrold woman required a cemented hemiarthroplasty for a pathologic left sub capital fracture. The patient's past medical history included stable angina, diet-controlled type II diabetes and metastatic breast cancer. During the cementing of the canal and insertion of the femoral prosthesis, desaturation, hypotension and cardiac arrest occurred. The patient underwent a successful intraoperative resuscitation and was transferred to the intensive care unit where she subsequently developed disseminated intravascular Coagulopathy. The patient died 24 hr later and autopsy confirmed the cause of death as fat embolization. The deleterious cardiovascular effects of methylmethacrylate have been discussed in the literature. However, clinical evidence supports fat embolization during arthroplasty surgery as a greater determinant of hemodynamic compromise. Surgical precautions are

paramount in minimizing the sequelae of Bone Implantation Syndrome and aesthetic treatment consists of supportive care.

Bone cement implantation syndrome is characterized by hypotension, hypoxemia, cardiac arrhythmias, cardiac arrest, or any combination of these complications. It may result from venous embolization that occurs in conjunction with intra medullary hypertension in the femur during insertion of the prosthesis in patients undergoing cemented total hip arthroplasty (THA). Intra medullary hypertension does not occur in patients undergoing non cemented THA. In this study, Ereth MH et al., [20] sought to compare embolization between patients undergoing cemented and non cemented THA and to determine whether this state resulted in cardio respiratory deterioration. In this prospective investigation of 35 patieundergoing elective THA, they used transesophageal echocardiography and invasive hemodynants mic monitoring, and in 12 of them, they monitored distribution of pulmonary ventilation and perfusion intraoperatively. Embolization was significantly greater after insertion of the prosthesis in patients undergoing cemented than in those undergoing non cemented THA. Cemented THA was also associated with decreased cardiac output and increased pulmonary artery pressure and pulmonary vascular resistance. Increases in ventilation-perfusion mismatching, however, could not be demonstrated 30 minutes after insertion of the femoral prosthesis. Intraoperative monitoring for embolism may help physicians assess patients in whom cardio respiratory function deteriorates during THA [20].

Intralipid prevents and rescues fatal pulmonary arterial hypertension and right ventricular failure and enhances the inflammatory response to endotoxin Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodelling leading to right ventricular (RV) hypertrophy and failure. Intralipid (ILP), source of-linolenicparenteralacidand nutrition for soy-derived phytoestrogens that are protective for lungs and heart. Umar S. et al., [21] investigated the therapeutic potential of ILP in preventing and rescuing monocrotaline-induced PAH and RV dysfunction. PAH was induced in male rats with monocrotaline (60 mg/kg). Rats then received daily ILP (1 mL of 20% ILP per day IP) from day 1 to day 30 for prevention protocol or from day 21 to day 30 for rescue protocol. Other monocrotaline-injected rats were left untreated to develop severe PAH by day 21 or RV failure by approximately day 30. Saline or ILP-treated rats served as controls. Significant increase in RV pressure and decrease in RV ejection fraction in the RV failure group resulted in high mortality. Therapy with ILP resulted in 100% survival and prevented PAH-induced RV failure by preserving RV pressure and RV ejection fraction and preventing RV hypertrophy and lung re modelling. In pre-existing severe PAH, ILP attenuated most lung and RV abnormalities. The beneficial effects of ILP in PAH seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and RV, appear to be major contributors. In conclusion, ILP not only prevents the development of PAH and RV failure but also rescues pre-existing severe PAH [21].

Novel anti-inflammatory effects of insulin have recently been described, and insulin therapy to maintain euglycemia suppresses the plasma levels of free fatty acids (FFA) and increases the survival of critically ill patients. Krogh-Madsen et al., [22] aimed to explore the effect of short-term high levels of plasma FFA on the inflammatory response to a low dose of endotoxin. Fourteen healthy male volunteers underwent the following two trials in a randomized crossover design: 1) continuous infusion of 20%Intralipid [0.7 ml.kg(-1).h(-1) (1.54 g/kg)] for 11 h, and 2) infusion of isotonic saline for 11 h (control). In each trial, heparin was given to activate lipoprotein lipase, and an intravenous bolus of endotoxin (0.1ng/kg) was given after 6 h of Intralipid/saline infusion.

Blood samples and muscle and fat biopsies were obtained before the Intralipid/saline infusion and before as well as after infusion of an endotoxin bolus. Plasma levels of FFA, triglycerides, and glycerol were markedly increased during the Intralipid infusion. Endotoxin exposure induced an increase in plasma levels of TNF-alpha, IL-6, and neutrophils and further stimulated gene expression of TNF-alpha and IL-6 in both skeletal muscle and adipose tissue. The systemic inflammatory response to endotoxin was significantly pronounced during Intralipid infusion. Short-term hyperlipidemia enhances the inflammatory response to endotoxin, and skeletal muscle and adipose tissue are capable of producing essential inflammatory mediators after endotoxin stimulation.

## Is Intralipid approved by the FDA for Local Anesthesia -induced cardiovascular collapse or resuscitation?

As with any FDA-labelled medication, the individual Intralipid products carry with them a set of contraindications to use and adverse effects. However, the extent to which these apply in the setting of Local Anesthesia -induced cardiovascular collapse or resuscitation, for which Intralipid is not FDA-approved, remains to be defined. The general contraindication to Intralipid use is the presence of disorders of fat metabolism; other contraindications not published in the product's package insert include egg allergy and acute myocardial infarction. The use of Intralipid is cautioned in patients with anaemia, severe liver disease, Coagulopathy, pulmonary disease, and in patients at risk for fat embolism. The most common adverse effects from general Intralipid use are those related to contamination of the administration site and irritation of the veins likely due to other solutions co-infused with Intralipid. Early or immediate adverse effects of Intralipid include allergic reactions, headache, somnolence, dizziness, diaphoresis, dysponea, nausea/vomiting, hyperthermia, and hypercoagulability.

More delayed adverse effects of Intralipid include thrombocytopenia, jaundice, overloading syndrome, increased liver function tests, leucopoenia, hepatomegaly, and splenomegaly; pancreatitis has rarely been associated

with Intralipid use. Those adverse effects that may be dose- or rate-related include pulmonary embolus or fat embolus, and pulmonary vasoconstriction may result from bolus administration of Intralipid. There were no adverse effects reported with Intralipid use in the four human case reports after Local Anesthesia -induced cardiovascular collapse, although further investigation is warranted [23].

## Conclusion

Intralipid treatment is a new treatment for BCIS which was never suggested before. Animal studies should be done in order to evaluate this new treatment modality.

## References


1. Weinberg GL, Vade Boncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ, et al. (1998) Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anesthesiology 88(4): 1071-1075.
2. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, et al. (2006) Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. Reg Anesth Pain Med 31(4): 296-303.
3. Weinberg G, Ripper R, Feinstein DL, Hoffman W (2003) Lipid emulsion infusion rescues dogs from bupivacaine-induced cardiac toxicity. Reg Anesth Pain Med 28(3): 198-202.
4. Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, et al. (2010) Lipid emulsions in the treatment of acute poisoning: a systematic review of human and animal studies. Clin Toxicol (Phila) 48(1): 1-27.
5. French D, Smollin C, Ruan W, Wong A, Drasner K, et al. (2011) Partition constant and volume of distribution as predictors of clinical efficacy of lipid rescue for toxicological emergencies. Clin Toxicol (Phila) 49(9): 801-809.
6. Mazoit JX (2012) Cardiac arrest and local anaesthetics. Presse Med.
7. Blaber MS, Khan JN, Brebner JA, McColm R (2012) Lipid rescue" for tricyclic antidepressant cardiotoxicity. J Emerg Med 43(3): 465-467.
8. Papadopoulou A, Willers JW, Samuels TL, Uncles DR (2012) The use of dye surrogates to illustrate local anesthetic drug sequestration by lipid emulsion: a visual demonstration of the lipid sink effect. Reg Anesth Pain Med 37(2): 183-187.
9. Mauch J, Jurado OM, Spielmann N, Bettschart-Wolfensberger R, Weiss M, et al. (2012) Resuscitation strategies from bupivacaine-induced cardiac arrest. Paediatr Anaesth 22(2): 124-129.
10. Mauch J, Martin Jurado O, Spielmann N, Bettschart-Wolfensberger R, Weiss M, et al. (2011) Comparison of epinephrine vs lipid rescue to treat severe local anesthetic toxicity - an experimental study in piglets. Paediatr Anaesth 21(11): 1103-1108.
11. Montiel V, Gougnard T, Hantson P: Diltiazem poisoning treated with hyperinsulinemic euglycemia therapy and intravenous lipid emulsion. Eur J Emerg Med 2011, 18:121-123.
12. Hamann P, Dargan PI, Parbat N, Ovaska H, Wood DM (2010) Availability of and use of Intralipid (lipid rescue therapy, lipid emulsion) in England and Wales. Emerg Med J 27(8):590-592.
13. West PL, McKeown NJ, Hendrickson RG (2010) Iatrogenic lipid emulsion overdose in a case of amlodipine poisoning. Clin Toxicol (Phila) 48 (4): 393-396.
14. Hiller DB, Di Gregorio G, Kelly K, Ripper R, Edelman L, et al. (2010) Safety of high volume lipid emulsion infusion: a first approximation of LD50 in rats. Reg Anesth Pain Med 35(2): 140-144.
15. high volume lipid emulsion infusion: a first approximation of LD50 in rats. Reg Anesth Pain Med 2010, 35:140-144.
16. Cave G, Harvey M (2009) Intravenous lipid emulsion as antidote beyond local anesthetic toxicity: a systematic review. Acad Emerg Med 16(9): 815-824.
17. Harvey M, Cave G (2007) Intralipid outperforms sodium bicarbonate in a rabbit model of clomipramine toxicity. Ann Emerg Med 49(2):178-185.e4.
18. Kotyra M, Houltz E, Ricksten SE (2010) Pulmonary haemodynamics and right ventricular function during cemented hemiarthroplasty for femoral neck fracture. Acta Anaesthesiol Scand 54(10): 1210-1216.
19. Motobe T, Hashiguchi T, Uchimura T, Yamakuchi M, Taniguchi N, et al. (2004) Endogenous cannabinoids are candidates for lipid mediators of bone cement implantation syndrome. Shock 21(1): 8-12.
20. Fallon KM, Fuller JG, Morley-Forster P (2001) Fat embolization and fatal cardiac arrest during hip arthroplasty with methylmethacrylate. Can J Anaesth 48(7): 626-629.
21. Ereth MH, Weber JG, Abel MD, Lennon RL, Lewallen DG, et al. (1992) Cemented versus noncemented total hip arthroplasty--embolism, hemodynamics, and intrapulmonary shunting. Mayo Clin Proc 67(11): 1066-1074.
22. Umar S, Nadadur RD, Li J, Maltese F, Partownavid P, et al. (2011) Intralipid prevents and rescues fatal pulmonary arterial hypertension and right ventricular failure in rats. Hypertension 58(): 512-518.
23. Krogh-Madsen R, Plomgaard P, Akerstrom T, Moller K, Schmitz O, Pedersen BK: Effect of short-term intralipid infusion on the immune response during low-dose endotoxemia in humans. Am J Physiol Endocrinol Metab 2008, 294:E371-379.
24. Felice K, Schumann H: Intravenous lipid emulsion for local anesthetic toxicity: a review of the literature. J Med Toxicol 2008, 4:184-191.

# Chapter 10

## Intralipid for Amniotic Fluid Embolism (AFE)?

## Abstract

In 1998 it was first showed that intravenous Intralipid could prevent or improve resuscitation from cardiovascular collapse by severe bupivacaine overdose in rats. Since then published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haldoperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others. Amniotic fluid embolism (AFE) is a rare but potentially catastrophic obstetric emergency. Despite earlier recognition and aggressive treatment, morbidity and mortality rates remain high. An estimated 5% - 15% of all maternal deaths in Western countries are due to AFE. The pathophysiology of AFE is not completely understood. AFE most commonly occurs during labor, delivery, or the immediate postpartum period. However, it has been reported to occur up to 48 h postpartum. Pulmonary hypertension and right heart strain/failure may be the result of physical amniotic fluid debris in the pulmonary vasculature or, perhaps more likely, result from circulating pulmonary vasoconstrictive mediators. Therapy with Intralipid in male rats resulted in 100% survival and prevented Pulmonary arterial hypertension-induced right ventricular failure by preserving right ventricular pressure and right ventricular ejection fraction and preventing right ventricular hypertrophy and lung remodeling. In preexisting severe Pulmonary arterial hypertension, Intralipid attenuated most lung and right ventricular abnormalities. The beneficial effects of Intralipid in Pulmonary arterial hypertension seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and right ventricular, appear to be major contributors. In conclusion, Intralipid not only prevents the development of Pulmonary arterial hypertension and right ventricular failure but also rescues preexisting severe Pulmonary arterial hypertension. Intralipid treatment is a new treatment for AFE (amniotic fluid embolism) which was never suggested before. Animal studies should be done in order to evaluate this new treatment modality.

## Introduction

Weinberg et al. [1] first showed in 1998 that an infusion of a soybean oil emulsion normally used as a total parenteral nutrition solution could prevent (by pretreatment) or improve resuscitation from cardiovascular collapse caused by severe bupivacaine overdose in the intact, anesthetized rat. Subsequent studies from the same laboratory confirmed these findings in isolated rat heart [2] and anesthetized dog [3]. Under the latter experimental model, return of spontaneous circulation after a bupivacaine challenge occurred in all animals receiving lipid, but in none of the saline controls [3]. This study was accompanied by an editorial asking whether lipid might be the long-sought "silver bullet" for local anesthetic systemic toxicity (LAST). Since then, the effectiveness of lipid emulsion infusion in reversing LAST has been confirmed in other laboratories and by systematic analysis [4] in the clinical setting as well. These publications, along with other animal studies, opened the door to more widespread use of lipid emulsion for emergency treatment of toxicities caused by a range of lipophilic drugs. Notably, published examples now include toxicities related to verapamil, diltiazem, amlodipine, quetiapine and sertraline, haldoperidol, lamotrigine, olanzapine, propranolol, atenolol, nevibolol, doxepin, dosulepin, imipramine, amitriptyline, glyosphate herbicide, flecainide, venlafaxine, moxidectin, and others.

## Material and Method

Tricyclic antidepressant (TCA) toxicity results predominantly from myocardial sodium-channel blockade. Subsequent ventricular dysrhythmias, myocardial depression, and hypotension cause cardiovascular collapse. Animal studies have demonstrated the effectiveness of intravenous lipid-emulsion in treating TCA cardiotoxicity. Blaber M. S. et al. [5] report a case of dothiepin (tricyclic antidepressant) overdose causing refractory cardiovascular collapse, which seemed to be successfully reversed with lipid-emulsion therapy (Intralipid□; Fresenius, Cheshire, UK). Lipid emulsions are a potentially novel therapy for reversing cardiotoxicity seen in TCA overdose. Research is required into the role of lipid emulsion in the management of poisoning by oral lipophilic agents. Lipid infusion is useful in reversing cardiac toxicity of local anesthetics, and recent reports indicate it may be useful in resuscitation from toxicity induced by a variety of other drugs. While the mechanism behind the utility of lipid rescue remains to be fully elucidated, the predominant effect appears to be creation of a "lipid sink". French D. et al. [6] tried to determine whether the extraction of drugs by lipid, and hence the clinical efficacy of lipid rescue in toxicological emergencies can be predicted by specific drug properties. Each drug investigated was added individually to human drug-free serum. Intralipid□ was added to this drugcontaining serum, shaken and then incubated at 37˚C. The lipid was removed by ultracentrifugation and the concentration of drug remaining in the serum was measured by high-pressure liquid chromatography. In this in vitro model, the ability of lipid emulsion to bind a drug was largely dependent upon the drug's lipid partition constant. Additionally, using a multiple linear regression model, the prediction of binding could be improved by combining the lipid partition constant with the volume of distribution together accounting for approximately 88% of the variation in the decrease in serum drug concentration with the administration of lipid emulsion. The lipid partition constant and volume of distribution can likely be used to predict the efficacy of lipid infusion in reversing the cardiac toxicity induced by anesthetics or other medications. Local anaesthetics may induce cardiac arrest, usually because of rapid absorption from the site of injection or because of an intended intravascular injection. Early central nervous system symptoms usually precede seizures. Cardiac arrhythmias follow the CNS signs. These arrhythmias often resolve with the i.v. bolus injection of 100 to 150 ml of a lipid emulsion (20% Intralipid□). Although long acting local anaesthetics (bupivacaine, ropivacaine, levobupivacaine) are predominantly involved in this cardiac toxicity, lidocaine may also induce cardiac arrhythmias and clinician must be aware of this risk. In case of cardiac arrest, resuscitation manoeuvres are of major importance. They need to be performed immediately and the efficacy of the lipid rescue requires a correct coronary flow to be efficacious. Finally, prevention is the key of a safe injection. It is important to control the dose, to inject slowly, without any excessive pressure and to verify that no blood reflux occurs [7]. Papadopoulou A. et al. [8] hypothesized that by substituting a dye surrogate in place of local anesthetic, they could visually demonstrate dye sequestration by lipid emulsion that would be dependent on both dye lipophilicity and the amount of lipid emulsion used. They selected 2 lipophilic dyes, acid blue 25 and victoria blue, with log P values comparable to lidocaine and bupivacaine, respectively. Each dye solution was mixed with combinations of lipid emulsion and water to emulate "lipid rescue" treatment at dye concentrations equivalent to fatal, cardiotoxic, and neurotoxic local anesthetic plasma concentrations. The lipid emulsion volumes added to each dye solution emulated equivalent intravenous doses of 100, 500, and 900 mL of 20% Intralipid in a 75-kg adult. After mixing, the samples were separated into a lipid-rich supernatant and a lipid-poor subnatant by heparin flocculation. The subnatants were isolated, and their colors compared against a graduated dye concentration scale. Lipid emulsion addition resulted in significant dye acquisition by the lipid compartment accompanied by a reduction in the color intensity of the aqueous phase that could be readily observed. The greatest amount of sequestration occurred with the dye possessing the higher log P value and the greatest amount of lipid emulsion. This study provides a visual demonstration of the lipid sink effect. It supports the theory that lipid emulsion may reduce the amount of free drug present in plasma from concentrations associated with an invariably fatal outcome to those that are potentially survivable.

## Statistical Analysis

Local anesthetic (LA) intoxication with cardiovascular arrest is a potential fatal complication of regional anesthesia. Lipid resuscitation has been recommended for the treatment of

LA-induced cardiac arrest. Aim of the study [9] was to compare four different rescue regimens using epinephrine and/or lipid emulsion and vasopressin to treat cardiac arrest caused by bupivacaine intoxication. Twenty-eight piglets were randomized into four groups (4 × 7), anesthetized with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via central venous catheter at a rate of 1 mg•kg–1•min–1 until circulatory arrest. Bupivacaine infusion and sevoflurane were then stopped, chest compression was started, and the pigs were ventilated with 100% oxygen. After 1 min, epinephrine 10 µg•kg–1 (group 1), Intralipid 20% 4 ml•kg–1 (group 2), epinephrine 10 µg•kg–1 + Intralipid 4 ml•kg–1 (group 3) or 2 IU vasopressin + Intralipid 4 ml•kg–1 (group 4) were administered. Secondary epinephrine doses were given after 5 min if required. Survival was 71%, 29%, 86%, and 57% in groups 1, 2, 3, and 4. Return of spontaneous circulation was regained only by initial administration of epinephrine alone or in combination with Intralipid. Piglets receiving the combination therapy survived without further epinephrine support. In contrast, in groups 2 and 4, return of spontaneous circulation was only achieved after secondary epinephrine rescue. In cardiac arrest caused by bupivacaine intoxication, first-line rescue with epinephrine and epinephrine + Intralipid was more effective with regard to survival than Intralipid alone and vasopressin + Intralipid in this pig model [9]. Local anesthetic (LA) intoxication with severe hemodynamic compromise is a potential catastrophic event. Lipid resuscitation has been recommended for the treatment of LA-induced cardiac arrest. However, there are no data about effectiveness of Intralipid for the treatment of severe cardiovascular compromise prior to cardiac arrest. Aim of this study was to compare effectiveness of epinephrine and Intralipid for the treatment of severe hemodynamic. Piglets were compromise owing to bupivacaine intoxication, anesthetized with sevoflurane, intubated, and ventilated. Bupivacaine was infused with a syringe driver via a central venous catheter at a rate of 1 mg•kg–1•min–1 until invasively measured mean arterial pressure (MAP) dropped to 50% of the initial value. Bupivacaine infusion was then 177 stopped, and epinephrine 3 µg•kg–1 (group 1), Intralipid 20% 2 ml•kg–1 (group 2), or Intralipid 20% 4 ml•kg–1 (group 3) was immediately administered. Twenty-one piglets (3 × 7), were recorded. All animals in group 1 (100%) but only four of seven (57%) piglets in groups 2 and 3, respectively, survived. Normalization of hemodynamic parameters (HR, MAP) and ET (CO2) was fastest in group 1 with all piglets achieving HR and MAP values. hemodynamic compromise owing to bupivacaine intoxication in piglets, firstline rescue with epinephrine was more effective than Intralipid with regard to survival as well as normalization of hemodynamic parameters and ET (CO2) [10]. Intravenous lipid emulsion (ILE) has been proposed as a rescue therapy for severe local anesthetic drugs toxicity, but experience is limited with other lipophilic drugs. An 18-year-old healthy woman was admitted 8 h after the voluntary ingestion of sustained-release diltiazem (3600 mg), with severe hypotension refractory to fluid therapy, calcium salts, and high-dose norepinephrine (6.66 µg/kg/ min). Hyperinsulinemic euglycemia therapy was initiated and shortly after was followed by a protocol of ILE (intralipid 20%, 1.5 ml/kg as bolus, followed by 0.25 ml/kg over 1 h). The main finding attributed to ILE was an apparent rapid decrease in insulin resistance, despite a prolonged serum diltiazem elimination half-life. Diltiazem is a lipophilic cardiotoxic drug, which could be sequestered in an expanded plasma lipid phase. The mechanism of action of ILE is not known, including its role in insulin resistance and myocardial metabolism in calcium-channel blocker poisoning [11]. There is increasing evidence for the use of Intralipid in the management of acute local anaesthetic toxicity. This is supported by the recent Association of Anaesthetists of Great Britain and Ireland (AAGBI) guidelines for the management of local anaesthetic toxicity. Acute hospitals in England and Wales were surveyed to determine the proportion that currently stocked Intralipid, the locations of stocks within the hospital, guidelines related to its use and previous use in the last 12 months. The majority of hospitals surveyed stocked Intralipid in multiple locations, although not in all areas using high volumes of local anaesthetics. Guidelines were typically in place, although these were often local rather than those from the AAGBI. Use in the last 12 months was uncommon, but typically information was not available on indications for its use. More systematic data collection is required on the safety and efficacy of Intralipid in the management of acute drug toxicity [12]. Intralipid therapy has been used successfully as “rescue therapy” in several cases of overdose. West P. L. et al. [13] present a case of iatrogenic lipid emulsion overdose because of a dosing error. A 71-year-old female overdosed on 27 tablets of 5 mg amlodipine. Although initially stable in the Emergency Department, she became hypotensive, oliguric, and respiratory failure developed despite medical therapy. The primary treating team felt that meaningful recovery was unlikely to occur without rapid improvement in clinical status, and 12.5 h after presentation, Intralipid rescue therapy was initiated. A protocol for Intralipid specifying a maximum infusion of 400 mL of 20% lipid emulsion was faxed, but the infusion was continued until 2 L of lipid emulsion was infused. There were no detectable adverse hemodynamic effects of the Intralipid infusion. After this time, laboratory values were difficult to obtain. Three hours after the infusion, a metabolic panel was obtained from ultracentrifuged blood showing hyponatremia. A white blood cell (WBC) was obtained from a complete blood count (CBC) performed 22 h after the infusion, hemoglobin and hematocrit could not be obtained from this blood. A platelet count was obtained by smear estimate. Hematocrits were obtained from centrifuged blood and appeared elevated. No oxygenation could be obtained on blood gas. The patient’s family chose to withdraw care on hospital day 2 and no further laboratory draws were obtained. Amlodipine was 1500 ng/mL (ref. 3 - 11 ng/mL). Lipid emulsion overdose caused no detectable acute adverse hemodynamic effects. The following laboratory values were unobtainable immediately after infusion: white blood cell count, hemoglobin, hematocrit, platelet count, and a metabolic

panel of serum electrolytes. Ultracentrifugation of blood allowed for detection of a metabolic panel 3 h after the infusion. Centrifuged hematocrits appeared to be higher than expected. Lipid infusion reverses systemic local anesthetic toxicity. The acceptable upper limit for lipid administration is unknown and has direct bearing on clinical management. Hiller D. B. et al. [14] hypothesize that high volumes of lipid could have undesirable effects and sought to identify the dose required to kill 50% of the animals (LD(50)) of large volume lipid administration. Intravenous lines and electrocardiogram electrodes were placed in anesthetized, male Sprague-Dawley rats. Twenty percent lipid emulsion (20, 40, 60, or 80 mL/kg) or saline (60 or 80 mL/kg), were administered over 30 mins; lipid dosing was assigned by the Dixon "up-anddown" method. Rats were recovered and observed for 48 h then euthanized for histologic analysis of major organs. Three additional rats were administered 60 mL/kg lipid emulsion and euthanized at 1, 4, and 24 h to identify progression of organ damage. The maximum likelihood estimate for LD(50) was 67.72 (SE, 10.69) mL/kg. Triglycerides were elevated immediately after infusion but returned to baseline by 48 h when laboratory abnormalities included elevated amylase, aspartate aminotransferase, and serum urea nitrogen for all lipid doses. Histologic diagnosis of myocardium, brain, pancreas, and kidneys was normal at all doses. Microscopic abnormalities in lung and liver were observed at 60 and 80 mL/kg; histopathology in the lung and liver was worse at 1 h than at 4 and 24 h. The LD (50) of rapid, high volume lipid infusion is an order of magnitude greater than doses typically used for lipid rescue in humans and supports the safety of lipid infusion at currently recommended doses for toxin-induced cardiac arrest. Lung and liver histopathology was observed at the highest infused volumes.

## Results

Cave G. and Harvey M. [15] evaluate the efficacy of lipid emulsion as antidotal therapy outside the accepted setting of local anesthetic toxicity. Literature was accessed through PubMed, OVID (1966- February 2009), and EMBASE (1947-February 2009) using the search terms "intravenous" and ["fat emulsion" or "lipid emulsion" or "Intralipid"] and ["toxicity" or "resuscitation" or "rescue" or "arrest" or "antidote"]. Additional author and conference publication searches were undertaken. Publications describing the use of lipid emulsion as antidotal treatment in animals or humans were included. Fourteen animal studies, one human study, and four case reports were identified. In animal models, intravenous lipid emulsion (ILE) has resulted in amelioration of toxicity associated with cyclic antidepressants, verapamil, propranolol, and thiopentone. Administration in human cases has resulted in successful resuscitation from combined bupropion/lamotrigine-induced cardiac arrest, reversal of sertraline/quetiapine-induced coma, and amelioration of verapamiland beta blocker-induced shock. Management of overdose with highly lipophilic cardiotoxic medications should proceed in accord with established antidotal guidelines and early poisons center consultation. Data from animal experiments and human cases are limited, but suggestive that ILE may be helpful in potentially lethal cardiotoxicity or developed cardiac arrest attributable to such agents. Use of lipid emulsion as antidote remains a nascent field warranting further preclinical study and systematic reporting of human cases of use. Previous investigators have demonstrated amelioration of lipid-soluble drug toxidromes with infusion of lipid emulsions. Clomipramine is a lipid-soluble tricyclic antidepressant with significant cardiovascular depressant activity in human overdose. Harvey M. and Cave G. [16] compare resuscitation with Intralipid versus sodium bicarbonate in a rabbit model of clomipramine toxicity. Thirty sedated and mechanically ventilated New Zealand White rabbits were infused with clomipramine at 320 mg/kg per hour. At target mean arterial pressure of 50% initial mean arterial pressure, animals were rescued with 0.9% NaCl 12 mL/kg, 8.4% sodium bicarbonate 3 mL/kg, or 20% Intralipid 12 mL/kg. Pulse rate, mean arterial pressure, and QRS duration were sampled at 2.5-minute intervals to 15 minutes. In the second phase of the experiment, 8 sedated and mechanically ventilated rabbits were infused with clomipramine at 240 mg/kg per hour to a mean arterial pressure of 25 mm Hg. Animals received either 2 mL/kg 8.4% sodium bicarbonate or 8 mL/kg 20% Intralipid as rescue therapy. External cardiac compression and intravenous adrenaline were administered in the event of cardiovascular collapse. Mean difference in mean arterial pressure between Intralipidand saline solution-treated groups was 21.1 mm Hg (95% confidence interval [CI] 13.5 to 28.7 mm Hg) and 19.5 mm Hg (95% CI 10.5 to 28.9 mm Hg) at 5 and 15 minutes, respectively. Mean difference in mean arterial pressure between Intralipidand bicarbonate-treated groups was 19.4 mm Hg (95% CI 18.8 to 27.0 mm Hg) and 11.5mm Hg (95% CI 2.5 to 20.5 mm Hg) at 5 and 15 minutes. The rate of change in mean arterial pressure was greatest in the Intralipid-treated group at 3 minutes (6.2 mm Hg/min [95% CI 3.8 to 8.6 mm Hg/min]. In the second phase of the experiment spontaneous circulation was maintained in all Intralipid-treated rabbits (n = 4). All animals in the bicarbonate-treated group developed pulseless electrical activity and proved refractory to resuscitation at 10 minutes (n = 4, P = 0.023). In this rabbit model, Intralipid infusion resulted in more rapid and complete reversal of clomipramine-induced hypotension compared with sodium bicarbonate. Additionally, Intralipid infusion prevented cardiovascular collapse in a model of severe clomipramine toxicity.

## Discussion

Amniotic fluid embolism (AFE) is a rare but potentially catastrophic obstetric emergency. Despite earlier recognition and aggressive treatment, morbidity and mortality rates remain high. An estimated 5% - 15% of all maternal deaths in Western countries are due to AFE [17]. Recent retrospective reviews of population-based hospital databases in Canada [18] and the United States [19] found AFE incidences of 6.1 - 7.7 cases per 100,000 births. Early studies revealed mortality rates as high as 61% - 86%, but more recent estimates suggest a case

fatality of 13% - 26% [19-21]. First reported by Meyer in 1926 [22], and then later identified as a syndrome in 1941 by Steiner and Lushbaugh [23]. The pathophysiology of AFE is not completely understood. AFE most commonly occurs during labor, delivery, or the immediate postpartum period. However, it has been reported to occur up to 48 h postpartum [24]. Once thought to be the result of an actual embolic obstruction of the pulmonary vasculature by components of amniotic fluid, AFE might result from immune activation and present as an anaphylactoid process. AFE likely involves a spectrum of severity from a subclinical process to a catastrophic event. Early recognition and prompt and aggressive resuscitative efforts enhance the probability of maternal and neonatal survival. Three phases in the clinical course of AFE have been described. The first or immediate phase is often characterized by altered mental status, respiratory distress, peripheral oxygen desaturation, and hemodynamic collapse. The second phase involves coagulopathy and hemorrhage and occurs in an estimated 4% - 50% of patients with presumed AFE. Although older studies of AFE required either sudden, unresuscitatable maternal death or the subsequent development of disseminated intravascular coagulation (DIC) for inclusion in the AFE database, it is now recognized that DIC does not develop in all cases of AFE. Tissue injury and end-organ system failure comprise the last phase of AFE. Clinical findings will vary depending on the organ system(s) predominantly affected. Ventilation-perfusion mismatching as a result of pulmonary vascular constriction at the onset of AFE may explain sudden hypoxia and respiratory arrest [25]. Pulmonary hypertension and right-heart strain/failure may be the result of physical amniotic fluid debris in the pulmonary vasculature or, perhaps more likely, result from circulating pulmonary vasoconstrictive mediators. The mechanisms for myocardial dysfunction that lead to early hypotension are multifactorial. Proposed explanations include myocardial failure in response to sudden pulmonary hypertension, a direct myocardial depressant effect of humoral mediators in amniotic fluid, deviation of the intraventricular septum due to right ventricular dilation, and/or ischemic myocardial injury from hypoxemia [26-28]. Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling leading to right ventricular (RV) hypertrophy and failure. Intralipid (ILP), a source of parenteral nutrition for patients, contains γ- linolenic acid and soy-derived phytoestrogens that are protective for lungs and heart. Umar S. et al. [29] investigated the therapeutic potential of ILP in preventing and rescuing monocrotaline-induced PAH and RV dysfunction. PAH was induced in male rats with monocrotaline (60 mg/kg). Rats then received daily ILP (1 mL of 20% ILP per day IP) from day 1 to day 30 for prevention protocol or from day 21 to day 30 for rescue protocol. Other monocrotaline-injected rats were left untreated to develop severe PAH by day 21 or RV failure by approximately day 30. Saline or ILP-treated rats served as controls. Significant increase in RV pressure and decrease in RV ejection fraction in the RV failure group resulted in high mortality. Therapy with ILP resulted in 100% survival and prevented PAH-induced RV failure by preserving RV pressure and RV ejection fraction and preventing RV hypertrophy and lung remodeling. In preexisting severe PAH, ILP attenuated most lung and RV abnormalities. The beneficial effects of ILP in PAH seem to result from the interplay of various factors, among which preservation and/or stimulation of angiogenesis, suppression and/or reversal of inflammation, fibrosis and hypertrophy, in both lung and RV, appear to be major contributors. In conclusion, ILP not only prevents the development of PAH and RV failure but also rescues preexisting severe PAH [29].

## Conclusion

Intralipid treatment is a new treatment for AFE which was never suggested before. Animal studies should be done in order to evaluate this new treatment modality.

## References


1. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ, et al. (1998) Pretreatment or Resuscitation with a Lipid Infusion Shifts the Dose-Response to Bupivacaine-Induced Asystole in Rats. Anesthesiology 88(4): 1071-1075.
2. Weinberg GL1, Ripper R, Murphy P, Edelman LB, Hoffman W, et al. (2006) Lipid Infusion Accelerates Removal of Bupivacaine and Recovery from Bupivacaine Toxicity in the Isolated Rat Heart. Reg Anesth Pain Med31(4): 296-303.
3. Weinberg G, Ripper R, Feinstein DL, Hoffman W (2003) Lipid Emulsion Infusion Rescues Dogs from Bupivacaine-Induced Cardiac Toxicity. Reg Anesth Pain Med 28(3): 198-202.
4. Jamaty C, Bailey B, Larocque A, Notebaert E, Sanogo K, (2010) Lipid Emulsions in the Treatment of Acute Poisoning: A Systematic Review of Human and Animal Studies. Clin Toxicol (Phila) 48(1): 1-27.
5. Blaber MS, Khan JN, Brebner JA, McColm R (2012) Lipid Rescue for Tricyclic Antidepressant Cardiotoxicit. J Emerg Med 43(3): 465-467.
6. French D, Smollin C, Ruan W, Wong A, Drasner K, et al. (2011) Partition Constant and Volume of Distribution as Predictors of Clinical Efficacy of Lipid Rescue for Toxicological Emergencies. Clinical Toxicology 49(9): 801-809.
7. J. X. Mazoit (2012) Cardiac Arrest and Local Anaesthetics La Presse Médicale in press.
8. Papadopoulou A, Willers JW, Samuels TL, Uncles DR (2012) The Use of Dye Surrogates to Illustrate Local Anesthetic Drug Sequestration by Lipid Emulsion: A Visual Demonstration of the Lipid Sink Effect. Reg Anesth Pain Med 37(2): 183-187.
9. Mauch J, Jurado OM, Spielmann N, Bettschart-Wolfensberger R, Weiss M, et al. (2012) Resuscitation Strategies from Bupivacaine-Induced Cardiac Arrest. Pediatr Anesth 22(2): 124-129.
10. Mauch J, Jurado OM, Spielmann N, Bettschart-Wolfensberger R, Weiss M, et al. (2011) Comparison of Epinephrine vs Lipid Rescue to Treat Severe Local Anesthetic Toxicity- An Experimental Study in Piglets. Paediatr Anaesth 21(11):1103-1108.
11. Montiel V, Gougnard T, Hantson P (2011) Diltiazem Poisoning Treated with Hyperinsulinemic Euglycemia Therapy and Intravenous Lipid EmulsionEur J Emerg Med 18(2): 121-123.
12. Hamann P, Dargan PI, Parbat N, Ovaska H, Wood DM, et al. (2010) Availability of and Use of Intralipid (Lipid Rescue

Therapy, Lipid Emulsion) in England and Wales. Emerg Med J 27(8): 590-592.

13. West PL, McKeown NJ, Hendrickson RG (2010) Iatrogenic Lipid Emulsion Overdose in a Case of Amlodipine Poisoning. Clin Toxicol (Phila) 48(4): 393-396.
14. Hiller DB, Di Gregorio G, Kelly K, Ripper R, Edelman L, et al. ( 2010) Safety of High Volume Lipid Emulsion Infusion: A First Approximation of LD50 in Rats," Regional Anesthesia and Pain Medicine 35(2): 140-144.
15. Cave G, Harvey M (2009) Intravenous Lipid Emulsion as Antidote beyond Local Anesthetic Toxicity: A Systematic Review. Acad Emerg Med16(9): 815-824.
16. Harvey M, Cave G (2007) Intralipid Outperforms Sodium Bicarbonate in a Rabbit Model of Clomipramine Toxicity. Annals of Emergency Medicine 49(2): 178-185.
17. Conde-Agudelo, Romero R (2009) Amniotic Fluid Embolism: An Evidence-Based Review. Am J Obstet Gynecol 201(5): 445. e1-13
18. Kramer MS, Rouleau J, Baskett TF, Joseph KS (2006) Maternal Health Study Group of the Canadian Perinatal Surveillance System: Amniotic-Fluid Embolism and Medical Induction of Labour: A Retrospective, PopulationBased Cohort Study. Lancet 368(9545): 1444-1448.
19. Abenhaim HA, Azoulay L, Kramer MS, Leduc L (2008) Incidence and Risk Factors of Amniotic Fluid Embolisms: A Population-Based Study on 3 Million Births in the United States. Am J Obstet Gynecol 199(1): 49. e1-8.
20. Knight M, Tuffnell D, Brocklehurst P, Spark P, Kurinczuk JJ, et al. (2010) UK Obstetric Surveillance System: Incidence and Risk Factors for Amniotic-Fluid Embolism. Obstet Gynecol 115(5): 910-917.
21. Gei AF, Vadhera RB, Hankins GD (2003) Embolism during Pregnancy: Thrombus, Air, and Amniotic Fluid. Anesthesiol Clin North America 21(1): 165-182.
22. Meyer JR (1926) Intralipid for Amniotic Fluid Embolism. Brasil Medico 2(4): 301-303.
23. Steiner PE, Lushbaugh CC (1986) Maternal Pulmonary Embolism by Amniotic Fluid as a Cause of Obstetric Shock and Unexpected Deaths in Obstetrics. Journal of the American Medical Association 255(16): 2187-2203.
24. Conde-Agudelo A, Romero R (2009) Amniotic Fluid Embolism: An Evidence-Based Review," Am J Obstet Gynecol 201(5): 445.e1-e13.
25. Clark SL, Hankins GD, Dudley DA, Dildy GA, Porter TF, et al. (1995) Amniotic Fluid Embolism: Analysis of the National Registry," American Journal of Obstetrics & Gynecology 172(4): 1158-1167.
26. Shea O, Eappen S (2007) Amniotic Fluid Embolism. International Anesthesiology Clinics 45(1): 17-28.
27. Stanten RD, Iverson LI, Daugharty TM, Lovett SM, Terry C, et al. (2003) Amniotic Fluid Embolism Causing Catastrophic Pulmonary Vasoconstriction: Diagnosis by Transesophageal Echocardiogram and Treatment by Cardiopulmonary Bypass. 102(3): 496-498.
28. James CF, Feinglass NG, Menke DM, Grinton SF, Papadimos TJ (2004) Massive Amniotic Fluid Embolism: Diagnosis Aided by Emergency Transesophageal Echocardiography. Int J Obstet Anesth 13(4): 279-283.
29. Umar S, Nadadur RD, Li J, Maltese F Partownavid P, et al. (2011) Intralipid Prevents and Rescues Fatal Pulmonary Arterial Hypertension and Right Ventricular Failure in Rats. Hypertension 58 (3): 512-518.

# Chapter 11

## Intralipid-Iodine for Imaging

## Field of the Invention

The present invention relates to the field of imaging using intralipid-based compounds. Specifically, the present invention relates to intralipid-iodine compounds and compositions for use in imaging, such as hysterosalpingography in infertile women, X-ray imaging with contrast materials.

## Summary of the Invention

The present invention provides a novel approach for imaging procedures that utilizes contrast materials, such as hysterosalpingography in infertile women, X-ray imaging, cardiac- and limbs-catheterization. Specifically, the present invention provides a multidisciplinary and multipotent intralipid-iodine compounds and compositions for use in imaging that utilizes contrast materials.

## Detailed Description of the Invention

Intravenous lipid emulsions have been used experimentally since at least the 19th century. An early product marketed in 1957 under the name Lipomul was briefly used in the United States but was subsequently withdrawn due to side effects. Intralipid was invented by the Swedish physician and nutrition researcher Arvid Wretlind, and was approved for clinical use in Sweden in 1962 [1]. In the United States, the Food and Drug Administration initially declined to approve the product due to prior experience with another fat emulsion. It was approved in the United States in 1972. Tubal disease is the cause of female infertility in approximately 30% of women [2] and 10-25% of these are due to proximal tubal obstruction [3]. Selective salpingography represent an approach in the diagnosis and treatment of proximal tubal abnormalities. Intralipid is a synthetic product composed of 10 % soybean oil, 1,2 % egg yolk phospholipids, 2.25 % glycerin, and water. When indicated, intralipid is infused 7-10 days prior to embryo transfer. Rates of ongoing pregnancy and live births were higher among women who underwent hysterosalpingography (HSG) with oil contrast than among women who underwent this procedure with water contrast.

It is the first time that intralipid-iodine is suggested to be used for HSG in infertile women. The common indications for selective salpingography are to differentiate spasm from true obstruction [4]. In addition to that, it allows clarifying findings from an equivocal hysterosalpingogram. Isthmic as well as intamural blockages were included. The tubal obstruction may be due to amorphous materials occluding the tubal lumen, inflammatory changes and adhesions [5]. The use of selective salpingography and fallopian tube recanalization has revolutionized the diagnosis and treatment of infertility. Diagnostic procedure has been used since 1980 [4]. It consists on opacification of the fallopian tube directly through a catheter placed in the tubal ostium. The objective is to differentiate spasm from true obstruction and to clear it with a catheter and guide wire system. Pregnancy rates among infertile women have been reported to increase after HSG, but it is unclear whether the type of contrast medium used (oil-based or water-soluble contrast) influences this potential therapeutic effect. Dreyer et al. [6] performed a multicenter, randomized trial in 27 hospitals in the Netherlands in which infertile women who were undergoing HSG were randomly assigned to undergo this procedure with the use of oil-based or water-based contrast. Subsequently, couples received expectant management or the women underwent intrauterine insemination. The primary outcome was ongoing pregnancy within 6 months after randomization. Outcomes were analyzed according to the intention-to-treat principle. A total of 1119 women were randomly assigned to HSG with oil contrast (557 women) or water contrast (562 women). A total of 220 of 554 women in the oil group (39.7%) and 161 of 554 women in the water group (29.1%) had an ongoing pregnancy (rate ratio, 1.37; 95% confidence interval, 1.16 to 1.61; P<0.001), and 214 of 552 women in the oil group (38.8%) and 155 of 552 women in the water group (28.1%) had live births (rate ratio, 1.38; 95% CI, 1.17 to 1.64; P<0.001). Rates of adverse events were low and similar in the two groups. Rates of ongoing pregnancy and live births were higher among women who underwent HSG with oil contrast than among women who underwent this procedure with water contrast. HSG, which should be done in the follicular phase of the cycle, evaluates the contour of the uterine cavity, cervical canal, and tubal lumina. Other than being diagnostic, it can prove to be therapeutic. The instrument used to introduce the radio-opaque medium should be chosen to give the least discomfort and to cause no leakage of dye from the cervix. Water-soluble medium is usually used rather than an oil-based medium. Fluoroscopy with image intensification gives the best results. Insufficient dye injection will give an incomplete study. Too much dye injection, especially under pressure, might cause extravasation of the dye into the vascular system or conceal the fimbrial ends of the tubes [7]. To determine whether hysterosalpingography (HSG) increases the conception rate and to compare the therapeutic effectiveness of oil and water soluble contrast media, the histories of 744 women who attended the Yale Infertility Clinic in 1965-69 were reviewed. Exclusion of women who had been infertile less than 1 year before coming to the clinic, in whom a HSG was done outside the clinic, and in whom there was no follow-up resulted in a study population of 460. The pregnancy rate for the study group (49%) was identical to that for the larger population. The study group was divided into women who became pregnant during their clinic attendance or within 1 year of terminating clinic care and women who did not become pregnant in this period. These 2 categories were then subdivided according to whether a HSG was done. To determine the relative effectiveness of different media, the conception rate following HSG in the Yale Clinic, where the iodized oil Ethiodol was used, was compared with that among 63 women who had HSG done by a private practitioner with the water soluble dye Salpix. 221 women were in the Ethiodol HSG group, 121 (55%) of whom conceived. Of the

239 Yale Clinic patients who did not have HSG, 103 (43%) became pregnant. Exclusion of couples with organic factors that could account for infertility resulted in a pregnancy rate of 58% for the HSG group and 47% for the non-HSG group. 25 (40%) of the 63 women in the Salpix group conceived, but exclusion of couples with organic factors lowered the pregnancy rate to 38% in this group. The average length of infertility was 0.5 years longer in the non-HSG group than in the Ethiodol HSG group, but shorter in the Salpix HSG group compared to the Ethiodol group. These results suggest that Ethiodol HSG may enhance fertility. Although the oil media has been criticized on the basis that it may cause granuloma formation or embolization, documented complications in the Yale series were rare. In contrast to earlier studies, unilateral nonpatency on x-ray was not found to affect eventual conception (58% pregnancy rate among women in the Ethiodol HSG group with normal tubes compared with 50% in those with filling of only 1 tube) [8].

Previous studies have suggested increased fertility rates following HSG using oil as compared with aqueous contrast medium. To compare the possible fertility-enhancing effects of two agents used for HSG, this prospective randomized study evaluated the subsequent fertility rates in 121 patients who underwent HSG, in which either oil or aqueous contrast medium was used. After random assignment to either agent, patients were observed for four menstrual cycles after HSG without resorting to any therapy other than clomiphene citrate where indicated. The pregnancy rates for the four cycles after HSG were compared with chi-square analysis in the total study and in the diagnostic subgroups. The subgroup with infertility of unknown cause had a significantly higher pregnancy rate after HSG with oil than after HSG with aqueous contrast medium. No significant difference was seen for any other subgroup or for the overall cohort [9]. In a prospective randomized study, the number of pregnancies after HSG was estimated in 398 patients who had been infertile for longer than 1 year. Iohexol was used in 101 patients, ioxaglate in 102 patients, diatrizoate meglumine in 97 patients, and ethiodized poppy-seed oil in 98 patients. Ten months after HSG, the patient, referring physician, and/or hospital department was consulted for information about pregnancies. Questionnaires were obtained from the patients who became pregnant during the waiting period of 3 months. No differences in demographic parameters, infertility status, or diagnosis made with HSG were detected among the four contrast media groups. Significantly more patients became pregnant after HSG in the ethiodized poppy-seed oil group than in the three water-soluble contrast media groups ($P<0.01$). When only intrauterine pregnancies resulting in full-term births were considered, significant differences in pregnancy rates between the oil-soluble and the water-soluble contrast media groups became more obvious. In the group that received ethiodized poppy-seed oil, almost one-third of the infertile women had normal pregnancies and childbirths after HSG [10].

Meta-analysis of four randomized clinical trials (RCTs) and six nonrandomized controlled studies evaluated pregnancy rates after the use of oil- or water-soluble contrast media during HSG. Four identified RCTs studied 800 patients and six nonrandomized studies comprised an additional 1,806 patients, all experiencing primary or secondary infertility. Pregnancy rates were significantly higher in the oil-soluble contrast media group compared with the water-soluble contrast media group in the RCTs. Inclusion of the six nonrandomized studies did not alter this conclusion. This apparent benefit was greatest for patients with unexplained infertility. Oil-soluble contrast media have a therapeutic effect compared with water-soluble media and this effect is greatest for patients who have been diagnosed as having unexplained infertility. New techniques for the evaluation of tubal patency support the hypothesis that tubal “plugs” may be involved in proximal tubal blockage [11].

Ninety-three patients with unilateral or bilateral proximal tubal occlusion confirmed by HSG or laparoscopy underwent FTR with use of water-soluble contrast material alone (n = 50) or also had an oil-based agent injected into each tube after recanalization (n = 43). Pregnancy rates and outcomes of the two groups were studied retrospectively. With respect to differences between groups, only the body mass index proved to be a significant predictor (oil, 28.4; water, 24.7; P = 0.008). Mean age, duration of infertility, type of infertility, and initial diagnosis were comparable. There was a weak trend toward a higher pregnancy rate in the oil-based contrast material group, but it was not significant (P = 0.64). The average time to pregnancy was 4.4 months with use of oil-based contrast material, compared to 7.7 months with use of only water-soluble contrast material (P = 0.03). The use of an oil-based agent had little effect on the rate of conception, but time to conception was reduced by more than 3 months [12]. HSG is used commonly in the evaluation of infertility and in the diagnosis of anomalies of the uterus and fallopian tubes. There is continued debate over the safety and diagnostic or therapeutic efficacy of water-soluble versus oil-based contrast media.

A 29-year-old woman with secondary infertility underwent HSG with both water-soluble and oil-based contrast. The fallopian tubes appeared normal. Six months later, a plain abdominal radiograph obtained at the occasion of a minor motor vehicle accident revealed evidence of retained loculated pelvic contrast material. Subsequent laparoscopy identified adhesions and cul-de-sac implants strongly suspicious for endometriosis. Biopsy and pathologic study documented lipogranuloma. Oil-based contrast media instilled into the pelvis at HSG can persist for prolonged periods and create granulomatous lesions mimicking endometriosis. In view of the controversy whether oil-based contrast materials are superior to water-soluble media, the routine use of oil-based contrast media should be considered carefully [13]. HSG has assumed a diagnostic and possibly therapeutic role in the evaluation of the infertile couple. The procedure is done using either an oil-based (OBCM) or a water-based (WBCM) contrast medium. Data from several retrospective studies suggest that higher pregnancy rates

may be achieved when OBCM is used. Interpretation of these results, however, may be confounded by various methodologic flaws in study design and comparisons of heterogeneous populations. Letterie and Rose [14] sought to compare the therapeutic benefit of OBCM and WBCM in a prospective randomized study of infertile patients, controlling for pelvic anatomy by laparoscopic assessment. They used ethiodized oil (Ethiodol) or iothalamate meglumine (Conray 60) for tubal lavage at the time of laparoscopy only in patients with normal pelvic anatomy. Of the 225 patients who had diagnostic laparoscopy in the evaluation of infertility, 40 (18%) had normal pelvic anatomy and an otherwise unremarkable evaluation. Adequate follow-up was available on 29 patients randomized to receive either OBCM (n = 15) or WBCM (n = 14). A significant difference in pregnancy rates was noted between OBCM (40%) and WBCM (14%) by chi-square analysis. No short- or long-term adverse reactions were noted. Results of this study suggest that in patients with normal pelvic anatomy as assessed laparoscopically, OBCM may offer a therapeutic benefit not evident with WBCM.

Moore et al. [15] evaluated the effect of different iodinated contrast agents on the fallopian tube and adnexal tissue in 15 rabbits. Ethiodized oil, an oil-soluble agent, was used in five rabbits. The following water-soluble agents were used: iothalamate meglumine 30% (n = 3), iothalamate meglumine 60% (n = 3), and ioxilan (n = 4). The agents were injected through catheters placed in the fallopian tubes. Fallopian tubes and peritoneal cavities were histologically evaluated. The contralateral tube served as a control. Ioxilan and iothalamate meglumine 30% produced no pathologic response in the tube or peritoneal cavity. Iothalamate meglumine 60% was associated with mild inflammatory infiltrate, mucosal edema, giant cell reaction, and periovarian adhesions that were bilateral but more pronounced on the injected side. Use of ethiodized oil resulted in papillary fibrous adhesions on the ovarian surface, and fat granulomas were seen in the periovarian tissues. The safety of oil-based contrast agents for use in HSG is therefore questioned. No significant differences were found among the water-soluble contrast agents.

HSG can be accomplished with either oil or water-soluble contrast medium. This randomized prospective study compared pregnancy rates in women who had HSG with either water- or oil-soluble contrast material and were followed for six months. 15 of 60 (25%) patients who received water-soluble dye conceived compared with 14 of 46 (30%) patients in the oil-soluble group, a statistically insignificant difference. Furthermore, no difference in pregnancy rates within each subgroup of fertility diagnosis was detected. Intravasation was more common in patients administered oil-based contrast materials (6 of 46 versus 1 of 60 patients, P = 0.02), although no serious consequences occurred. No difference in the amount of pain as assessed by pain scoring was experienced by patients in each group. The authors conclude that pregnancy rates are similar after HSG with oil- and water-soluble contrast material, during at least the first six months after the procedure [16].

Aspects of the immunological relationship between mother and conceptus still remain a mystery, although the recent advances in molecular biology have enlightened some of the parameters that participate in feto-maternal cross-talk during implantation [17]. The atypical expression of major histocompatibility complex, the specific roles of some hormones and cytokines, as well as the temporal and spatial distributions of uterine natural killer cells, represent substantive parameters of feto-maternal immunotolerance during implantation [18]. Although human maternal and fetal immunology is difficult to investigate, aberrant immune responses and an imbalanced cytokine network may be related to infertility, implantation failures after IVF, and recurrent pregnancy losses (Makrigiannakis et al., 2011). Patients with recurrent implantation failure (RIF) should be tested for inherited and acquired thrombophilias. Each patient should be individually assessed and counseled regarding management with low-molecular-weight heparin (LMWH). Empirical treatment with LMWH, aspirin, or corticosteroids is not effective for women with RIF who have negative thrombophilic tests [20]. If thrombophilic tests are normal, patients should be tested for immunological causes. The findings of a recent study suggest that increases in the percentage of CD56(dim) cells and NK cytotoxicity in peripheral blood may be important contributing factors for both RSA and IVF failure [21]. Human leukocyte antigen (HLA)-DQA1*0505 sharing or the maternal killer immunoglobulin-like receptor (KIR) repertoire is associated with recurrent spontaneous abortion (RSA) or repeated implantation failure (RIF) [22] and if abnormal, the patient might then benefit from intravenous immunoglobulin (IVIg) therapy [22]. IVIg has been successful in the treatment of recurrent miscarriage and recurrent implantation failure among women with elevated anti-phospholipid antibodies (APA) and/or NK cell activity [23]. When the pregnancy outcomes of women with a history of reproductive failure and elevated NK cell cytotoxicity treated with intralipid were compared with women treated with IVIg, no differences were seen. Side-by-side comparison showed that synthetic pre-implantation factor (sPIF) is equally effective to inhibit NK cell toxicity at a lower dose than intravenous gamma immunoglobulin or intralipid treatment currently used [24]. sPIF is not yet available commercially, but intralipid infusions are available globally. Intralipid is a synthetic product composed of 10 % soybean oil, 1,2 % egg yolk phospholipids, 2.25 % glycerin, and water. When indicated, IL is infused 7-10 days prior to embryo transfer, and one more time again after a positive pregnancy in women whose NKa is due to an autoimmune causes (antiphospholipid antibodies and/or antithyroid antibodies) (CARE Fertility Forum Index). In cases of alloimmune implantation dysfunction (DQa and/HLA matching between the embryo recipient and the male partner), the same applies, but in this situation, the infusion is repeated at 2-4 week intervals until the 24th week of pregnancy. IL costs about 10 times less than IVIg, is not a blood product, and is without significant side effects (CARE Fertility Forum Index).

## Conclusion

It is the first time that intralipid-iodine is suggested to be used for HSG in infertile women.

## References


1. Hallberg D, Holm I, Obel AL, Schuberth O, Wretlind A, et al. (1967) Fat emulsions for complete intravenous nutrition. Postgrad Med J 43(498): 307-316.
2. Juras J et al. (2002) Imaging in tubal infertility. Rev Prat 2: 1768-1774.
3. Elstein M (2008) Tubal disease and fertility outcome. Reprod Biomed Online 16(2): 167-169.
4. Thurmond AS, Machan LS, Maubon AJ, Rouanet JP, Hovsepian DM, et al. (2000) A Review of Selective Salpingography and Fallopian Tube Catheterization. Radio Graphics 20(6): 1759-1768.
5. Papaioannou S, Afnan M, Girling AJ, Ola B, Olufowobi O, et al. (2003) Diagnostic and therapeutic value of selective salpingography and tubal catheterization in an unselected infertile population. Fertil Steril 79(3): 613-617.
6. Dreyer K, Joukje van Rijswijk, Velja Mijatovic, Mariëtte Goddijn, Harold R. Verhoeve, et al. (2017) Oil-Based or Water-Based Contrast for Hysterosalpingography in Infertile Women. 376(21): 2043-2052.
7. Baramki TA (2005) Hysterosalpingography. Fertil Steril 83(6): 1595-15606.
8. Mackey RA, Glass RH, Olson LE, Vaidya R (1971) Pregnancy following hysterosalpingography with oil and water soluble dye. Fertil Steril 22(8): 504-507.
9. Schwabe MG, Shapiro SS, Haning RV (1983) Hysterosalpingography with oil contrast medium enhances fertility in patients with infertility of unknown etiology. Fertil Steril 40(5): 604-606.
10. Rasmussen F, Lindequist S, Larsen C, Justesen P (1991) Therapeutic effect of hysterosalpingography: oil- versus water-soluble contrast media--a randomized prospective study. Radiology 179(10): 75-78.
11. Watson A, Vandekerckhove P, Lilford R, Vail A, Brosens I, et al. (1994) A meta-analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result? Fertil Steril 61(3): 470-477.
12. Pinto AB, Hovsepian DM, Wattanakumtornkul S, Pilgram TK (2003) Pregnancy outcomes after fallopian tube recanalization: oil-based versus water-soluble contrast agents, J Vasc Interv Radiol 14(1): 69-74.
13. Grosskinsky CM, Clark RL, Wilson PA, Novotny DB (1994) Pelvic granulomata mimicking endometriosis following the administration of oil-based contrast media for Hysterosalpingography. Obstet Gynecol 83(5 Pt 2): 890-892.
14. Letterie GS, Rose GS (1990) Pregnancy rates after the use of oil-based and water-based contrast media to evaluate tubal patency. South Med J 83(120): 1402-1403.
15. Moore DE, Segars JH Jr, Winfield AC, Page DL, Eisenberg AD, et al. (1990) Effects of contrast agents on the fallopian tube in a rabbit model. Radiology 176(3): 721-724.
16. Alper MM, Garner PR, Spence JE, Quarrington AM (1986) Pregnancy rates after hysterosalpingography with oil- and water-soluble contrast media. Obstet Gynecol 68(1): 6-9.
17. Gautam N Allahbadia (2015) Intralipid Infusion is the Current Favorite of Gynecologists for Immunotherapy. J Obstet Gynaecol India 65(4): 213-217.
18. Oreshkova T, Dimitrov R, Mourdjeva M (2012) A cross-talk of decidual stromal cells, trophoblast, and immune cells: a prerequisite for the success of pregnancy. Am J Reprod Immunol 68(5): 366-373.
19. Makrigiannakis A, Petsas G, Toth B, Relakis K, Jeschke U, et al. (2011) Recent advances in understanding immunology of reproductive failure. J Reprod Immunol 90(1): 96-104.
20. Fatemi H, Popovic-Todorovic B (2013) Implantation in assisted reproduction: a look at endometrial receptivity. Reprod Biomed Online 27(5): 530-538.
21. Karami N, Boroujerdnia MG, Nikbakht R, Khodadadi A (2012) Enhancement of peripheral blood CD56(dim) cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure. J Reprod Immunol 95(1-2): 87-92.
22. Varla-Leftherioti M, Keramitsoglou T, Parapanissiou E, Kurpisz M, Kontopoulou-Antonopoulou V, et al., (2010) HLA-DQA1*0505 sharing and killer immunoglobulin-like receptors in sub fertilecouples: report from the 15th International Histocompatibility Workshop. Tissue Antigens 75(6): 668-672.
23. Coulam CB, Acacio B (2012) Does immunotherapy for treatment of reproductive failure enhance live births? Am J Reprod Immunol 67(40): 296-304.
24. Roussev RG, Dons'koi BV, Stamatkin C, Ramu S, Chernyshov VP, et al. (2013) Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy, Reprod Biomed Online 26(1):79-87.
25. CARE Fertility Forum Index. Intralipids- All you need to know. http://www.carefertility.com/ivf/viewtopic.php?t=39200.

# Chapter 12

## Intralipid use in Trauma

## Abstract

Hemorrhage is responsible for most deaths that occur during the first few hours after trauma. Current evidence indicates that initial liberal fluid resuscitation strategies may be associated with higher mortality in injured patients. In the prehospital arena, intravenous fluids have been associated with worse patient outcomes due to increased coagulopathy and time to definitive care. Once in the trauma bay, damage control resuscitation principles apply to the severely injured patient. Large volume crystalloid infusion increases mortality. The best patient outcomes have been found with transfusion of blood products in a ratio that closely mimics whole blood. Intralipid increased renal blood flow, carotid vascular resistance and mesenteric vascular resistance. In the presence of intralipid, L-NMA-induced pressor response and systemic, carotid and renal vasoconstriction were more pronounced than in control dogs. Except for the coronary and carotid circulations, intralipid modulates the NO pathway in cardiac and regional blood flow. Intralipid infusion in trauma patients is first suggested in the medical literature.

**Keywords:** Intralipid, Trauma

## Introduction

The use of intralipid therapy has been gaining traction as a treatment option for an ever expanding range of toxicities. Veterinary literature has reviewed intravenous lipid emulsion therapy (ILE) [1,2] and published case reports or studies are available noting efficacy in toxicities including macrocyclic lactones [3,4], baclofen [5], beta-blockers, calcium channel blockers [6], NSAID [7,8], bromethalin [9], lidocaine [10], permethrin toxicity [11,12], tricyclic antidepressants [13]. Intravenous lipid emulsion (ILE) in human literature has been reported as a therapy for local anesthetic [14,15] calcium channel blocker [16,17], psychotropic medication [18] , glyphosatesurfactant herbicide toxicities [19] and even cocaine overdosage [20]. Original work performed by Weinberg noted a response in rats with bupivacaine induced asystole with lipid emulsion [21]. How exactly ILE works is not certain but two theories are considered.

The “lipid sink” theory is most commonly considered the primary mode of action. In this theory, the formation of a lipid compartment within the intravascular space can serve as a “sink” into which the lipophilic drug will be drawn into. The drug is then excreted/metabolized. Determination of a drug’s lipophilicity may be noted by its log P value. A value >1 indicates lipophilic compound which may move into the temporary lipid phase and be less distributed throughout the body. The formulation of ILE utilized may play a role and supports the “lipid-sink” theory based on one study [22]. This theory has been supported in two case reports that followed plasma ropivacaine [15] and serum verapamil concentrations [17].

An alternate theory is that the lipid provides an energy source for the cardiac myocytes by increasing the availability of FFA. The increase of FFA may also aid in increasing the activation of voltage-gated calcium channels in the myocardium, increasing cytosolic calcium channels This mechanism may be most important in cases of calcium-channel blockade [23,24].

## Intralipid Use In Rapid Blood Loss

The following were studied in a perimortem mouse model of rapid blood loss: (a) efficacy of a prototypical micellar colloid, Intralipid 20%, (IL20), compared to albumin (b) comparison of intra-arterial and intravenous resuscitation, (c) efficacy of IL20 at a volume 2 × the volume of blood removed, and (d) efficacy of oxygenated IL20 after clinical death (CD). CD, the absence of breathing and zero blood pressure (BP), was produced by removing 55% of the blood volume within 3 minutes. After CD, the chest was opened to observe ventricular contraction. IL20, Ringer’s lactate (RL), or albumin was infused perimortem. Without resuscitation CD occurred in 2.85 ± 0.40 minutes. Ventricular contraction persisted 20.50 ± 1.11 minutes after CD. RL infused immediately after CD restored breathing if given intra-arterially but not intravenously. IL20 was superior to the prototypical colloid, albumin in maintaining the BP. Increasing the volume of IL20 further increased BP. Delayed RL infusion after CD failed to restore breathing. Delayed resuscitation after CD with oxygenated IL20 restored breathing and BP. Micellar colloid is superior to the prototypical colloid albumin and can possibly be of use when signs of life are no longer present. In extremis, intra-arterial infusion is superior to intravenous infusion [25]. Fat emulsions, Intralipid 30% and Intralipid 10% were compared in terms of the resulting plasma levels of different lipid components and clinical tolerance in critically-ill patients with multi-injuries. Sixteen critically-ill patients with severe systemic inflammatory response were randomly assigned to two groups, each one comprised of eight patients. Each group was administered the same quantity of fat/Kg/day either Intralipid 30% or Intralipid 10%. The infusion lasted 12 h daily for 6 days. During the infusion of the fat emulsion, a lower median plasma concentration of triglycerides, phospolipids and free cholesterol was observed in patients who received Intralipid 30% compared with those who received Intralipid 10%. The above observations were sustained 4 h after the termination of the infusion. Free fatty acids had a higher mean plasma concentration in the group of patients who received Intralipid 30%. There were no differences between the two groups as far as the median plasma concentration of cholesterol and lipoproteins (LDL, HDL, VLDL) are concerned. On the contrary, there was an increase in LpX in the Intralipid 10% group. From the above findings, we draw the conclusion that Intralipid 30% revealed better profiles of different lipid components than Intralipid 10% in critically-ill patients. The new emulsion of higher concentration in triglyceride was proved clinically safe and its use is suggested for critically-ill patients who require total parenteral nutrition [26]. Intralipid modulates the NO pathway in cardiac and regional blood flow The commercial propofol preparation in an intralipid solution causes marked vasodilatation. Both propofol and its solvent seem to stimulate the nitric oxide (NO) pathway. The role of intralipid in cardiac and regional haemodynamic changes induced by propofol and their respective interactions with the NO pathway was assessed.

Dogs were instrumented to record arterial pressure, heart rate, cardiac output, dP/dt (the first derivative of left ventricular pressure) and vertebral, carotid, coronary, mesenteric, hepatic, portal and renal blood flows. Experimental groups were as follows. Group 1 (control; n = 11): N-methyl-L-arginine (L-NMA) 20 mg kg-1 i.v.; Group 2 (n = 8): propofol (10 mg ml-1) 4 mg kg-1 i.v. bolus followed by 0.6 mg kg-1 min-1; Group 3 (n = 6): intralipid 0.25 ml kg-1 bolus followed by 0.06 ml kg-1 min-1. After 60 min, L-NMA was injected in Groups 2 and 3. Propofol induced increases in heart rate, coronary and carotid blood flows, and decreases in systemic vascular resistance and dP/dt. Intralipid increased renal blood flow, carotid vascular resistance and mesenteric vascular resistance. In the presence of intralipid, L-NMA-induced pressor response and systemic, carotid and renal vasoconstriction were more pronounced than in control dogs. Except for the coronary and carotid circulations, intralipid modulates the NO

pathway in cardiac and regional blood flow [27]. Propofol is a commonly used anesthetic. Despite its favourable safety profile, propofol causes hypotension which can result in end-organ hypoperfusion. Intralipid is a lipid emulsion that has been shown to reverse the vasodilatory effects of propofol in isolated vessels; however, whether these effects are recapitulated in vivo is not known. The objectives of this study were to determine if intralipid reverses the hypotensive and anesthetic effects of propofol in rats. Under isoflurane anesthesia, male Sprague Dawley rats were instrumented with indwelling catheters for mean arterial pressure (MAP) assessments as well as subdural electrodes for cortical activity assessments by electroencephalography (EEG). Propofol (10 mg/kg IV) caused hypotension (55±2% drop in MAP, P<0.001) and intralipid (4mL/kg IV) caused greater reversal (80±9%) of blood pressures compared to saline (19±1%; P<0.001). Blockade of the autonomic nervous system with chlorisondamine (2.5 mg/kg IV) caused marked hypotension (56±3% lowering of MAP, P<0.001) which could be reversed with a constant infusion of phenylephrine (300 µg/kg/hr); under these conditions, propofol nevertheless caused hypotension (12±4% lowering of MAP) which was completely reversed by intralipid. Propofol-induced cortical burst suppression was not affected by intralipid (2±3%), saline (-4%) or 20% BSA (-2±1%; P=0.27). These results demonstrate that intralipid reverses propofol-mediated hypotension with minimal effects on its anesthetic profile. Intralipid could be particularly useful as a rescue against propofol in patients prone to hemodynamic instability such as the elderly [28]. Sepsis is one of the most serious complications that can occur during total parenteral nutrition (TPN) procedures. In this experimental study, we investigated the effects of TPN, with or without lipid emulsion, on vascular endothelial damage.

In total, 50 rabbits were used, divided into 5 groups of 10 each. TPN with lipids (group 1), TPN without lipids (group 2), and 0.09% saline (group 3) were given for 10 days via a central venous catheter. Group 4 received no treatment other than placement of a central venous catheter for 10 days. Group 5 was a control group. At the end of day 10, rabbits were sacrificed and tissue samples of liver, kidney, and inferior vena cava were prepared and examined by immunohistochemical methods for vascular cellular adhesion molecule (VCAM)-1 expression. In tissue sections of liver, kidney, and inferior vena cava, VCAM-1 activity was increased prominently in TPN with and without lipids compared with the control group. VCAM-1 activity in the TPN with lipids group was decreased versus the TPN without lipids group (P>0.05). The TPN procedure results in vascular endothelial cell damage not only in the vein where the solution is introduced but also in other parts of the vascular system. Even if it is not statistically significant, lipids in the TPN formula may decrease this endothelial cell damage, as shown by immunohistochemistry [29]. Ischemia-reperfusion injury is a determinant in liver injury occurring during surgical procedures, ischemic states, and multiple organ failure. The pre-existing nutritional status of the liver, i.e., fasting, might contribute to the extent of tissue injury. This study investigated whether Intralipid, a solution containing soybean oil, egg phospholipids, and glycerol, could protect ex vivo perfused livers of fasting rats from anoxia-reoxygenation injury. The portal vein was cannulated, and the liver was removed and perfused in a closed ex vivo system. Isolated livers were perfused with glucose 5.5 and 15 mM, and two different concentrations of Intralipid, i.e., 0.5:100 and 1:100 (v/v) Intralipid 10%:medium (n = 5 in each group). The experiment consisted of perfusion for 15 min, warm anoxia for 60 min, and reoxygenation during 60 min. Hepatic enzymes, potassium, glucose, lactate, bilirubin, dienes, trienes, and cytochrome-c were analyzed in perfusate samples. The proportion of glycogen in hepatocytes was determined in biopsies. Intralipid attenuated transaminases, lactate dehydrogenase, potassium, diene, and triene release in the perfusate (dose-dependant) during the reoxygenation phase when compared with glucose-treated groups. The concentration of cytochrome-c in the medium was the highest in the 5.5-mM glucose group. The glycogen content was low in all livers at the start of the experiment. Intralipid presents, under the present experimental conditions, a better protective effect than glucose in anoxia-reoxygenation injury of the rat liver [30].

## The effect of intralipid on protein consumption in severe burned patients

Rong et al. [31] investigated the effect of intralipid on protein consumption in severe burned patients. Sixty-seven nonoperative patients with severe burns were divided into Intralipid treatment group and non-intralipid treatment group (control group), and the former was treated with 20% intralipid (500 ml once a day) from postburn day 4 for 10 consecutive days. Venous blood samples were collected from these patients for testing total protein, albumin, total cholesterol and triglyceride on postburn days 1, 7 and 14, respectively. The levels of total protein, albumin, total cholesterol and triglyceride were within normal range on postburn day 1 in both groups, and only the albumin level was lowered in the groups on day 7 but at comparable magnitudes (32+/- 4.83 vs 31+/-5.04 g/L, P<0.05). In contrast, the levels of total protein, albumin, total cholesterol and triglyceride were below the normal range on postburn day 14 in both groups, but intralipid treatment group showed more albumin loss than the control group (28+/-6.46 vs 23+/-7.03 g/L, P<0.01). Intralipid (20%) provides good energy source to ameliorate albumin loss in severe burned patients [31]. A concentrated fat emulsion (Intralipid 30%) with a phospholipid/triglyceride ratio of 0.04 was tested for clinical tolerance and metabolic effects in the short-term parenteral nutrition of septic and trauma critically ill patients and compared with Intralipid 20% (phospholipid/triglyceride ratio of 0.06). This was a prospective, randomized, multicenter study in the intensive care units in 10 university hospitals, including 90 adult patients in 2 groups: 55 septic and 35 trauma patients. Patients in each

group were randomly divided into 2 subgroups according to the fat emulsions administered (1.4 g/kg per day) as part of the calories for at least 6 days of continuous total parenteral nutrition (TPN). One subgroup was treated with 30% long-chain triglycerides (phospholipid/ triglyceride ratio: 0.04) and the other with 20% long-chain triglycerides (phospholipid/ triglyceride ratio: 0.06). The parenteral nutrition formula was isocaloric and isonitrogenous with 0.25 g of nitrogen/kg per day and 40% of the nonprotein calories as fat. Clinical tolerance was assessed during the study. At baseline and after 3 and 6 days of TPN, the following biochemical parameters were measured: prealbumin, retinol-binding protein, serum albumin, hematologic, hepatic and renal function variables, triglycerides, phospholipids, total and free cholesterol, nonesterified cholesterol, nonesterified fatty acids, and lipoproteins. At baseline, no differences in age, gender, severity of the condition [Acute Physiology and Chronic Health Evaluation (APACHE II) score], or clinical chemistry were found between the subgroups. The levels of plasma proteins studied and the renal, hematologic, or hepatic function variables did not vary during the study period. Total cholesterol increased significantly, owing to esterified cholesterol, with 20% long-chain triglyceride in septic patients (baseline: 2.1 +/- 0.8 mmol/L, day 6: 2.8 +/- 0.6 mmol/L, p = .026). In septic patients receiving 20% long-chain triglycerides, plasma triglycerides had a similar behavior (baseline: 1.4 +/- 0.6 mmol/L, day 3: 2.2 +/- 0.8 mmol/L, p < .05). The very-low-density lipoprotein content of cholesterol, triglycerides, and phospholipids showed a tendency to decrease in septic patients treated with 30% long-chain triglycerides (NS). None of the emulsions induced the synthesis of lipoprotein X. The results indicate that while both fat emulsions used in the TPN of critically ill patients are clinically safe, the 30% long-chain triglyceride fat emulsion with a phospholipid/triglyceride ratio of 0.04 causes fewer lipid metabolic disturbances [32].

A 4-year-old boy with an abdominal trauma had lipiduria following treatment with total intravenous nutrition for 4 days. Renal function was normal throughout the course and the lipiduria ceased after withdrawal of the intravenous nutrition. The lipids were possibly excreted through the kidneys or entered the urine through a traumatic communication between the lymphatic vessels and the urinary system. Control of renal function and lipiduria after 1 year revealed normal conditions [33].

## Current fluid resuscitation and the vasopressor support in severe trauma patients

Harrois et al. [34] discuss the fluid resuscitation and the vasopressor support in severe trauma patients. A critical point is to prevent a potential increase in bleeding by an overly aggressive resuscitative strategy. Indeed, large-volume fluid replacement may promote coagulopathy by diluting coagulation factors. Moreover, an excessive level of mean arterial pressure may induce bleeding by preventing clot formation.

Fluid resuscitation is the first-line therapy to restore intravascular volume and to prevent cardiac arrest. Thus, fluid resuscitation before bleeding control must be limited to the bare minimum to maintain arterial pressure to minimize dilution of coagulation factors and complications of over fluid resuscitation. However, a strategy of low fluid resuscitation needs to be handled in a flexible way and to be balanced considering the severity of the hemorrhage and the transport time. A target systolic arterial pressure of 80-90 mmHg is recommended until the control of hemorrhage in trauma patients without brain injury. In addition to fluid resuscitation, early vasopressor support may be required to restore arterial pressure and prevent excessive fluid resuscitation. It is crucial to find the best alchemy between fluid resuscitation and vasopressors, to consider hemodynamic monitoring and to establish trauma resuscitative protocols [34]. The ideal strategy for prehospital intravenous fluid resuscitation in trauma remains unclear. Fluid resuscitation may reverse shock but aggravate bleeding by raising blood pressure and haemodilution. We examined the effect of prehospital i.v. fluid on the physiologic status and need for blood transfusion in hypotensive trauma patients after their arrival in the emergency department (ED). Retrospective analysis of trauma patients (n=941) with field hypotension presenting to a level 1 trauma centre. Regression models were used to investigate associations between prehospital fluid volumes and shock index and blood transfusion respectively in the emergency department and mortality at 24h. A 1L increase of prehospital i.v. fluid was associated with a 7% decrease of shock index in the emergency department ($p<0.001$). Volumes of 0.5-1L and 1-2L were associated with reduced likelihood of shock as compared to volumes of 0-0.5L: OR 0.61 ($p=0.03$) and OR 0.54 ($p=0.02$), respectively. Volumes of 1-2L were also associated with an increased likelihood of receiving blood transfusion in ED: OR 3.27 ($p<0.001$). Patients who had received volumes of >2L have a much greater likelihood of receiving blood transfusion in ED: OR 9.92 ($p<0.001$). Mortality at 24h was not associated with prehospital i.v. fluids. In hypotensive trauma patients, prehospital i.v. fluids were associated with a reduction of likelihood of shock upon arrival in ED. However, volumes of >1L were associated with a markedly increased likelihood of receiving blood transfusion in ED. Therefore, decision making regarding prehospital i.v. fluid resuscitation is critical and may need to be tailored to the individual situation. Further research is needed to clarify whether a causal relationship exists between prehospital i.v. fluid volume and blood transfusion. Also, prospective trials on prehospital i.v. fluid resuscitation strategies in specific patient subgroups (e.g. traumatic brain injury and concomitant haemorrhage) are warranted [35]. Feinman et al. [36] explore the recent literature regarding the optimal type and amount of intravenous fluids for the trauma patient from the time of injury through their ICU stay. It discusses damage control principles as well as targeted resuscitation utilizing new technology.

In the prehospital arena, intravenous fluids have been associated with worse patient outcomes due to increased coagulopathy and time to definitive care. Once in the trauma bay, damage control resuscitation principles apply to the severely injured patient. Large volume crystalloid infusion

increases mortality. The best patient outcomes have been found with transfusion of blood products in a ratio that closely mimics whole blood. Thrombelastography is a useful adjunct in resuscitation and can help guide the judicious use of blood products. New technology can help providers ascertain when a patient is appropriately resuscitated by determining adequate global and regional perfusion. During the resuscitation of the acutely injured patient, crystalloids should be limited in favor of blood components. Damage control principles apply until definitive hemostasis is obtained, at which point the focus should change to targeted resuscitation using traditional global endpoints of resuscitation in conjunction with determinants of regional perfusion [36]. Hemorrhage is responsible for most deaths that occur during the first few hours after trauma. Animal models of trauma have shown that restricting fluid administration can reduce the risk of death; however, studies in patients are difficult to conduct due to logistical and ethical problems. To maximize the value of the existing evidence, we performed a meta-analysis to compare liberal versus restricted fluid resuscitation strategies in trauma patients. Medline and Embase were systemically searched from inception to February 2013. We selected randomized controlled trials and observational studies that compared different fluid administration strategies in trauma patients. There were no restrictions for language, population, or publication year. Four randomized controlled trials and seven observational studies were identified from 1,106 references. One of the randomized controlled trials suffered from a high protocol violation rate and was excluded from the final analysis. The quantitative synthesis indicated that liberal fluid resuscitation strategies might be associated with higher mortality than restricted fluid strategies, both in randomized controlled trials (risk ratio, 1.25; 95% CI, 1.01-1.55; three trials; I(2), 0) and observational studies (odds ratio, 1.14; 95% CI, 1.01-1.28; seven studies; I(2), 21.4%). When only adjusted odds ratios were pooled for observational studies, odds for mortality with liberal fluid resuscitation strategies increased (odds ratio, 1.19; 95% CI, 1.02-1.38; six studies; I(2), 26.3%). Current evidence indicates that initial liberal fluid resuscitation strategies may be associated with higher mortality in injured patients. However, available studies are subject to a high risk of selection bias and clinical heterogeneity. This result should be interpreted with great caution [37]. Massive transfusion protocol seems to improve outcome in massively bleeding trauma patients, but not pelvic fracture patients. The aim of this study was to evaluate the effect of massive transfusion protocol on the mortality and fluid resuscitation of shocked pelvic fracture patients. This is a trauma register study from a single hospital. From the trauma registry patients with pelvic fracture, injury severity score >15, admission base excess below -5, age >15 years, blunt trauma, and primary admission from the scene were identified. Patients were divided into two groups: Group 1-pre-massive transfusion protocol (2006-2009) and Group 2-post-massive transfusion protocol (2010-2013). Basic characteristics and intensive care unit length of stay, mortality, and fluid resuscitation data were retrieved from the registry. Standardized mortality ratio was assessed using revised injury severity classification, version II methodology. Altogether, 102 patients were identified. Group 1 ( n = 56) and Group 2 ( n = 46) were comparable in their basic characteristics. The observed mortality was 35.7% and 26.1% in Groups 1 and 2, respectively. The standardized mortality ratio failed to reveal any difference between observed and expected mortality in either group. In the emergency room, the use of crystalloids decreased from 5.3 ± 3.4 to 3.3 ± 1.8 L ( p = 0.002) with increased use of fresh frozen plasma (2.9 ± 4.4 vs 5.1 ± 5.3, p = 0.007). No improvement in the adjusted survival of shocked pelvic fracture patients is apparent after implementation of massive transfusion protocol. Implementation of massive transfusion protocol is associated with a higher use of fresh frozen plasma and improved ratio of fresh frozen plasma: red blood cell toward the targeted 1:1 and decreased use of crystalloids [38].

## Conclusion

Intralipid increased renal blood flow, carotid vascular resistance and mesenteric vascular resistance. In the presence of intralipid, L-NMA-induced pressor response and systemic, carotid and renal vasoconstriction were more pronounced than in control dogs. Except for the coronary and carotid circulations, intralipid modulates the NO pathway in cardiac and regional blood flow. Intralipid infusion in trauma patients is first suggested in the medical literature.

## References


1. Fernandez, AL, Lee JA, Rahilly L, Hovda L, et al. (2011) Use of intravenous lipid emulsion as an antidote in veterinary toxicologyJ Vet Emerg Crit Care (San Antonio) 21(4): 309-320.
2. Gwaltney-Brant S, Meadows I (201) Use of intravenous lipid emulsions for treating certain poisoning cases in small animals. Vet Clie North Am Small Animal Pract 42(2): 251-62, vi.
3. Clarke DL, Lee JA, Murphy LA, Reineke EL (2011) Use of intravenous lipid emulsion to treat ivermectin toxicosis in a Border Collie. J Am Vet Med Assoc 239(10): 1328-1333.
4. Epstein SE, Hollingsworth SR (2013) Ivermectin-induced blindness treated with intravenous lips therapy in a dog. J Vet Emerg Crit Care(San Antonio) 23(1): 58-62.
5. Bates N, Chatterton J, Robbins C, Wells K, Hughes J, et al. (2013) Lipid infusion in the management of poisoning: a report of 6 canine cases. Vet Rec 172(13): 339.
6. Maton BL, Simmonds EE, Lee JA, Alwood AJ, et al. (2013) Use of high-dose insulin therapy and intravenous lipid emulsion to treat severe, refractory diltiazem overdose in a dog. J Vet Emerg Care 23(3): 321-327.
7. Herring JM, McMichael MA, Corsi R, Wurlod V (2015) Intravenous lipid emulsion therapy in three cases of canine naproxen overdose. J Vet Emerg Crit Care (San Antonio) 25(5): 672-678.
8. Bolfer L, McMichael M, Ngwenyama TR, O'Brien MA (2014) Treatment of ibuprofen toxicosis in a dog with IV lipid emulsion. J AM Anim Host Assoc 50(2): 136-140.

9. Heggem-Perry B, McMichael M, O'Brien M, Moran C (2016) Intravenous lipid emulsion therapy for bromethalin toxicity in a dog. J Am Anim Hosp Assoc 52(4): 265-268.

10. O'Brien TQ, Clark-Price SC, Evans EE, Di Fazio R, McMichael MA, et al. (2010) Infusion of a lipid emulsion to treat lidocaine intoxication in a cat. J Am Vet Med Assoc 237(12): 1455-1458.

11. Peacock R, Hosgood G, Swindells KL, Smart L (2015) Randomized, controlled clinical trial of intravenous lipid emulsion as an adjunctive treatment for permethrin toxicosis in cats. J Vet Emerg Crit Care 25(5): 597-605.

12. Seitz MA, Burkitt-Creedon JM (2016) Persistent gross lipemia and suspected corneal lipidosis following intravenous lipid therapy in a cat with permethrin toxicosis.J Vet Emerg Crit Care 26(6): 804-808.

13. Cave G, Harvey M, Shaw T, Damitz R, Chauhan A (2013) Comparison of intravenous lipid emulsion, bicarbonate, and tailored liposomes in rabbit clomipramine toxicity. Acad Emerg Med 20(10): 1076-1079.

14. Litz RJ, Roessel T, Heller AR, Stehr SN (2008) Reversal of central nervous system and cardiac toxicity after local anesthetic intoxication by lipid emulsion injection. Anesth Analg 106(5): 1575-1577.

15. Mizutani K, Oda Y, Sato H (2011) Successful treatment of ropivacaine induced central nervous system toxicity by use of lipid emulsion: effect on total and unbound plasma fractions. J Anesth 25(3): 442-445.

16. Young AC, Velez LI, Kleinschmidt KC (2009) Intravenous fat emulsion therapy for intentional sustained-release verapamil overdose. Resuscitation 80(5): 591-593.

17. French D, Armenian P, Ryan W, Wong A, Drasner K, et al. (2011) Serum verapamil concentrations before and after Intralipid therapy during treatment of overdose. Clin Toxicol (Phila) 49(4): 340-344.

18. Nair F, Paul FK, Protopapas M (2013) Management of near fatal mixed tricyclic antidepressant and selective serotonin reuptake inhibitor overdose with Intralipid 20% emulsion. Aneasth Intensive Care 41(2): 264-265.

19. Han SK, Jeong J, Yeom S, Ryu J, Park S (2010) Use of a lipid emulsion in a patient with refractory hypotension caused by glyphosate-surfactant herbicide. Clin Toxicol (Phila) 48(6): 566-568.

20. Jakkala-Saibaba R, Morgan PG, Morton GL (2011) Treatment of cocaine overdose with lipid emulsion. Anaesthesia 66(12): 1168-1170.

21. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ (1998) Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivicaine-induced asystole in rats. Anesthesiology 88(4): 1071-1075.

22. Mazoit JX, Le Guen R, Beloeil H, Benhamou D (2009) Binding of long-lasting local anesthetics to lipid emulsions. Anesthesiology 110(2): 380-386.

23. Turner-Lawrence DE, Kerns WI (2008) Intravenous fat emulsion: a potential novel antidote. J Med Toxicol 4(2): 109-114.

24. Rothschild L, Bern S, Oswald S, Weinberg G (2010) Intravenous lipid emulsion in clinical toxicology. Scan J Trauma Resusc Emerg Med 18: 51.

25. Simpkins C, Talluri K, Williams M (2016) Intra-Arterial Perimortem Resuscitation Using a Micellar Colloid. Mil Med 181(5 Suppl): 253-258.

26. Kalfarentzos F, Kokkinis K, Leukaditi K, Maroulis J, Onoufriou A, et al. (1998) Comparison between two fat emulsions: Intralipid 30 cent vs intralipid 10 cent in critically ill patients. Clin Nutr 17(1): 31-34.

27. Doursout MF, Joseph PM, Liang YY, Hartley CJ, Chelly JE (2002) Role of propofol and its solvent, intralipid, in nitric oxide-induced peripheral vasodilatation in dogs. Br J Anaesth 89(3): 492-498.

28. Bourque A, Panahi S, Mittal R, Moher C, Gragasin F (2015) Intralipid Reverses Propofol-Mediated Hypotension in Rats. The FASEB Journal 29(1 Supplement624): 3.

29. Demircan M, Gurunluoglu K, Karaman A, Mizrak B (2015) Damaging Effects of Total Parenteral Nutrition Formula on Vascular Endothelium. J Pediatr Gastroenterol Nutr 61(4): 464-468.

30. Stadler M, Nuyens V, Boogaerts JG (2007) Intralipid minimizes hepatocytes injury after anoxia-reoxygenation in an ex vivo rat liver model. Nutrition 23(1): 53-61.

31. Rong XZ, Zhang T, Li QH, Yang RH, Hua R, Huang XH (2006) Effect of intralipid for ameliorating protein loss in severe burned patients. Nan Fang Yi Ke Da Xue Xue Bao 26(4): 500-501.

32. García-de-Lorenzo A, López-Martínez J, Planas M, Chacón P, Montejo JC, et al. (2003) Safety and metabolic tolerance of a concentrated long-chain triglyceride lipid emulsion in critically ill septic and trauma patients. JPEN J Parenter Enteral Nutr 27(3): 208-215.

33. Nielsen HK, Lybecker H (1991) Lipiduria after intralipid infusion of a lipid emulsion in a boy with an abdominal trauma. JPEN J Parenter Enteral Nutr 15(5): 572-573.

34. Harrois A, Hamada SR, Duranteau J (2014) Fluid resuscitation and vasopressors in severe trauma patients. Curr Opin Crit Care 20(6): 632-637.

35. Geeraedts LM, Pothof LA, Caldwell E, de Lange-de Klerk ES, D'Amours SK (2015) Prehospital fluid resuscitation in hypotensive trauma patients: do we need a tailored approach? Injury 46(1): 4-9.

36. Feinman M, Cotton BA, Haut ER (2014) Optimal fluid resuscitation in trauma: type, timing, and total. Curr Opin Crit Care 20(4): 366-372.

37. Wang CH, Hsieh WH, Chou HC, Huang YS, Shen JH, et al. (2014) Liberal versus restricted fluid resuscitation strategies in trauma patients: a systematic review and meta-analysis of randomized controlled trials and observational studies. Crit Care Med 42(4): 954-961.

38. Söderlund T, Ketonen T, Handolin L (2017) Bleeding Pelvic Fracture Patients: Evolution of Resuscitation Protocols. Scand J Surg 106(3): 255-260.