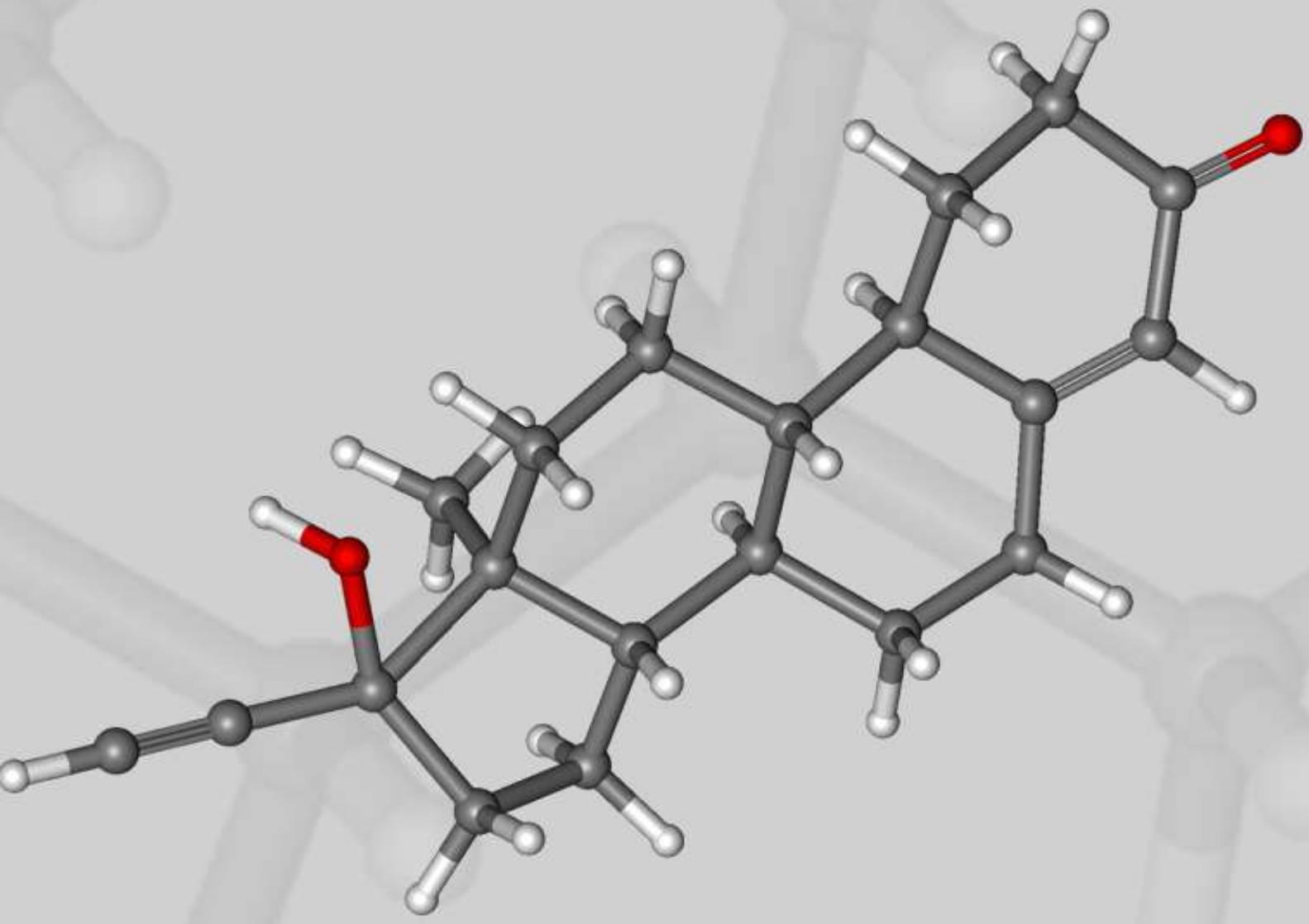
Norethindrone Acetate:
Pharmacokinetics, Potency and Alternative Clinical Applications



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Norethindrone Acetate: Pharmacokinetics, Potency and Alternative Clinical Applications

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Contents

Abstract

Therapeutic uses of norethindrone (NE) and norethindrone acetate (NEA) have been longstanding and widely accepted. While primary indications for NE and NEA remain oral contraception and hormone replacement therapy (HRT), clinical research has shown the compounds to be effective in treating a variety of gynecological disorders. Because of its wide-range of effects, clinical and mechanistic research has focused on elucidating the physiological effects and therapeutic potential of NE and NEA. This manuscript provides a cumulative prospective on the pharmacokinetics, potency, mechanisms of action, and alternative clinical uses of NE and NEA through a review of literature starting from NE's use in the late 1960s to the present day. A literature search was conducted using PubMed and Google Scholar. Once manuscripts were selected and reviewed, additional sources were identified using the selected articles' references. The most current articles were given priority. This review clarifies current understanding of NE and NEA and demonstrates the benefits and drawbacks of NE and NEA treatment for a wide variety of pathology and symptomatology. Furthermore, the paper identifies gaps in our molecular understanding of NE's mechanisms of action, which is important for possible treatment application and to evaluate side effect profiles in specific patients.

Keywords: **Norethindrone acetate; Norethidnrone; Progesterone; Progestin; Potency**

List of Abbreviations

NE	Norethindrone
NEA	Norethindrone Acetate
PR	Progesterone Receptor
GR	Glucocorticoid Receptors
MR	Mineralocorticoid Receptors
AR	Androgen Receptor
NFKB	Nuclear Factor Kappa B
AP-1	Activator Protein-1
RBA	Relative Binding Affinity
DHT	5a-Dihydrotestosterone
MPA	Medroxyprogesterone Acetate
SHBG	Steroid Hormone Binding Globulin
EE	Ethinyl Estradiol
BMD	Bone Mineral Density
GnRHa	Gonadotropin-Releasing Hormone Agonists
ESCs	Endometrial Stromal Cells
SDF-1	Stromal Cell-Derived Factor 1
VEGF	Vascular Endothelial Growth Factor
MMP	Matrix Metalloproteinases
ECM	Extracellular Matrix

Introduction

Terminology

Progestogens are a class of steroid hormones, which activate the progesterone receptor (PR). Progesterone is the most abundant progestogen, however endogenous progestogens also include 17α-hydroxyprogesterone, 20α- dihydroprogesterone, 5α- dihydroprogesterone, 11-deoxycorticosterone, and 5α –dihydrodeoxycorticosterone. Synthetic progestogens are referred to as progestins, and they are further sub-classified based on their derivative compound as well acetylation status (Figure 1).

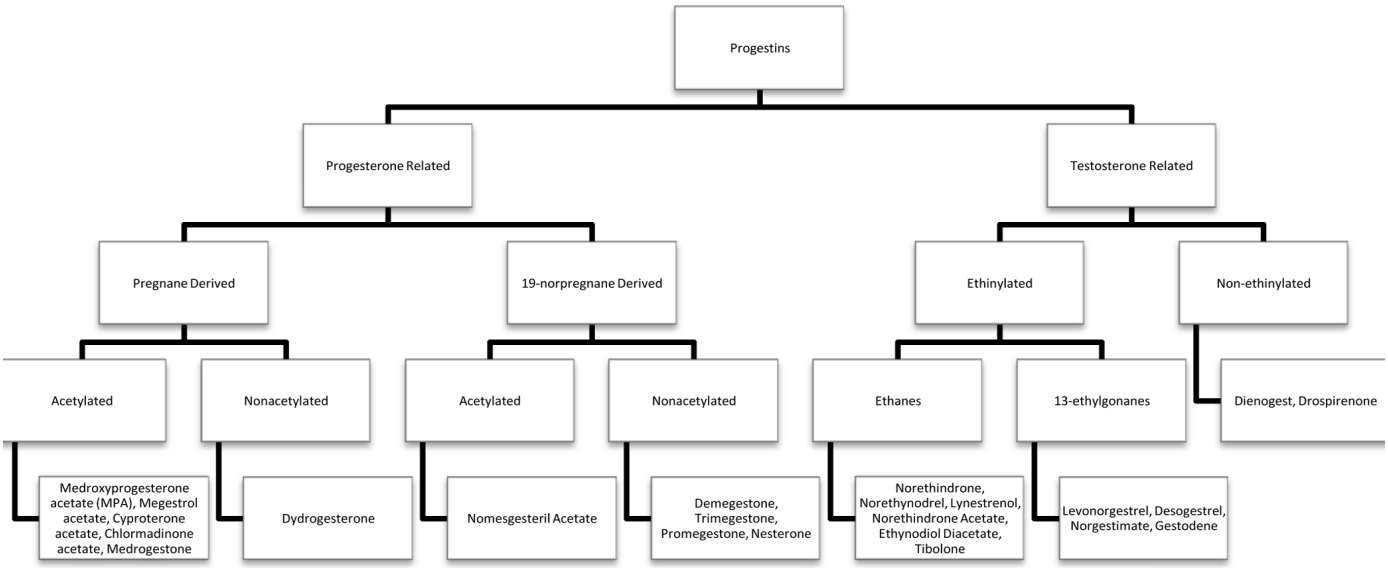

Figure 1: Classification of Progestins.

Progestins are classified based on chemical structures, either related to progesterone or related to testosterone. Of those related to progesterone, compounds are either pregnane derived or 19-norpregnane derived. They are further subdivided based on acetylation. Of the pregnane derivatives, those that are acetylated include medroxyprogesterone acetate (MPA), megestrol acetate, cyproterone acetate, chlormadinone acetate, medrogestone, and the nonacetylated compounds include dydrogesterone. Of the 19-norpregnane derivatives, acetylated is nomegestril acetate and non-acetylated is demegestone, trimegestone, promegestone, nesterone. Those related to testosterone are either ethinylated, including estranes, specifically norethindrone (NE), norethynodrel, lynestrenol, norethindrone acetate (NEA), ethynodiol diacetate, tibolone, and 13-ethylgonanes, specifically levonorgestrel, desogestrel, norgestimate, gestodene, or non-ethinylated, dienogest, drospirenone. (5)

The norethindrone (NE) family of progestins is commonly referred to as estranes, due to their 18 Carbon nucleus, as ethinyl derivatives. NE and norethindrone acetate (NEA) differ in chemical structure from testosterone by an ethinyl group at carbon 17 and absence of methyl group at carbon 10 (Figure 2A & 2B). These differences give the compounds progestogenic and oral activity, as well as reduce their androgenic effects [1,2].

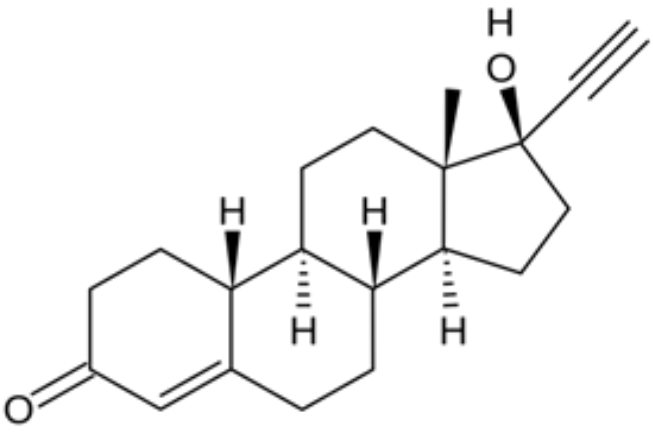

Figure 2A: Chemical Structure of Norethindrone.

Mechanism of Action

Progestogens enact their intracellular effects by binding the progesterone receptor (PR), along with other steroid receptors, including glucocorticoid receptors (GR), mineralocorticoid receptors (MR), and androgen receptors (AR), with varying affinities. Affinity for PR accounts for effects in female reproductive tissue, and affinity for GR, MR, and AR accounts for noted side effects (Table 1).

Table 1: Relative binding affinities (RBAs) and biological activity of progesterone and progestins.

Progestogene	PR		AR			GR		MR	
	RBA%	Progestogenic	RBA%	Agonistic	Anti-androgenic	RBA%	Glucocorticoid	RBA%	Anti-mineralcorticoid
Progesterone	100	+	n/d	±	(+)	0.84-35	±	9-1000	+
MPA	65-298	++	36	±	-	21.6-79	+	0.1-160	-
NE	134	+	55	+	-	0-1.4	-	0-2.7	-
NEA	27-43	++	n/d	+	-	0.88-1.6	-	0.07	-

Biological activities were determined in vivo (in rats and rabbits) using a range of assays. Progestogenic activity was measured by endometrial transformation and/or pregnancy maintenance and/or ovulation inhibition. Androgenic activity was measured by increase in weight of ventral prostate and levator ani of immature castrated male rats. Anti-androgenic activity: weight of seminal vesicles and prostate of castrated rats and/or feminization-inducing activity in male rats. Glucocorticoid activity was measured by production of glycogen and tyrosine transaminase in rat liver. Anti-mineralocorticoid activity was measured by sodium and potassium excretion from ovariectomized rats fed with low sodium diet. (- not effective; (+) weakly effective; + effective; ++ strongly effective; ± literature inconsistent; n/d not determined); Progesterone receptor (PR), Androgen receptor (AR), Glucocorticoid receptor (GR); Mineralocorticoid receptor (MR) [3].

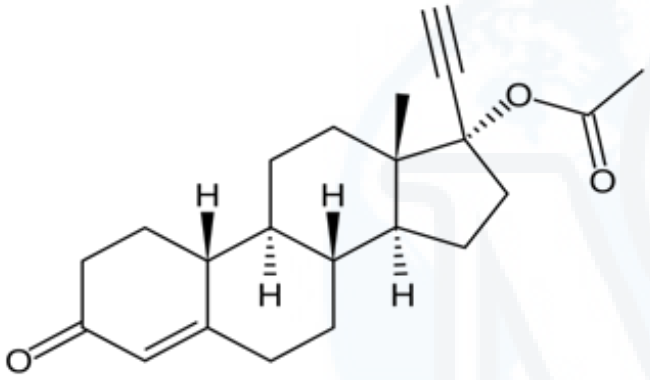

Figure 2B: Chemical Structure of Norethindrone Acetate.

Steroid receptors, generally speaking, are ligand-activated transcription factors. First, a hormone binds the inactive receptor, and the hormone-receptor complex translocates to the nucleus where it binds specific DNA sequences in the promoter regions of target genes, thus activating gene expression. Target genes can also be repressed via protein-protein interactions between the steroid receptor and other transcription factors, including nuclear factor kappa B (NFKB) and activator protein-1 (AP-1). Relative binding affinity (RBA) found NE and NEA to be effective for PR and AR, with no effect on GR and MR [3]. Pharmacokinetics of NE and NEA are reported in Table 2.

A study exploring the Ki values for progesterone and progestins, toward human AR COS-1 cells found that NEA and NE had similar binding affinity for AR as 5a-dihydrotestosterone (DHT) [4]. NEA was found to be a strong AR transactivation agonist, comparable to DHT, unlike progesterone, which was only weak a partial agonist. While DHT on AR inhibits inflammatory transcription factors NFKB, NEA shows only partial transrepression activity on AP-1. Further molecular studies suggest that NEA competes with DHT and exerts significant and promoter-specific off-target transcriptional effects via AR [4]. It is hypothesized that NEA has a similar conformation as DHT when bound to AR, unlike progesterone and other progestins. This confirms that NEA differs from progesterone on a molecular level and supports further study to elucidate the RBA of different progestins for different steroid receptors, as it has profound physiological and pharmacological consequence [4].

Table 2: Pharmacokinetics of NEA, including absorption, distribution, metabolism, and elimination [21].

Pharmacokinetics of NEA	Description and Parameters
Absorption	Rapid; C_{max}= 26.19ng/mL; T_{max}= 1.83 hrs; AUC= 166.9ng/mL
Distribution	V_d=4L/kg; Sex hormone binding globulin (SHBG) -36%; Albumin binding- 61%; Found in breast milk
Metabolism	Extensive; via reduction followed by sulfate and glucuronide conjugation
Elimination	Urine and feces (primarily as metabolites); $T_{1/2}$= 8.51 hrs

Potency and Efficacy

Potency is defined as the concentration of substrate that induces half the maximal response, or EC_{50}, while efficacy is the maximal effect elicited by a substrate under defined experimental conditions (Table 1). However, potencies have been described in many studies with regards to RBA, relative response to fixed constant doses in animal or clinical assays, or dose required for defined response [5,6]. Measurements of potency are based on the effect on nuclear estradiol receptor, DNA synthesis, isocitric and estradiol dehydrogenases, delay of menses, inhibition of ovulation, and morphological features of endometrium, especially glandular proliferation and glycogen deposition in endometrial glands [5,9-12].

One study assessed the potency of progestins as relative delay of menses, subnuclear vacuolization, glycogen deposition, and lipids/lipoproteins, and found NE and

NEA to be roughly equivalent in potency [7]. In terms of other progestins, comparison of progestin potencies in bioassays measuring endometrial transformation in rabbits rank ordered as medroxyprogesterone acetate (MPA)>dydrogesterone>NE, whereas an ex vivo assay measured transactivation in rabbits and found MPA≥NE>dydrogesterone [8].

The quantification and qualification of the potency and efficacy of progesterone and different progestins in the current literature suggest that parameters vary significantly amongst cell, tissue, animal, and clinical assays. Additionally, there are far fewer studies that measure the independent effect of progestins without the subsequent use of estrogen. Future studies aiming to clarify these principles will be paramount to NE's clinical application.

Metabolism

Progestins are more stable to first-pass metabolism than naturally occurring progesterone, which allows for greater bioavailability at lower doses. Although they can be administered intramuscularly, vaginally, percutaneously, intranasally, sublingually, and rectally, oral use is most common. Upon oral consumption, NEA is quickly converted to NE during intestinal and hepatic first pass metabolism, during which it undergoes significant transformation. The principle metabolite is 5-a-dihydro-norethisterone. Both NE and NEA have relatively high oral bioavailability of 46%-73%, approximated at 64%, 36% bound to steroid hormone binding globulin (SHBG), 61% to albumin, and 3% free in circulation, and a half life of 8 hours after oral administration [13-15] (Table 2).

NE undergoes substantial ring A reduction to form 5 alpha dihydro- and 3 beta, 5 alpha tetrahydro- NE metabolites, which undergo conjugation [16,17]. These dihydro- and tetrahydro NE metabolites can also undergo aromatization and form ethinyl estradiol (EE), a potent synthetic estrogen [18]. The *in vivo* conversion ratio of NE and NEA to EE in postmenopausal women is 0.7% ± 0.2% and 1.0% ± 0.4% at oral doses of 5mg and 10mg, respectively. This translates to about 6μg of EE per milligram of NEA. For NE, the conversion ratio was 0.4%± 0.4%, 4μg of EE per milligram of NE [18]. The conversion ratio of NEA to EE in premenopausal women is 0.20-0.33%, depending on dosage [19]. The difference in conversion rate is hypothesized to be due to menopausal status and BMI [20]. Both studies show that a significant amount of NEA is converted to EE, which exerts effects on bone and CNS [2]. Earlier studies deemed that the estrogenic effects of metabolically derived EE on the liver, in terms of synthesis of transport proteins, were likely compensated by the androgenic activity of NE [18].

FDA Indications

FDA indications of NE and NEA are limited to secondary amenorrhea, endometriosis, and abnormal uterine bleeding due to hormonal imbalance. However, its clinical use has expanded to a variety of endocrinologic and gynecologic disorders including but not limited to dysmenorrhea, ovarian endometriotic cysts, and tubal obstruction. As alternative medications have entered the market, NEA use has fluctuated, but there is very limited data on the superiority of alternative treatments. Its cost effective nature and effective symptom relief makes NEA an ideal agent for the management of various reproductive endocrinology disorders.

NEA is commonly used in combination with EE in OCP and menopausal hormone replacement therapies. It has also been combined with GnRH agonists.

Dosage

Recommended dosages depend on the indication. For secondary amenorrhea, the dosage is 2.5-10mg/day for 5-10 days; for endometriosis, initial dosage starts at 5mg/day, with the maximum dosage at 15mg/day; abnormal uterine bleeding, usually 2.5-10mg/day for 5-10 days [21]. A study in Milan found that 71% of women on 2.5 mg/day of NEA had relief for symptomatic endometriosis [22]. When used in combination with EE, the dosage of NA or NEA is as low as 0.1mg.

In comparison, MPA, dosed orally for dysfunctional uterine bleeding or amenorrhea, is administered at 5-10 mg per day for 5-10 days. Likewise, progesterone, when used for amenorrhea, has an oral dosage of 400 mg per day for 10 days, while vaginal dosage form in gel is 45 mg-90 mg, once every day for up to six doses [21].

Side Effects

According to PDR2016, known NE side effects include breakthrough bleeding, spotting, change in menstrual flow, amenorrhea, edema, weight changes, cholestatic jaundice, rash, melasma, chloasma, clinical depression, acne, breast enlargement, headache, and urticaria [21].

Contraindications

According to PDR 2016, NEA and NE are contraindicated with known/suspected pregnancy, undiagnosed vaginal bleeding, known/suspected/history of breast cancer, active or history of deep vein thrombosis or pulmonary embolism, active or recent arterial thromboembolic disease (e.g., stroke, myocardial infarction), liver dysfunction or disease.

Additionally, patients with risk factors for arterial vascular disease (e.g., hypertension, diabetes mellitus, tobacco use, hypercholesterolemia, obesity) and/or venous thromboembolism (e.g., personal history or family history of venous thromboembolism, obesity, systemic lupus erythematous) should be managed appropriately. PDR recommends careful diagnosis of breakthrough bleeding and irregular vaginal bleeding, and to use caution with patients' with a history of clinical depression. It is recommended to monitor patients with hyperlipidemias and/or diabetes mellitus during therapy for potential adverse effects on carbohydrate and lipid metabolism. Lastly, NE may affect certain endocrine tests, liver function tests, and blood components in laboratory tests; patients on thyroid

replacement therapy may require higher doses of thyroid hormone.

Metabolic and Cardiovascular effects

Investigations with different animal models identified PRs involved in cell growth and gene transcription in vascular endothelium and vascular smooth muscle. NEA mimics progesterone in acting as a vasodilator in coronary vasculature, whereas other progestins, including MPA, causes vasoconstriction [2,23].

Effect on Coagulation

It is suggested that NEA and its metabolites alter expression of coagulation genes in cultured human cells, specifically liver and vascular endothelium. In human hepatocytes and umbilical vein endothelial cells, NEA was found to down-regulates fibrinogen expression twofold, as well as factor VII and prothrombin expression. NEA up-regulates gene expression of tissue factor, tissue factor pathway inhibitor, and tissue plasminogen activator. The expression of the estrogen receptor was unchanged by NEA [24]. Another study examined this phenomenon in postmenopausal women and found that NEA mediates the effect of estradiol in improving fibrinolysis, specifically shortening euglobulin clot lysis time, decreasing plasminogen activator inhibitor-1 activity, and increasing D-dimer antigen levels [25].

An early study examined the effect of NEA on blood coagulation and fibinolytic parameters in female monkeys and found an increase in antithrombin-III and plasminogen, but concluded that NEA did not contribute to thrombotic events [26]. Comparison of combined oral contraceptives with different generations of progestin on hemostatic variables identified NE as having the smallest increase in protein C activity, as compared to drospirenone, norgestimate, gestodene, and levonorgestrel [27]. This finding suggests that NE might be superior to other progestins in its safety profile in regards to risk of thromboembolic events.

Effect on Lipid Profile

A recent study examining hormone therapy in postmenopausal women found that the addition of NEA to estradiol treatment negated improved lipid profile achieved by estradiol alone, specifically the magnitude of increase in HDL and decrease in LDL. However, the addition of NEA did cause a greater reduction in the LDL:HDL ratio than treatment with estradiol alone, primarily due to the significant increase in HDL with estradiol [28]. It's suggested that NEA lowers HDLs and triglycerides, but doesn't have a large impact on estrogen-mediated decrease in total cholesterol and LDL [29,30].

In swine models, NEA had a hypolipemic effect due to inhibition of splanchnic triglyceride [31]. When evaluating specific cardiovascular parameters, including HDL subtypes, NEA had no effect on HDL2, the subtype responsible for cardioprotective effect, but decreased HDL3. Likewise, lipoproteins and ApoA1 were unchanged, while ApoA2 was decreased; increased ApoA2 is associated with development of vascular fatty streaks in animal studies. Moreover, NEA decreased cell adhesion markers E-selectin, vascular cell adhesion molecule, and angiotensin converting enzyme activity [32].

An observational study in women with endometriosis-associated pain treatment compared Leuprolide and NEA. NEA negatively impacted women's lipid profiles with HDL, total cholesterol, LDL and no change in triglyceride levels. These findings were statistically significant and highlight the importance of evaluating cardiovascular risk factors and discussing lifestyle modifications [33].

Carbohydrate metabolism

An early study found that NE and its derivatives moderately effected carbohydrate metabolism, causing impaired glucose tolerance with definite hyperinsulinemia. The investigators concluded that this effect could be minimized with triphasic preparations [30,34]. Addition of NEA to estrogen treatment in postmenopausal women had a negative effect on insulin resistance, despite the benefits observed with estrogen use alone [28]. It is clear that additional studies should be conducted to further elucidate the role of NE alone.

Use of NEA as add back therapy and effect on bone

There is limited data on the effect of NE and NEA, not in combination with estradiol or as add-back therapy, on bone. There is documentation of decreased bone quality in young women who begin progestin-only oral contraception shortly after pubertal development, although the study did not specify the type of progestin or explore the physiological mechanism [35]. An early study found that high dose NEA prevented bone reabsorption when administered without additional estrogen in postmenopausal women [36]. Similarly, several studies suggest that 1-5 mg NE has beneficial effects on bone mineral density (BMD) [37-40]. A recent 2-year randomized double-blinded placebo controlled study found that NE therapy didn't significantly affect BMD from baseline values and was comparable to the placebo. However, 1 mg of NE over 2 years attenuated bone loss at the spine site [29]. A randomized clinical trial compared oral dosage of NEA versus treatment with leuprolide acetate in premenopausal women with symptomatic endometriosis and assessed changes in BMD. Women taking NEA had no significant change in lumbar or hip BMD while patients taking leuprolide had significant decrease in lumbar and hip BMD [41].

Gonadotropin-releasing hormone agonists (GnRHa) have been shown to be effective in reducing endometriosis-associated pelvic pain. However, GnRHa treatment is associated with hypoestrogenic side effects, including progressive bone loss and vasomotor symptoms, such as hot flashes, headache, and vaginal dryness [2]. NE and NEA have been shown to beneficially mediate an increase

in bone mass [42]. This noted beneficial effect is attributed to its lack of affinity for glucocorticoid receptor. Measuring BMD changes, fracture incidence, and biochemical markers of bone turnover (i.e. bone specific alkaline phosphatase, serum osteocalcin, and urinary N-telopeptide of type 1 collagen), it was found that NE supplementation with hormone replacement therapy significantly improved BMD and inhibited the occurrence of new fractures. It is suggested that progestins, specifically NE, regulates bone turnover and promotes bone formation by directly acting on osteoblasts via the PR [42].

It is important to note, however, that recent clinical trials have not found evidence that progesterone or pure progestins, those that act solely on PR, improve BMD in postmenopausal, hysterectomized, or amenorrheic women [35]. NE's androgenic and estrogenic activity may also contribute to beneficial effect on bone metabolism. It is thought that NEA may exert its antiresorptive effect on bone via its ethinyl estradiol metabolites [42].

CNS effects

In recent years, there has been increasing interest in the cognitive effects of estrogen and progesterone in menopausal women. One study explored these effects through functional MRI and neuropsychological measures in postmenopausal women, and progesterone was associated with changes in patterns of regional brain activation during visual memory tasks, with greater activation of the left prefrontal cortex and right hippocampus compared to placebo. Progesterone was also associated with significant improvement in verbal working memory compared to placebo [43]. These results highlight the potential of progestins as a potential therapeutic means to improving memory.

FDA Indications

Endometriosis: The use of NE and NEA in endometriosis has been longstanding, for more than 5 decades, however mechanistic studies regarding its therapeutic effect have been lacking. It is generally accepted that progestins induce decidualization and atrophy in endometrium and lower the serum estradiol levels by inhibiting the hypothalamic-pituitary-ovarian axis [44].

Progestins, including NE and NEA, inhibit the release of luteinizing hormone from the anterior pituitary, thus preventing ovulation and cyclic ovarian steroid production. Patients on NE and NEA develop amenorrhea, due to the absence of estrogen-mediated mitogenic and proliferative effects, with eventual decidualization and atrophy of the endometrium [16,45].

NE was shown to inhibit the proliferation of endometrial stromal cells (ESCs) in an in vitro study. NE's antiproliferative effect suppresses ovulation and is thus relieves endometriosis symptoms and dysmenorrhea [46]. Also, NE is effective on endometriotic lesions via inhibition of proliferation of ESCs. NE treatment increases the activity of caspase 3/7, downstream effectors in the apoptotic cascade, thus increasing the number of apoptotic cells in the endometrial stroma. Moreover, this inhibition of proliferation and induction of apoptosis was accomplished without cytotoxicity, as measured by leakage of lactate dehydrogenase [46]. NEs' ability to induce apoptosis is unique, as the natural occurring progesterone only exerts activity on the PR, thus only mediating an antiproliferative effect.

Angiogenesis plays a significant role in gynecologic disorders, including endometriosis and abnormal uterine bleeding. Endometrial angiogenesis is controlled primarily by vascular endothelial growth factor (VEGF). Stromal cell-derived factor 1 (SDF-1) is also induces angiogenesis, stimulating endometrial cell proliferation and maintaining cell survival via the endothelial cell receptor. Together, SDF-1 and VEGF synergistically promote vascular endothelial cells angiogenesis. Although the mechanisms are not well elucidated, progestins are suggested to enhance blood vessel fragility and change vasculature in the endometrium, decreasing the number of spiral arterioles, reducing the density of normal capillaries and increasing the number of defects in small vessels. It's suggested that progestins attenuate 17β- estradiol stimulated VEGF and SDF-1 production in cultured endothelial stromal cells. This effect may be mediated via progestins' binding of progesterone receptor, which inhibits estrogen-receptor -dependent gene activation, potentially through competition for common limiting coactivators. Additionally, this effect is mediated by progestin's' down-regulation of ER in endometrium [47]. While this is significant, a separate study found progestins to increase certain isoforms of VEGF, which could account for the breakthrough bleeding noted during continuous use of progestins. Specifically, it noted NE dose-dependent increase in mRNA for VEGF 165 and VEGF 121 [48].

Matrix metalloproteinases (MMP), along with serine proteases, cause proteolytic digestion of the extracellular matrix (ECM) to allow for successful implantation of ectopic endometrium in the peritoneal cavity. Multiple studies have found different MMPs upregulated in ectopic endometrial tissue in patients with endometriosis [49-51]. It is suggested that progestins, including NEA, inhibit the production of MMP-1 and MMP-3 via suppression of their mRNA, while they increase the production of their tissue inhibitors [52]. This demonstrates the multifaceted benefits of NEA in endometriosis treatment.

Clinical studies have found confirm NEA after Lupron depot, a GNRH agonist, reduced break through bleeding compared to the use of NEA alone [44,53-55]. In a prospective study, patients with colorectal endometriosis received 2.5 mg/day NEA and found significant improvement in the intensity of chronic pelvic pain, deep dyspareunia, dyschezia, as well as severity of diarrhea, intestinal cramping and passage of mucus. Improvement in pelvic pain and other self-reported symptoms was replicated in similar observational studies [56-58]. While it is thought that the symptom relief

is mediated through the mechanisms discussed above, further research is needed to illustrate the main mechanism of action. Recent evidence suggests that progestin therapy decreases nerve fiber density in endometrial tissue, specifically sympathetic, parasympathetic, and sensory nerve fibers in deep infiltrating, rectovaginal endometriosis [59].

Alternative Clinical Applications:

a) **Endometrial Neoplasia**

Progestins are an attractive option for treatment of endometrial hyperplasia because of their inhibitory effect on epithelial proliferation. They reduce estrogenic receptors and increase their catabolism through activation of 17β-hydroxysteroid dehydrogenase enzyme, which converts active estradiol to inactive estrone, and estrone-sulfotransferase enzyme, which causes conjugation of estrone thereby diminishing the estrogenic effect that leads to endometrial abnormalities. Additionally, in the case of a simple or complex hyperplasia, progestins can preserve fertility in young women and be used in older patients who aren't surgical candidates. In a clinical study, nearly two-thirds of premenopausal women with complex hyperplasia treated with 5mg NEA showed regression of lesion, assessed with fractional re-curreting, after three months [60].

Wnt genes are a family of signaling molecules that regulate cell-to-cell interactions during development and tissue homeostasis. One study found that NEA upregulated Wnt-7a gene expression in estrogen treated normal endometrial epithelial cells. This upregulation of Wnt-7a is hypothesized to contribute to the antineoplastic properties of progestins [61].

b) **Adenomyosis**

Adenomyosis is characterized by intramyometrial presence of ectopic endometrial glandular tissue and stromal cells surrounded by reactive hyperplastic myometrium [62]. Adenomyosis usually presents in 40-50 year olds as painful menstruation and heavy bleeding, and possibly chronic pelvic pain. Clinical studies of use of NEA for adenomyosis have suggested its efficacy in relieving dysmenorrhea and menorrhagia. Maximum effect was noted at 3 months on NA and was maintained throughout treatment; a three week on and one week off treatment regimen minimized break-through bleeding [63].

Treatment of adenomyotic stromal cells (ASCs) in human primary cultures with endogenous progesterone and another testosterone-derived progestin, dienogest, increased apoptosis, as measured by annexin V-positive/7-AAD- negative cells and caspase 3/7 activity. Additionally, the treatments both inhibited proliferation, assessed by decreased ASCs in S phase, through decreased oxidative stress via decreased reactive oxygen species production, increased expression of antiapoptotic genes p53 and Bax, decreased expression of Bcl-2, and inhibiting production of prostaglandin E2, which inhibits apoptosis. However, the molecular actions of NE and NEA are unknown and more research is needed [62].

Breast and Breast Cancer: There is limited information on the use of NEA and risk of breast cancer in premenopausal women. One older cohort study examining 1150 premenopausal women with benign breast disease found that women treated with NE had a 50% reduction in breast cancer risk. The authors suggested that androgenic progestins, but not progesterone derivatives, have potential beneficial effects on breast tissue in premenopausal women [67].

One observational study in postmenopausal women suggested that hormonal therapy with NEA increases mammographic breast density and risk of breast cancer, although more studies are needed to discern the effect of dose, treatment duration, and route of administration [64,65].

Additionally, there is discussion of progestins as a therapeutic strategy for breast cancer. Some progestins have been shown to inhibit estrone sulfatase and 17B-hydroxysteroid dehydrogenase, which are essential for the conversion of estrone to biologically active estradiol: they mitigate the effect of estrogen on breast tissue [68]. More recent studies have confirmed the estrogenic effect of NEA, demonstrating its ability to increase cell replication in MCF-7:WS8 cells, to elevate estrogen target genes, generate apoptosis in MCF-7:5C cells, a stable cell line derived from parental MCF-7 cells by long-term estrogen deprivation, increase estrogen receptor transcriptional activity, and have its action blocked by 4-hydroxytamoxifen and ICI [69]. Further research is needed to elucidate this.

A) **Scleroderma**

An early study suggested the use of NEA in the treatment of scleroderma [70]. A follow-up study demonstrated that NEA increased excretion of hydroxyproline and glycosaminoglycans, indicating a therapeutic potential in scleroderma, specifically through effect on collagen and ground substance components. The trial conducted found improvements in skin elasticity, skin biopsy, calorimetry, and respirator function tests, as well as several minor side effects. The study concluded, however, that NEA treatment of scleroderma had "doubtful" beneficial effect [71].

B) **Catamenial Epilepsy and Catamenial Headache**

One case study treated 2 patients with catamenial epilepsy and catamenial headaches. NEA was found to alleviate symptoms [72]. However, it is important to note that NEA is contraindicated in migraines with an aura as well as vascular headaches.

C) **Catamenial Pneumothorax**

Catamenial pneumothorax is a rare complication of

endometriosis. A case study of two patients with catamenial pneumothorax found reduction of pneumothorax recurrence with extended treatment of NEA [73]. An older case study also found that continuous treatment with NE 0.7 mg/day with monthly intramuscular injections of leuprolide acetate prevented recurrent pneumothorax, dependent on the maintenance of amenorrhea [74].

D) **Tubal Obstruction**

Proximal tubal obstruction (PTO) is a major cause of infertility; causes include endometriosis, post-infection fibrosis, salpingitis, isthmica nodosa, and mechanical obstruction [75]. One study explored the use of NEA as medical suppressive treatment in a subgroup of infertile patients with PTO; tubal patency was achieved in 50% of patients treated with NEA, compared to 90% with GnRH analog [76].

Limitations

There are few studies, which evaluate the physiological effects of NE or NEA, especially in regards to biochemical and hemodynamic variables. This is because NE and NEA are often observed in conjunction with GnRH agonists or estradiol. Moreover, there are limited studies that explore the long-term effects of use of NEA and NE, which contributes to lack of understanding. Additionally, the majority of studies conducted are retrospective reviews rather than prospective studies.

Lastly, in surveying the available literature, it is important to note discrepancies among studies in their use of different animal and clinical models, comparison with different forms of progestins, small size of study participants, and recruitment of premenopausal versus postmenopausal women. These parameters limit how much the study findings can be generalized.

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