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Review

Pharmacology of conjugated equine estrogens: Efficacy, safety and mechanism of action

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ABSTRACT

Oral conjugated equine estrogens (CEE) are the most used estrogen formulation for postmenopausal hormone therapy either alone or in combination with a progestin. CEE is most commonly used for the management of early menopausal symptoms such as hot flashes, vaginitis, insomnia, and mood disturbances. Additionally, if used at the start of the menopausal phase (age 50–59 years), CEE prevents osteoporosis and may in some women reduce the risk of cardiovascular disease (CVD) and Alzheimer's disease (AD). There appears to be a common mechanism through which estrogens can protect against CVD and AD.

CEE is a natural formulation of an extract prepared from pregnant mares' urine. The product monogram lists the presence of only 10 estrogens consisting of the classical estrogens, estrone and 17β-estradiol, and a group of unique ring B unsaturated estrogens such as equilin and equilenin. The ring B unsaturated estrogens are formed by an alternate steroidogenic pathway in which cholesterol is not an obligatory intermediate. Both the route of administration and structure of these estrogens play a role in the overall pharmacology of CEE. In contrast to 17β-estradiol, ring B unsaturated estrogens express their biological effects mainly mediated by the estrogen receptor β and not the estrogen receptor α.

All estrogen components of CEE are antioxidants, and some ring B unsaturated estrogens have several fold greater antioxidant activity than estrone and 17β-estradiol. The cardioprotective and neuroprotective effects of CEE appear to be, to some extent, due to its ability to prevent the formation of oxidized LDL and HDL, and by inhibiting or modulating some of the key proteases involved in programmed cell death (apoptosis) induced by the excess neurotransmitter glutamate and other neurotoxins.

Selective combinations of ring B unsaturated estrogens have the potential of being developed as novel therapeutic agents for the prevention of cardiovascular disease and Alzheimer's disease in both aging women and men.

This article is part of a Special Issue entitled 'Menopause'.

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1. Introduction

The average age at which menopause occurs is approximately 50 years, and with an average life expectancy of over 85 years, a woman will live for more than a third of her life in the postmenopausal state. During this phase, the ovary essentially stops secreting 17β-estradiol and progesterone, although small amounts of estrogen are still being produced by the peripheral aromatization of mainly adrenal androgens [1,2]. The reduced levels of circulating estrogen in postmenopause compared to premenopause results in a deficiency affecting the genitourinary tract, central nervous system, bone, skin and cardiovascular system. Afflictions frequently associated with the onset of menopause are vasomotor symptoms (hot flashes), vaginal dryness, sleep disorders, and mood changes. While it is difficult to separate the effects of aging from those due to lack of estrogen, it is well established that estrogen replacement therapy (ERT), more recently termed estrogen therapy (ET), can relieve many of the menopausal symptoms. Substantial clinical and epidemiological evidence from observational and some randomized control trials show that ET can relieve vasomotor symptoms and lower the risk of osteoporosis. ET could also possibly reduce the risk of cardiovascular disease (CVD) and Alzheimer's disease (AD) in women who initiated hormone therapy (HT) in the very early phase or preferably at the start of menopause. The objective of this review is to discuss the pharmacology of CEE and its individual components with special emphasis on their mechanism of action in the etiology of CVD and AD in healthy postmenopausal women. The use of CEE in animal models is outside the scope of this brief review. Detailed epidemiological aspects of these benefits and risks as well as a number of other effects associated with ET and HT are discussed by several others in this special issue.

2. Estrogen preparations used for ET

Numerous estrogen preparations are available on the market for ET; however, in this brief review, we will discuss the pharmacology of conjugated equine estrogens (CEE) only, with emphasis on the unique ring B unsaturated estrogenic components. For more than 70 years, CEE has been and still is the most prescribed drug for estrogen replacement, with over 3000 publications dealing with its safety profile and efficacy. The drug CEE is a complex 'natural' extract of pregnant mares' urine containing 10 different estrogens, as depicted in Fig. 1. These estrogens are the sulfate esters of ring B saturated estrogens (classical estrogens): estrone (E_1; 3-hydroxy-1,3,5(10) estratrien-17-one), 17β-estradiol (17β-E_2; 1,3,5(10) estratrien-3,17β-diol) and 17α-estradiol (17α-E_2; 1,3,5(10) estratriene-3,17α-diol); and the unique ring B unsaturated estrogens: equilin (Eq; 3-hydroxy-1,3,5(10),7-estratetraen-17-one), equilenin (Eqn; 3-hydroxy-1,3,5(10) 6,8-estrapentaen-17-one), 17α-dihydroequilin (17α-Eq; 1,3,5(10) 7-estratetraen-3,17α-diol), 17β-dihydroequilin (17β-Eq; 1,3,5(10) 7-estratetraen-3, 17β-diol), 17α-dihydroequilenin (17α-Eqn; 1,3,5(10) 6,8-estrapentaen-3,17α-diol), 17β-dihydroequilenin (17β-Eqn; 1,3,5(10)6,8-estrapentaen-3,17β-diol), and delta-8-estrone (Δ^8-E_1; 3-hydroxy-1,5(10)8-estratetraen-17-one). In Fig. 1, an additional estrogen, Δ^8-17β-estradiol (1,5(10)8-estratetraen-3,17β-diol), is also a ring B unsaturated estrogen that is formed in humans from Δ^8-E_1 (Section 3.3). Its pharmacology is also discussed in this review.

Ring B unsaturated estrogens differ from classical ring B saturated estrogens by the presence of one or two additional double bonds in the B ring of the steroid nucleus (Fig. 1). A series of early in vivo studies in the pregnant mare [3], indicate that ring B saturated estrogens E_1, 17β-E_2 and 17α-E_2 are formed by the classical pathway of steroidogenesis: acetate → mevalonate → isopentenyl pyrophosthate → 3,3-dimethyl-allyl pyrophosphate → geranyl pyrophosphate → farnesyl pyrophosphate → squalene → lanosterol → cholesterol → pregnenolone → dehydroepiandrosterone → androstenedione → estrogens (Fig. 2). In contrast, ring B unsaturated estrogens such as equilin and equilenin are formed by an alternate pathway of steroidogenesis where both squalene and cholesterol are not the obligatory precursors. Furthermore, a clear bifurcation in the classical pathway of steroidogenesis occurs before the formation of squalene and cholesterol (Fig. 2). In vivo metabolic studies [4,5] in the pregnant mare also established that the ring B unsaturated estrogen Eq is the precursor of other ring B unsaturated estrogens such as 17α-Eq, 17β-Eq, Eqn, 17α-Eqn, and 17β-Eqn. The biosynthetic origin of Δ^8-E_1 in the pregnant mare has not yet been established. The exact composition of CEE is not fully known, however the approximate concentration of the ten estrogens present in the form of sulfate esters are: E_1 (50%), Eq (22%), 17α-Eq (14%), 17α-E_2 (4.5%), Δ^8-E_1 (3.5%), Eqn (2%), 17β-Eq (1.7%), 17α-Eqn, 17β-E_2 and 17β-Eqn (≤1%). Although the estrogens present in the drug CEE are in their sulfate conjugated form, it is generally accepted that all of these estrogens exert their pharmacological effects after hydrolysis through their unconjugated form. All ten estrogens are biologically active and have varying degrees of uterotropic activity [4]. Moreover, additional studies in postmenopausal women show that some ring B unsaturated estrogens such as EqS are several-fold more potent than E_1 sulfate in suppressing urinary gonadotropins, and in the stimulation of sex hormone-binding globulin (SHBG), corticosteroid binding globulin (CBG) and angiotensinogen [6,7]. Potency comparisons of CEE with micronized 17β-E_2 and piperazine estrone sulfate, in terms of serum levels of hepatic proteins, indicate that in postmenopausal women CEE is 2–5-fold more potent than E_1 sulfate and 17β-E_2 [8]. Daily oral administration of 0.125 mg of Δ^8-E_1 sulfate to postmenopausal women for 8 weeks lowers plasma levels of FSH and the urinary bone turnover marker n-telopeptide [9]. Thus, the ring B unsaturated estrogen components of CEE contribute to its overall activity. All known estrogen components of CEE interact with both estrogen receptors α and β (to be discussed in a subsequent section). Therefore, the total pharmacological effects of CEE are a result of the sum of these known individual activities plus those of yet-to-be-identified compounds present in the pregnant mares' urine extract [3].