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Research/Clinical Overview

Review of the Effects of 17α-Estradiol in Humans: A Less Feminizing Estrogen With Neuroprotective Potential

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ABSTRACT 17α-Estradiol is a less feminizing isomer of the potent hormonal estrogen, 17β-estradiol. 17α-Estradiol is an orally active small molecule with conflicting reports of efficacy in preclinical models of degenerative diseases. A number of studies suggest neuroprotective potential in human neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease. Several studies have established an antioxidant effect of 17α-estradiol in humans. The sodium salt of 17α-estradiol 3-sulfate is a minor component (2.5–9.5%) of several widely marketed estrogen hormone replacement products, such as Premarin®, that are approved by the U.S. Food and Drug Administration and have been prescribed and studied in women and men for more than 65 years. Most of the more than 100 published reports on the neurological effects of feminizing estrogens found positive responses in at least one measure relating to cognition or prevention and treatment of AD, notwithstanding the negative results in the Women's Health Initiative studies. Whether these limited, and often not statistically significant, findings are clinically meaningful remains unknown. In many in vitro and in vivo preclinical neuroprotection and related studies, 17α-estradiol and 17β-estradiol are active at similar concentrations and doses. However, 17α-estradiol is less pleiotropic than 17β-estradiol, and thus its potential toxicity might be lower. Given decades of mixed reports regarding the potential efficacy and safety of strongly feminizing hormones in neurodegenerative diseases, the weakly feminizing 17α-estradiol might be a suitable candidate for clinical testing of the neuroprotective potential of this chemical class because it avoids, or significantly reduces, the adverse effects of potent hormonal compounds. Drug Dev Res 70:1–21, 2009. © 2009 Wiley-Liss, Inc.

Key words: estradiol; 17α-estradiol; 17β-estradiol; estrogen; Premarin®; hormone replacement therapy; Women's Health Initiative; Phase I clinical trial; human studies; neuroprotection; mitochondria; Alzheimer's disease; Parkinson's disease; stroke; Friedreich's ataxia; Huntington's disease; retinitis pigmentosa

INTRODUCTION

The estrogen 17α-estradiol is a less feminizing isomer of the most potent known natural hormonal estrogen, 17β-estradiol [Moos et al., 2008]. Exposure of humans to 17α-estradiol in various forms and mixtures, including the single agent itself as a sodium salt of the 3-sulfate conjugate (also known as MX-4509; MITO-4509; ABP-150), has been extensive, as outlined herein. A brief introduction to various data and considerations

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in regard to 17α-estradiol and its sulfate conjugate follows.

17α-Estradiol is an orally active small molecule with mixed reports of efficacy in preclinical neuroprotection models that are predictive of therapeutic potential for neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) [Blanchet et al., 1999; Merchenthaler and Shughrue, 2005], and for stroke [Yang et al., 2000], as well as for orphan or neglected indications such as Friedreich's ataxia and retinitis pigmentosa [Dykens et al., 2003]. There are, however, no reported studies of 17α-estradiol as a single agent in neurodegenerative diseases, such as AD, or as a cognition activator in humans. With regard to a possible mechanism of action, there is evidence of an antioxidant effect in humans dosed with 17α-estradiol [Oettel, 1999], and oxidative stress is implicated in the etiology of all the diseases noted above. Indeed, antioxidants may be beneficial for a wide variety of central nervous system diseases.

The sodium salt of 17α-estradiol 3-sulfate is a component of several approved and widely marketed estrogen hormone products that have been prescribed and studied in women and men and have been approved by the U.S. Food and Drug Administration (FDA) since 1942. As a single agent, however, neither 17α-estradiol nor its sulfated form is approved in the United States or Canada for commercial distribution. 17α-Estradiol is commercially available in Germany, under the brand name Pantostin®, as a solution applied to the scalp for the treatment of alopecia.

The majority of published human neurological studies of feminizing estrogens—more than 100 studies—report positive responses in at least one measure relating to cognition or the prevention and treatment of AD [Howell et al., 2005a], although negative results were obtained in the large Women's Health Initiative (WHI) studies [see Grady, 2003; Mulnard et al., 2000; Rapp et al., 2003; Shumaker et al., 2003, 2004; Wassertheil-Smoller et al., 2003]. Whether these limited, and in many cases not statistically significant, positive findings are clinically meaningful is unknown [Howell et al., 2005b]. In this context, it bears reiteration that the WHI conclusions continue to be debated; one example is an article in *The Wall Street Journal* bearing the provocative headline: "How NIH Misread Hormone Study in 2002" [Parker-Pope, 2007]. Additional comments on the WHI studies follow, and a wide range of opinions and possible explanations for the results can be found in several reports [Bluming, 2004; Harman et al., 2004, 2005a,b; Maki, 2004; Manson et al., 2007; Mitka, 2007; Naftolin et al., 2003; Rossouw et al., 2007; Wickelgren, 2003]. While complex, the cumulative results indicate that feminizing estrogens, whatever their benefits, are associated with adverse side effects in some subpopulations of women, especially postmenopausal women who have not been exposed to feminizing estrogens for a number of years. As a result, there is increasing interest in estrogen compounds that retain clinical benefit but that have reduced risk of those adverse side effects. Given the substantial cumulative experience with 17α-estradiol, which we review here, its further testing in clinical trials should be considered.

BACKGROUND

17α-Estradiol and its conjugates are endogenous in humans; the compound is found in low levels in our food and in our environment, so humans are exposed to it naturally. While this observation might not provide much comfort in terms of safety, it does distinguish the molecule from other wholly xenobiotic drugs. At least 10 studies of the administration of 17α-estradiol or the sulfate conjugate form to humans by oral (PO), sublingual, subcutaneous (SC), intravenous (IV), or topical routes, including studies at relatively high oral doses ($\leq$25 mg/day), have been published, and no untoward effects have been reported.

The published human studies provide evidence that orally administered 17α-estradiol is rapidly and extensively conjugated, deconjugated, and reconjugated [Hobe et al., 2002], but there are no reports of other metabolic events such as modification of the estrogen steroid ring structure. While 17α-estradiol is orally available in humans, its degree of bioavailability is unknown, and the bioavailability of this class of chemical compounds has not been well characterized to date. Standard toxicology testing has not been performed on this drug, although it has been studied as an individual agent or as a component of other products in a large number of published preclinical and clinical studies on estrogens. There is no evidence of significant adverse effects or feminizing activity with 17α-estradiol in humans as a single agent, at single doses of 0.05–0.2 mg and at a repeated daily oral dose of 2 mg for 12 weeks [Dykens et al., 2005a; Schröder et al., 1997; reviewed in Moos et al., 2008]. Other clinical studies have been conducted with higher doses of 17α-estradiol, with no reports of adverse effects. However, it is unclear if potential side effects were adequately documented; that is, whether they were observed but not reported, or simply were not observed.

As mentioned above, most publications on the effects of feminizing estrogens in human neurological studies report positive responses for at least one measure, although the effects are typically modest

and often lack statistical significance. Howell et al. [2005a] have summarized this literature, which amounts to well over 100 studies on cognition or on the prevention and treatment of AD. However, the primary findings from the large WHI and related studies, which used Premarin and Prempro™ as the source of estrogens (see further comment below), have raised concerns that estrogens may slightly increase the risk of dementia in postmenopausal women aged ≥65 years. Because of the numerous positive studies published previously, the highly publicized results of the WHI study, including the WHI Memory Study (WHIMS), took most people by surprise. Among the initial conclusions from these studies were a lack of improvement in cognitive function, a small increased risk of cognitive decline, no prevention of mild cognitive decline, an increased risk of probable dementia, and an increased risk of ischemic stroke. The many positive effects of estrogen therapies, especially in postmenopausal women, had been widely accepted by medical practitioners and biomedical researchers based on the cumulative knowledge of estrogens, the related hormonal and neuronal pathways, and the effects of aging on endocrine and other physiological systems.

The initial conclusions from the WHI caused controversy and rifts between patients, physicians, and scientists, some discarding altogether the notion that estrogens have positive effects against AD and a host of other disorders, but with others steadfastly defending the myriad positive effects of estrogens. After all, the WHI conclusions contradicted not only prevailing wisdom, but also numerous positive epidemiological and other studies spanning several decades, including large prospective studies that convincingly showed that prior estrogen use was associated with reduced risk of AD (for example, see Zandi et al. [2002]). In retrospect, given the complicated pathways affected by estrogen and the importance of its hormonal actions, it should not have been a surprise that the estrogen story turned out to be more complex than anticipated [see, e.g., Gruber et al., 2002, and www.uspharmacist.com/NewLook/CE/er/lesson.htm]. In defense of estrogen therapies, many pointed to methodological issues with the WHI trials, including timing of dosing (recently menopausal or years postmenopause), negative effects of certain progestins such as medroxyprogesterone (as in opposed estrogen formulations such as Prempro, in contrast to the unopposed Premarin), treatment biases including education and health status, and the preferential inclusion of older patients perhaps suffering from cerebral thromboses and multi-infarct dementia rather than AD (i.e., neurovascular complications of feminizing hormones resulting in a rapid onset cognitive decline atypical of AD). Furthermore, the natural extract that is the basis of Premarin contains at least 10 major components, including 2.5–9.5% of the sodium salt of 17α-estradiol 3-sulfate, and at least 200 other constituents, many of whose actions are far from understood [Dey et al., 2000]. That is, the WHI trials were carried out with a complex mixture of estrogens, rather than a single, well-characterized compound.

Even the WHI authors [Shumaker et al., 2003, 2004] noted that "on balance, most studies support the protective effects of estrogen in both in vitro and in vivo studies." Nevertheless, while the development of feminizing estrogens as drugs for neurodegenerative disorders was sharply curtailed, other researchers began or continued their work on less feminizing or nonfeminizing estrogens, in the hope of retaining the neuroprotective effects of feminizing hormones while minimizing their negative effects [see, e.g., Toran-Allerand et al., 2005].

Compared with 17β-estradiol, 17α-estradiol is active at similar concentrations and doses in numerous in vitro and in vivo model systems of neuroprotection [see, e.g., Howell et al., 2005a; Moos et al., 2008]. Given that 17α-estadiol is (1) as good as or better than 17β-estradiol in some preclinical studies, (2) more selective in its other physiological effects, (3) apparently less toxic, and (4) less feminizing than the hormonal estrogens that are the focus of many published human studies [Moos et al., 2008], it represents an attractive compound for studying the efficacy and safety of members of this chemical class in humans. At present, however, this possibility has not been adequately tested, and as noted above, the available studies are inconsistent, and some yield no significant evidence of neuroprotection. Other estrogen and related compounds have been studied in disorders of the central nervous system, including antiestrogens, such as the anticancer drugs tamoxifen [O'Neill et al., 2004] and raloxifene, and brain-selective estrogen receptor modulators (SERMs) in AD [Brinton, 2004], as well as the pregnancy hormone estriol in multiple sclerosis [see, e.g., Sicotte et al., 2002; Morales et al., 2006]. Although various studies are continuing, and some are promising, no conclusive evidence has been presented to date that would lead to the use of estrogen and related compounds as approved drugs for treatment of neurodegenerative disorders.

Before describing the human studies on exposure to 17α-estradiol and its sulfate conjugate, we should comment in more detail on neuroprotection and mechanism of action. Numerous studies have shown that treatment with estrogens, especially feminizing hormones like 17β-estradiol, provide neuroprotection [see, e.g., Zhao and Brinton, 2005, 2006]. Neuroprotective effects have been

demonstrated in cell culture and animal models, and in human studies [reviewed in Howell et al., 2005a,b], yet not all studies have been consistent, for reasons that are unclear [see, e.g., Levin-Allerhand et al., 2002; Chakrabarti et al., 2005]. Estrogens appear to exert their neuroprotective actions via multiple pathways [see, e.g., Singh et al., 2006], and new ones are regularly suggested [see, e.g., seladin-1 as a potential target of estrogen receptors in the brain, in Peri et al., 2005]. One major pathway involves estrogen binding directly to synaptic membranes, where it triggers multiple signaling cascades that have rapid (within minutes) effects on synaptic transmission and on plasticity [reviewed in Woolley, 2007; Vasudevan and Pfaff, 2007; see also Norman et al., 2004; Wu et al., 2005; Dominguez et al., 2007; Hart et al., 2007; Jelks et al., 2007]. In addition, estrogens have anti-inflammatory effects on the brain, and those could be a major mechanism underlying their neuroprotective activity [Rodriguez et al., 2007; Vegeto et al., 2006; Suzuki et al., 2007]. Moreover, estrogens reduce free radical production and oxidative stress, and this activity could result in neuroprotection at several levels. As a key component of neurotoxicity, oxidative stress involves a compromise of mitochondrial structural and functional integrity [Howell et al., 2005a,b; Nilsen et al., 2006]. Oxidative stress in AD and the potential implications for prevention and therapy have been reviewed elsewhere [Behl, 2005]. See also Chiasson et al. [2006] for differential oxidative effects on neurofilaments, Dykens [2007] for a review of the redox properties of estrogens and estrogen analogues, Shintani and Klionsky [2004] for a discussion of nonapoptotic cell death pathways including autophagy, and Viña et al. [2007] for a discussion of mitochondrial oxidant signaling. It has been proposed that estrogens and other steroids intercalate into cell membranes, where they block lipid peroxidation by scavenging free radicals, and are then catalytically recycled via glutathione, thereby preserving mitochondrial function and, ultimately, cell viability [Dykens et al., 2003; Simpkins et al., 2005; Cegelski et al., 2006; Prokai et al., 2005].

A number of studies have shown that the classical estrogen receptor (ER) subtypes ERα and ERβ are located on nerve cell membranes [Vasudevan and Pfaff, 2007], and that neuroprotection involves, in at least some experimental systems, estrogen binding to membrane-associated ERα [see, e.g., Hart et al., 2007; Jelks et al., 2007; Toran-Allerand et al., 2002]. A thorough review of ligands and the binding specificity for ER subtypes can be found in Kuiper et al. [1997, 1998]. The additional possibility that certain ERs are localized to mitochondria has been suggested by a number of groups [e.g., Yang et al., 2004; Chen et al., 2007; Yager and Chen, 2007; Milner et al., 2008]. However, mechanism of action is undoubtedly complicated, and several studies have shown neuroprotection with estrogens that exhibit little, if any, binding to classical ERs [Howell et al., 2005a,b; Perez et al., 2005, 2006]. For example, the synthetic estrogen analog, MX-4565 (previously known as ZYC-5 and MITO-4565) is a potent neuroprotective agent in several model systems at doses leading to little or no binding to ERα and ERβ receptors [Xia et al., 2002; Dykens et al., 2003, 2005a], and similar results have been found for other synthetic estrogens [Cordey et al., 2005; Honjo et al., 2005]. In fact, on a molar basis, agents such as MX-4565 have significantly more potent neuroprotective activity than 17β-estradiol. It is now known that various forms of ERs and other estrogen-binding proteins are present at the cell surface [Vasudevan and Pfaff, 2007; Woolley, 2007]; these results provide a possible explanation for neuroprotection by nonfeminizing estrogens.

The following sections summarize the published literature, describing doses and concentrations of 17α-estradiol and its sulfate conjugate in women and men, exposure in food and the environment, and how these levels relate to other estrogens in the body. This summary is followed by a description of Phase I results and other human studies dealing with the pharmacokinetics and metabolism of 17α-estradiol, and finally its safety and efficacy. For clarity, we will describe the components of approved human therapies containing 17α-estradiol or its sulfate conjugate and summarize briefly the safety and efficacy studies conducted with products containing these compounds, indicating when appropriate the relevance of these studies to a target indication such as AD and other neurodegenerative disorders.

HUMAN EXPOSURE TO 17α-ESTRADIOL AND ITS CONJUGATES AS AN ENDOGENOUS SUBSTANCE AND IN FOOD AND THE ENVIRONMENT

17α-Estradiol and its conjugates are endogenous substances, and humans are further exposed to 17α-estradiol or its conjugates from food and from the environment. These compounds are, however, present at very low levels, as summarized in Table 1. In comparison, the highest dose and plasma C_{max} in the Dykens et al. [2005a] Phase I single, rising oral dose clinical trial of the sodium salt of 17α-estradiol 3-sulfate were 0.2 mg and 619 pg/ml, respectively, an order of magnitude or two higher than endogenous and environmental exposure levels. Although these data might not establish much comfort regarding the safety of 17α-estradiol, they do distinguish the drug from xenobiotics and provide an important historical context.

Proof that 17α-estradiol and its conjugates were endogenous substances in humans was difficult for

TABLE 1. Maximum Concentrations of 17α-Estradiol and Other Estrogens.

Exposure	Endogenous or other sources	17α-Estradiol	17β-Estradiol and/or estrone sulfate
Women and men	Urine	''Low levels''	
Pregnant women	Urine	21 μg/day	376 μg/day
Premenopausal women	Serum or plasma		1,246 pg/ml
Postmenopausal women	Serum		300 pg/ml
Men	Plasma		460 pg/ml
Adult women and men	Daily production		140–630 μg/day
Food	Various	0.03–0.78 μg/kg	
Food in adult women and men	Daily intake		0.08–0.10 μg/day
Waste water treatment	Effluents	5 pg/ml	

researchers to establish in early studies, but more advanced analytical techniques have allowed detection of 17α-estradiol and its conjugates by multiple laboratories at low levels in both women and men [see, e.g., Gerhardt et al., 1989]. Hobe et al. [2002] concluded from the available studies that 17α-estradiol had been found naturally in the blood and urine of many animal species, but in human urine only in limited cases or at low concentrations. Hobe et al. [2002] further suggested that this paucity of 17α-estradiol is due to the low activity of the enzyme steroid 17α-hydroxy oxidoreductase in humans, and they also noted that other investigators have suggested the possibility of 17α-estradiol arising from aromatization of epi-testosterone. Estrogens have been studied analytically in samples from adult humans using a variety of techniques, including gas chromatography and mass spectrometry (GC/MS) [Gerhardt et al., 1989]. In an early study, 17α-estradiol was not detected in pooled samples of human pregnancy urine, although there was some evidence of its presence in one individual [Schott and Katzman, 1964]. In another study, Luukkainen and Adlercreutz [1965] were unable to detect 17α-estradiol in the urine of pregnant women, though they did find 11-dehydro-17α-estradiol (of unknown origin; that is, it may not have arisen via 17α-estradiol). More advanced analytical techniques showed 17α-estradiol to be present as a minor component of 50 or more steroids identified by capillary GC/MS in urine from men and from both pregnant and nonpregnant women [Gerhardt et al., 1989]. Adlercreutz and Luukkainen [1969, 1970] also identified a number of estrogens in various biological materials from pregnant women. During the pregnancies of women with single, with twin, and with anencephalic fetuses, as well as one woman with Addison's disease, 17α-estradiol was found using GC/MS. These investigators also identified 17α-estradiol following oral administration of estrone sulfate to several subjects and in pregnancy urine. Levels of 17α-estradiol in the urine of 12 pregnant women ranged from 3 to 21 μg excreted per day. In comparison, 17β-estradiol levels in these women were 60–376 μg/day. These authors also reported finding 17α-estradiol during pregnancy in human bile, and speculated that it was formed by aromatization of a neutral 17α-hydroxylated steroid. GC/MS data suggested the presence of multiple unsaturated forms of 17α-estradiol in pregnancy urine, including 11-dehydro-17α-estradiol, although its biosynthetic route is unknown. Following what the authors qualitatively termed "huge" oral doses of estrone sulfate to four women, small amounts of 17α-estradiol were found in their urine, but 17α-estradiol was not detected in 1 liter of pooled normal pregnancy plasmas obtained at delivery.

Maume et al. [2001] assessed the presence of estradiol and its metabolites in meat, and found various classes of free estradiol and conjugates to be present, including 17α-estradiol. Hartmann et al. [1998] reported finding 17α-estradiol in goose fat, hens' eggs, milk, and cheese at levels ranging from 0.03 to 0.78 μg/kg, but none in foods of plant origin. Paris and Dolo [1993] showed that the main extractable metabolites in calves implanted with 17β-estradiol were 17α-estradiol-glycopyranoside and 17α-estradiol in the liver and kidney, with 17α-estradiol being the major glucuronidated metabolite in the kidney, liver, and muscle. However, per these articles, the daily production of estrogens in humans was estimated to range within 54–630 μg compared with a daily intake via food of 0.07–0.1 μg, hence the conclusion that the exposure to estrogens in food was insignificant.

Most samples in a study conducted on surface and wastewater in the Netherlands contained concentrations of 17α-estradiol below the limits of detection [Belfroid et al., 1999]. However, in samples where it was found, 17α-estradiol reached levels of 5 ng/L (5 pg/ml), 17β-estradiol was found at levels of $\leq$12 ng/L, and estrone was found at levels of $\leq$47 ng/L.

For perspective, consider that the major estrogens in human blood are 17β-estradiol, estrone, and estrone sulfate [Fotherby, 1996]. Serum concentrations of estrogens vary during the menstrual cycle of normal women, as might be expected [Gruber et al., 2002; Ruder et al., 1972]. The potent feminizing estrogen 17β-estradiol reaches the highest serum levels in premenopausal women, at 250–500 pg/ml, in the preovulatory phase. However, after menopause, estrone sulfate achieves the highest serum concentrations, with a mean value of 300 pg/ml, whereas 17β-estradiol drops to <20 pg/ml. These values are summarized in Table 2. Again for comparison, note that the plasma C_{max} from the highest dose (0.2 mg/subject) in the Dykens et al. [2005a] Phase I clinical study of the sodium salt of 17α-estradiol 3-sulfate was 619 pg/ml, and that postmenopausal women dosed with 0.625 mg Cenestin® (a branded blend of synthetic conjugated estrogens) achieve total estrone C_{max} levels of 2,500–3,000 pg/ml [Stevens et al., 2002].

PHARMACOKINETICS AND METABOLISM IN HUMANS

Following oral or sublingual doses, 17α-estradiol is present only at low levels as free drug [Hobe et al., 2002] because it is extensively conjugated in humans, as it is in all animal studies reported to date. Conversion of 17α-estradiol to 17β-estradiol has only been detected in humans after large doses. 17α-Estradiol is also readily found after large doses of other estrogens such as estrone. For additional details on this topic, see the review of the pharmacokinetics and pharmacodynamics of conjugated equine estrogens by Bhavnani [1998] and the studies in Kuhnz et al. [1993].

Pharmacokinetics in Humans

Several studies have evaluated the pharmacokinetic (PK) parameters of 17α-estradiol or its sodium sulfate conjugate in humans after PO, sublingual, and IV dosing. Table 3 summarizes these data. The half-life and clearance of 17α-estradiol suggest that once daily dosing might be possible.

TABLE 2. Approximate Serum and Plasma Concentrations (pg/ml) of Estrogens in Normal Women and Men.

Phase	17β-Estradiol	Estrone	Estrone sulfate	Estriol
Women				
Follicular	40–200	30–100	654	3–11
Preovulatory	250–500	50–200		
Luteal	100–150	50–115	1,246	6–16
Premenstrual	40–50	15–40		
Postmenopausal	<20	15–80	300	3–11
Men				
Ages 21–48	34	47	460	

The Dykens et al. [2005a] Phase I clinical study was conducted in the U.K. on a total of eight postmenopausal female volunteers. (Although the results of this Phase I trial have been reported previously [Dykens et al., 2005a], we include the basic data and conclusions here for completeness and ease of comparison with other studies.) This Phase I study evaluated the safety, tolerability, and pharmacokinetics of rising oral doses, administered once daily, of solutions of the sodium salt of 17α-estradiol 3-sulfate. All eight volunteers successfully completed the study. Six subjects received the sodium salt of 17α-estradiol 3-sulfate in oral solution (rising doses of 0.05, 0.1, and 0.2 mg), and two subjects received placebo. At least 7 days elapsed between dose administrations, and each study period was 24 h in duration. The plasma concentrations of 17α-estradiol (measured after enzymatic cleavage of sulfate) increased with the rising doses, and they were proportional to the peak plasma concentrations, but they were not proportional with respect to the overall extent of absorption as measured by area under the curve (AUC). There were no statistically significant differences in T_{max} between the dose levels ($P = 0.61$). The elimination half-life of the drug was dose dependent, and varied from 16.5 to 19.2 h. See Table 4 for summary data.

In the clinical study conducted by Hobe et al. [2002], male volunteers 20–46 years old received 17α-estradiol at 4 mg orally ($n = 3$) or at 0.4 mg sublingually ($n = 4$); both regimens were supplemented with a tracer dose of tritium-labeled 17α-estradiol. The serum concentrations of 17α-estradiol (free and liberated by enzymatic hydrolysis) were quantified by GC/MS, and the serum total radioactivity and urinary radioactivity excretion were determined. After oral administration, 17α-estradiol was rapidly and extensively conjugated, and less than 1% of the free steroid appeared in serum. The 17α-estradiol conjugates were eliminated from serum with a half-life of >10 h. Sublingual administration resulted in significantly higher serum levels of the free compound (5–15%). The drug levels reported in this publication are compared with those in the Dykens et al. [2005a] Phase I trial in Table 5.

Bioavailability

The bioavailability of 17α-estradiol is not known with precision. The absolute human bioavailability of a 2-mg dose of 17β-estradiol has been reported to be about 5%, with a range of <1% to 12% between subjects [see Fotherby, 1996, and references cited therein].

Specific Effects on Population Subgroups

There are no known data relating to specific effects of 17α-estradiol by sex, pregnancy, ethnic

TABLE 3. Summary of Human Pharmacokinetics Data With 17α-Estradiol.

Studies	Results	Comments	Subjects	Dose/Duration	References
Phase I study to evaluate safety, tolerability, and PK of single rising PO doses of 17α-estradiol sodium sulfate	Plasma concentrations from all dose levels proportional to C_{max} but not to AUC • C_{max} (free+conjugated) ~154–619 pg/ml • T_{max} ~24 h • $T_{1/2}$ ~1725 h	• Conducted in compliance with ICH/GCP guidelines	• 8 healthy postmenopausal women • 6 treated with ascending doses • 2 treated with placebos	• 0.05, 0.1, 0.2 mg PO solution • single rising dose; administration with ≥7-day washout period between doses	Dykens et al., 2005a
PO and sublingual dosing of 17α-estradiol	4 mg oral dose yielded: • C_{max} free 76 pg/ml • C_{max} conjugated 16.5 ng/ml 0.4 mg sublingual dose yielded: • C_{max} free 652 pg/ml • C_{max} conjugated 5.8 ng/ml	• Conducted in compliance with ICH/GCP guidelines • <1% free steroid following oral dosing • 5–15% free steroid following sublingual dosing	• 12 male volunteers • 2 to 4/group	• 4 mg PO • 0.4 mg sublingually • single dose administration	Hobe et al., 2002
IV dosing of tritiated 17α-estradiol	• Excreted at relatively rapid rate • 58–66% of radioactivity recovered in urine over 4 days		• 2 nonpregnant women • aged 40 and 45	• IV dose of ~25 μCi of material with specific activity 108 μCi/μg • single dose administration	Williams and Layne, 1967

TABLE 4. Summary of Pharmacokinetic Parameters After Single Rising Oral Doses of 17α-Estradiol Sodium Sulfate to Postmenopausal Women*.

Dose level (μg)	Parameter	C_{max} (pg/ml)	T_{max}[a] (h)	AUC 0-t (pg/ml · h)	AUC 0-infinity (pg/ml · h)	Lambda z (h-1)	$T_{1/2}$ (h)
50	Mean	154.5	2.07	869.85	3312.19	0.1118	19.254
	SD	94.46	0.5–4.02	949.56	3016.88	0.0964	25.2161
100	Mean	258.17	1.5	1937.58	3612.21	0.0502	17.177
	SD	186.95	1.0–4.0	1145.58	1534.2	0.0257	8.6402
200	Mean	619.33	4	4707.62	7005.53	0.0467	16.5389
	SD	444.94	1.0–4.05	2469.97	3554.23	0.0207	4.7754

*Drug levels are total (free plus conjugated material after enzymatic cleavage and basic workup).
[a]Median values and range are presented for T_{max}.

subgroups, children, the elderly, or impaired organ function.

Interactions

There are no known drug–drug interactions for 17α-estradiol, and no data on the effects of food have been found.

Excretion and Metabolism in Humans

The excretion and metabolism of 17α-estradiol and/or its conjugates have been investigated in several clinical studies, as summarized in Tables 6 and 7. Evidence for human metabolic conversion of 17α-estradiol to 17β-estradiol, the latter being a more potent feminizing hormone, has been reported, but only in trace amounts after high doses of 17α-estradiol.

After oral dosing, 17α-estradiol has been found to be rapidly conjugated, with only small amounts of free steroid found in serum. Sulfate and sugar conjugates have been identified after administration of 17α-estradiol, with evidence for doubly conjugated forms and mixed conjugation. Certain conjugates are deconjugated and reconjugated before urinary excretion. No metabolism of the central steroid ring structure has been reported after administration of 17α-estradiol. The extent of plasma protein binding has not been reported (Fig. 1). For example, Hobe et al. [2002] reported that the amount of unconjugated 17α-estradiol in serum is

TABLE 5. Drug Levels in Serum or Plasma After Oral Dosing of 17α-Estradiol or 17α-Estradiol Sodium Sulfate to Men or Postmenopausal Women*.

Sex	Dose	No. of subjects	Route	Where measured	Peak Free (ng/ml)	Peak conjugated (ng/ml)	Peak total (ng/ml)	Peak time (min)	Ratio of peak total/dose
Male	4 mg 17α-estradiol	4	PO	serum	0.08	16.52	16.60	90	4.15
Female	0.20 mg 17α-estradiol sodium sulfate	6	PO	plasma			0.62	240	3.10
Female	0.10 mg 17α-estradiol sodium sulfate	6	PO	plasma			0.26	90	2.58
Female	0.05 mg 17α-Estradiol Sodium Sulfate	6	PO	plasma			0.15	124	3.09

*Drug levels are total (free plus conjugated material after enzymatic cleavage and acidic or basic workup).

TABLE 6. Urinary Excretion of Estrogens in Two Men After Oral 50 mg 17α-Estradiol.

	Levels in urine (μg/24 h)		
Day[a]	Estrone	17α-/17β-Estradiols Fraction	Estriol
1	2–4	2	5–7
2	15–38	565–657	15–25
3	11–30	460–532	17–23
4	7–16	279–355	11–14
5	6–8	92–190	5–12
6	5	59–73	0–18

[a]17α-Estradiol was administered on day 2.

less than 1% at all time points over the 24-h period after oral dosing in humans, and that it is conjugated rapidly and extensively. Significantly higher levels of free 17α-estradiol (5–15%) were evident during the first 12 h following sublingual administration. While conjugation was extensive, no evidence was found of metabolism of the steroid ring structure. Evidence of sulfate conjugation was found, including mixed conjugates (sulfates and glucuronides). Evidence of the presence of 3- and 17-monosulfate and 3,17-disulfate metabolites of 17α-estradiol was also reported. Hobe et al. [2002] found no evidence of administered 17α-estradiol being converted into estrone or 17β-estradiol (or their conjugates) in humans. Similarly, in the Dykens et al. [2005] Phase I clinical study, no measurable conversion of 17α-estradiol to 17β-estradiol was found.

In a clinical pilot study, six men over age 50 were treated with 2 mg/day of 17α-estradiol orally for 12 weeks [see Schröder et al., 1997; Oettel, 1999]. There were no signs of metabolism of 17α-estradiol to estrone or to 17β-estradiol, as reflected by serum levels of 17β-estradiol and estrone and unchanged hepatic estrogenicity; that is, no changes were found in serum hormone binding globulin (SHBG), corticosteroid binding globulin (CBG), prolactin, testosterone, or subjectively assessed breast tenderness.

Williamson et al. [1972] studied the metabolism of tritium-labeled 17α-estradiol-17-glucoside administered IV to two normal young women. Within 72 h, 46–60% of the radioactivity was excreted in urine. During the first 6 h, 1.4–4% of the dose was excreted as unchanged glucoside, along with 5–14% of a derivative identified as the doubly conjugated 3-glucuronide, 17-glucoside of 17α-estradiol. During the following 42 h, none of the injected glucoside and only minor amounts of the 3,17-double conjugate were present in urine. After the initial 6 h, the main excretory product was the 17-glucuronide of 17α-estradiol.

Williams and Layne [1967] studied the metabolism of tritiated 17α-estradiol administered IV to two nonpregnant female subjects, aged 40 and 45 years. The material was excreted at a relatively rapid rate, mostly as the glucuronoside conjugate of 17α-estradiol. The major metabolite was 2-methoxy-17α-estradiol, although levels were not reported. The presence of radioactive estrone in the urine could not be established, and polar metabolites were present in amounts less than 1% of recovered radioactivity. These investigators concluded that 17α-hydroxyphenolic steroid dehydrogenase activity is not extensive in humans, and that the presence of a 17α-hydroxy group almost completely inhibits metabolism of steroid ring D.

Breuer and Schott [1966] reported on the metabolism of 17α-estradiol following administration of 50 mg orally to two male subjects, aged 35 and 40 years. In these subjects, 2.2–3.4% of the administered dose was recovered in the combined fractions containing urinary estrone, estradiol, and estriol, in contrast to 20% following the oral administration of 50 mg of 17β-estradiol. 17α-Estradiol was metabolized to a much greater extent than was 17β-estradiol during incubation with human liver slices.

TABLE 7. Summary of Data on Product Metabolism in Humans.

Study	Results	Comments	Subjects	Dose/Duration	Reference
Phase I study to evaluate safety, tolerability, and PK of single rising PO doses of 17α-estradiol sodium 3-sulfate	• No apparent conversion to 17β-estradiol	• Samples treated enzymatically and subjected to basic workup • Conducted in compliance with ICH/GCP guidelines	• 8 healthy postmenopausal women • 6 treated with ascending doses • 2 treated with placebos	• 0.05, 0.1, 0.2 mg, PO • single rising dose administration with ≥7-day washout period	Dykens et al., 2005a
Metabolism of 17α-estradiol	• 17α-estradiol rapidly and extensively conjugated • 4 mg PO yielded C_{max} free of 76 pg/ml and C_{max} conjugated of 16.5 ng/ml 0.4 mg, sublingual yielded C_{max} free of 652 pg/ml and C_{max} conjugated of 5.8 ng/ml • Evidence of mono- and di-conjugation with sulfates and glucuronides, including mixed conjugates • No evidence of conversion to estrone or 17β-estradiol was found	• Samples treated enzymatically and subjected to acidic workup • <1% free steroid found in serum following PO dosing at 20 min–24 h postdosing • 5–15% free steroid found in serum at 10 min–12 h postdosing • No evidence of metabolism of core steroid ring structure	• 12 male volunteers • 2 to 4/group	• 4 mg PO • 0.4 mg sublingually • single dose administration	Hobe et al., 2002
Metabolism study of 17α-estradiol	• No signs of metabolism to estrone or 17β-estradiol		• 6 men • >50 years old	• 2 mg PO daily for 12 weeks	Schröder et al., 1997, and Oettel, 1999
Metabolism study of tritiated 17α-estradiol-17-glucoside	• Within 72 h, 4–60% of radioactivity excreted in urine • In 1st 6 h, 1–4% was excreted as unchanged glucoside • In 1st 6 h, 5–14% excreted as doubly conjugated 3-glucuronide, 17-glucoside • In 6–48 h period, none of injected glucoside and minor amounts of double conjugate present in urine • After 1st 6 h, main excretory product was 17-glucuronide	17α-estradiol-6,7-^{3}H-glucoside injected	• 2 women • normal and young	• tracer levels, IV	Williamson et al., 1972
Metabolism study using tritiated 17α-estradiol	• 58–66% of radioactivity recovered in urine over 4 days • >90% of deconjugated radioactive urinary isolates in 1st 24 h were unchanged 17α-estradiol • Most excreted as glucuronoside • Major metabolite was 2-methoxy-17α-estradiol (amount not given) • No estrone detected in urine (<0.1% if any) • Polar metabolites <1% of recovered radioactivity	17α-estradiol-6,7-^{3}H injected	• 2 women • not pregnant • ages 40 and 45	• ~25 μCi of 17α-estradiol (108 μCi/μg), IV • single dose administration	Williams and Layne, 1967

TABLE 7. Continued.

Study	Results	Comments	Subjects	Dose/ Duration	Reference
Metabolism of 17α-estradiol	• Estrone isolated as metabolite in urine • Trace amounts, at most, of 17β-estradiol detected	• Samples treated enzymatically and subjected to strong acid • Only 2.2–3.4% of dose recovered in estrogen fractions (vs. 20% when 17β-estradiol administered)	• 2 men • 35 and 40 years old	• 50 mg PO • single dose administration	Breuer and Schott, 1966
Metabolism in human liver homogenates	• Formation of 17α-estradiol-17β-D-glucoside observed • No glucosides of 17β-estradiol or estrone observed under similar conditions	Done in the presence of UDP-glucose	In vitro	• Human liver homogenates	Williamson and Layne, 1971
Properties of a human adrenal steroid alcohol sulfotransferase	• Only monosulfates are formed from 3,17-diols • Phenolic hydroxyl sulfurylated at very low rates	• Sulfurylated at 17-position 6x faster than 17β-estradiol	In vitro	• Human adrenals	Adams and McDonald, 1981

The formation of 17α-estradiol-17β-D-glucoside in vitro by human liver homogenates was shown in the presence of uridine diphosphate (UDP) glucose. No glucosides of 17β-estradiol or estrone were observed under similar conditions [Williamson and Layne, 1971].

Adams and McDonald [1981] reported on the in vitro properties of a human adrenal steroid alcohol sulfotransferase. This sulfotransferase sulfurylates hydroxyl groups on different positions of the steroid ring. Although only monosulfates are formed from substrates such as 3,17-diols, the position of the sulfate group depends on the relative configuration of the hydroxyl groups. 17α-Estradiol was found to be sulfurylated at the 17-position at a much higher rate than 17β-estradiol (6:1), and the phenolic group of estrogens was sulfurylated at very low rates.

SAFETY AND EFFICACY

Therapeutic Effects

To date no published studies of 17α-estradiol or its 3-sulfate conjugate have evaluated the compound as a single agent in a neurodegenerative disorder such as AD, or as a cognition activator. Instead, some studies have assessed the activity of the compound in non-neurological models. For example, in a clinical pilot study [see Schröder et al., 1997; Oettel, 1999], researchers evaluated the therapeutic potential of 17α-estradiol vs. 17β-estradiol in several in vitro and in vivo model systems. No differences in nongenomic actions of 17α-estradiol and 17β-estradiol were observed, based on the evaluation of antioxidant potential in vitro, inhibition of iron-catalyzed lipid peroxidation in synaptosomal membranes, copper-induced low-density lipoprotein (LDL) oxidation, and uptake of oxidatively modified LDL in human blood macrophages, including influence on human endothelial function (i.e., nitric oxide release, adhesion proteins, and differentiation of monocytes to macrophages). On the other hand, clear differences in genomic hormone action were observed between the two estradiol isomers. The estrogenic effects of 17α-estradiol in a human breast cancer cell line (MCF-7/2A) were at least ten times lower than those of 17β-estradiol. In the human pilot study, treatment with 17α-estradiol resulted in a significant delay in the lag phase of LDL oxidation. These results show that the anti-oxidative activity of 17α-estradiol is not mediated by classical ER-linked pathways.

The controlled, randomized, double-blind trial conducted by Orfanos and Vogels [1980] evaluated the effects on alopecia androgenetica of daily application of Pantostin (0.025 17α-estradiol hair lotion) for an average of 6.5 months. Trichograms were taken and evaluated under standardized conditions, and in 63% of the treated patients, a reduction in the amount of telogen hairs was recorded, whereas in the control (placebo) group, the same reduction was found in only 37% of the cases. Similarly, in the treatment group, only 11% of the patients worsened, in contrast to 50% in the control group who showed an increased telogen rate ($>10\%$). The amount of growing anagen hairs and of seborrhea did not change significantly in either group, and no side effects were observed.

Munster et al. [2003] studied testosterone metabolism in human skin cells in vitro and its interaction with other steroids. They assessed cell-type-specific androgen metabolism in human skin, and the inhibition of this metabolism by drugs. Cultured human foreskin and scalp skin keratinocytes and fibroblasts, as well as

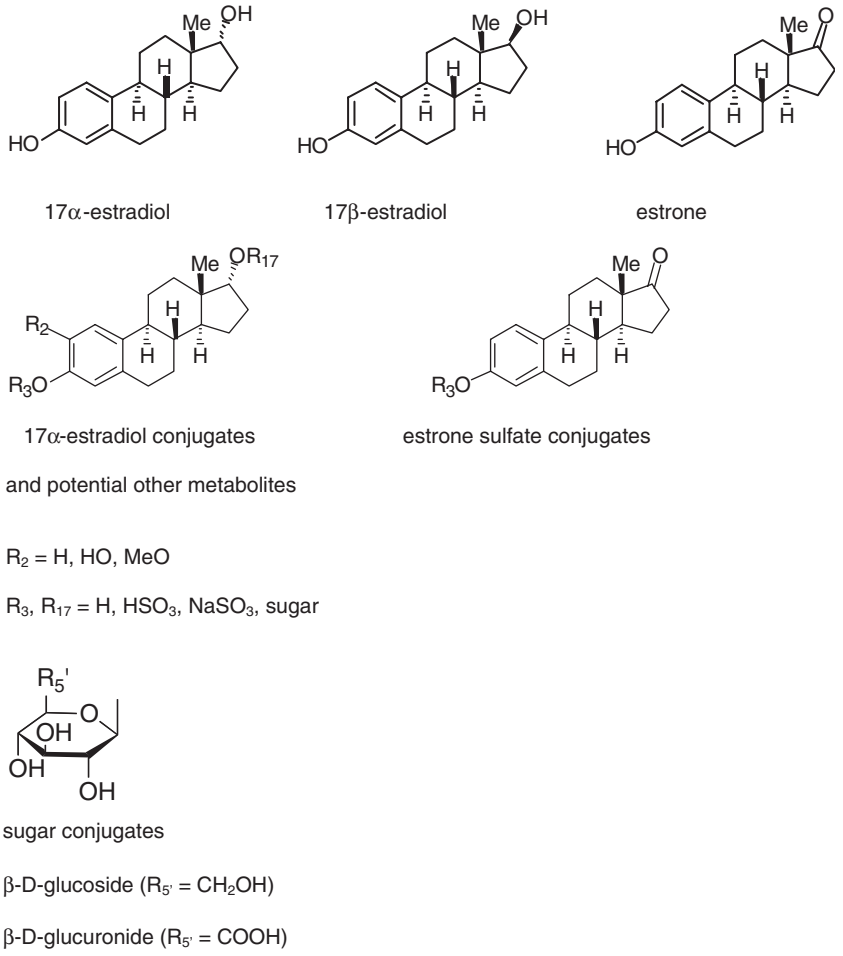

Fig. 1. Chemical structures of estradiol isomers, conjugates, and metabolites.

occipital scalp dermal papilla cells (DPC), were incubated with testosterone alone and in the presence of 17α-estradiol, 17β-estradiol, or another steroid. At nontoxic concentrations, 17α-estradiol and 17β-estradiol decreased 17-ketometabolites. The authors concluded that the effects of 17α-estradiol in androgenetic alopecia are not due to 5α-reductase inhibition.

Niiyama et al. [2001] investigated the influence of estrogens on androgen metabolism in human hair follicles to study the pathways involved in estrogen-mediated induction of hair growth in androgenic alopecia. In contrast to the potent 5α-reductase inhibitor finasteride, the estradiols were relatively weak. At 100 nM in dermal papillae, 17α-estradiol inhibited the synthesis of dihydrotestosterone (DHT) by only 20%, and 17β-estradiol inhibited DHT synthesis by 60%, whereas finasteride inhibited DHT synthesis by 86% at 1 nM.

The influence of 17α-estradiol, referred to by the authors as "a hormonally almost inactive isomer" of 17β-estradiol, on testosterone metabolism in rat liver slices was studied by Schriefers et al. [1991]. The relative inhibitory effects of 17α-estradiol:17β-estradiol:17α-ethinylestradiol were 100:73:58, the inverse of their hormonal potencies.

The significance of these limited nonneurological studies is not overwhelming, but they cannot be ignored either. The results show that, in more than one system, 17α-estradiol has significant therapeutic effects relative to the more feminizing 17β isomer.

Safety Data

Several human studies of 17α-estradiol or its 3-sulfate conjugate that are relevant from the perspective of safety are described below.

The sodium salt of 17α-estradiol 3-sulfate has been evaluated in a Phase I clinical study [Dykens et al., 2005a], conducted at single doses of ≤ 0.2 mg (peak plasma concentrations of 619 pg/ml). In addition, 17α-estradiol has been the subject of eight published clinical studies, and no significant adverse effects were

reported, even at single oral doses as high as 50 mg and after oral doses of 2 mg administered daily for as long as 12 weeks. In reports of doses of 17α-estradiol administered IV (single dose radiotracer metabolism studies), SC (daily for 3–6 weeks), and topically (for at least 6 months), no systemic hormonal responses or other significant safety issues have been reported. Doses used in human studies of 17α-estradiol that may have some bearing on safety are compared briefly in Table 8, and more detailed information is provided in Table 9.

In the Phase I study [Dykens et al., 2005a], all doses of the sodium salt of 17α-estradiol 3-sulfate were well tolerated. There was no vaginal bleeding and no breast tenderness. Three adverse effects were reported: (1) sensations of altered body temperature after administration of the 0.1-mg dose, (2) rhinitis 1–3 days postdose, lasting 5 days after administration of the 0.1-mg dose, and (3) urinary tract infection 1 day postdose, lasting 12.9 days after administration of a placebo dose. All were classified as mild and unlikely to be related or not related to study medication. All biochemistry, hematology, and urinalysis results, as well as vital signs and electrocardiogram (ECG) measurements, remained within normal ranges or the deviations were not considered clinically significant by the investigating physician.

In another clinical pilot study [see Schröder et al., 1997; Oettel, 1999], 17α-estradiol prolonged the lag time significantly for ex vivo oxidation of LDL by copper, suggesting an in vivo antioxidant effect. There were no signs of feminizing estrogenic activity as reflected by serum levels of 17β-estradiol and estrone and by unchanged hepatic estrogenicity (no change in SHBG, CBG, prolactin, testosterone, or subjectively assessed breast tenderness).

In the study by Meyer et al. [1976], there were no significant changes in serum electrolytes, lipids, glucose, basic urea nitrogen (BUN), creatinine, liver enzymes, SHBG, albumin, globulin, calcium, phosphate, or other blood chemistry measures. There were also no complaints of headache, nausea, diarrhea, modification of menstrual cycles, gynecomastia, tingling of the breasts, or breast tenderness, and no findings of edema, altered blood pressure, changes in respiratory rate, body temperature, or body weight. One man reported a decrease in libido, which persisted for 1 year after 17α-estradiol treatment was discontinued. In contrast, the potent feminizing isomer 17β-estradiol causes a host of changes in humans, including breast development, growth of uterine lining, suppression of gonadotropins, changes in skin texture, elevation of plasma growth hormone, insulin, liver enzymes, proteins, triglycerides, copper, and ceruloplasmin, and

TABLE 8. Comparison of Doses of 17α-Estradiol in Published Human Studies.

Study	Subjects	Duration	Highest dose and route	Reference
Phase I study to evaluate safety, tolerability, and PK of single rising doses with 17α-estradiol sodium sulfate	• 8 healthy postmenopausal women • 6 treated • 2 placebos	3 rising single doses	0.2 mg PO single dose	Dykens et al., 2005a
Clinical pilot study	• 6 men • >50 years old	12 wks	2 mg PO	Schröder et al., 1997, and Oettel, 1999
Estrogenicity of 17α-estradiol	Subset of: • 16 women with secondary amenorrhea • 2 castrated patients • 29 women with menopausal complaints (hot flushes)	Single dose or multiple dose	>3 mg/day PO for 14 days	Lauritzen, 1969
Metabolism of 17α-estradiol	• 2 men • 35 and 40 years old	Single dose	50 mg PO	Breuer and Schott, 1966
Metabolism of 17α-estradiol	• 12 male volunteers • 2 to 4/group	Single dose	20 mg PO	Hobe et al., 2002
Separation of effect on collagen from other clinical and biochemical effects with 17α-estradiol	• 5 patients • all with well established cystinuria • D-Penicillamine co-administered	Daily injections for 3–6 weeks	15 μg/kg/day SC	Meyer et al., 1976

TABLE 9. Safety and Efficacy of 17α-Estradiol in Human Subjects.

Study	Results		Subjects	Dose/Duration	Reference
Phase I study to evaluate safety, tolerability, and PK of 17α-estradiol sodium sulfate	• Well tolerated • Biochemistry, hematology, urinalysis, vital signs, and ECG normal or insignificant change • No signs of feminizing effects found	• 3 mild adverse events • 2 considered unlikely to be related to therapy (temperature change sensation and rhinitis) • 1 considered unrelated (urinary tract infection)	• 8 healthy postmenopausal women • 6 per group • 2 placebos per group	• 0.05, 0.1, 0.2 mg PO solution • single rising dose administration with ≥7-day washout period	Dykens et al., 2005a
Clinical pilot study of 17α-estradiol	• No side effects found • No signs of feminizing effects found	Significant delay of lag phase of LDL oxidation	• 6 men • >50 years old	• 2 mg PO QD for 12 weeks	Schröder et al., 1997, and Oettel, 1999
Separation of effect on collagen from other clinical and biochemical effects with 17α-estradiol	• No detectable changes found in any of a large number of parameters, including breast development, menstruation, blood pressure, and serum clotting factors • 1 man reported reduced libido for 1 year after stopping therapy • Stinging at injection site for 1–2 min	Increases in skin prolyl hydroxylase activity, soluble collagen content in the skin, and urinary hydroxyproline excretion	• 5 patients • all had well-established cystinuria • D-penicillamine co-administered	• 10–15 μg/kg/day SC for 3–6 weeks	Meyer et al., 1976
Estrogenicity of 17α-estradiol	Ineffective at stimulating atrophic endometrium	Effects on vaginal smear, cervical mucus, and gonadotropin excretion corresponded to relative estrogenic activity	Subset of: • 16 women with secondary amenorrhea • 2 castrated patients • 29 women with menopausal complaints	• >3 mg//day PO for 14 days	Lauritzen, 1969
Local application of 17α-estradiol for androgenetic alopecia	No systemic side effects observed during 6.5 mo (e.g., no gynecomastia or changes in libido found)	Like 17βestradiol, may reduce androgenetic hair loss, but not regrow new hair	• 69 patients • 51 evaluated • controlled, double blind, randomized	• 0.025% alcoholic solution applied topically to scalp for ≥6 months	Orfanos and Vogels, 1980

salt and water retention. None of these changes were observed after 3–6 weeks of daily 17α-estradiol administration. In this study, 17α-estradiol increased skin prolyl hydroxylase activity, increased soluble collagen content in the skin, and increased urinary hydroxyproline excretion [Meyer et al., 1976].

Lauritzen [1969] reported that single oral doses of 17α-estradiol of >3 mg for 14 days in women were ineffective in stimulating the proliferation of an atrophic endometrium or affecting the amenorrhea index. In contrast, potent feminizing hormone preparations, such as ethinyl-estradiol and conjugated estrogens, are effective at 0.04–40 mg. The effects on vaginal smear, cervical mucus, and gonadotropin excretion corresponded to the estrogenic activity. These results again demonstrate the lack of feminizing effects of 17α-estradiol in humans.

The package insert for Pantostin describes no adverse reactions. 17α-Estradiol, 0.025%, is the active ingredient in this product, which is approved and marketed in Germany for topical use. In the controlled, randomized, double-blind trial conducted by Orfanos and Vogels [1980], daily application of Pantostin for an average of 6.5 months resulted in no local or systemic adverse effects. No systemic hormonal effects such as gynecomastia or changes in libido were noted at 6.5 months, suggesting that systemic levels of 17α-estradiol are not high enough to induce feminizing effects, that it

is not converted significantly into more potent feminizing estrogens, and/or that it does not have systemic feminizing effects at this dose. The primary findings of this study indicated that hair lotions containing 17α-estradiol might have therapeutic value similar to that of 17β-estradiol in reducing androgenetic hair loss if applied topically for a long period of time. However, no regrowth of new hair was found.

Additional clinical studies have been conducted with 17α-estradiol [Breuer and Schott, 1966; Hobe et al., 2002], but it is unclear whether potential side effects were adequately documented; that is, they may have been observed but not reported, or simply not observed. As a result, we do not detail those studies here.

Finally, the package insert for Premarin describes its indications and usage, contraindications, warnings, precautions, adverse reactions, and other relevant information (see www.premarin.com). However, most or all of its side effects are believed to be driven by the potent feminizing actions of the mixture's many hormonal components and their metabolites. Thus, 17α-estradiol would not generally be expected to have these effects, although one cannot be certain without further study.

Taken together, these reports indicate that 17α-estradiol is not significantly metabolized to potent feminizing hormones in humans, does not have feminizing hormone actions on its own, even at elevated doses, and, finally, does not produce any adverse side effects.

Marketing Experience

Multiple FDA-approved conjugated estrogen preparations contain sub-milligram quantities of the sodium salt of 17α-estradiol 3-sulfate. These include Premarin and Prempro, in which 2.5–9.5% of the conjugated estrogen component is 17α-estradiol. Examples of other FDA-approved and marketed drugs include Cenestin®, which may contain up to 1.2% of 17α-estradiol 3-sulfate, and Pantostin, which contains 0.025% 17α-estradiol.

Most estrogen-based hormone replacement products are a combination of multiple estrogens, commonly referred to as estrogen or hormone replacement therapy (ERT or HRT). Premarin has been one of the most commonly prescribed drugs in recent years, with more than 26 million prescriptions dispensed in the United States in 2003 (the nineteenth most commonly prescribed product in the United States in 2003 and the 22nd most commonly prescribed in 2006; see www.rxlist.com). As mentioned previously, ten major components are commonly noted in extracted products like Premarin, derived from the urine of pregnant mares, and more than 200 components can be separated using modern analytical techniques. Another HRT product, Cenestin, is a blend of nine synthetic conjugated estrogens. In a number of estrogen products, estrone is the principal constituent, present at levels in the range of approximately 50–85%, according, for example, to *The Physician's Desk Reference* (PDR) [2006]) (see Fig. 2 and Tables 10–13).

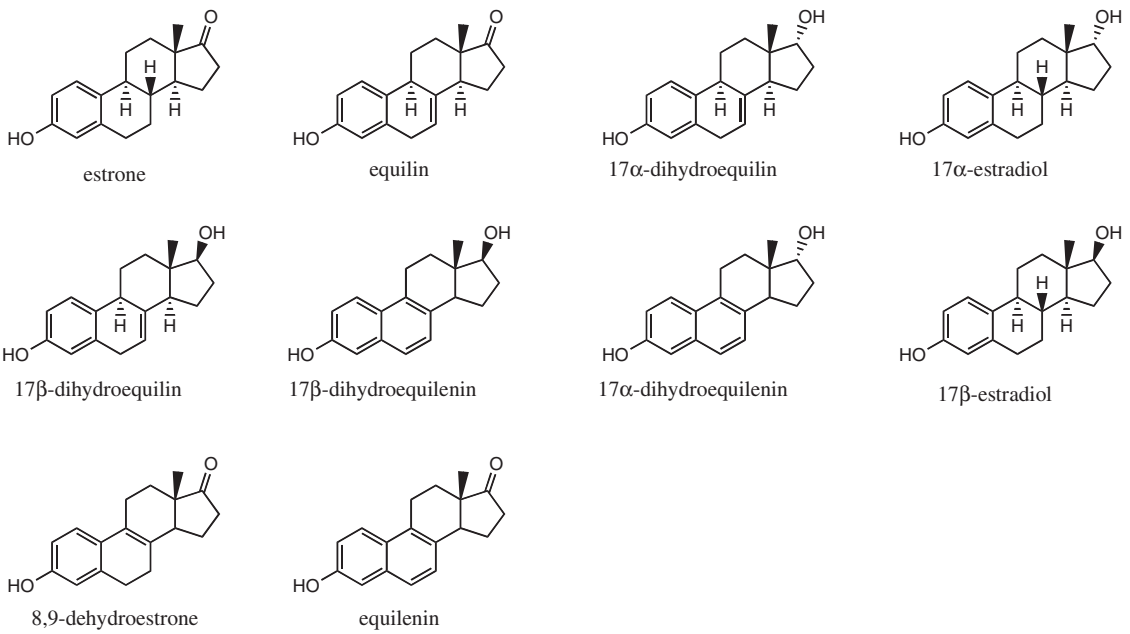

Fig. 2. Chemical structures of major components of premarin (without sulfates).

TABLE 10. Estrone in Representative HRT Products.

Brand names	Description	Major estrogen components (%)	Company
Cenestin®	Blend of synthetic conjugated estrogens (sodium sulfates)	Estrone (~50–60%)	Duramed/ Barr
Estradiol	Estradiol	17β-estradiol (100%)	Watson
Estratest®	Esterified estrogens (sodium sulfates) and methyl testosterone	Estrone (75–85%, not counting progestin)	Solvay
Gynodiol®	Estradiol	17β-estradiol (100%)	Novavax
Menest®	Esterified estrogens (sodium sulfates)	Estrone (75–85%)	Monarch
Prefest®	Estradiol and norgestimate	17β-estradiol (100%, not counting progestin)	Monarch
Premarin®	Conjugated estrogens (sodium sulfates)	Estrone (52.5–61.5%)	Wyeth
Prempro™ and Premphase®	Conjugated estrogens (sodium sulfates) and medroxyprogesterone acetate	Estrone (52.5–61.5%, not counting progestin)	Wyeth

TABLE 11. Major Components of Premarin*.

Component (sodium sulfates)	Component (mg)	% of total
Estrone	0.370	59.20
Equilin	0.168	26.88
17α-Dihydroequilin	0.102	16.32
17α-Estradiol	0.027	4.32
17β-Dihydroequilin	0.011	1.76
17α-Dihydroequilenin	0.011	1.76
17β-Dihydroequilenin	0.021	3.36
Equilenin	0.015	2.40
17β-Estradiol	0.005	0.80
D8,9-Dehydroestrone	0.026	4.16

*Based on average of two 0.625-mg tablets.

The specifications of maximum 17α-estradiol (as sodium sulfate) levels in Premarin are ≤9.5% of a 2.5-mg tablet, or ≤0.2375 mg of the sodium salt of 17α-estradiol 3-sulfate (~0.6 μmole). However, Premarin doses of 30 mg/day have been prescribed for certain indications and uses described therein for "breast cancer, for palliation only, in appropriately selected women and men with metastatic disease" [see PDR, 2006], making potential daily exposure to the sodium salt of 17α-estradiol 3-sulfate as high as 2.85 mg. As a component of Premarin, the sulfate conjugate of 17α-estradiol has been administered to both women and men for 3 months or longer. In one long-term study, the effectiveness of estrogens for therapy of myocardial infarction at doses up to 10 mg/day was studied in 275 middle-aged men during the 1950s and 1960s [see, e.g., Stamler et al., 1963].

17α-Estradiol is present as the 3-position sodium sulfate salt (molecular weight 374.44) in marketed hormone replacement products such as Premarin and Cenestin. The actual amount of 17α-estradiol (as sodium sulfate) in different products has been analyzed by various groups, which have generally found quantities in the middle of the typical range. For example, the FDA analyzed two Premarin 0.625-mg tablets and found an average of 0.027 mg (4.3%) of 17α-estradiol to be present (see Table 11; FDA Memo, 1997). Adams et al. [1979] analyzed five batches of 1.25-mg Premarin tablets and found 0.045–0.060 mg 17α-estradiol. Three generic 1.25-mg estrogen tablets showed 0.011 mg of 17α-estradiol (and with daily 2.5-mg tablets, 17α-estradiol levels in urine ranged from 7.0 to 24.6 μg/day in 25 physiologically or surgically menopausal women, increasing 2- to 3-fold over 3–4 weeks). Roos and Lau-Cam [1985] analyzed 20 tablets of conjugated or esterified estrogens and found 17α-estradiol levels of 0.49–3.40% and estrone levels of 46.6–88.9%.

U.S. Pharmacopeia (USP) standards have been set for estrogens, including Premarin, and monographs for other estrogen products. (USP works closely with the FDA, the pharmaceutical industry, and the health professions to establish authoritative drug standards. These standards are enforceable by the FDA and the governments of other countries, and are recognized worldwide as a hallmark of quality. About 4,000 standard monographs are published in the USP and the National Formulary, the official drug standards compendia.) Recent USP guidelines [see the United States Pharmacopoeia, 1982, p. 627, in particular] state the following:

> Conjugated Estrogens is a mixture of sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from Estrone and Equilin. It contains other conjugated estrogenic substances of the type excreted by pregnant mares. It is a dispersion of the estrogenic substances on a suitable powdered diluent.
> Conjugated Estrogens contains not less than 52.5% and not more than 61.5% of sodium

TABLE 12. Comparison of Two Estrogen Products and Recommended Daily Prescribing Dosages vs. 17α-Estradiol Exposure.

	Tablet Strengths (mg)						Maximum dose (mg)
	0.3	0.45	0.625	0.9	1.25	2.5	30
Premarin							
Minimum 17α-estradiol 2.5%	0.0075	0.01125	0.015625	0.0225	0.03125	0.0625	0.75
Maximum 17α-estradiol 9.5%	0.0285	0.04275	0.059375	0.0855	0.11875	0.2375	2.85
Cenestin							
Average 17α-estradiol 1.2%	0.0036		0.0075	0.0108	0.015		

TABLE 13. Examples of Marketed Estrogen Tablets.

Brand names	Description	Company
Estradiol tablets	Estradiol	Mylan and Watson
Estratest tablets	Esterified estrogens and methyl testosterone	Solvay
Cenestin tablets	Blend of synthetic conjugated estrogens	Duramed
Premarin tablets	Conjugated estrogens	Wyeth
Prempro tablets	Conjugated estrogens and medroxyprogesterone acetate	Wyeth

Source: PDR [2006].

estrone sulfate and not less than 22.5% and not more than 30.5% of sodium equilin sulfate, and the total of sodium estrone sulfate and sodium equilin sulfate is not less than 79.5% and not more than 88.0% of the labeled content of Conjugated Estrogens. Conjugated Estrogens contains as concomitant component as sodium sulfate conjugates not less than 13.5% and not more than 19.5% of 17α-dihydroequilin, not less than 2.5% and not more than 9.5% of 17α-estradiol.

Bioequivalence became an area of controversy among Wyeth, potential generic product manufacturers, and others, as summarized in the quote below [U.S. Congress, Office of Technology Assessment, 1992].

Bioequivalence for Generic Conjugated Estrogens

The Food and Drug Administration (FDA) Generic Drugs Advisory Committee made recommendations in early 1991 concerning bioequivalence testing and approval of generic conjugated estrogen products. The comparison product is Premarin, and part of what makes demonstrating bioequivalence to Premarin so difficult is that Premarin itself is quite complex. Premarin is derived from the urine of pregnant mares and has 10 components: estrone sulfate, equilin sulfate, 17-α dihydroequilin, 17-βdihydroequilin, 17-α estradiol, 17-β estradiol, delta (8,9) dehydroestrone, equilenin, 17-α dihydroequilenin, and 17-β dihydroequilenin. Nine of these components are currently commercially available, but delta (8,9) dehydroestrone is not. Premarin contains ~50–60% estrone sulfates, 22.5–32.5% sodium equilin sulfate, and 7.5–20% unspecified conjugated estrogens. The United States Pharmacopoeia (USP), the legal standard for drugs in the United States, specifies properties, action, use, dosages, strength, and purity. Conjugated estrogens are derived, either in whole or in part, from equine urine, or they may be produced synthetically using estrone and equilin. Currently, the USP requires only two compounds to be present in a conjugated estrogen product: sodium estrone sulfate and sodium equilin sulfate. Additionally, such products "may contain other conjugated estrogenic substances of the type excreted by pregnant mares." In February 1991, the FDA Generic Drugs Committee recommended that changes be made to the USP monograph for conjugated estrogens to make the required contents more specific, which would result in generic products (when approved) that are closer in composition to Premarin. The committee recommended that the 10 components of Premarin be divided into several categories: therapeutic moieties, which independently demonstrate therapeutic activity and are required components; concomitant components, which are present in a substantial amount in the innovator product but for which independent therapeutic activity has not been established—these components are required to be present in quantities that fall within set upper and lower limits; components requiring a limit test, Which [sic] allows no more than a specific percentage, which can be zero, to be present; and signal

impurities, which must not exceed a set upper limit but which may be zero, provided the product is adequately stable.
The committee proposed that the generic product contain two therapeutic moieties present in Premarin-estrone sulfate and equilin sulfate; three concomitant components—17-α dihydroequilin, 17-β dihydroequilin, and 17-α estradiol; two limit tests for 17-β estradiol and delta (8,9) dihydroestrone; and signal impurities for equilenin, 17-α dihydroequilenin, and 17-β dihydroequilenin. The other five components of Premarin are not required in generic products. The USP is currently revising its monograph for conjugated estrogen tablets and considering a redefinition of content requirements. The FDA issued a revised guidance for bioequivalence studies in August 1991, and several companies have subsequently pursued approval of generic products.

Premarin was first approved by the FDA in 1942 for the treatment of menopausal symptoms, and it is the subject of more than 3,500 publications [Baumgartner, 1999]. Because Premarin was approved before the 1962 amendments to the Food, Drug, and Cosmetic Act, Wyeth-Ayerst was required to prove safety but not efficacy to continue selling the product. Premarin was later evaluated under the Drug Efficacy Study Implementation (DESI) program and judged to be effective in 1972. Since the introduction of Premarin, the FDA has approved other estrogens, both natural and synthetic, for the treatment of menopausal symptoms, and by the early 1990s in the United States, there were more than 10 manufacturers of estrogen products used in HRT. Today, estrogens are marketed in the form of oral tablets by a number of companies (see Table 13). FDA correspondence, in the context of approving new generic estrogen products, refers to 17α-estradiol as "not active," "a concomitant component," or even an "excipient." For background, see a 41 page FDA Memo on the "Approvability of a Synthetic Generic Version of Premarin" (www.fda.gov/cder/news/celetterjw.htm), dated May 5, 1997, from Janet Woodcock, Director, Center for Drug Evaluation and Research, to Douglas L. Sporn, Director, Office of Generic Drugs. See also Duramed's Cenestin New Drug Application (NDA no. 20-992), dated March 24, 1999 (see, in particular, Part 1 of the Administrative Documents section, which can be found at www.fda.gov/cder/foi/nda/99/20992.htm). In this context, the FDA proposed that three of the additional estrogens in Premarin be recommended for inclusion as "concomitant components" in the USP monograph for conjugated estrogens. These particular "concomitant components" would be required to be in the product, but would not be considered active ingredients and, thus, would not need to be included in bioequivalence testing.

Additional Perspectives Regarding the Safety and Efficacy of Estrogens

Several major reviews of the pharmacology and toxicology of estrogens can be found in the literature [see, e.g., Hart, 1990] (see also the Cenestin NDA excerpts below), and many research publications have appeared describing the pharmacology and toxicology of 17α-estradiol. Further information is available on the Internet and through the Freedom of Information Act (e.g., Ames mutagenicity results for 17α-estradiol). From these many reports, one could conclude that 17α-estradiol has the potential to be safer than more feminizing estrogens like 17β-estradiol, which is a much more potent hormone and a natural estrogen that is a component of all marketed estrogens. Because the pharmacology and toxicology of estrogens in animals has not proved to be as predictive as actual human experience (see Cenestin NDA, and excerpts from an FDA Division Director memo below), the extensive past exposure in humans is probably more important in assessing the safety of 17α-estradiol.

As alluded to above, animal safety data on estrogens, either in vitro or in vivo, have not proven to be quantitatively predictive of the effects of these products in women [Stern, 1982; Heywood and Wadsworth, 1980; Westerholm, 1980]. Consequently, one view would be that the most confident conclusions can be drawn from human experience. Nonetheless, extensive animal safety data are available in the published literature on estrogens. Animal data are intended to be a screen to identify gross toxicities, such as whether or not a drug product is a potential human carcinogen. Animal tests cannot definitively determine human clinical effects, but they are useful in screening compounds for activity. The following quotes, taken from the Cenestin NDA (www.fda.gov/cder/foi/nda/99/20992.htm), describe the situation well:

Conjugated estrogens and estrogens in general have been the subject of substantial toxicological evaluation · · · . Safety studies in humans, animals, and in vitro have examined the mechanism of action of estrogens, their binding to estrogen receptors, activation of estrogen response elements metabolism, pharmacokinetics, and relative potencies · · · .
Because of the volume of available data on estrogens for menopausal symptoms, the FDA Center for Drug Evaluation and Research (CDER) does not require new safety studies in

> animals prior to testing in humans or prior to drug product approval. For example, no long-term animal safety testing has been required for any of the estrogen-alone products for menopausal therapy approved through the NDA or abbreviated NDA (ANDA [Abbreviated NDA]) process · · · ·
> As is the case with other approved drug products in this class, existing animal safety data for estrogens may be reasonably extrapolated to new estrogen drug products.

CONCLUSIONS

As summarized above, the less feminizing (see, e.g., Littlefield et al. [1990] and Moos et al. [2008]) 17α isomer (in its free and sulfate conjugate form) of the potent natural hormonal estrogen 17β-estradiol has had extensive exposure in humans, and the literature records a long history of sometimes contradictory animal and human studies. Nonetheless, 17α-estradiol is an orally available, small-molecule drug, with efficacy in some relevant preclinical models that may be predictive of therapeutic potential in human neurodegenerative diseases. These diseases include AD, PD, and stroke, as well as in orphan indications such as Friedreich's ataxia and retinitis pigmentosa. Taking the various studies reviewed here into consideration, it seems warranted to test 17α-estradiol in animal models of neurodegenerative disorders. Furthermore, it would seem prudent to test 17α-estradiol at doses at which it is not feminizing, so that any positive effects could be separated from the known neuroprotection provided by feminizing estrogens. This admonition is simple in concept, but more difficult in practice because the estrogenicity of 17α-estradiol relative to that of 17β-estradiol varies among studies for reasons that are not always clear [Moos et al., 2008]. In spite of the potential technical challenges of such studies, the cumulative information on safety, efficacy, and PK of 17α-estradiol—especially given the lack of drugs for neuroprotection—make them attractive.

Despite the continuing controversy regarding how to interpret WHI studies with potent hormonally active estrogens, published human data appear to support the safety of the less feminizing isomer, 17α-estradiol, recommending it as a potentially worthwhile candidate for evaluating the nonhormonal neuroprotective actions of this chemical class of drugs in humans. Perhaps Mendelsohn and Karas [2007] have described this field post-WHI as well as anyone, quoting Shopenhauer: "Opinion is like a pendulum and obeys the same law. If it goes past the center of gravity on one side, it must go a like distance on the other; and it is only after a certain time that it finds the true point at which it can remain at rest." May it finally come to rest where it best serves patients and caregivers.

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