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Clinical effects of the fetal estrogen estetrol in a multiple-rising-dose study in postmenopausal women☆

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ABSTRACT

Objective: Estetrol (E4) is a natural fetal estrogen. The safety of increasing doses of E4 and its preliminary effects on the vagina, endometrium and menopausal vasomotor symptoms were investigated.
Study design: This was a partly randomized, open-label, multiple-rising-dose study in 49 postmenopausal women. Subjects with an intact uterus were randomized to receive either 2 mg E4 or 2 mg estradiol-valerate (E2V) for 28 days. Subsequent dose-escalation groups (non-randomized) were: 10 mg E4 (intact uterus and ≥35 hot flushes/week); and 20 mg and 40 mg E4 (hysterectomized subjects).
Main outcome measure: Adverse events (AEs) and vaginal cytology were evaluated in all treatment groups; hot flushes/sweating and endometrial proliferation were analyzed with 2 and 10 mg E4 and 2 mg E2V.
Results: Estetrol appeared to be safe, without serious drug-related AEs. In all the groups there was a clear shift from parabasal to superficial vaginal cells, indicating an estrogenic effect and a potential for the treatment of vulvovaginal atrophy. The endometrial thickness remained stable in the 2 mg E4 group and increased with E2V and 10 mg E4. A decrease in the mean number of hot flushes and sweating was seen with 2 and 10 mg E4 and 2 mg E2V.
Conclusions: Estetrol in a dose range of 2–40 mg per day improved vaginal cytology and vasomotor symptoms in postmenopausal women. Endometrial proliferation occurred with the 10 mg dose. Estetrol seems a safe and suitable candidate to develop further for hormone therapy.

1. Introduction

Estetrol (E4) is an estrogenic steroid molecule produced exclusively by cooperativity between the human fetal liver and the fetoplacental unit during pregnancy [1,2]. The molecule was discovered in 1965 at the Karolinska Institute in Stockholm by the group of Diczfalusy [1]. The structure of E4 differs from the other natural estrogens estrone (E1), estradiol (E2) and estriol (E3), by an additional alpha-hydroxy (OH) group at position 15 of the molecule. It is known as E4 since it has four OH groups. Originally, E4 was of less interest since it has (compared to E2) a low estrogen receptor affinity, and it also appeared to be unsuitable as a marker for fetal wellbeing due to the large variability of E4 levels during complicated human pregnancy [2,3].

In 2001, pharmacological *in vitro* and *in vivo* studies showed that E4 has remarkably little interaction with the liver, both kinetically and dynamically [4], and since then interest in the compound recurred. In 2008, the preclinical studies performed so far have been summarized in a review paper [2]. In contrast to other estrogens, these new studies showed that E4 does not bind to the carrier protein sex-hormone binding globulin (SHBG), and does not induce its synthesis in hepatocytes cultured in vitro [5]. Moreover, E4 does not change the activity of relevant cytochrome P-450 related liver enzymes [3]. Additional *in vivo* preclinical studies revealed that E4 inhibits ovulation dose-dependently, and acts as an estrogen on the vagina, uterus and bone [6–8]. In an experimental hot flush model, E4 was found to suppress the naloxone-induced tail skin temperature increase [9]. In the rat, high oral bioavailability and a relatively long half-life were observed [6]. Both were confirmed in a first-in-human single oral dose study in postmenopausal women with doses of 0.1, 1, 10 and 100 mg E4 [10]. E4 was dose-dependently

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absorbed and the elimination half-life was 28 h, important prerequisites for its development as a once daily oral drug. Even single doses of E4 had a profound central inhibitory and dose-dependent effect on gonadotropins, confirming its biological potency [10].

In vitro studies in human breast cancer cell lines showed that E4 is a weak estrogen agonist, but in the presence of E2, E4 behaves as an antagonist on the breast [11–13]. Dimethylbenzanthracene (DMBA) *in vivo* studies showed that E4 is able to prevent breast tumor development in a dose-dependent way and also caused regression of existing tumors in this model dose-dependently [14].

All this data support the potential use of E4 for both oral contraception and hormone therapy (HT). A phase II development program for the use of E4 as the estrogen in a combined oral contraceptive has recently been completed. Dose-finding revealed that 15 mg E4 combined with 3 mg drospirenone is the optimal doses for inhibition of ovulation and acceptable bleeding patterns [15,16]. A phase III program has therefore been initiated.

For the further development of E4 for HT, a multiple-rising-dose study with doses of 2, 10, 20, and 40 mg E4 has been performed in postmenopausal women to investigate the safety, tolerability, and pharmacokinetics/dynamics of E4, including its effect on the number of hot flushes/sweatings, the endometrium and vaginal cytology. This paper reports the clinical results of this study; the pharmacokinetic and pharmacodynamics outcomes have been submitted for publication elsewhere [17,18].

2. Methods

2.1. Study design

This partly randomized, open-label, multiple-rising-dose study in healthy postmenopausal women was conducted between July 2005 and August 2007 at the Clinical Pharmacology Unit (CPU) of INC Research (formerly Kendle International Inc.), Utrecht, The Netherlands. The study was approved by an independent ethics committee and was conducted in accordance with the Declaration of Helsinki and the ICH guideline for Good Clinical Practice. All participants gave written informed consent.

This paper reports on the primary objective, which was to investigate the safety and tolerability of E4, and the secondary objectives to investigate the effect of E4 on the number of hot flushes/sweating, vaginal cytology and endometrial thickness and proliferation. Other secondary objectives were to investigate the steady state pharmacokinetics and the pharmacodynamic effects of E4, reported separately [17,18].

2.2. Treatments

Ten subjects were intended to be allocated per each of the five treatment groups, for a treatment duration of 28 consecutive days. Subjects in the first two groups were randomized (open-label fashion) to receive once daily either 2 mg E4 or 2 mg E2-valerate (E2V), a standard HT in the treatment of climacteric symptoms. Subsequently, there were three consecutive 28-day dose-escalation periods assessing higher dose levels of E4 (10 mg, 20 mg and 40 mg E4). Women, different subjects in each group, were consecutively allocated to the next higher dose only after review of adverse events, levels of SHBG, hemostatic variables of the previous dose level, and after approval by an external independent advisor and the principal investigator.

For endometrial protection, subjects in the 2 mg E4, 2 mg E2V and 10 mg E4 groups received a progestogen (5 mg lynestrenol), once daily for 14 days, starting three days after the last intake of the estrogen treatment.

E4 was supplied as a solution (propylene glycol/water mixture), and delivered in separate bottles per daily dose for each participant. E2V (Progynova®; 2 mg; Bayer Health Care) and lynestrenol (Orgametril®, 5 mg; Merck Sharp & Dohme) were supplied as commercial tablets, packed in blisters.

2.3. Participant selection

Subjects assigned to the 2 mg E2V and 2 and 10 mg E4 groups were not older than 70 years of age at the time of screening, and postmenopausal, defined as ≥6 months amenorrhea with serum follicle-stimulating hormone (FSH) levels ≥40 IU/L, and serum estradiol (E2) levels <73 pmol/L. In addition, in the 2 mg E4 and E2V groups, five subjects needed to have >50 and five subjects <10 hot flushes per week, whereas subjects in the 10 mg E4 group needed to have had >50 hot flushes per week. Since endometrial proliferation was observed in the 10 mg E4 group, subjects assigned to the 20 mg and 40 mg E4 groups had to be 50–70 years of age, hysterectomized (total hysterectomy) for more than 6 months before study entry, and postmenopausal, defined by FSH levels ≥40 IU/L, and serum E2 levels <110 pmol/L. Additional inclusion criteria for all subjects were a body mass index of 18–30 kg/m^2; no contraindications to estrogen and/or progestogen use; and without clinically significant findings on the breast, ovaries and uterus.

2.4. Assessments

The subjects were screened before the start of the study medication. Study visits were scheduled on Day 1, 2, 7, 14; and on Day 28 which was the last day of E4 or E2V treatment. Hereafter, study visits were scheduled on Day 29, 30, 31; and on Day 56, which was a follow-up visit. In addition, there was daily telephone contact during the treatment period.

Safety and tolerability assessments included the collection of adverse events (AEs), physical and gynecological examinations, vital signs, body weight, electrocardiograms (ECGs), and safety laboratory parameters (hematology, biochemistry, urinalysis). AEs were solicited from the subjects at the study visits but could be voluntarily reported at any time. Subjects graded the severity of the AEs (mild, moderate, severe). The principal investigator assessed whether the AEs were related to treatment.

Vaginal cytology (vaginal maturation index [VMI]) was performed at screening and on Day 28; the percentage of parabasal, intermediate, and superficial cells was counted on 100 single cells according to the method of Hustin and Van der Eynde [19]. The VMI is a popular measure of vulvovaginal atrophy, whereby the percentages of the different cell types in the vaginal epithelium are evaluated; increases in superficial and intermediate cells and a decrease in parabasal cells indicate an improvement in vulvovaginal atrophy.

Endometrial thickness was measured in the women with a uterus (the 2 mg E2V group and 2 and 10 mg E4 groups) by ultrasound at screening, on Days 14 and 28, and at follow-up on Day 56. An endometrial biopsy was performed at screening and on Day 28. For the 2 mg E2V and 2 mg E4 groups, a biopsy was only performed on Day 28 if the endometrial thickness had increased by more than 50%.

The number of hot flushes and episodes of sweating were recorded in a diary by subjects in the 2 mg E2V and the 2 and 10 mg E4 groups from the first day of dosing throughout the study including the period of lynestrenol treatment, up to Day 56. Hot flushes/sweating were not required as inclusion in the 20 mg and 40 mg E4 groups.