CLIMACTERIC 2008;11(Suppl 1):15–21

Preventive effect of oral estetrol in a menopausal hot flush model

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Key words: ESTETROL, E_4, HOT FLUSH, VASOMOTOR SYMPTOMS

ABSTRACT

Objective To evaluate the efficacy of estetrol (E_4) in alleviating hot flushes in an experimental animal model considered representative for menopausal vasomotor symptoms.

Methods Recording of the thermal responses in the tail skin of morphine-dependent ovariectomized rats after morphine withdrawal by administration of naloxone. Six groups of rats were treated orally for 8 days as follows: vehicle (negative) control; E_4: 0.1, 0.3, 1.0 and 3.0 mg/kg/day; and, as active (positive) control, ethinylestradiol 0.3 mg/kg/day. On day 8, tail skin temperature was recorded at baseline and for 60 min at 5-min intervals following naloxone administration.

Results In control animals, tail skin temperature increased sharply by about 4.5°C after naloxone treatment and reverted to baseline by 60 min. Estetrol suppressed the tail skin temperature increase in a dose-dependent fashion. The highest dose of E_4 tested (3 mg/kg/day) was equipotent to a 10-fold lower dose of ethinylestradiol. Both fully suppressed tail skin temperature changes.

Conclusion Estetrol is effective in alleviating hot flushes in an experimental animal model considered representative for studying menopausal hot flushes (vasomotor symptoms). In this model, the potency of estetrol is 10-fold lower compared to ethinylestradiol.

INTRODUCTION

Vasomotor symptoms (hot flushes and sweats) occur in the majority of perimenopausal and postmenopausal women as a consequence of fluctuations in estrogen levels and decrease in ovarian estrogen production[1]. Symptoms are manifested by rapid, regional elevations in skin temperature, a decrease in core temperature, an increase in heart rate without changes in blood pressure and surges in the release of luteinizing hormone (LH)[2–4]. In many women, the symptoms are severe and disruptive to daily activity. Although the exact cause of the vasomotor symptoms is not known, estrogen replacement therapy or estrogen/progestin hormone replacement therapy (HRT) is the highly effective standard benchmark treatment.

In view of the potential complications and side-effects related to HRT, efforts have been made to lower the estrogen dose[5] and find alternative routes of administration (transdermal, intravaginal, intranasal) as well as new treatments[6], both hormonal, e.g. tibolone[7] and phytoestrogens[8], as

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DOI: 10.1080/13697130701822807

well as non-hormonal[9]. A so far unrecognized possibility for the hormonal treatment of vasomotor symptoms is the use of estetrol (E_4).

Estetrol (1,3,5(10)-estratrien-3,15α,16α,17β-tetrol) is an estrogenic hormone, discovered in 1965 by Egon Diczfalusy and co-workers at the Karolinska Institute in Stockholm[10]. This steroid is produced by the fetal liver only and found exclusively during human pregnancy[11]. Estetrol has been isolated in maternal urine as early as week 9 of gestation[12,13]. At term, the hormone is found at high concentrations (in the low nanomolar range) in maternal plasma and at over ten times higher levels in fetal plasma[14,15]. The hormone has been shown to have a relatively low affinity to nuclear and cytosolic estrogen receptors[16–18]. It acts as a weak estrogen in the rodent uterus[19,20] and in growth promotion of cultured estrogen-responsive MCF-7 cells[21]. More details concerning the history of E_4 and data from studies in the period 1965–1985 have been summarized in a review paper[22]. After 20 years of experimental work, E_4 research was virtually abandoned. The physiologic role of E_4 during pregnancy is unknown and no clinical research to address this question has been performed.

Although estrogen-related vasomotor instability appears to be restricted to higher primates, a rat model is available for experimental purposes. The model has been successfully used to study the mechanisms of human vasomotor symptoms[23] and estrogen treatment[24]. The present study was designed to investigate the effects of E_4 on thermoregulation in this model to estimate the potential effectiveness of E_4 in the prevention and treatment of human vasomotor symptoms (hot flushes).

METHODS

The study was performed at the laboratories of SkeleTech Inc., Bothhell, WA, USA, according to the United States Food and Drug Administration (FDA) Good Laboratory Practices (GLP) for non-clinical laboratory studies, after approval had been obtained from the Experimental Animal Committee.

Hormones

Ethinylestradiol (EE) was purchased from Steraloids Inc., Newport, RI, USA. Estetrol was synthesized by Syncom BV, Groningen, The Netherlands. Estetrol was >98% pure and did not contain a detectable level of 17β-estradiol (E2), as determined by analysis with high performance liquid chromatography/mass spectrometry, nuclear magnetic resonance and differential scanning calorimetry (results not shown). Estetrol and EE were dissolved in the vehicle (hydroxy propyl-β-cyclodextrin in deionized water; 20% wt/vol) within 72 h prior to initiation of drug treatment.

Animals

A total of 36 virgin female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA. The animals were approximately 7 weeks old at the time of arrival. They were acclimatized for about 1 week prior to commencement of the experimental procedures. Animals were housed two per cage under controlled temperature and humidity conditions on a 12-h light cycle. They had *ad libitum* access to deionized water and rodent chow (Certified Rodent Diet 5002; PMI Feeds, Inc., Richmond, IN, USA).

Experimental procedure

A graphic summary of the experimental procedure is presented in Figure 1. Three days prior to dosing, animals were ovariectomized. Animals (six per group) were assigned to one of six treatment groups, as follows: vehicle; E_4: 0.1, 0.3, 1.0 or 3.0 mg/kg/day and EE 0.3 mg/kg/day. Vehicle and test compounds were administered at 3.0 ml/kg body weight for 8 consecutive days prior to and including the day of the hot flush (HF) session. Dosing by oral gavage was started 3 days after surgery. Morphine dependency was induced by subcutaneous implantation of pellets containing 75 mg morphine (Murty Pharmaceuticals, Lexington, KY, USA). The first pellet was implanted under light inhalation anesthesia 5 days prior to the HF session. Two additional pellets were implanted under the same conditions 3 days prior to the HF session.

For the HF session, animals were transported to a procedure room. After an adaptation period of 5–10 min, the animals were anesthetized with ketamine HCl (80 mg/kg, intramuscular) approximately 10 min prior to the HF session. A temperature-sensitive electrode was fixed with tape to the ventral surface of the tail and connected to a multi-channel temperature recorder. Tail skin temperature (TST) was recorded until it stabilized (approximately 5 min) and

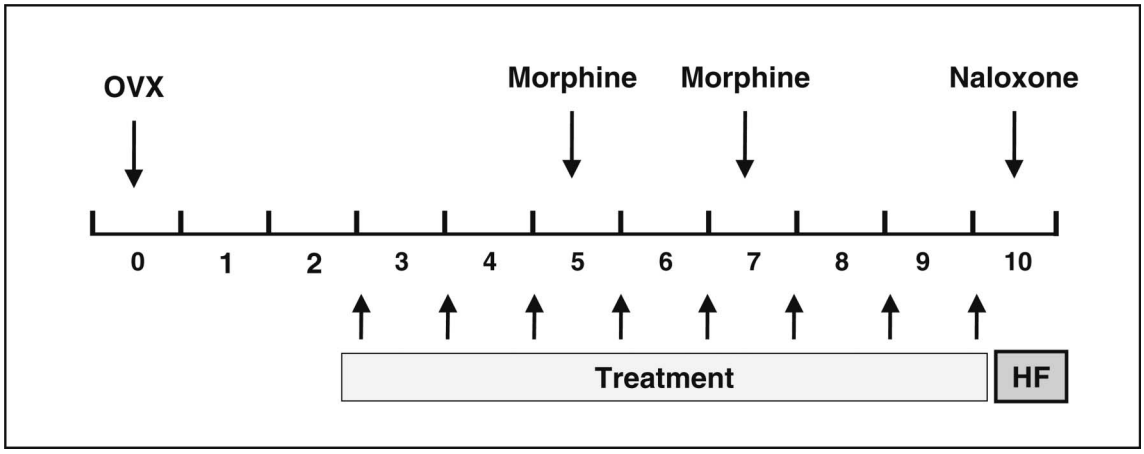

Figure 1 Schedule of treatments and procedures for the 10-day rat model of the menopausal hot flush. OVX, ovariectomy; HF, hot flush

thereafter the animals were injected with naloxone HCl (1 mg/kg). Temperature recordings were continued for 1 h and the temperature was reported in 5-min intervals.

Statistical analysis

A two-way analysis of variance (ANOVA) with repeated measures was performed, with the between-subjects factor of *dose* or *group*, and the within-subjects factor of *time*, i.e. the different time points between $t = 0$ and $t = 60$, using SAS software (SAS Institute, Inc., Car, NC, USA). Where appropriate, additional one-way ANOVA procedures were performed with the between-subjects factor *time* only for each separate dose group, followed by Dunnett's post hoc analyses comparing treatment with the control group. In addition, corresponding time points in two groups were compared by using independent samples *t* tests. The dependent variable was TST.

RESULTS

Body weight increases were observed in all groups over an observational period of about 3 weeks (Table 1). By day 10, the body weights of the rats in the vehicle-treated control group increased by about one-third, largely due to growth of the 7-week-old animals. The weight gain was reduced by estrogen treatment. Estetrol resulted in a dose-dependent body weight reduction on day 10, with weights in the high-dose E_4 group (3.0 mg/kg/day) comparable to those of the EE group and about 15% below those of the control group. These changes were not significant.

Table 1 Mean body weights (in grams) of the experimental animals

	Mean body weight (g)				
Treatment (mg/kg/day)	*Arrival*	*Day 0*	*Day 5*	*Day 7*	*Day 10*
Vehicle	183	215	228	235	240
0.1 E_4	182	212	223	226	236
0.3 E_4	182	214	221	216	224
1.0 E_4	182	215	222	224	229
3.0 E_4	182	215	215	214	205
0.3 EE	182	213	213	210	207

E_4, estetrol; EE, ethinylestradiol

Table 2 presents a summary of the mean TSTs just prior to naloxone treatment ($t = 0$ min), at or around the time of the maximum effect ($t = 15$ min) and at the end of the observation period ($t = 60$ min).

Figure 2 illustrates TST changes in rats after different therapies. The mean baseline temperature prior to naloxone administration ranged from 22.3°C to 22.8°C across the different treatment groups. Following naloxone administration, the mean TST in the vehicle-treated control group increased by about 4.5°C to a mean of 27.3 ± 1.1°C (mean ± SEM) about 15 min after naloxone treatment. Mean TST reverted to baseline at the end of the 1-h observation time point. The temperature rise in the lowest E_4 group (0.1 mg/kg/day) was similar to that of the control group and had no significant effect on TST. Treatment with EE (0.3 mg/kg/day) significantly prevented the naloxone-induced increase in TST ($p < 0.05$), with mean TST values between 21.7°C

Table 2 Effect of 8-day oral treatment with estetrol (E_4, 0.1–3.0 mg/kg/day), ethinylestradiol (EE, 0.3 mg/kg/day) or vehicle on mean (±SEM) tail skin temperature at 0, 15 and 60 min after naloxone administration to morphine-dependent ovariectomized Sprague-Dawley rats

Treatment (mg/kg/day)	*n*	*t = 0 min*		*t = 15 min*		*t = 60 min*	
		Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Vehicle	6	22.59	0.33	27.33	1.07	22.07	0.58
0.3 EE*	5	22.32	0.09	21.96	0.12	21.67	0.16
0.1 E_4	6	22.75	0.23	28.39	1.22	22.29	0.44
0.3 E_4	6	22.60	0.18	23.68	1.05	21.93	0.41
1.0 E_4	6	22.43	0.24	24.09	1.44	22.14	0.39
3.0 E_4	6	22.38	0.33	22.38	0.36	22.13	0.23

*One animal in the EE group was sacrificed on day 9 because of severe signs of distress

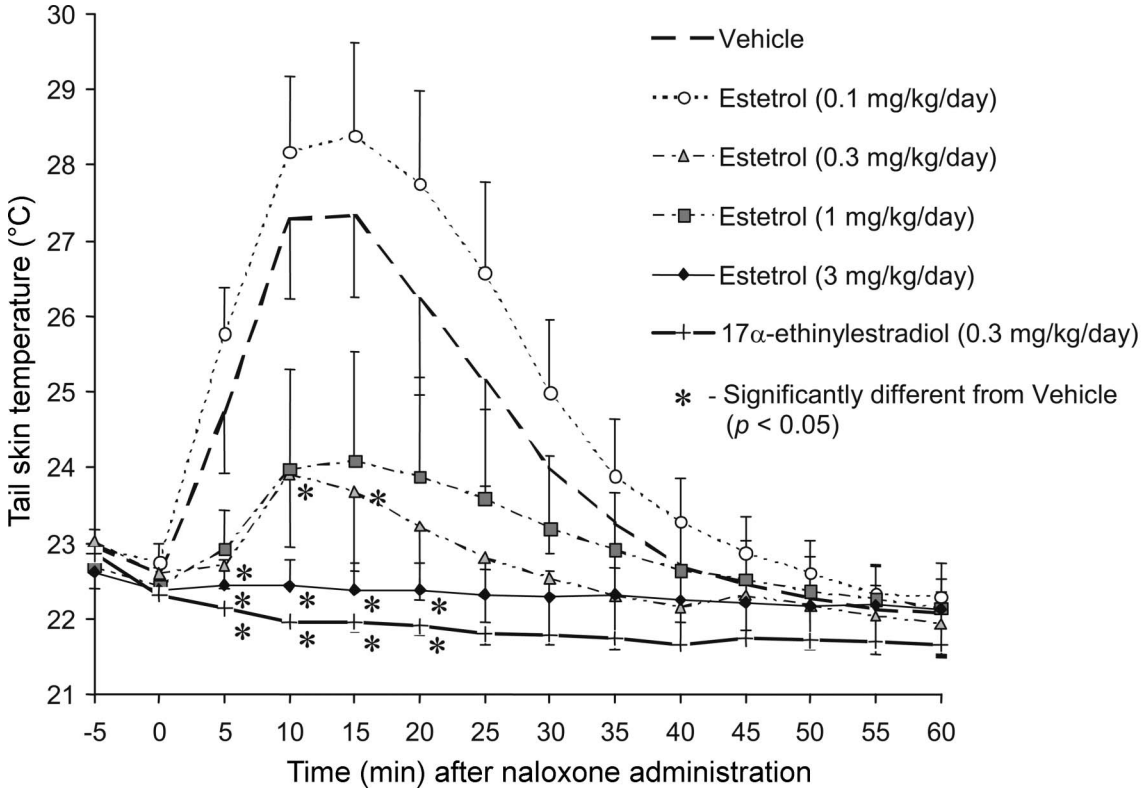

Figure 2 The effects of estetrol and 17α-ethinylestradiol on the naloxone-induced hot flush response in female ovariectomized rats

and 22.3°C at the 5–20-min interval. Treatment with E_4 at the three highest dose levels, i.e. 0.3, 1.0 and 3.0 mg/kg/day, inhibited the naloxone-induced temperature rise in a dose-dependent fashion. Analysis by two-way ANOVA revealed a significant time and treatment effect ($p < 0.05$). A one-way ANOVA indicated that the three higher doses of E_4 significantly attenuated the hot flush response, as did treatment with EE. Further analyses by *t* tests indicated that the highest dose of E_4 (3.0 mg/kg/day) was similar in efficacy to EE and was statistically significantly different from vehicle treatment at the 5–20-min time interval. The lowest effective dose of E_4 (0.3 mg/kg/day) was significantly different from vehicle during the 5–15-min time period.

DISCUSSION

Estetrol is a steroid hormone, exclusively produced by the fetal liver during human pregnancy.

Based on the outcome of studies in various experimental systems in the period between 1965 and 1985, E_4 was considered to be a very weak estrogen[22]. Its physiological significance was not investigated and further research was not performed. Interest in human treatment indications of E_4 has been raised after the demonstration in recent years of high oral bioavailability and long elimination half-lives in the rat (2–3 h)[25] and in early postmenopausal women (28 h)[26]. One of the questions related to the profile of a new estrogenic steroid is the effect on vasomotor symptoms.

The concept of the rat hot flush model is based on the observation that, in rats, morphine dependency results in increased core temperature. The increase is considered to be a consequence of diminished heat loss, which is not compensated by a proportional reduction in heat production. Sudden morphine withdrawal, as produced by naloxone treatment, produces a short phase of heat dissipation, as apparent by transient temperature increases in the TST and by a small drop in core temperature. The increases in TST were found to be similar in magnitude (4.8–7.2°C) and duration (60–90 min) to peripheral skin temperature changes reported during menopausal hot flushes[23]. In addition, as during the human hot flush, transient increases in heart rate and hypersecretion of LH were observed in rats during morphine withdrawal.

Estrogen therapy suppresses the rise in rat TST and is accompanied by physiological changes similar in magnitude, duration and temporal patterns to those associated with the menopausal hot flush[24]. Because of these similarities, the rat model is considered an appropriate tool for the study of thermoregulation in humans[23,24,27,28].

The present study evaluated the oral efficacy of the human pregnancy hormone E_4 for preventive effects on hot flushes in this well-validated pharmacological model[23]. The potent orally bioavailable synthetic estrogen EE was chosen as comparator and positive control, since EE is effective in this model[28].

The data from this study show that, following morphine deprivation, a swift rise in TST was induced by naloxone. The increases in TST reported here were similar in magnitude (4.8–7.2°C) and duration (60–90 min) to peripheral skin temperature changes during the menopausal hot flush[23]. The data show a significant dose-dependent effect of E_4. The effect of the highest dose of E_4 (3 mg/kg/day) is similar to the effect of the positive control EE at a 10-fold lower dose (0.3 mg/kg/day), as is the effect on body weight. Since only one dose of EE was tested as active control, one may therefore conclude that the potency of EE is at least 10 times higher than the potency of E_4.

Estrogens, e.g. EE and E_2, are effective in the rat hot flush model[24,28] and are highly effective in the treatment of hot flushes and other vasomotor symptoms in women[6]. Tibolone is also effective in the rat[29] and in women[7]. Clonidine has some effect in the rat[29] and in women[9]. Phytoestrogens (soy) have no effect in this rat model[30] and have no or questionable effects in women[8]. The estrogen antagonist tamoxifen for the treatment of breast cancer in the human has significant estrogen agonist activity in the rat model when tested alone, but is an antagonist in the presence of EE[28]. The selective estrogen receptor modulator raloxifene, prescribed for the prevention of osteoporosis in women, does not demonstrate any estrogen agonistic activity, whereas it exhibits significant antagonistic activity. Both tamoxifen and raloxifene are known to cause hot flushes as a side-effect of treatment. These data from the literature demonstrate the remarkable predictive value of the rat hot flush model for the clinical effects of drugs for vasomotor symptoms. Therefore the data reported in this study support the likelihood that E_4 will be effective in peri- and postmenopausal women for the treatment of vasomotor symptoms in general, and hot flushes in particular. Appropriate placebo-controlled studies in women with vasomotor symptoms are required to test the effectiveness of E_4 for this treatment indication.

In summary, we have shown an estrogenic effect of E_4 by preventing the occurrence of hot flushes in the pharmacological rat hot flush model. Based on these data and in conjunction with other reports, including those in this Supplement, we conclude that E_4 is a potential candidate for human therapy of hot flushes and other vasomotor symptoms[31].

ACKNOWLEDGEMENT

We are grateful to Steven Bain who supervised the study at SkeleTech Inc, Bothell, USA.

Conflict of interest H.C.B. is CEO and shareholder of Pantarhei Bioscience; C.F.H. has financial interest in estetrol.

Source of funding This study was funded by Pantarhei Bioscience.

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