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Comparative evaluation of Estredox, a brain-targeted estradiol delivery system versus traditional estrogen replacement therapy

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Estredox is a novel brain-targeted delivery system for estradiol (E_2). The mechanism of this estradiol-chemical delivery system (E_2-CDS) is based on an interconvertible dihydropiridine ↔ pyridinium salt carrier (targetor) attached to E_2. After administration of the E_2-CDS, the targetor moiety is oxidized to a quaternary pyridinium salt (E_2-Q^+). Here we demonstrate that a single i.v. injection with E_2-CDS (3 mg/kg) resulted in sustained presence of E_2-Q^+ in three various brain regions. The sustained and gradual release of estradiol from E_2-Q^+ is reflected by the time-course of plasma estrogen level. At the end of repeated administration of E_2-CDS (daily once 0.3 mg/kg i.v. for 10 consecutive days) we found a sharp decrease in the levels of plasma estradiol followed by a gradual decrease. The levels of E_2-Q^+ in the investigated brain regions decreased gradually from the first post-treatment day, however, a detectable amount of E_2-Q^+ was still present in the hypothalamus, striatum, and cortex even on the 24th post-treatment day. Strikingly different plasma estradiol levels were found in the groups of orchidectomized rats that received daily i.v. injections of estradiol benzoate (E_2-BZ). The plasma estradiol levels in these animals were much higher compared to E_2-CDS-treated animals throughout the treatment period but the level sharply dropped immediately after the treatments. In contrast to the E_2-CDS-treated animals there was no estradiol in any of the brain regions of E_2-BZ-treated rats on the 1st and 2nd post-treatment day. All of these data are in line with the long-lasting pharmacological effects of E_2-CDS-treatment on estrogen-mediated functions in castrated rats and give further experimental support for brain-targeting estrogen-treatment approach as opposed to the traditional estrogen replacement therapy.

1. Introduction

Moderate to severe vasomotor symptoms like sensation of heat with sweating causing cessation of activity, commonly known as hot flushes occur in the majority of postmenopausal women. Further menopausal symptoms like mood disturbances, memory loss, reduced sexuality, insomnia are also of central origin, while reduced bone mass, vaginal dryness, skin thickening are peripheral problems related to the decrease of the ovarian function.

Estrogen replacement is a consistent and satisfactory therapy to improve the quality of life and relieve the symptoms in postmenopausal women: estrogen replacement elevates mood, improves short-term memory, delays/prevents dementia, reduces depression, increases sexual activity and combats osteoporosis. Nowadays estrogen alone is mostly used in women who have had hysterectomy, in those who have uterus, progestin is added to avoid endometrial thickening induced by high peripheral estrogen level: albeit estrogen easily crosses the blood brain barrier, it is poorly retained in the brain. In order to assure a relevant brain level, higher constant peripheral exposure is necessary which leads to complications like cancer, hypertension, thromboembolism and altered metabolism. Thus, an enhanced central versus peripheral estrogen concentration is the ideal therapeutical approach.

The brain-targeted estradiol chemical delivery system, Estredox (E_2-CDS) is a prominent example for rational drug design to deliver and target estradiol to the central nervous system (Bodor and Brewster 1991). The CDS moiety is designed to undergo an enzymatic oxidation similarly to the NAD(P)H-$NAD(P)^+$ system. This process converts the lipophylic E_2-CDS to a hydrophilic, membrane impermeable trigonelline derivative E_2-Q^+ which is blocked into the brain where then slowly releases estradiol. Due to this enhanced central efficacy Estredox is supposed to be useful in the therapy of hot flashes, it can stimulate sexual behavior, cognitive functions and memory, prevents dementia.

As a pharmacological proof of the CNS delivery concept, we demonstrated earlier the positive effect of E_2CDS on the copulatory behavior in castrated male and ovariectomized female rats with concomitant determination of blood luteinizing hormone (LH) and estradiol (E_2) levels. In males, single doses of E_2CDS (0.3 and 3 mg/kg iv.) re-established mounting and intromission potential. In females, five-day treatment with a low dose of 0.03 mg/kg i.v. with E_2CDS normalized the sexual behavior (lordosis, female reproductive behavioral pattern) in ovariectomized animals. This effect was three times stronger and lasted 10 days longer than that of estradiol benzoate (E_2BZ). More-

over, E_2BZ was unable to reduce LH levels contrary to E_2CDS which suppressed LH production up to two weeks after stopping the treatment (Tapfer et al. 2004).
Here we present the results of our recent studies where estradiol/E_2Q^+ levels from plasma and brain tissue samples were determined in orchidectomized male rats treated either with a single dose (3 mg/kg i.v.) of E_2CDS/E_2BZ or with repeated injections of E_2CDS/E_2BZ at a dose of 0.3 mg/kg i.v. for 10 consecutive days.

2. Investigations and results

2.1. Determination of estradiol levels in plasma and brain tissue samples of orchidectomized male rats treated with single dose of E_2-CDS or E_2BZ:

A single i.v. injection of E_2-CDS (3.0 mg/kg) caused a sharp increase in the levels of plasma estradiol in orchidectomized rats one day after the treatment (Fig. 1A). From plasma samples obtained between the 12th and the 16th post-treatment days the plasma estradiol levels dropped below the detection limit in about half of the animals. Twenty, 21 and 23 days after the E_2-CDS-treatment the plasma estradiol levels were below detection limit in each animal tested at this time interval. In contrast, a detectable amount of E_2-Q^+ was present in hypothalamus, striatum, and cortex even at the 21st and 23rd post-treatment days (Fig. 1B).
In another set of experiments a group of orchidectomized male rats was treated with a single dose of E_2-BZ (3 mg/kg). Plasma estradiol levels were determined in samples taken on the first, second and third post-treatment day or 1, 2, 4, 8 and 24 hours after the treatment. Plasma estradiol levels in samples obtained 48 and 72 hours after the treatment were below the detection limit in all animals (data not shown). Estradiol levels from plasma samples obtained at shorter time after a single injection of E_2-BZ are shown in the Table. Detectable amount of estradiol was present in the tissue samples (hypothalamus, striatum, and cortex) taken only one and four hours after the treatment (Table).

Table: Estradiol levels of plasma and brain samples at various post-treatment times from rats treated with E_2BZ

Plasma estradiol levels* (pg/ml) Time after treatment (h)					
	1	2	4	8	24
mean	>20.000	8853	1106	117	25
SEM		1268	189	11	6.5
Tissue estradiol levels (pg/mg) Time after treatment (h)					
	1	4		24	
HT	53.2 ± 3.4	12.9		<5	
CX	46.3 ± 5.4	9.3 ± 32.6		<5	
STR	10.6 ± 0.4	5.1 ± 31.7		<5	

* Estradiol levels of plasma samples taken five days prior to the treatment with E_2BZ were below the detection limit

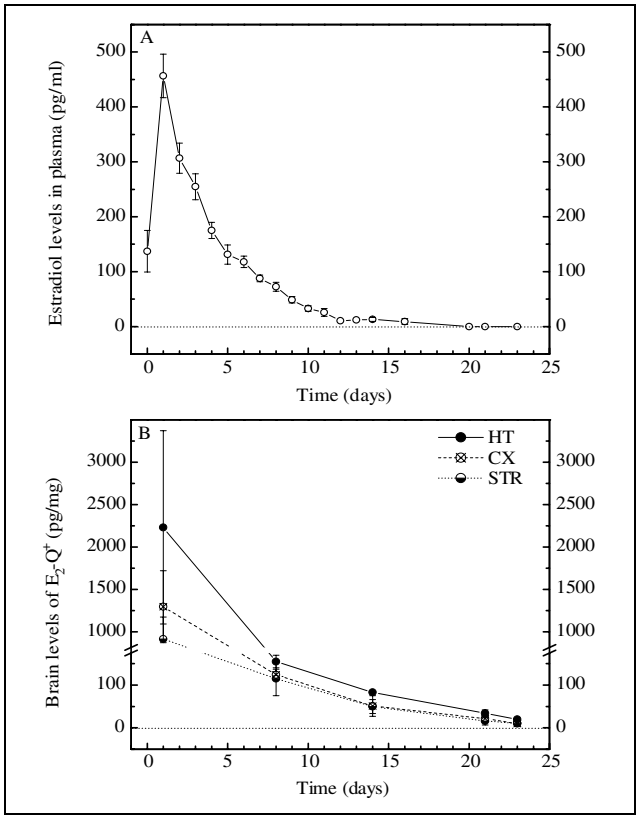

Fig. 1: Effects of a single dose of E_2-CDS (3 mg/kg, i.v.) on plasma estradiol levels (panel A) and E_2-Q^+ levels of different brain regions (panel B). (HT: hypothalamus; CX: cortex; STR: striatum) Each point represents the group mean ± SEM. n = 2–13 in the case of plasma samples, 3–4 in the case of brain tissue samples

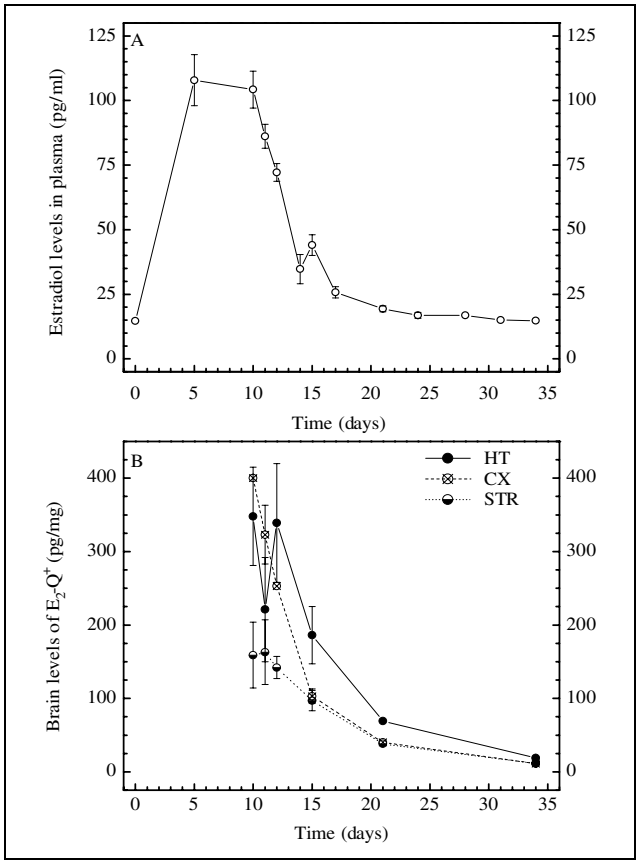

Fig. 2: Effects of repeated administration of E_2-CDS (once per day on 10 consecutive days; 0.3 mg/kg, iv) on plasma estradiol levels (panel A) and E_2-Q^+ levels of different brain regions (panel B). (HT: hypothalamus; STR: striatum; CX: cortex). Each point represents the group means ± SEM. n = 8–17 in the case of plasma samples collected between the 5th and 15th experimental days, and 3–4 in the case of brain tissue samples

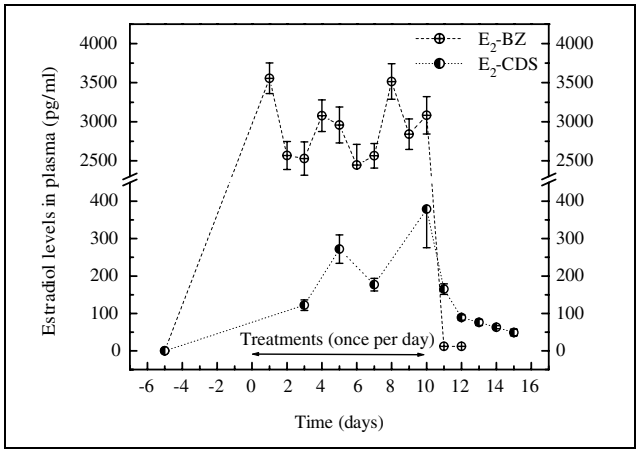

Fig. 3: Effects of repeated administration of E_2-CDS and E_2-BZ (10 consecutive days; 0.3 mg/kg, i.v.) on plasma estradiol levels. The horizontal bar shows the length of treatment period. Each point represents the group means ± SEM. In the E_2-BZ-treated group blood samples were collected from 15 animals in the first half (day 1st-day 4th) and from 13 animals in the second half of the treatment period. On days 11th and 12th, the blood samples were taken from 13 and 6 animals, respectively. In the E_2-CDS-treated group blood samples were collected from 7 animals during the treatment period

2.2. Determination of estradiol levels in plasma, and brain tissue samples of orchidectomized male rats after repeated administration of E_2-CDS or E_2BZ:

After repeated administration of E_2-CDS (one i.v. injection of 0.3 mg/kg per day on 10 consecutive days) in orchidectomized rats there was a sharp decrease in the levels of plasma estradiol followed by a gradual decrease (Fig. 2A). From plasma samples obtained between the 11th and the 14th post-treatment days the plasma estradiol levels dropped below the detection limit in about half of the animals. Even on the 18th post-treatment day there was one animal out of three with detectable level of plasma estradiol. The levels of E_2-Q^+ in the three brain regions decreased gradually from the first post-treatment day (Fig. 2B). Detectable amounts of E_2-Q^+ were present in hypothalamus, striatum, and cortex even on the 24th post-treatment day.

Striking differences were found in the levels of plasma estradiol between groups of orchidectomized rats that received 10 daily injections of 0.3 mg/kg of E_2-CDS or E_2-BZ respectively (Fig. 3). The plasma estradiol levels in E_2-BZ-treated animals were much higher compared to E_2-CDS-treated animals throughout the treatment period but sharply dropped immediately after the treatments. In the E_2-CDS-treated group the plasma estradiol levels were approximately ten times lower than in the E_2-BZ-treated animals but after the treatments the levels of plasma estradiol remained well over the detection limit (5 pg/ml) of the RIA kit used in our experiments.

3. Discussion

The most frequent and troublesome symptoms in menopause are the brain-mediated hot flushes which may be alleviated with hormone or estrogen replacement therapy (HRT, ERT). High peripheral estrogen levels are not necessary to calm hot flushes. In addition, systemic estrogen replacement may induce a number of other side effects like hypertension, thromboembolic events, fluid retention, gallstones etc. Moreover, in non-hysterectomized women estrogen cannot be applied without progestin because of the enhanced risk of uterine cancer induced by the high peripheric estrogen level. Even the combination therapy has numerous risks; recently, the world's largest study involving several thousands of women was stopped because of the significantly enhanced risk of heart attack, stroke and breast cancer, all being related to the high peripheral estrogen level.

Various drugs can be selectively delivered to the brain by a dihydropyridine in equilibrium pyridinium salt chemical delivery system (Bodor and Simpkins 1983; Bodor and Farag 1983; Brewster et al. 1988). The redox-based chemical estrogen delivery system offers a good alternative to classical systemic ERT or HRT because it assures sustained central estrogen concentration with a minimal peripheral level. Its central efficacy was demonstrated in numerous animal studies. Brewster et al. (1988) reported that administration of E_2-CDS in castrated female Sprague-Dawley rats resulted in a sustained suppression of serum levels of LH. In another series of experiments, single dose administration E_2-CDS caused a dose- and time-dependent suppression of LH and FSH with maximal effect at day 7, while equimolar E_2 were not significantly different from control values (Rahimy et al. 1990). The same authors have found that in an animal model for menopausal hot flush, in the tail-skin temperature assay multiple injections with E_2-CDS significantly attenuated the temperature rise while E_2 pellets were without any effect. At the same time, plasma E_2 levels were two times higher compared to E_2-CDS (Rahimy et al. 1991). As a further evidence for central efficacy Anderson et al. (1987) have demonstrated that E_2-CDS dissolved in DMSO reestablished male's copulatory behavior for 28–35 days at a single i.v. dose of 3 mg/kg. Recently we have shown that application of E_2-CDS cyclodextrin complex (E_2-CDS-CD) in neutered male and female rats leads to a persistent reestablishment of copulatory behavior (Tapfer et al. 2004). In addition, treatment with E_2-CDS-CD caused a long-lasting suppression of blood LH levels.

Estrogen is suggested to be associated with the protection of various brain structures. Wise et al. (2001) found that estrogen protected against stroke-like injury, most prominently in the cortex in ovariectomized rats. Similar findings of Saleh et al. (2001) in a rat MCAO model further support the neuroprotective nature of central estrogen therapy. In the global ischemia model in gerbils, estrogen at physiological levels attenuated hippocampal injury (Jover et al. 2002). Estrogen also beneficially influences anxiety and cognition as it has been recently demonstrated using the open field and radial arm maze tests in ovariectomized rats (Bowman et al. 2002).

Analysis of plasma and brain tissue samples revealed that following a single i.v. injection (1 mg/kg) with E_2-CDS brain levels of E_2-Q^+ exceeded its serum level by 33-, 70-, and 294-fold at the 1st, 7th and 14th post-treatment days (Rahimy et al. 1989). In the present study we confirm the sustained presence of E_2-Q^+ in three distinct areas of the brain of orchidectomized rats after a single injection of E_2-CDS (3 mg/kg; i.v.). Detectable amounts of E_2-Q^+ were present 23 days after the treatment in the samples from hypothalamus, striatum and frontal cortex. By this time the plasma levels of estradiol were below the detection limit in all of the samples. However at the 14th and 16th post-treatment days a detectable amount of estradiol was present in some of the samples indicating the sustained release of estradiol from E_2-Q^+ locked-in the brain. Similar results were obtained with orchidectomized rats treated with a lower dose of E_2-CDS (0.3 mg/kg) on ten consecutive days. In contrast, plasma and brain levels of

estradiol decreased vary rapidly in orchidectomized rats after either a single injection or repeated administration of E_2-BZ.

In conclusion, our present data provide further experimental support for the brain targeting estradiol chemical delivery system treatment approach as opposed to the traditional hormone replacement therapy. Brain-targeted estradiol delivery using E_2-CDS results not only in better efficacy but also in increased safety because the peripheral effects are considerably reduced due to the low, steady state blood level of E_2-CDS.

4. Experimental

4.1. Animals and drug administration

Sprague-Dawley male rats (120–150 g) were orchidectomized under ether anesthesia. One month later the animals were treated with E_2-CDS or with E_2-benzoate (E_2-BZ) dissolved in 20 w/v% cyclodextrin solution via tail vein injection(s) in a volume of 0.1 ml/100 g body weight. Prior to the treatments and then on the experimental days blood samples were taken by retro-orbital sinus puncture under light ether anesthesia. The samples were stored at 4 °C for one hour then centrifuged at 1000 × g for 10 min. Plasma was separated and stored at −80 °C until assayed.

4.2. Determination of estradiol

Estradiol levels were determined by double antibody I^{125} isotope-RIA kits obtained from ADALTIS ITALIA. Estradiol levels from plasma samples were determined by direct injections. E_2-Q^+ levels from tissue samples were determined after extraction with acetone/water (Rahimy et al. 1989) followed by a hydrolysis with NaOH and concentration using a Savant SpeedVac concentrator.

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