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SYNTHESIS AND BIOLOGICAL ASSESSMENT OF LONG-ACTING ESTRADIOL FATTY ACID ESTERS IN OVARIECTOMIZED RATS

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Summary—Diesters of 17β-estradiol using palmitic acid (16:0) and oleic acid (18:1, *cis*-9-Octadecenoic acid) have been synthesized for potential evaluation as long-acting compounds. Female castrated rats were injected 1 μmol of estradiol dipalmitate and estradiol dioleate, using estradiol benzoate and estradiol enanthate as controls. Biological activity was determined by uterine wet weight and uterine diameter as well as on the suppression of serum anterior pituitary gonadotropins. A delayed absorption of estradiol was observed after administration of both diesters which was well correlated with the duration of biological effects. The data demonstrate that esterification with palmitic or oleic acids at 3 and 17 positions provides long-acting properties to 17β-estradiol, which could be applied in substitutive therapy and or in hormonal contraception.

INTRODUCTION

It has been well recognized that esterification of steroids having a hydroxyl group prolongs their action [1, 2]. It has also been demonstrated that esterification at 17-position provides longer duration than when 3-hydroxyl group is chosen for esterification [3]. Short chain fatty acids have been previously employed in order to demonstrate the relationship between the size of the fatty acid and the duration of effects. The longer the fatty acid chain, the longer the effect obtained [1].

Recently it has been shown that 17β-estradiol exists in the human blood not only as free or conjugated forms, but also as fatty acid esters [4]. The physiological role of these naturally occurring estradiol esters is still unclear, however, the possibility of using long chain fatty acid esters for prolonging the duration of steroid action has been advocated [5]. With the purpose to study whether long-acting effects of estradiol could be achieved by the esterification at 3 and 17 position, we designed the present investigation with the aim to evaluate the long-acting properties of estradiol dipalmitate and estradiol dioleate in spayed female rats.

EXPERIMENTAL

Reagents

Estradiol-17β (1,3,5(10)-estratrien-3, 17β-diol), and estradiol-3-benzoate (1,3,5(10)-estratrien-3,17β-diol 3-benzoate) were kindly provided by Syntex (Mexico City), estradiol-17 enanthate (1,3,5(10)-estratrien-3-17β diol 17-enanthate) was a gift of Aplicaciones Farmacéuticas de Mexico (Mexico City). Fatty acids, palmitic (hexadecanoic acid 16:0) and oleic (18:1, *cis*-9-Octadecenoic acid) were purchased from Sigma Co. (St Louis, MO, U.S.A.), thionyle chloride was kindly provided by Bayer (Mexico City). Reagents for the radioimmunoassay of gonadotropins were provided by NIAMDD rat pituitary hormone distribution program (Baltimore, MA, U.S.A.). Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were measured in serum by the double antibody radioimmunoassay technique following protocols supplied by NIAMDD.

Results are expressed as ng/ml of the rLH-Rp-1 and rFSH-Rp-1. Estradiol-17β was measured in serum by specific radioimmunoassay with antiserum provided by the World Health Organization Matched Reagents Programme [6]. Cross reaction of the 17β-estradiol antiserum was 4.0 and 1.4% for estradiol benzoate and estradiol enanthate, respectively. Radioactivity measurements were determined in a Packard Tri-Carb 300 and Auto Gamma 500 (Packard Instruments). Results are expressed as pg/ml.

Chemical synthesis

The dipalmitate and dioleate estradiol diesters (1,3,5(10)-estratrien-3,17β-diol di-palmitate and (1,3,5(10)-estratrien-3,17β-diol, dioleate) were synthesized by the chloride acid formation, prior to esterification with the corresponding acid, following the technique described previously [7].

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Table 1. Physico-chemical properties of estradiol dipalmitate and estradiol dioleate

Method	Estradiol dipalmitate	Estradiol dioleate	Estradiol-17β
NMR (ppm)	(s) 1.3 (d) 0.9, 7.5 (t) 4.9, 7.0	(s) 1.3 (d) 0.9, 7.5 (t) 4.9, 5.5, 7.0	(s) 0.9 (d) 7.3 (t) 3.8, 6.8
i.r. (cm^{-1})	pellet KBr 2900, 2840, 1760, 1730, 1460, 1170	film $CHCl_3$ 3000, 2900, 2840, 1755, 1730, 1130	pellet KBr 3500, 3250, 2940, 2880, 1250, 1050
u.v. (λ max in nm)	in $CHCl_3$ 275, 269	in $CHCl_3$ 275, 269	in $CHCl_3$ 286, 280
M.P. (°C)	65–67	oil	173–179

The purification of the diesters was performed using Silicagel column G-60 (Merck, Mexico City), and eluted with a solvent system of benzene–hexane, 50:50 and 100:0, (v/v).

The purity of each estradiol diester was evaluated by hydrogen nuclear magnetic resonance (NMR), infrared spectroscopy (i.r.), melting point (M.P.) and thin-layer chromatography (TLC).

Biological assessment

Female Wistar rats weighing between 150–250 g were bilaterally ovariectomized 1 month before treatment and subsequently allocated in 6 groups of 20 rats each as follows:

Group I, control ovariectomized, received no treatment (OVX); Group II, controlled animals which received only the subcutaneous injection of vehicle castor oil (V); Group III received 1 μmol of estradiol benzoate (EB); Group IV received 1 μmol of estradiol enanthate (EE), Groups V and VI were injected with 1 μmol of estradiol dipalmitate (EDP) and estradiol dioleate (EDO), respectively. The volume of the vehicle was 0.3 ml in each group and all compounds were administered on a single occasion.

All animals had free access to food and water and had a 12 h dark–light cycle. Rats were sacrificed at 0, 3, 5, 12 and 15 days after treatment.

Blood samples were collected and the serum obtained stored at -20°C until hormone assays were performed. The results are expressed as mean $\pm$ SD.

RESULTS AND DISCUSSION

Chemical synthesis

The chemical purity of estradiol fatty acid esters was evaluated by several methods as shown in Table 1. The hydrogen nuclear magnetic resonance showed the presence of ester functionality as demonstrated by the chemical shifts of hydrogen in C-17 position (4.9 ppm) and aromatic hydrogens (7, 7.5 ppm).

The infrared spectroscopy, showed the absence of absorption bands at 3,500 and 3250 cm^{-1} corresponding to hydroxyl groups, and confirms the esterification at C-3 and C-17 positions. The purity of each compound was further confirmed by thin-layer chromatography and melting point.

Biological assessment

The organotropic effects of estradiol esters are shown, in Fig. 1 and Fig. 2. Acute effects in uterine weight and uterine diameter were found in the EB group, whereas the EE group showed a sustained effect on the same parameters. The EDP and EDO groups showed a physiological response comparable with the intact group. This effect was maintained throughout the experiment. After administration of castor oil (vehicle), no effect was observed in any of the parameters studied.

The antigonadotropic effect was taken as a parameter of estrogenic action. As expected, LH and FSH serum levels rose after castration and when the steroid derivatives were injected both gonadotropins decreased in the serum. The inhibitory effect on gonadotropins was more pronounced in the estradiol diesters groups (EDO and EDP). As shown in Fig. 3, there was a sustained inhibition of LH when EDP and EDO were administered. Estradiol serum levels (Fig. 4), indicated a rapid and irregular absorption of estradiol in EB group. A delayed absorption was evident after treatment with enanthate and both estradiol diesters. Indeed maximum levels of estradiol were observed 12 days after administration of either estradiol diesters.

The synthesis and biological assessment of several estradiol mono and diesters has been previously reported, and their potential use as long acting compounds advocated [1–3]. Recently, the isolation of lipoidal derivatives of estradiol (estradiol 17-stearate and estradiol 17-arachidonate) from human female serum rose numerous questions regarding their physiological role as well as their potential application as naturally occurring long-acting estrogens [4, 5]. These observations prompted

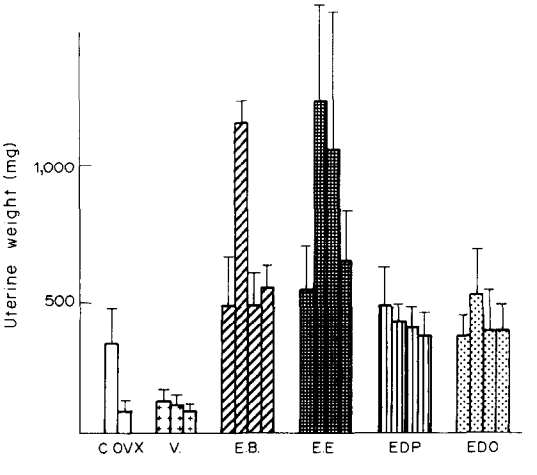

Fig. 1. Organotropic effects of estradiol after a single subcutaneous injection of 1 μmol of each estradiol ester. Female intact rats (C), ovariectomized rats (OVX), vehicle (V), estradiol dipalmitate (EDP) and estradiol dioleate (EDO). Results are expressed as mean $\pm$ SD of 5 animals at 3, 5, 12 and 15 days after treatment.

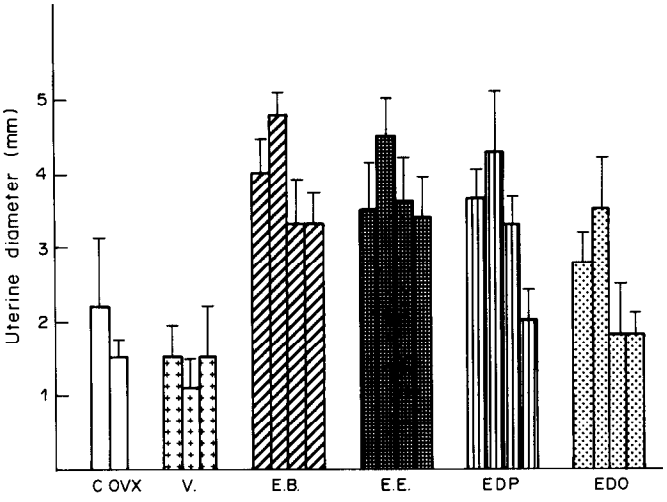

Fig. 2. The effects of estradiol administered as single dose of 1 μmol of mono or diesters on the uterine diameter is shown. Values are given as the mean $\pm$ SD of 5 animals. The groups and sampling are the same as in Fig. 1.

us to evaluate the long-acting properties of estradiol dipalmitate and estradiol dioleate in the ovariectomized rat model. The results obtained in the present investigation confirm previous observations on the long-acting properties of estradiol alkyl derivatives [1], and demonstrate that esterification in 3 and 17 position provides a sustained effects when compared with those observed after administration of the estradiol mono esters (EB and EE).

No correlation was found between the serum estradiol levels and the maximum organotropic responses in all groups. Furthermore a sustained effect of the estradiol diesters derivatives was evident regarding the uterine weight (Fig. 2).

The gonadotropin serum levels remained low 15 days after the administration of all preparations, these findings prevent us to establish the exact length of the effects produced by the administration of estradiol diesters, however, the estradiol serum levels (Fig. 4) suggest that the hydrolysis of EDP and EDO occurs at a slower rate than the hydrolysis of estradiol mono esters.

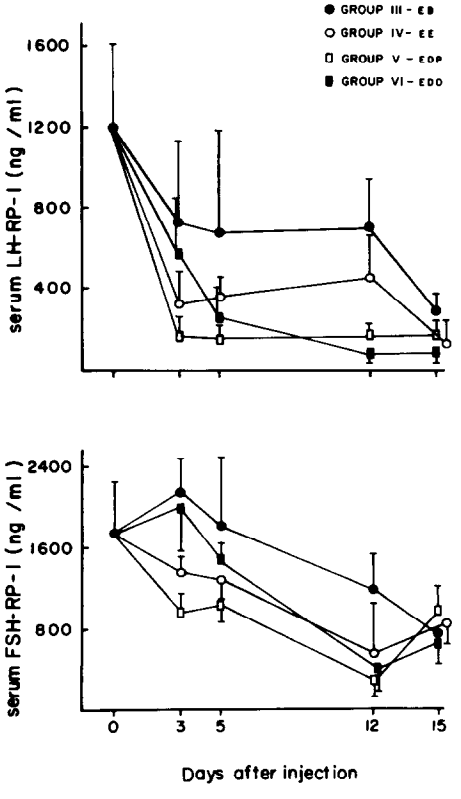

Fig. 3. Serum LH and FSH after administration of estradiol esters to ovariectomized adult rats. The values of LH, and FSH of female intact rats without treatment were 74 $\pm$ 43 and 135 $\pm$ 42 ng/ml respectively. Each point represents the mean $\pm$ SD of 5 animals.

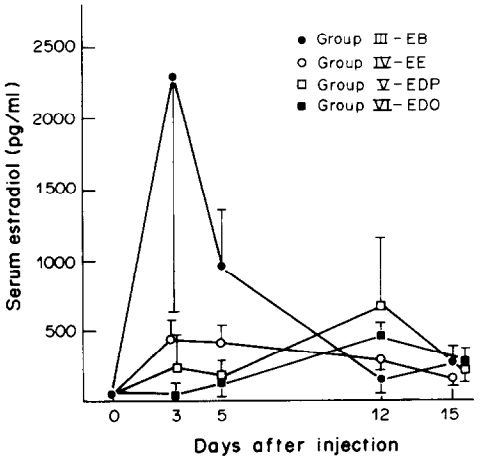

Fig. 4. Estradiol serum after a single s.c. injection of 1 μmol of estradiol mono and diesters to ovariectomized rats, the values of female intact rats without treatment was 115 $\pm$ 41 pg/ml. Values are mean $\pm$ SD of 5 animals.

The overall results suggest that both estradiol diesters (EDP and EDO) show long-acting properties which could be applied in estrogen substitutive therapy and or injectable hormonal contraception.

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