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Estrogenic effects of 17β-aminoestrogens assessed in uteri of rats and mice

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Abstract

Administration of exogenous estrogens has been associated with an increase of thromboembolic events. The 17β-aminoestrogens produce anticoagulant effects contrasting with the procoagulant effects of the natural occurring estradiol in rodents. This work compares the estrogenic effects induced by 17β-aminoestrogens prolame, butolame, pentolame, and estradiol in vivo models. Dose–response curves were performed using immature CD1 mice and Wistar rats. The animals were injected with estradiol or 17β-aminoestrogens (0.01 to 1000 μg/kg), or vehicle. The uterine wet and dry weights were determined. The 17β-aminoestrogens increased uterine weight in a dose-dependent manner. The uterotrophic effect produced by estradiol induced lower ED_{50} (6.5 and 4 μg/kg) and higher E_{max} values (+523–350%) in mice as compared with those from the rat, indicating more susceptibility of the mice model. The 17β-aminoestrogens are partial estrogenic agonists with a relative uterotrophic effect of estradiol (100%) from 9–86%. Only the ED_{50} values of 17β-aminoestrogens in CD1 mice showed a direct correlation to the length of the amine group substitution in C-17 since their efficacy and potency were in the order: prolame>butolame>pentolame.

Keywords: 17β-aminoestrogen; Estradiol; Uterotrophic effect; Estrogenic effect

1. Introduction

Women's protection against cardiovascular disease during their reproductive life has been documented and attributed to the modulating actions of the endogenous estrogens. The exogenous hormone replacement therapy and oral contraceptives used by millions of women worldwide has been claimed as beneficial during menopause based on the observed decreased risk of arteriosclerosis and myocardial infarction (Gordon et al., 1978; Kalin and Zumoff, 1990). These effects have been mainly explained because of their influence on lipoproteins metabolism, prevention of arteriosclerosis, vasodilator effects, and decreases in blood pressure with a subsequent improvement of blood flow (Henderson et al., 1988; Wren, 1992). Another proposed mechanism is the participation of estrogen receptors as modulator markers of inflammation and coagulation, influencing cardiovascular episodes (Cushman, 2002).

Other authors have demonstrated that oral contraceptives and hormonal replacement therapy are associated with an increase of thromboembolic events. Oral contraceptives use is the major cause of thrombotic disease in young women, whose highest risk occurs during the first year of use (up to 1 per 1000 per year), and is even higher among women who course with coagulation abnormalities or with prothrombotic predisposition (Rosendaal et al., 2001, 2002).

Alterations in blood clotting, inducing hypercoagulability are the most important factors in thrombosis genesis (Gembitskii and Begunov, 1994). The estrogen component in oral contraceptives and hormonal replacement therapy has been considered to be the main responsible for the

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elevated risk of thromboembolic diseases. Ethinylestradiol, contained in oral contraceptives, increases coagulation and fibrinolysis affecting hepatic and vascular functions. A daily dose intake of 10 μg of ethinylestradiol significantly increases factors VII, II–VII–X, VIII, von Willebrand factor, tissue plasminogen activator, plasminogen activator inhibitor-1, and big-endothelin1 (Mammen, 2000).

The thrombogenic effects of estrogens have been associated with dose dependence; high-dose estrogen therapy in men with prostatic cancer resulted in a higher rate of cardiovascular complications related to thrombosis, such as myocardial infarction, stroke, and venous thromboembolism (Gembitskii and Begunov, 1994). A low-dose combination of oral contraceptives markedly decreases the incidence of thromboembolic events (Rosendaal et al., 2001, 2002).

We have demonstrated in previous experimental studies on rodents that estrogens of clinical use, such as ethinylestradiol and 17β-aminoestrogens, produce changes in blood coagulation (Jaimez et al., 2000). The 17β-aminoestrogen pentolame produced anticoagulant effects opposite to the hypercoagulant effects observed with estradiol, in ovariectomized Wistar rats (Lemus et al., 1998). The anticoagulant selective effect of the 17β-aminoestrogens is related to the aromaticity of the A ring of the steroid molecule, since other 17β-aminoandrostane derivatives do not produce any anticoagulant effects (Rubio-Póo et al., 1993). These compounds induce mice vaginal cornification and increase uterine weight in adult rats, due to the interaction between α and β estrogen receptors similarly to that produced by estradiol (Jaimez et al., 2000).

The study of the estrogenic effects of 17β-aminoestrogens and estradiol in different animal models could provide more information and may contribute to a better understanding of the structure–activity relationships of these compounds to develop new and safer alternatives of estrogenic agents, especially directed to those patients with predisposition to thromboembolism.

The objective of this work was to assess the effects of 17β-aminoestrogens prolame, butolame, and pentolame on immature CD1 mice and Wistar rats, comparing their uterotrophic effects with that elicited by estradiol.

2. Materials and methods

2.1. Materials

All solvents and reagents used were of analytical reagent grade, and were used without further purification. Estrone (3-hydroxy-1,3,5(10)-estratrien-17-one) and 17β-estradiol (1,3,5(10)-estratrien-3,17β-diol) were purchased from Syntex (Mexico). The 17β-aminoestrogens (Fig. 1) prolame [17β-(3′-hydroxy-1′-propylamino)-1,3,5(10)-estratrien-3-ol], butolame [17β-(4′-hydroxy-1′-butylamino)-1,3,5(10)-estratrien-3-ol], and pentolame [17β-(5′-hydroxy-1′-pentylamino)-1,3,5(10)-estratrien-3-ol] were prepared from estrone according to the methods previously described (Fernández-G. et al., 1985; Lemini et al., 1993). Their characterization and chemical purity were obtained by the usual spectroscopic methods (IR, MNR) and analytical (TLC, MS and chemical analysis) techniques. All chemicals used were of the highest purity available from Baker Co. (Mexico).

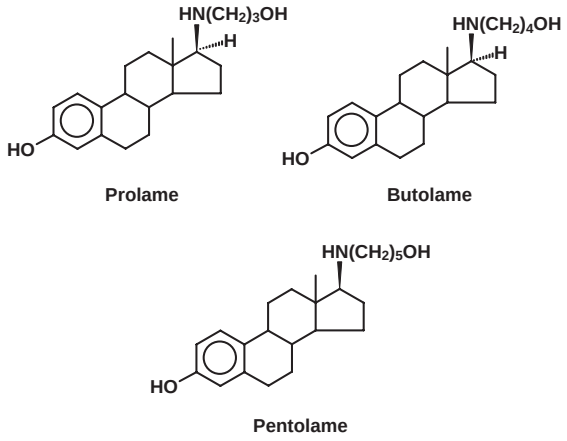

Fig. 1. Structure of the 17β-aminoestrogens: prolame, butolame, pentolame.

2.2. Animals

Immature female CD1 mice (10–15 g, 21 days old) and Wistar rats (35–40 g, 21 days old), from the animal facilities of the School of Medicine, National University of Mexico, were used. All experimental studies were conducted in accordance to the Mexican National Protection Laws on Animal Protection and the General Health Law Related to Health Research (NOM-062-Z00-1999).

2.3. Experimental design

The effects on uterine wet (U_{ww}) and dry weights (U_{dw}) were assessed. The animals were distributed among groups according to a balanced design based on body weight (6–8 animals per group in each experiment) and randomly assigned to treatment groups. Room temperature was kept constant (20–22 °C) with 12–12 h, light–dark cycles. Different groups of animals were subcutaneously (s.c.) injected once a day for 3 consecutive days with prolame, butolame, pentolame, or estradiol (0.01 to 1000 μg/kg of body weight), the control group received the vehicle (3 ml/kg) only. On the fourth day, the animals were weighed and the uterotrophic activity induced by the tested compounds was evaluated by the gain in uterine wet and dry weights. The uteri were dissected, blotted, and weighed to obtain the wet weight. Afterwards, the organs were dried at 70 °C for 24 h, and weighed again to obtain the dry uterine weight. The uterine weights of all the groups were expressed in milligrams and the percent of response was calculated with relation to the vehicle (100%).

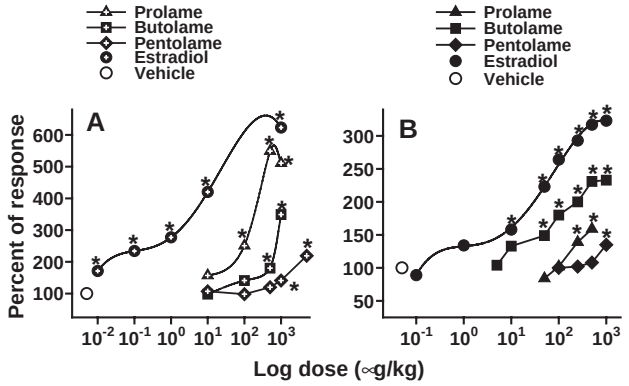

Fig. 2. Effects on uterine wet weight of the 17β-aminoestrogens (prolame, butolame, pentolame) and estradiol in immature CD1 mice (A) N=10, and Wistar rats (B) N=11. *P<0.05.

2.4. Data analysis

All experiments were repeated twice. The results were expressed in means±standard error (S.E.M.). To calculate the difference with respect to the vehicle group, the relation: $[U_w(100)/U_wV]-100$ was used. U_w indicates the wet or dry uterine weight (U_{ww} or U_{dw}). The relative uterotrophic effect to estradiol was calculated by the relation: $[E_{max}$17β-aminoestrogens$]\times100/(E_{max}$ estradiol). The effective dose 50 (ED_{50}), the maximum response value (E_{max}), and confidence limits were calculated from the dose–response curves using the sigmoid fitting model. Slope values were obtained by linear regression. Correlation analysis of the evaluated parameters was also performed. These data analysis was done with the Origin® 6.1 program (Copyright© 1991–2000 Origin Lab Co, Northampton, USA).

2.5. Statistical analysis

Statistical significance among the different treated groups with respect to the control was analyzed. The significance of the differences between control (vehicle) and treated groups was assessed by the Dunn's or Dunnet's methods as required (Zar, 1984). P<0.05 was considered as limit for statistically significant data. The analysis was performed using the Sigma Stat statistical software 2.0 Copyright© 1992–1995, Jandel Corporation.

3. Results and discussion

3.1. Uterotrophic effects of 17β-aminoestrogens

Dose–response curves were obtained to determine the estrogenic efficacy and potency of 17β-aminoestrogens as compared with estradiol. The results expressed in percent of the uterine weight are shown in Fig. 2. Prolame, butolame, and pentolame significantly increased the uterine wet weight (P<0.05) in a dose-dependent manner similarly to estradiol but with lower efficacy and potency. The dose–response curves allowed us to obtain the ED_{50}, and the E_{max} of all the compounds. From the data regression analysis, we calculated the slopes and their corresponding regression coefficients (r) as indicated in Table 1.

3.1.1. Uterotrophic effects of 17β-aminoestrogens on immature CD1 mice and immature Wistar rats. Dose–response curves

Fig. 2A shows that estradiol induced significant uterine weight increases (P<0.05) starting with the administration of 0.01 μg/kg of body weight in CD1 mice. Table 1 shows the ED_{50} and E_{max} values obtained with estradiol and the 17β-aminoestrogens. The response of CD1 mice to estradiol showed lower ED_{50} (6.5 and 4 μg/kg) and higher E_{max} values (+523–350%) as compared with those from the rat model, indicating that the mice model is more susceptible than that of the rat. The 17β-aminoestrogens elicited the same behavior as estradiol in relation to the E_{max} values. They produced a relative uterotrophic effect to estradiol (100%) from 23% to 86%. Nonetheless, their ED_{50} in immature CD1 mice were higher. The ED_{50} parameters of

Table 1
Uterotrophic effect, ED_{50} and E_{max} of prolame, butolame, and pentolame and estradiol in immature CD1 mice and Wistar rats

	Immature CD1 mice			Immature Wistar rats		
Treatment	ED_{50} U_{ww} (confidence limits 20–80) slope and r	ED_{50} U_{dw} (confidence limits 20–80) slope and r	E_{max}(%) $U_{ww}-U_{dw}$	ED_{50} U_{ww} (confidence limits 20–80) slope and r	ED_{50} U_{dw} (confidence limits 20–80) slope and r	E_{max}(%) $U_{ww}-U_{dw}$
Prolame	141 μg/kg (93–214) 203; 0.9372	143 μg/kg (74–278) 122; 0.9325	449–275[a] 86–79[b]	163 μg/kg (97–273) 77; 0.9967	138 μg/kg (55–349) 43; 0.8699	59–20[a] 20–9[b]
Butolame	532 μg/kg (471–601) 103; 0.8285	516 μg/kg (451–591) 76; 0.8795	249–180[a] 48–51[b]	73 μg/kg (21–252) 57; 0.9848	98 μg/kg (33–297) 66; 0.9730	133–140[a] 46–61[b]
Pentolame	1758 μg/kg (751–4114) 38; 0.8079	1286 μg/kg (795–2080) 44; 0.8005	119–105[a] 23–30[b]	569 μg/kg (491–660) 33; 0.8574	516 μg/kg (496–537) 43; 0.8542	35–56[a] 12–24[b]
Estradiol	6.5 μg/kg (0.613–68) 92; 0.9858	4 μg/kg (0.433–39) 51; 0.9674	523–350[a] 100	52 μg/kg (6–482) 63; 0.9782	22 μg/kg (2–287) 59; 0.9899	288–230[a] 100

U_{ww}=uterine wet weight; U_{dw}=uterine dry weight.
[a] Related to the V (0).
[b] Related to estradiol (100%).

Table 2
Correlation analysis of ED_{50}, E_{max} and RBA of the 17β-aminoestrogens

Immature mice and rats	Regression coefficient r and P values
ED_{50} CD1 U_{ww} vs. ED_{50} CD1 U_{dw}	0.959
	0.040
ED_{50} Wistar U_{ww} vs. ED_{50} Wistar U_{dw}	0.993
	0.007
ED_{50} CD1 U_{ww} vs. ED_{50} Wistar U_{ww}	0.953
	0.046
ED_{50} CD1 U_{dw} vs. ED_{50} Wistar U_{dw}	0.893
	0.125
E_{max} CD1 U_{ww} vs. E_{max} CD1 U_{dw}	0.993
	0.007
E_{max} Wistar U_{ww} vs. E_{max} Wistar U_{dw}	0.930
	0.065
Wistar[a] rats	
RBA vs. ED_{50} Wistar U_{ww}	−0.436
	0.564
RBA vs. ED_{50} Wistar U_{dw}	−0.518
	0.482
RBA vs. E_{max} Wistar U_{ww}	0.931
	0.069
RBA vs. E_{max} Wistar U_{dw}	0.984
	0.013

U_{ww}=uterine wet weight; U_{dw}=uterine dry weight.
Taken from Jaimez et al., (2000).
[a] RBA=relative binding affinity.

17β-aminoestrogens were directly related to the length of the amine group substituted in C-17 of the steroid molecule. In contrast, E_{max} values displayed an inverse relation (r=0.9999). From these data it can be inferred that prolame is the most efficacious 17β-aminoestrogen in eliciting the uterotrophic effect.

Fig. 2B shows the dose–uterine weight curves obtained after administration of 0.1 to 1000 μg/kg of prolame, butolame, pentolame, and estradiol to immature Wistar rats. Here estradiol significantly increased the uterine weight (P<0.05) starting with the 10 μg/kg dose. Comparison between estradiol E_{max} values (288–230%) as 100% with the 17β-aminoestrogens (prolame, butolame, and pentolame) indicated that they were able to induce uterotrophic effects from 9 to 61% (relative uterotrophic effect of estradiol). The 17β-aminoestrogens slope and r coefficient values were close in the cases of estradiol, prolame, and butolame curves; however, pentolame's slope had a lower value, indicating the lowest efficacy of this compound. In the immature Wistar rat model, butolame showed to be the most efficacious of the three assayed 17β-aminoestrogens. Table 2 depicts the correlation analysis of the ED_{50}, and E_{max} values of uterine weight of rats and mice, including the regression coefficients and P values.

The E_{max} values relative to the uterotrophic effect of the 17β-aminoestrogens in the immature CD1 mice showed an inverse relation to the substitution of the chain length of the amino group on C-17 (Fig. 1). These observations agree with those we described recently in the ovariectomized Wistar rat model and binding studies, where an inverse correlation of their relative binding affinities (RBA) with the length of the substitution on the amino group was also found (Jaimez et al., 2000). The uterotrophic effect of the 17β-aminoestrogens decreased as the length of the chain substitution on the amine group increased.

ED_{50} data obtained in immature CD1 mice (Table 1) revealed: prolame is 22- to 36-fold less potent than estradiol; butolame: 82- to 129-fold less potent than estradiol, and pentolame 270- to 322-fold less potent than estradiol.

The correlation analysis derived from the obtained ED_{50}, and the E_{max} values showed that these values resulted in high regression coefficients: r=0.93 to 99 with significant P values from 0.04 to 0.007 (Table 2). The immature Wistar rats data compared with our previous results related to the 17β-aminoestrogens in ovariectomized Wistar rat and the relative binding affinities to the estradiol receptor values (RBA; Jaimez et al., 2000) showed a high correlation values (r=0.94 to 0.984; P<0.05).

The 17β-aminoestrogens administered to the immature CD1 mice and Wistar rats increased the uterine weight revealing that these compounds behave as partial agonists to estradiol. They were able to induce uterotrophic effects from 9% to 86% related to estradiol (100%).

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