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Contrasting effects of estradiol and 17β-aminoestrogens on blood clotting time in rats and mice

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Abstract

Estrogens have been associated with thromboembolic events. Our group has described the anticoagulant effect of 17β-aminoestrogens in rodents, potentially new alternative estrogenic agents without thrombogenic risk. This work compares the contrasting effects of estradiol and the 17β-aminoestrogens (prolame, butolame, and pentolame) on blood clotting time. Ovariectomized CD1 mice received a single injection of 17β-aminoestrogens, estradiol (20 to 80 mg/kg), or vehicle. Estradiol decreased blood clotting time from −10% to −25% (48 h; $P<0.01$) and 17β-aminoestrogens increased it, differing in latency (~12 h; +48%, $P<0.01$) and duration (~72 h +58%, $P<0.01$). In male Wistar rats, similar effects (pentolame +45%; estradiol −31%; $P<0.01$) were observed 48 h after five consecutive daily injections of 1000 μg/animal/day. The maximum procoagulant effect of estradiol was obtained after 72 h with 10 μg/animal/day (−45%; $P<0.01$). 17β-Aminoestrogens always produced opposite effects to those of estradiol on blood coagulation.

Keywords: 17β-Aminoestrogen; Estradiol; Anticoagulant effect; Thrombogenic effect

1. Introduction

Women's protection against cardiovascular disease during their reproductive life has been attributed to the modulating actions of endogenous estrogens. In the last years, exogenous hormone therapy in oral contraceptives and hormone replacement therapy has been used by millions of women worldwide (Kalin and Zumoff, 1990). The beneficial effect among estrogens users, during menopause, has been claimed based on the observation of a reduced risk of arteriosclerosis and myocardial infarction (Gordon et al., 1978). These effects have been mainly explained based on their influence on lipoprotein metabolism, which leads to prevent arteriosclerosis by producing vasodilator effects; hence, inducing a fall in blood pressure with the subsequent improvement in blood flow (Henderson et al., 1988; Wren, 1992). These lipid-lowering effects of hormonal replacement therapy can partially explain the possible decrease in cardiovascular risk observed in postmenopausal women. Another proposed mechanism is the participation of estrogen receptors as modulator markers of inflammation and coagulation, influencing cardiovascular episodes (Cushman, 2002).

However, conflicting results have emerged from the use of estrogens. Other authors have demonstrated that oral contraceptives and hormonal replacement therapy are associated with an increase in thromboembolic events. It has been reported that the oral contraceptives use is the major cause of thrombotic disease in young women, with the highest risk during the first year of use (up to 1 per 1000 per year). The venous thrombotic events of oral contraceptives usage is higher among women who course with coagulation abnormalities or women with prothrombotic predisposition (Rosendaal et al., 2001, 2002).

The progestogen component included in oral contraceptives and hormonal replacement therapy has not yet been

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considered to be a risk factor for either venous thromboembolic events or detrimental for hemostasis. Instead, the concern for fibrinolysis and platelet aggregability is related to the endothelium and platelets reactivity (Blomback et al., 1997). In the endothelium, estrogens and progestogens influence collagen, elastin synthesis, release of vasoactive compounds, and/or factors controlling fibrinolysis. In predisposed women, progestogens may as well increase the distensibility and capacitance of veins, decreasing blood flow that might then lead to venous stasis and thrombosis. In the arteries, progestogens may act as vasoconstrictors, enhancing vasospasms at injured endothelium sites that might, at the end, lead to ischemic diseases (Kuhl, 1996).

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 elevated risk of thromboembolic diseases. It is known that the estrogens widely used in oral contraceptives, as ethinylestradiol, hasten coagulation and fibrinolysis by affecting the hepatic and vascular functions. A daily dose 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 since a low-dose combination of oral contraceptives markedly decreases the incidence of thromboembolic events (Rosendaal et al., 2001, 2002). In this regard, a 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).

Experimental studies on rodents have shown that estrogens of common clinical use and 17β-aminoestrogens produce changes in blood coagulation (Mandoki et al., 1983). Interesting is the anticoagulant selective effect of the 17β-aminoestrogens observed in blood clotting time. The anticoagulant effect of these group of compounds 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). Our in vivo studies have shown that these compounds elevate fibrinogen concentration by means of a mechanism not yet identified (García-Manzano et al., 2002). In the evaluation of the estrogenic activity of these compounds, it was shown that they induced vaginal cornification in CD1 mice and an increase of the uterine weight in adult Wistar rats, due to the interaction between the α and the β estrogen receptors in a similar way to that produced by estradiol (Jaimez et al., 2000).

The study of the anticoagulant effects of 17β-aminoestrogens and estradiol in different animal models could provide more information about their actions on blood coagulation and contribute to a better understanding of the structure–activity relationships of these compounds. This knowledge could also contribute to develop new and safer alternatives of estrogenic agents, especially directed to those patients prone to thromboembolism.

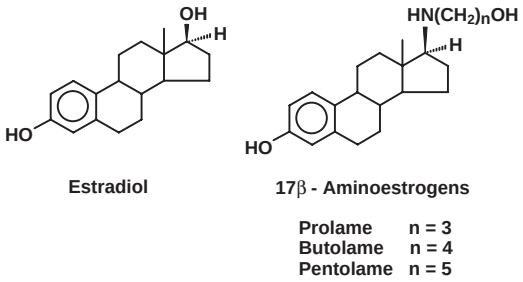

Fig. 1. Structure of the 17β-aminoestrogens, prolame, butolame, pentolame, and of estradiol.

We demonstrated that the 17β-aminoestrogen, pentolame, produces anticoagulant effects opposite to the procoagulant effects observed with estradiol, in ovariectomized Wistar rats (Lemus et al., 1998). We are interested to know whether other 17β-aminoestrogens can behave similarly to pentolame and whether these opposite effect of pentolame could also be produced in other species, such as the ovariectomized CD1 mice. Additionally in the present work, we compare the results obtained in mice with those produced in the male Wistar rat, which is a suitable animal model, since larger blood volume samples can be withdrawn for blood tests to determine the hemostatic changes required to elucidate the mechanism of the anticoagulant and thrombogenic effects of estrogens.

The present study was aimed at comparing the effects of single and multiple administrations of the 17β-aminoestrogens: prolame, butolame, and pentolame (Fig. 1) on blood clotting time with those produced by estradiol in ovariectomized CD1 mice and male Wistar rats.

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 chem-

icals used were of the highest purity available from Baker Co. (Mexico).

2.2. Animals

Adult ovariectomized CD1 mice (30–35 g) and adult male Wistar rats (200–250 g), from the animal facilities of the School of Medicine, National University of Mexico (UNAM), 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. Blood clotting time

2.3.1. Experimental design

The experiments were performed in ovariectomized CD1 mice and male Wistar rats. CD1 mice were ovariectomized under chloral hydrate anesthesia. Three weeks after ovariectomy, vaginal smears were taken and only the animals that showed abundant leukocytes were subsequently used to assure the efficacy of the ovariectomy. Animals were distributed among groups according to a balanced design based on body weight (6–8 animals per group in each experiment). Room temperature was kept constant (20–22 °C) with 12–12 h, light–dark, cycles, estrogens were dissolved in propyleneglycol (vehicle) and administered subcutaneously (s.c.); control animals received the vehicle only.

2.3.2. Blood clotting time assessment

Blood clotting time was measured according to earlier reports (García-Manzano et al., 2002). Groups of ovariectomized CD1 mice received a single s.c. administration of prolame, butolame, pentolame, estradiol (20, 80, 80, 60 mg/kg of body weight, respectively) or the vehicle (5 ml/kg). Temporal course of blood clotting time was assessed at 12, 24, 48, 72, 96, and 120 h after the last injection. The male Wistar rats received s.c. pentolame or estradiol (0.1 to 1000 μg/animal/day) or the vehicle (1 ml/kg/day) for five consecutive days. The evaluation was performed at 12, 24, 48, and 72 h after the last injection. Evaluation of blood clotting time also followed a balanced, latin square, block design, so that there were no differences in the testing times among the groups of the blind assay experiments. The blood clotting time data were normalized with respect to the vehicle group (100%).

2.4. Statistical analysis

All experiments were repeated at least twice. Results are expressed as means±standard error (S.E.M.) Statistical significance among the different treated groups with respect to the control (vehicle) was assessed by the Dunn's or Dunnet's method 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 (USA).

3. Results and discussion

3.1. Time course effects on blood clotting time of ovariectomized CD1 mice after a single administration of prolame, butolame, pentolame, or estradiol

All the 17β-aminoestrogens administered in a single s.c. injection produced blood clotting time dose-dependent increases lasting for several days. The effects on blood clotting time of a single administration of prolame (20 mg/kg), butolame (80 mg/kg), pentolame (80 mg/kg), and estradiol (60 mg/kg) to the ovariectomized CD1 mice are shown in Fig. 2. The blood clotting times of the animals treated with the 17β-aminoestrogens, estradiol, and vehicle groups were daily measured until the anticoagulant effect had weaned off and could no longer be detected. Fig. 2 shows the blood clotting time's normalized data as compared to the individual control group of the corresponding experiment. Prolame had to be administered at 20 mg/kg because of its low solubility. The increased blood clotting time observed after 24 h from the administration of prolame, butolame, or pentolame gradually diminished during the following three days. It was also observed that the latency and E_{max} of the anticoagulant effect varied. Prolame increased significantly the blood clotting time 12 h after

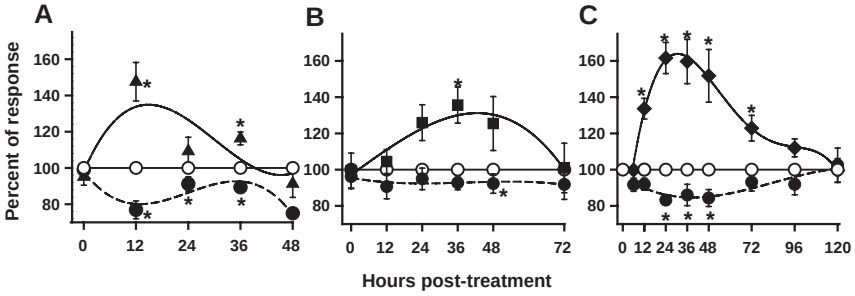

Fig. 2. Time course of the effect of a single s.c. injection of prolame (A: 20 mg/kg) butolame (B: 80 mg/kg), pentolame (C: 80 mg/kg), or estradiol (60 mg/kg) in ovariectomized CD1 mice. *$P<0.05$.

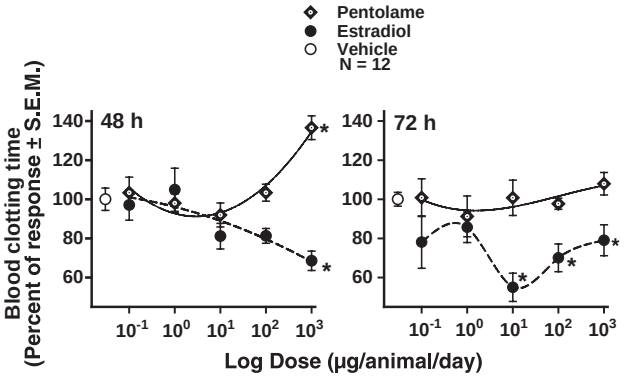

Fig. 3. Log dose–response relationships of pentolame and estradiol (0.1–1000 μg) on blood clotting time of male Wistar rats, 48 and 72 h after five consecutive s.c. administrations. *P<0.05.

its injection (+48%, P<0.01) maintaining it for 36 h (+16%; P<0.01). Butolame showed its maximum effect 36 h after its administration (+36%; P<0.01), and pentolame produced sustained anticoagulant effects for 12, 24, 36, 48, and 72 h after having been administered (+34%, +62%, +60%, +58%, +23%, respectively; P<0.01). A single estradiol administration of 60 mg/kg always reduced significantly the blood clotting time with respect to the control group from −10% to −25%, being significant from 12 to 48 h (P<0.01).

3.2. Effects of repeated administrations of pentolame or estradiol on blood clotting time in the male Wistar rat. Dose–response relationships

Daily s.c. administration for 5 days of the 17β-amino-estrogen, pentolame, in the adult male Wistar rat (Fig. 3) produced an anticoagulant effect 48 h after the last administration of the compound; for estradiol similar effects were found. The blood clotting time was increased in a 45% (P<0.01) after pentolame administration. At this same time, estradiol shortened it −31% (P<0.01) and sustained it for 72 h; the maximum effect was obtained with the 10 μg/animal daily dose (−45%; P<0.01). These findings agree with those from our previous report on ovariectomized Wistar rats (Jaimez et al., 2000). The effect of estradiol at 96 h did not reveal any significant difference when compared to the vehicle group (data not shown).

4. Conclusions

We observed that in the ovariectomized CD1 mice and in the adult male Wistar rat, the 17β-aminoestrogens, prolame, butolame, and pentolame, always produced opposite effects on blood clotting time to those induced by estradiol. The estradiol procoagulant effects and the anticoagulant effects of the 17β-aminoestrogens were both sustained and significant (P<0.01). In the CD1 mice model, the 17β-aminoestrogen, pentolame, showed the largest anticoagulant effect followed by butolame and then by prolame. The main differences of the 17β-aminoestrogens effects were in their latency and duration, probably due to their distinct solubility properties leading to different bioavailabilities. Additional work is already in progress to ascertain the involved mechanisms of this interesting effect, especially in relation to the hemostatic parameters and fibrinogen concentration, which are mainly affected by these compounds (García-Manzano et al., 2002).

The anticoagulant effect and the apparent absence of a procoagulant phase of the group of 17β-aminoestrogens, herein reported, suggest that these compounds are very interesting and need to be deeply studied, especially the mechanism involved in the resultant anticoagulant effect. A deeper knowledge of them could shed some light on the estrogen mechanism of blood coagulation and might lead to the development of estrogens for clinical applications aimed at reducing, instead of increasing, the risk of thromboembolic accidents.

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