Impact of 8-Isoestrone Analogs on Estradiol Binding in Uterine Tissue from Ovariectomized Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 2, pp. 165-167, February, 1995
Original article submitted March 15, 1994

8-Isoanalogs of estrone were studied for their ability to influence estradiol binding in the cytosolic fraction of uterine tissue from ovariectomized rats and for their uterotropic activity 24 h after injection into such rats. Two groups of estrone 8-isoanalogs with opposite biological effects were identified: those increasing estradiol binding in the cytosolic fraction of uterine tissue and those decreasing this binding. Uterogenic activity was exhibited by all of the compounds tested, with the exception of compound I. No correlation was found between the uterogenic activity of the isoanalogs and hormone-receptor interactions.

Key Words: *uterus; estrogen receptors; rats*

Estrogens exert their biological activity through high-affinity and selective interaction with cellular receptors [5-7]. They have been shown capable of producing hypocholesterolemic effects by reducing vessel permeability for atherogenic lipoproteins and the cholesterol/phospholipid ratio and by raising blood levels of high-density lipoproteins and lowering those of low-density lipoproteins [4,9]. Using the scheme proposed by Torgov-Ananchenko, 8-isoestrone analogs were synthesized in Saint Petersburg University's Department of Chemistry of Naturally Occurring Compounds. Their structure was demonstrated by spectral methods and confirmed by thin-layer chromatography (Fig. 1) [8]. Interest in compounds of this series stems from their marked ability to improve lipid and lipoprotein metabolism when the estrogenic potential is diminished. The objectives of this work were to study molecular mechanisms of action of the newly synthesized 8-isoestrone analogs and compare their uterogenic and receptorotropic activities.

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MATERIALS AND METHODS

A total of 50 female rats weighing 120-140 g were used, 6-9 animals in each of the test groups. 8-Isoestrone analogs were injected intraperitoneally in an oil solution in a dose of 0.1 or 1 mg/kg on day 7 after ovariectomy performed as described [3]. Rats injected with the solvent served as controls. Euthanasia was carried out under ether anesthesia 24 h postinjection. Uteri were scraped clean of fatty and connective tissues and homogenized in liquid nitrogen. Isolation of the cytosol and assays of the compounds for their effect on estradiol binding in the cytosolic fraction of uterine tissue were performed as detailed [1]. Protein was determined using a modification [10] of Lowry's method [12]. The nonparametric Wilcoxon-Mann-Whitney *U* test was used for statistical treatment of the results.

RESULTS

The results are summarized in Table 1. Compound I in the doses of 0.1 and 1.0 mg/kg reduced estradiol binding in the cytosolic fraction by 50%

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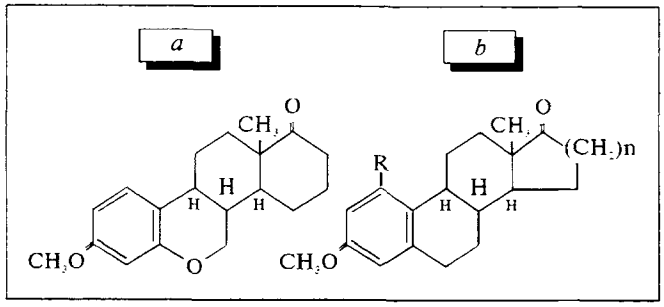

Fig. 1. Structural formulas of the four tested compounds. *a*) 6-oxa-D-homo-8-isoestrone methyl ether; *b*) D-homo-8-isoestrone methyl ether: R=H, $n=2$; 8-isoestrone methyl ether: R=H, $n=1$; 1-methyl-D-homo-8-isoestrone methyl ether: R=CH_3, $n=2$.

and 40%, respectively, without showing uterotropic activity. Compound II reduced estradiol binding 1.5-fold at 0.1 mg/kg and increased it 2.4-fold at 1 mg/kg. Compounds III and IV increased estradiol binding 2.7-fold and 2.4-fold, respectively, at 0.1 mg/kg and 2.9-fold and 3.1-fold at 1 mg/kg, and they both exhibited pronounced uterotropic activity at these dose levels.

In our previous studies, a single injection of estradiol into ovariectomized rats at 0.1 or 2.5 mg/kg was found to increase the number of estradiol receptors in uterine tissue by a factor of 3.2 and 3.3, respectively, as compared to the control group. This suggests that, similarly to estradiol, compounds III and IV at both dose levels (0.1 and 1 mg/kg) and compound II in the dose of 1 mg/kg exert their uterotropic activity by increasing the number of specific binding sites for estrogens. The lack of uterotropic activity in compound I may be explained by its allosteric interaction with estradiol receptors, with the result that chromatin is not activated and there is no substantial increase in protein synthesis, including the synthesis of receptor proteins, which is believed [10] to deplete the cytoplasmic pool of estrogen receptors. This effect is similar to that exerted by antiestrogens, whose antiestrogenic activity depends on their ability to maintain a low level of receptors in the cytosol [11]. The introduction of oxygen in position 6 of an 8-isoanalog (as in compound I) appears to incur an absence of uterotropic activity and a reduction in the density of cytosolic estradiol receptors. In our view, compounds of the 8-isoseries may be looked upon as promising agents in the treatment of postmenopausal syndrome (compounds II, III, and IV) and of estrogen-dependent tumor growth (compound I).

In summary, two groups of compounds with opposite biological effects have been identified by examining the cytosolic fraction of uterine tissues from ovariectomized rats 24 h after injection of the respective preparations: those that reduce estradiol binding (compound I in both doses [0.1 and 1 mg/kg] and compound II in the dose of 0.1 mg/kg) and those that increase this binding (compounds III and IV in both doses and compound II at 1 mg/kg). All compounds, with the exception of I, exhibit uterotropic activity.

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TABLE 1. Effects of Estrone Isoanalogs on Estradiol Binding in the Cytosolic Fraction of Uterine Tissue from Ovariectomized Rats and the Uterotropic Activity of the Analogs 24 h after Injection

Compound	Dose, mg/kg	Percentage content of cytosolic estradiol receptors in uterine tissue	Uterotropic activity (% of control value)
I (6-oxa-D-homo-8-isoestrone methyl ether)	0.1	48.7*	84.0
	1.0	60.6*	102.6
II (D-homo-8-isoestrone methyl ether)	0.1	62.9*	135.2*
	1.0	235.8*	205.0*
III (8-isoestrone methyl ether)	0.1	270.0*	153.7*
	1.0	239.1*	151.6*
IV (1-methyl-D-homo-8-isoestrone methyl ether)	0.1	293.9*	149.0*
	1.0	306.9*	94.0

Note. Uterotropic activity was calculated as the ratio of uterus weight to body weight×100%. The asterisk denotes a significant difference from the control ($p=0.05$).

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Correlation of Antiinflammatory and Hormonal Activity of Derivatives of the 8,16-Diazasteroid Series

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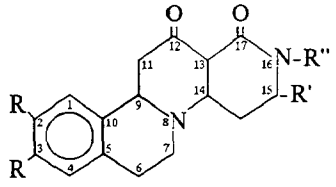
Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 2, pp. 168-171, February, 1995
Original article submitted March 9, 1994

Antiinflammatory activity and mechanism of action are studied for seven compounds of the 8,16-diazasteroid series. It is established that the antiinflammatory activity of the compounds is increased on the whole due to the reduced ketofunction in the 12 position of 8,16-diazasteroid as well as for the introduction of methoxy groups in the 2 and 3 position or phenol substitute in the 16 position. The activity of compounds VI and VII also depends on the inflammation model or on the pain reaction and differs significantly from the effectiveness of diclofenac sodium and prednisolone. Unlike the latter, the compounds under study are virtually devoid of hormonal activity.

Key Words: *8,16-diazasteroid; diclofenac sodium; prednisolone; antiinflammatory activity; hormonal effects; structure-activity correlation*

The heterosteroid compounds are of interest as a useful tool for distinguishing antiinflammatory, antiallergic, and immunomodulating effects from hormonal activity [1]. A more profound modification of the structure of 8-azasteroids by the substitution carbon for nitrogen in the 16 position can either boost or weaken the pharmacological effects and makes it possible to perform a purposeful synthesis of compounds with preassigned properties. The aim of the present investigation was to identify the compounds with pronounced antiinflammatory properties among the known 8,16-diazasteroids [2] and to study the mechanisms of their biological effects. The general structure of the compounds under study was as follows:



where I: R=H, R'=R"=H; II: R=H, R'=R"=H, at C_{12}-H; III: R=H, R'=CH_3, R"=H; IV: R=H,

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0007-4888/95/0002-0159$12.50