Effect of Ethanol on Uterotropic Action of Estrogens

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Female rats aging 3 months at the beginning of experiments received 5 or 15% ethanol and then were subjected to bilateral ovariectomy 2 weeks before end of the experiment. During the last 11 days they were daily injected intramuscularly with 2 μg estradiol. Drinking of 5% ethanol combined with injections of estrogens induced DNA damage in the uterus detected by comet assay and abolished induction of progesterone receptors, changes in peroxidase activity, proliferation index, endometrium thickness, and other indices reflecting the hormonal effect of estradiol on the uterus. Drinking of 15% ethanol was accompanied by an increase in DNA-damaging effects of estrogens and a decrease in their hormonal uterotropic effects. It is concluded that unlike tobacco smoking, drinking of moderate ethanol concentrations modifies primarily genotoxic, but not the hormonal effect of estrogens.

Key Words: *estrogens; ethanol; DNA damage; modifiers of estrogen-induced carcinogenesis*

Peculiarities of estrogen action on tissues sensitive to these hormones are important for understanding the mechanisms of estrogen-induced carcinogenesis and anticarcinogenesis. It was recently established that estrogens not only promote carcinogenesis, but also can induce it by damaging DMA directly or indirectly via estrogen metabolites. In light of this, the two major types of hormonal carcinogenesis were distinguished: promotor and genotoxic [2]. It is important to study the conditions potentiating the genotoxic component in the general effects of estrogens (specifically, on the uterine tissues), because such conditions or factors can determine the type of hormonal carcinogenesis and the phenotype (with more or less favorable prognosis) of hormone-depending tumors.

Previously we showed that tobacco smoke modifies the uterotropic effect of estrogens, in particular, it moderated the hormonal component and potentiates the genotoxic component [2,3]. This phenomenon was called estrogen toggle (re-targeting) effect. Here we analyzed combined effects of estrogens and ethanol. The effects of ethanol on the endocrine system was described previously [4,8], but modulation of uterotropic (hormonal and genotoxic) effects of estrogens was not previously studied.

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MATERIALS AND METHODS

The study was carried out on 64 female rats (Rappolovo Breeding Center). At the beginning of the experiment, their age was 3 month. The animals were maintained under standard vivarium conditions. Series I rats (n=28) were divided into control and experimental groups. The control rats drank tap water during 4 months, the experimental rats drank 5% ethanol in tap water. All rats were weighed 2.5 weeks before the end of the experiment and subjected to bilateral ovariectomy. The rats were daily injected intramuscular by with estradiol (E2, 2 μg/day) for 11 days until the end of the experiment. At the morning of the last day, the rats were weighed and sacrificed with lethal ether dose. The blood was drawn from the heart, serum concentration of estradiol was determined with a radioimmunological kit (Beloris, Minsk) and cholesterol content with enzymocolorimetric kits (Randox). The uteri were isolated, weighed, and the content of progesterone receptors [9] and peroxidase activity were assayed

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TABLE 1. Serum Concentration of Estradiol and Cholesterol and EDH Activity in Bilaterally Ovariectomized Rats (*M*±*m*)

Index	Series I		Series II	
	control (H_2O)	test (5% ethanol)	control (H_2O)	test (5% ethanol)
Estradiol concentration, pmol/liter	31.2±4.6	42.7±5.9	315.2±94.3	367.8±73.7
Cholesterol, mg/100 ml	35.4±1.9	38.0±4.3	72.7±3.4	65.3±4.2
Activity of 2-hydroxyamilase, nmol/mg protein for 30 min	15.1±3.6	22.2±3.9	28.4±7.0	25.2±2.9

[12]. Flow cytometry was used to evaluate fractions of S- and G2/M-phase cells and proliferation index [5]. DNA abnormalities were assayed by gel-electrophoresis (comet assay) in a modification suitable for cells isolated from solid tissues [11]. To measure the thickness of the epithelium, the samples were fixed in 10% formalin, embedded in paraffin, and analyzed by histological and morphometric methods [10]. To evaluate the possible effect of ethanol on estrogen metabolism, in some rats hepatic estradiol-2-hydroxylase (EDH) activity was determined in the liver [6], because uterine EDH activity is very low. In series II, the rats were divided into 2 control (intact and reference) and 2 experimental groups. The control and experimental rats drank tap water and 15% ethanol for 2 months. All rats were subjected to bilateral ovariectomy 2.5 weeks before the end of the experiment. During 11 days before the end of the experiment, some control and experimental rats were daily injected intramuscularly with estradiol (E2, 2 μg/day). Body and uterus weight, the content of progesterone receptors, peroxidase activity, comet assay parameters of the uterine tissue, serum concentration of estradiol and cholesterol, and hepatic EDH activity were analyzed. The data were processed statistically using Student's *t* test.

RESULTS

Combined administration of 5 or 15% ethanol with E2 did not change serum cholesterol and estradiol. These parameters did not differ from those in reference rats treated with E2 only (Table 1), which suggests that ethanol did not affect E2 clearance and its non-reproductive effects. No differences in EDH activity were revealed between the experimental and control rats (Table 1), which indicated that in contrast to tobacco smoke [2], alcohol did not promote 2-hydroxylation and synthesis of the corresponding catecholestrogens.

Ethanol (5% solution) produced no significant changes in the proliferative potential of E2-stimulated endometrium (in control rats the fractions of S- and G2/M-phase cells were 9.68±0.90% and 8.96±0.60%, respectively, and the proliferation index was 18.60±

TABLE 2. Hormonal and DNA-damaging Effect of Estradiol in Bilaterally Ovariectomized Rats Drinking Ethanol in Various Concentrations (*M*±*m*, *n*=9-14)

Index	Series I		Series II			
			without E2		with E2	
	control (H_2O)	test (5% ethanol)	control (H_2O)	test (5% ethanol)	control (H_2O)	test (5% ethanol)
Uterus weight, mg	373±21	378±23	111±7	108±6	336±14	298±11*
Peroxidase activity, U/mg protein	3975±150	4022±222	477±154	603±165	3198±451	3412±449
Progesterone receptor content, fM/mg protein	365±25	335±28	13.9±1.0	9.0±0.6*	88.5±37.4	44.5±7.6
Endometrium thickness, μ	17.5±1.81	21.7±2.34	—	—	—	—
Comets						
mean number, %	19.1±9.1	38.6±9.4	—	—	32.9±10.2	83.1±5.2*
mean length, arb. units						
per one cell with a comet	3.60±0.32	4.5±0.3	—	—	5.3±1.1	9.8±1.4*
per 100 analyzed cells	0.52±0.17	2.40±0.49*	—	—	3.5±0.8	8.8±1.3*

Note. *$p<0.05$ compared to the corresponding control.

1.05%; the corresponding values in experimental rats were 7.24±0.90%, 8.35±0.33%, and 15.60±1.06%; $p>0.05$ in all cases), and did not modulate other hormonal effects of estradiol: the dynamics of body weight (data not shown), uterus weight, thickness of the endometrium, and the content of progesterone receptors and peroxidase activity in the uterus. At the same time, appearance of "comets" (this phenomenon characterizes DNA damage) in cells isolated from the endometrium of rats subjected to combined effect of E2 and 5% ethanol was more expressed than in rats receiving E2 only. Combined administration of E2 and 15% ethanol weakened the hormonal and augmented the DNA-damaging effects of E2 (Table 2). The data of comet analysis in reference rats receiving no E2 is not presented in Table 2, because in these rats the uteri were small, and sufficient amount of tissue could not be obtained.

Moderate alcohol consumption is a risk factor for the development of some hormone-dependent tumors, such as breast cancer (mainly due to tumors expressing no estrogen and progesterone receptors, and therefore characterized by poor prognosis) [7]. While 15% alcohol is optimal for modeling chronic alcoholism, administration of 5% alcohol serves as a model of moderate alcohol consumption [1,4]. Out findings indicate that in contrast to tobacco smoking [2,3], chronic consumption of low (moderate) alcohol concentrations affects only the DNA-damaging (genotoxic) component of the uterotropic effect of estrogens. In addition, the estrogen toggle effect (*i.e.*, increasing the genotoxic and decreasing the hormonal estradiol effects [2]) occurs more frequently at 15% than at 5% ethanol, although it cannot be excluded that this phenomenon is related to not only phasic, but also tonic toxic effect of ethanol on functional properties of biological tissues [1,4].

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