Modification of Uterotropic Effect of Estrogens by Whole-Body γ-Irradiation

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The effect of γ-irradiation on the realization of the effects of estrogens was studied on rats treated with N-acetylcysteine, vitamins C and E, melatonin, and carnosine or subjected to forced swimming in a training mode. Irradiation (0.2 Gy) in combination with estrogens and without correction therapy induced genotoxic changes in the uterus, while irradiation in a higher dose (2 Gy) predominantly potentiated the hormonal effect of estrogens. Correction of the revealed abnormalities was achieved mainly with carnosine. The peculiarities of "estrogen toggle (re-targeting) effect" under the effect of γ-irradiation and its elimination differed from those induced by ethanol intake or tobacco smoking, which is important for understanding the mechanisms of hormone-induced carcinogenesis.

Key Words: *estrogens; effects; γ-irradiation; DNA damage; hormonal carcinogenesis*

Peculiarities of the effect of estrogens on the tissues sensitive to this hormone are important for understanding the mechanisms of estrogen-induced carcinogenesis and anticarcinogenesis. In the uterus, estrogens induce a proliferative response accompanied by expression of a large number of genes and proteins, *e.g.,* peroxidase and progesterone receptors (PR). Normally, the effect of estrogens is mediated by their interaction with specific receptors and is not accompanied by damage to DNA. However, there is evidence that estrogens can promote and even induce hormonal carcinogenesis. They can damage DNA both directly or indirectly (via production of free radical catecholestrogen metabolites). Correspondingly, two major types of hormonal carcinogenesis are distinguished: promotor and genotoxic [1,15]. Elucidation of factors and conditions potentiating the genotoxic component in the general effects of estrogens (specifically, on the uterus) is of crucial importance, because these factors can determine both the type of hormonal carcinogenesis and biological properties of induced hormone-dependent tumors.

We previously showed that drinking of 15% ethanol and long-term smoking modify the uterotropic action of estrogens: the hormonal component is weakened (uterus weight, PR induction), while damage to DNA is potentiated (DNA unwinding in alkaline medium and comet formation) [2,3]. This phenomenon was termed as estrogen toggle (re-targeting, ET) [1]. The search for new factors inducing ET and agents preventing or attenuating ET is now an actual problem.

Our aim was to elucidate whether the effect of estrogens can be modulated by whole-body γ-irradiation with two doses differing by an order of magnitude and what stimuli (pharmacological or non-pharmacological) could be used for preventive treatment.

MATERIALS AND METHODS

The study was carried out on 112 female rats (Rappolovo Breeding Center) aged 3 month at the start of the experiment. The animals were maintained under standard vivarium conditions.

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The rats were divided into 8 groups. The rats of 6 experimental groups were subjected to single whole-body γ-irradiation on an IGUR-1 setup (^{137}Cs, 0.2 Gy, 33 sec), while the group 7 rats were irradiated with a dose of 2 Gy (exposure 330 sec). Groups 1 and 7 rats received no correction therapy. Group 2 rats were treated with N-acetylcysteine (NAC) 5 times a week starting from day 1 of the experiment (100 mg/kg, through a tube); group 3 rats was injected with ascorbic acid (50 mg/kg, intraperitoneally) and α-tocopherol (40 mg/kg, intramuscularly); group 4 rats were subcutaneously injected with melatonin (1 mg/kg); group 5 rats were injected with carnosine (100 mg/kg intraperitoneally); group 6 rats swam 5 times a week in a training mode (*i.e.* the duration of the swimming sessions gradually increased from 5 to 60 min). Nonirradiated rats served as the control.

The animals were subjected to bilateral ovariectomy 2.5 weeks before the end of the experiments and were treated with estradiol (2 μg/day, intramuscularly) for 11 days until the end of the experiment.

The rats were weighed before the start of experiment, before irradiation, before ovariectomy, and before decapitation. The blood was rapidly collected, serum concentration of estradiol (E_2) was measured with a radioimmunoassay kit (Beloris, Minsk) and cholesterol content with enzymocolorimetric kits (Randox). The uteri were isolated and weighed. When necessary, they were homogenized in cold physiological saline or Tris buffer (0.05 M, pH 7.4). The samples were divided into aliquots to assay PR content [11] and peroxidase activity [10]; nuclear DNA was measured by flow cytometry (fractions of S- and G_2/M-phase cells and proliferation index [5]); DNA abnormalities were assayed by gel-electrophoresis (comet assay) adapted for solid tissues [14]. To measure the thickness of intrauterine epithelium, the samples were fixed in 10% formalin, embedded in paraffin, and analyzed by histomorphometrical methods [12]. The effect of ethanol on estrogen metabolism was assessed in some rats by hepatic estradiol-2-hydroxylase (EDH) activity [7], because uterine EDH activity is very low. Protein content was determined by the method of Lowry. The data were processed statistically using Student's *t* test.

RESULTS

Single whole-body γ-irradiation in both doses in combination with estradiol had no effect on the body weight and blood cholesterol (data not shown). Hence, γ-irradiation under chosen conditions did not modify the so-called non-reproductive action of estrogens. In addition, irradiation had practically no effect on EDH activity (106.5±18.4, 129.6±23.3, and 133.4±25.7 nM/mg protein per 30 min in the control and in rats irradiated with 0.2 and 2 Gy doses, respectively; $p>0.2$ in both cases). Hence, γ-irradiation had no effect on estrogen metabolism (specifically, on 2-hydroxylation). Finally, whole-body γ-irradiation did not modify estradiol-induced thickening of the uterine epithelium and peroxidase activity in the uterus (data not shown; it cannot be excluded that the dose of estradiol used in these experiments was above optimal for peroxidase activity [12]).

Irradiation with a dose of 0.2 Gy produced no significant changes in blood estradiol concentration, uterus weight, and PR content. However, PR content trended to decrease ($p=0.106$); this tendency became significant after irradiation in a dose of 2 Gy ($p=0.02$, Table 1). In rats irradiated with a dose of 0.2 Gy, the hormonal effects of estradiol were significantly potentiated, when the rats were treated with combination of vitamins C and E (uterus weight), melatonin (uterus weight and PR content), and carnosine (PR content). At the same time, plasma E_2 content increased in rats receiving vitamins C and E and carnosine increased, and slightly decreased in animals treated with melatonin (Table 1).

According to DNA flow cytometry data, single whole-body irradiation in a dose of 0.2 Gy did not induce aneuploidy (a sign of genome imbalance) in intrauterine lining samples. The distribution of endometrial cells by phases of the cell cycle and proliferation index in irradiated rats little differed from the normal, but the mean length of comet tail significantly increased in comparison with the control (Table 1), which attested to aggravation of DNA damage. It is noteworthy that irradiation in a 10-fold dose produced no such changes in DNA. Carnosine and forced swimming in a training regimen weakened this effect of γ-irradiation in a dose of 0.2 Gy. Carnosine was more effective and did not decrease proliferation index in the uterine epithelium (Table 1). The effects of these two agents on PR content in the uterus were also different (Table 1).

The ET phenomenon can be complete (potentiation of genotoxic and inhibition of hormonal component of estrogen action) or partial (increase of damage to DNA), so ET inductors can be classified correspondingly. According to our data, inductors of complete ET are tobacco smoking and drinking of 15% ethanol (a model of chronic alcoholism), in moderate doses alcohol acts as an inductor of partial ET [2,3]. γ-Irradiation in a dose of 0.2 Gy induced partial ET, while irradiation in a dose of 2 Gy did not induce ET (in this case, no increase in the degree of estrogen-induced damage to DNA occurred in the uterus, Table 1). This paradox can be explained by the fact that irradiation in high doses is accompanied by more intensive postradiation reparative processes compared to those induced by small doses [6,9]. It should be noted

TABLE 1. Effects of γ-Irradiation on Uterotropic Action of Estradiol, Proliferation Indices, and Degree of DNA Damage in Ovariectomized Rats (*M*±*m*)

Index	Control	Irradiation						
		0.2 Gy						2 Gy
		without correction	+N-AC	+vitamins C and E	+melatonin	+carnosine	+swimming	
Serum E_2 content, pmol/liter	205.4±31.0 (9)	150.7±40.3 (10)	171.5±43.2 (8)	305.0±32.7*+ (9)	113.0±27.1* (10)	279.3±31.0+ (8)	131.6±34.8 (11)	165.9±24.5 (12)
Uterus weight, mg	359.3±26.0 (14)	341.4±13.3 (13)	367.3±18.4 (11)	425.0±18.7+ (12)	404.6±17.2+ (14)	384.0±24.2 (9)	363.4±19.4 (11)	357.3±14.0 (12)
PR content°, fmol/mg protein	194.8±32.5 (4)	132.8±3.0 (4)	139.5±21.3 (4)	125.5±9.8 (4)	148.3±4.6+ (4)	202.0±12.6+ (4)	142.5±12.9 (4)	90.7±6.8*+ (4)
Flow cytometry								
Cell fraction, %								
in S-phase	16.3±3.7 (14)	17.0±1.5 (13)	13.3±1.0 (10)	16.7±3.1 (12)	12.2±1.1+ (14)	12.6±1.4+ (8)	13.5±0.6+ (13)	16.7±1.4 (11)
in G_2/M-phase	5.6±1.2 (14)	6.2±0.6 (13)	4.3±0.6+ (10)	7.5±1.3 (12)	6.8±0.7 (14)	6.1±1.0 (8)	3.8±0.3+ (13)	4.6±0.5 (11)
Proliferation index, %	21.9±4.8 (14)	23.2±1.8 (13)	17.6±1.4+ (10)	24.2±3.3 (12)	19.0±1.8 (14)	18.7±1.7 (8)	17.3±0.7+ (13)	21.3±1.6 (11)
Comets								
Mean number, %	58.8±13.1 (7)	87.1±6.9 (7)	93.0±2.6* (7)	80.1±10.5 (7)	66.6±12.6 (7)	47.4±12.6+ (8)	63.5±8.6 (8)	74.2±10.0 (7)
Comet tail Mean length, μ								
per one cell with a comet	47.1±4.9 (7)	91.0±17.5* (7)	62.0±14.4 (7)	48.5±10.4 (7)	49.3±10.5 (7)	29.2±3.5*+ (8)	43.6±7.7+ (8)	29.7±4.2*+ (7)
per 100 cells	27.5±7.1 (7)	84.0±19.3* (7)	56.8±14.1 (7)	45.4±10.7 (7)	37.8±9.4 (7)	15.9±5.0+ (8)	31.6±7.9+ (8)	25.2±4.7+ (7)

Note. Number of measurements is given in parentheses. $p<0.05$ *compared to the control, +compared to irradiated rats (0.2 Gy) without correction. °Material for assay was taken from 2-3 rats.

that our experimental paradigm cannot assess the damage to DNA produced by irradiation alone compared to that induced by irradiation+estradiol, because the uteri in ovariectomized rats is too small to collect material for comet assay.

In contrast to elimination of the ethanol-induced ET (combination of NAC with vitamins C and E was the most efficient [3]), correction of estrogen-modulating effect of irradiation was achieved in our experiments predominantly by swimming or carnosine treatment. The latter was a unique agent that prevented inhibition of hormonal action of estradiol (assessed by PR induction) and impeded potentiation of its DNA-damaging effect (Table 1). Taken together, these data attest to certain differences in the mechanisms of ET induced by various modifying factors and to a variety of biochemical reactions preventing the development of this phenomenon. Specifically, carnosine (considered for a long period as the agent with predominant antioxidant action) is also a natural antiglycosylation substance [4,8]. Potentiation of glycosylation processes, which is characteristic of aging and the response to irradiation, is probably involved into transmission of estrogen signal [13].

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