Modification of the Uterotropic Effect Produced by Estrogens in Aging Rats

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We studied the influence of various methods for correction of age-related changes (administration of N-acetyl-L-cysteine, vitamins C and E, melatonin, and carnosine and swimming training) on the realization of estrogens effects in ovariectomized rats. The proliferation index in the endometrium decreased in 12-14-month-old control animals. The weight of the uterus, percentage of "comets", and average length of their tail in estradiol-treated rats far surpassed the corresponding parameters in control animals. Administration of melatonin and N-acetyl-L-cysteine and swimming training corrected these genotoxic abnormalities. Our results indicate that aging induces incomplete variant of the phenomenon for switching of estrogen's effects (increase in the severity of genotoxic damage without facilitation of the hormonal effect). These methods for correction of age-related changes have not only common, but also distinguishing characteristics compared to correction of changed induced by drinking of ethanol in various concentrations, whole body γ-irradiation, and exposure to tobacco smoke.

Key Words: *estrogens; effects; age; DNA damage; hormonal carcinogenesis*

The development of many noninfectious human diseases (*e.g.*, malignant neoplasms, cardiovascular disorders, disturbances in the central nervous system, and osteoporosis) is directly or indirectly related to an excess and deficiency of estrogens in the body. The incidence of these diseases markedly increases during aging [7, 11]. The rate of various pathological processes related to a deficiency or strong stimulation with estrogens increases in the same age period. This problem raises many questions. We hypothesized that the cause of estrogen-dependent nosological dichotomy is bifunctionality of these hormones. For example, estrogens can produce the hormonal and genotoxic effect. This contributes to the phenomenon for switching of estrogen's effects (PSE). Changes in the mechanisms of hormonal carcinogenesis are followed by a variety of serious clinical complications [1,2].

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Our previous experiments demonstrated 2 variants of PSE. The complete variant suggests potentiation of the genotoxic effect and facilitation of the hormonal effect produced by estrogens. The incomplete variant reflects the formation of DNA damage without modification of the hormonal effect. We showed that tobacco smoke, ethanol, and whole-body γ-irradiation induce various variants of PSE. Pharmacological and other factors facilitating this phenomenon were studied [3,4]. The present work completes this division of research. We evaluated the role of aging in the realization of PSE (experiments with rat endometrium) and studied methods for the prevention of changes in adult and old animals.

MATERIALS AND METHODS

Experiments were performed on 77 female rats obtained from the Rappolovo nursery. Experimental rats aging 10-12 months received N-acetyl-L-cysteine (100 mg/kg through a gastric tube), ascorbic acid (50 mg/kg

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intraperitoneally) and α-tocopherol (40 mg/kg intramuscularly), melatonin (1 mg/kg subcutaneously), or carnosine (100 mg/kg intraperitoneally) 5 times a week for 2.5 months. Other rats were subjected to swimming training 5 times a week. The duration of this exercise gradually increased from 5 to 60 min. Young and old animals of control groups (1-1.5 and 10-12 months, respectively) received no corrective treatment. Bilateral ovariectomy was performed 2.5 weeks before the end of experiments. Estradiol in a dose of 2 μg was injected intramuscularly over 11 days before decapitation. The animals were weighted before the start of experiments, before ovariectomy, and in the last day. After decapitation the blood was rapidly collected. Plasma estradiol concentration was measured by radioimmunological methods using Beloris kits. Cholesterol content was estimated by enzyme colorimetry with Randox kits. The uteri were weighted and homogenized in cold 0.9% NaCl or 0.05 M Tris buffer (pH 7.4). The number of receptors for progesterone was estimated in samples from 2-3 rats [12]. Peroxidase activity was measured [10]. The content of nuclear DNA was determined by flow cytometry (ratio of cells in S and G_2/M phases and proliferation index) [15]. The degree of DNA damage was estimated by gel electrophoresis ("comet" analysis) with modifications for cells from solid tissues [14]. Samples were fixed in 10% formalin, embedded into paraffin, and subjected to histomorphometry to estimate the thickness of the intrauterine epithelial layer. We evaluated the possible effect of aging on estrogen metabolism. Since activity of estradiol-2-hydroxylase [5] in uterine tissue is extremely low, it was measured in the liver from some rats. Protein content in tissue homogenates was determined by the method of Lowry.

We calculated the average values and standard errors. The results were analyzed by Student's *t* test (Statistica for Windows 4.0 and SigmaPlot softwares).

RESULTS

We studied differences between young and old control rats and evaluated factors that modify the uterotropic effect of estrogens. In old control rats the weight of the uterus and length of "comet" tail were greater than in young animals (Tables 1 and 2). These data indicate that in old rats genotoxic damage was more pronounced than in young animals. Young and old animals did not differ in the number of progesterone receptors, blood estradiol concentration, height of epithelial cells in the endometrium (Table 1), blood cholesterol level, and activities of liver peroxidase and estradiol-2-hydroxylase (data not shown). The ratio of cells in S and G_2/M phases of the cell cycle and proliferation index tended to decrease in the endometrium from old rats. In these animals the average number of "comets" markedly increased (Table 2).

We studied 5 potential modifiers of the effect produced by estrogens. None of the factors affected the weight of the uterus (Table 1), proliferation index in the endometrium (Table 2), cholesterol concentration, and activities of peroxidase and estradiol-2-hydroxylase in old rats (data not shown). Carnosine markedly increased the thickness of the intrauterine epithelium and promoted an increase in the degree of estrogenemia (Table 1). Melatonin stimulated, while N-acetyl-L-cysteine suppressed the induction of progesterone receptors in the uterus. Moreover, N-acetyl-L-cysteine tended to decrease blood estradiol concentration and number of "comets" in the endomet-

TABLE 1. Blood Estradiol Content (E_2) and Its Uterotropic Effect under Various Approaches to the Correction of Age-Related Changes in Rats (*M*±*m*)

Parameter	Control		N-acetyl-L-cysteine	Vitamins C and E	Melatonin	Carnosine	Swimming
	young	old					
E_2, pmol/liter plasma	70.0±20.2 (10)	93.0±19.9 (10)	63.00±35.8 (5)	79.0±25.6 (6)	29.0±5.3$^+$ (5)	124.0±27.7 (5)	28.0±2.2$^+$ (5)
Weight of the uterus, mg	333.0±14.8 (15)	405.0±29.6* (11)	411.0±26.6 (9)	380.0±14.8 (10)	377.0±25.0 (10)	350.0±16.9 (11)	377.0±21.4 (11)
Thickness of the intrauterine epithelial layer, μ	24.20±0.59 (15)	23.90±1.96 (11)	24.30±0.99 (9)	28.20±1.56 (10)	26.80±1.55 (10)	29.50±1.47$^+$ (11)	23.40±1.13 (11)
Progesterone content, fmol/mg protein	86.0±4.4 (4)	92.0±3.0 (4)	64.2±3.1$^+$ (4)	120.0±10.8 (4)	212.0±19.0$^+$ (4)	71.5±4.2 (4)	112.0±7.1 (4)

Note. Here and in Table 2: $p<0.05$: *compared to young control rats; $^+$compared to old control rats. The number of measurements is shown in brackets.

TABLE 2. Flow Cytometry of DNA and Degree of DNA Damage Estimated by the Method of "Comets" in the Uterus from Rats of Different Age (*M*±*m*)

Parameter	Control		N-acetyl-L-cysteine	Vitamins C and E	Melatonin	Carnosine	Swimming
	young	old					
Ratio of cells, %							
S phase	15.8±3.9	11.2±1.2	12.5±1.2	12.4±1.3	13.7±1.2	13.8±1.2	11.1±1.5
	(11)	(10)	(6)	(10)	(10)	(11)	(11)
G_2/M phase	9.1±1.1	7.0±0.6	9.9±0.8+	8.5±0.8	8.5±0.8	6.8±0.6	7.2±0.5
	(11)	(10)	(6)	(10)	(10)	(11)	(11)
Proliferation index, %	24.90±4.21	18.20±1.64	22.40±1.93	20.90±1.31	22.20±1.77	20.60±1.64	18.30±1.57
	(11)	(10)	(6)	(10)	(10)	(11)	(11)
Average number of "comets", %	7.7±5.5	21.0±6.2	6.3±4.0+	22.5±10.0	7.6±3.4	21.7±5.6	6.2±2.1+
	(15)	(11)	(9)	(10)	(10)	(10)	(11)
Average length of tail, μ							
per 1 cell	6.32±2.04	14.80±1.08*	11.6±2.0	12.44±2.04	9.12±1.80+	12.88±3.24	8.48±1.84+
with "comet"	(15)	(11)	(9)	(10)	(10)	(10)	(11)
per 100	4.80±0.88	7.24±1.52	5.80±1.08	8.00±0.92	5.52±0.76	7.88±1.32	4.44±0.84
analyzed cells	(15)	(11)	(9)	(10)	(10)	(10)	(11)

rium. Melatonin and swimming training significantly decreased estradiol concentration and length of the "comet" tail (Tables 1 and 2).

These results show that administration of estradiol to young and old rats was not accompanied by significant changes in the hormonal effect of estrogens, which is consistent with published data [6,13]. The weight of the uterus is not a reliable criterion in this respect. In 12-14-month-old ovariectomized rats not receiving estradiol the weight of the uterus surpasses that in 3-4-month-old animals to a similar extent. It should be emphasized that these findings concern adult, but not old animals that were not characterized by blockade of the cycle. We revealed 2 specific characteristics of the endometrium from old rats receiving estradiol. In these animals proliferative activity tended to decrease, while the number/length of the "comet" tail increased. These results agree with published data that intensification of cell division is not necessary for the formation of DNA damage [1,8]. The question arises: whether genotoxic damage results from age-related changes or it is associated with the effect of estradiol [3,4]? The scheme of our experiments does not allow evaluating the degree of DNA damage in animals not receiving estradiol. In ovariectomized rats the size of the uterus is small, and the amount of samples is not sufficient for the analysis of "comets". To solve this problem it is necessary to use "pure" antiestrogens not possessing activity of estrogenic agonists [9].

DNA damage in uterine tissues associated with aging or treatment with estradiol was not observed in rats subjected to physical exercises (swimming) or receiving melatonin and N-acetyl-L-cysteine. Previous experiments showed that melatonin and N-acetyl-L-cysteine produce similar effects on animals drinking 5% ethanol [3]. Moreover, swimming training prevented damage after treatment with estradiol and total γ-irradiation [4]. In our experiments these factors produced an antitoxic effect and decreased blood estradiol level (Table 1). It should be emphasized that there are two approaches to the prevention or suppression of PSE [2-4]. The first approach suggests the use of preparations possessing antigenotoxic activity and partially normalizing the decreased hormonal effect of estrogens. The second approach suggests combination treatment with preparations that have only one of these properties.

Our results indicate that the induction of PSE under the influence of modifying factors is realized via different mechanisms. The prevention of this phenomenon involves various biochemical reactions [2,4].

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