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Radioprotective and Apoptotic Properties of a Combination of α-Tocopherol Acetate and Ascorbic Acid

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We studied radioprotective and apoptotic properties of a combination of α-tocopherol acetate and ascorbic acid. α-Tocopherol acetate (10 mg/kg body weight) or ascorbic acid (20 mg/kg) or combination of these agents in the same doses was orally administered to male rats at various terms before and after single whole-body exposure to γ-irradiation in the doses of 2 and 8 Gy. Irradiation increased the frequency of chromosome aberrations in bone marrow cells and plasma level of low-molecular-weight DNA. Vitamin combination administered before or after irradiation significantly reduced the frequency of chromosome aberrations by 2-2.5 times. Administration of this combination 10 min before irradiation 1.5-fold increased the content of low-molecular-weight DNA in blood plasma in comparison with the control animals exposed to radiation. The combination of α-tocopherol acetate and ascorbic acid produced radioprotective effects and enhanced apoptosis in irradiated cells.

Key Words: *α-tocopherol acetate; ascorbic acid; radioprotective substances; low-molecular-weight DNA; apoptosis*

Genotoxic exposures, including ionizing radiation, stimulate apoptosis of damaged cells and DNA release into the blood [2]. Dose-dependent increase in DNA concentration in the blood plasma was observed in rodents exposed to whole-body irradiation in doses of 2-100 Gy. Estimation of DNA in blood plasma is suggested to use to radiation-monitoring purposes [2,10]. Total content of extracellular DNA and its various fractions in the blood also characterizes apoptosis as a response of a tumor to radiotherapy [1]. It is known that α-tocopherol acetate (TA) and ascorbic acid (AA) have radioprotective properties [8,9].

In our experiments, the radioprotective and apoptotic properties of a combination of TA and AA were evaluated by the frequency of chromosome aberrations in bone marrow cells and level of low-molecular-weight DNA (lmwDNA) in blood plasma of rats exposed to ionizing radiation.

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MATERIALS AND METHODS

Experiments were performed on 12-week-old male Wistar rats (n=350) weighing 210±35 g obtained from the Rappolovo breeding center of laboratory animals (Leningrad Region). The animals were kept under standard conditions of a vivarium. Uniform single whole-body γ-irradiation with cesium-137 in doses from 2 to 100 Gy was conducted using IGUR-1 instrument (Institute of Biophysics, Moscow; dose rate of 1.9 Gy/min). The combination of TA (10% oil solution, Kiev Vitamin Plant) in a dose of 10 mg/kg body weight and AA (powder, St. Petersburg Pharmaceutical Factory) in a dose of 20 mg/kg body weight was administered once orally through a gastric tube 10 min or 1 h before irradiation and 10 min, or 1 h, or 3 h after irradiation. In special groups, AT alone or AA alone in the same doses were orally administered. Controls received drinking water (once, orally).

Chromosome aberrations were estimated in 24 h after irradiation. Colchicine (0.2 ml 0.025%; Sigma-Aldrich) was intraperitoneally injected to animals 2 h before sacrifice by cervical dislocation under ether narcosis. The tibiae were washed with medium 199

(I. I. Mechnikov Biomed) at 37ºC. Cell suspension was centrifuged at 150*g* for 6 min, resuspended in 0.56% KCl solution, and fixed with glacial acetic acid–methanol (1:3) mixture. The samples were stained after Romanowsky-Giemsa and analyzed under a light microscope. At least 100 metaphase plates were analyzed for each rat.

For isolation of extracellular DNA, the rats were decapitated 5 h after irradiation and blood samples were collected. The blood from 3 rats was pooled. Blood plasma was separated by centrifugation at 900*g* for 10 min at 4ºC and twice re-centrifuged (10 min at 2200*g*) for removal of cell detritus. Extracellular nucleic acids were isolated from the samples by phenol/chloroform extraction [3].

The data were processed using Student's test for unrelated samples (Statistica 17.0).

RESULTS

The combination of TA and AA administered both before and after irradiation protected the bone marrow from cytogenetic damages (Table 1).

Whole-body single irradiation in a dose of 2 Gy significantly increased the fraction of cells with chromosome aberrations by 11.1 times in comparison with non-irradiated control. The spectrum of chromosome aberrations varied. In irradiated rats, the frequency of single and paired fragments and dicentric chromosomes increased. Dicentric chromosomes appeared only after irradiation and were not found in animals not exposed to radiation. The count of dicentric chromosomes is the key component of cytogenetic biodosimetry in humans exposed to ionizing radiation [5]. TA or AA administered separately before and after irradiation did not affect the frequency of chromosome aberrations in comparison with irradiated control, which indicates the absence of radioprotective effects of these substances. The combination of TA and AA administered 1 h or 10 min before radiation, and 10 min or 3 h after radiation significantly reduced the total frequency of chromosome aberrations by 2-2.5 times in comparison with irradiated control. The frequency of individual types of chromosome aberrations also tended to decrease under these conditions.

Our previous studies showed that single irradiation induces an increase in the level of extracellular DNA in rat blood with the maximum within 5 h after exposure and the following decrease to the initial level [4]. Irradiation in doses from 2 to 100 Gy significantly increased plasma level of lmwDNA starting from the dose of 4 Gy in comparison with non-irradiated control (the effect was estimated in 5 h after the exposure) in a dose-dependent manner: by 4.6 times after irradiation in a dose of 4 Gy, and by 61.5 times after irradiation in a dose of 100 Gy (Table 2).

For estimation of the effects of TA–AA combination on lmwDNA level in the blood of irradiated rats, we chose the dose of 8 Gy, because it induces bone marrow type of radiation sickness [3]. TA–AA combination administered 1 h prior to irradiation did not significantly increase in lmwDNA level in blood plasma of rats in comparison with non-irradiated control. However, administration of this combination 10

TABLE 1. Effects of TA, AA, and Their Combination of the Frequency of Chromosome Aberrations in Bone Marrow Cells of Rats Exposed to Radiation in a Dose of 2 Gy (*M±m*)

Group		Frequency of chromosome aberrations, %			
		total	single fragments	paired fragments	dicentrics
Non-irradiated control (*n*=6)		0.9±0.3*	0.3±0.2*	0.6±0.3*	0*
Irradiated control (*n*=20)		10.0±1.4	3.2±1.3	4.7±1.4	2.1±1.2
TA	10 min before irradiation (*n*=6)	11.0±1.4	3.0±1.0	5.0±1.4	2.0±0.9
	10 min after irradiation (*n*=6)	10.0±1.3	2.8±1.1	4.0±1.3	3.2±1.2
AA	10 min before irradiation (*n*=6)	9.0±1.3	3.5±1.2	4.0±1.3	2.5±1.1
	10 min after irradiation (*n*=6)	10.0±1.3	2.0±0.8	5.0±1.3	5.0±1.1
TA+AA	1 h before irradiation (*n*=6)	4.0±1.2*	1.1±0.6	2.0±1.3	0.9±0.3
	10 min before irradiation (*n*=6)	5.0±1.2*	1.7±0.8	2.1±0.9	1.2±0.7
	10 min after irradiation (*n*=6)	5.0±1.2*	1.7±0.9	2.3±1.2	1.0±0.5
	3 h after irradiation (*n*=6)	5.0±1.0*	1.3±0.7	2.9±1.0	0.8±0.4

Note. *n*: number of rats. *p<0.05-0.001 in comparison with irradiated control.

TABLE 2. Effects of Ionizing Radiation on lmwDNA Level (ng/ml) in Blood Plasma of Rats in 5 h after Irradiation (n=7, $M\pm m$)

Non-irradiated control (n=18)	Irradiation					
	2 Gy	4 Gy	8 Gy	20 Gy	50 Gy	100 Gy
5.5±1.5	11.9±5.7	25.5±7.5*	53.2±15.5*	138.6±28.6*	271.6±42.4*	338.5±49.9*

Note. n: number of samples, each contained the blood from 3 rats. *p<0.02-0.001 in comparison with non-irradiated control.

TABLE 3. Effects of Combination of TA and AA on the Level of lmwDNA in Blood Plasma of Rats 5 h after Exposure to Radiation in a Dose of 8 Gy ($M\pm m$)

Group		lmwDNA level, ng/ml
Irradiated control (n=21)		53.2±6.6
TA+AA	1 h before irradiation (n=7)	49.8±9.7
	10 min before irradiation (n=7)	78.5±11.4*

Note. n: number of samples, each contained the blood from 3 rats. *p<0.005 in comparison with irradiated control.

min before irradiation significantly increased lmwDNA level (by 1.5 times; Table 3).

Administration of TA–AA combination in the same doses to rats not exposed to radiation (n=7) increased (p<0.01) the level of lmwDNA in blood plasma to 10.2±2.1 ng/ml in 5 h after administration (*vs.* 5.5±1.5 ng/ml in non-irradiated rats receiving no vitamins).

For evaluation of the radioprotective and apoptotic properties of the TA and AA combination in rats, we used doses corresponding to the preventive doses of these vitamins in humans. The combination of TA and AA in these doses was proposed as the radioprotective agent for irradiation in low doses [4]. It was found that combination of TA and AA in preventive doses administered at various terms before and after irradiation exhibits radioprotective properties and reduces the frequency of chromosome aberrations in the bone marrow of irradiated mice. Taking into account the absence of radioprotective properties in TA and AA administered separately, we can conclude that these vitamins in low preventive doses reveal synergic interactions at the terms from 1 h before irradiation to 3 h after irradiation.

Vitamins E and C can have both pro-apoptotic and anti-apoptotic effects depending on the medicinal form and doses [8]. It is shown that a combination of TA and AA in preventive doses induces apoptosis of irradiated cells when administered 10 min before radiation and does not induce cell death when administered 1 h before irradiation. It can be hypothesized that the combination of TA and AA enhances natural removal of cells with damaged chromosomes, as they are more sensitive to apoptosis [6]. In intact animals, the combination of TA and AA increases removal of cells with spontaneous chromosome aberrations, while in animals exposed to radiation, this combination enhances removal of cells with radiation-induced chromosome aberrations. However, this effect is found only after administration the substances in 10 min before the damaging exposure. These data support current hypothesis on the crucial effects of the first 10 min in the cell response to environmental factors inducing programmed death of this cell [7]. We believe that the combination of TA and AA in preventive doses enhances selection processes in tissues for elimination of severely damaged cells and improves defense from spontaneous and ionized radiation-induced mutations.

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