Hemostatic System in Wistar Rats in Different Types of Oxidative Stress

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We studied parameters of hemostatic homeostasis in isolated and combined exposure to low-intensity γ-irradiation in a low dose, hyperbaric oxygenation, and antiorthostatic hypokinesia. Complex effects of the above stress factors are accompanied by a pronounced hypercoagulable shift with signs of thrombinemia against the background of depressed fibrinolysis, which indicates the risk of intravascular blood coagulation.

Key Words: *stress; antiorthostatic hypokinesia; hyperbaric oxygenation; hypercoagulable shift; disseminated intravascular coagulation*

Oxidative stress (OS), *i.e.* accumulation of peroxidation products leading to the exhaustion of compensatory reserve of the antioxidant system and therefore to disruption of homeostatic regulation [2,4,5,8], underlies the pathogenesis of many diseases and extreme conditions. Balanced function of the hemostatic system playing an important role in the development of stress reactions and adaptation processes can be impaired under conditions of abnormal oxidative status [3,7].

OS inductors have different nature, therefore, the search for their application and evaluation of possible interactions modifying the final effect is an important problem.

Here we present an experimental study of isolated and combined effects of low dose ionizing radiation, hyperoxia, and antiorthostatic hypokinesia on hemostasis. Such a combination of stress factors may occur during medical procedures and radiotherapy of cancer.

MATERIALS AND METHODS

We used Wistar male rats weighing 220-250 g. All manipulations with animals were carried out in accordance with the requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the protection of experimental animals (86/609/EEC; 1986). Experimental rats were subjected to prolonged γ-irradiation in a dose of 4.4 μGy/min for 4 days (4.5 h per day) using an irradiation facility with panoramic Cs^{137} source; the total dose was 4.8 mGy. Antiorthostatic hypokinesia (AH) lasting from 3 h to 3 days was modeled by negative tilt at -45° using a mobile carriage in specially designed cages with access to water and food. Hyperbaric oxygenation was performed once for 3 h by creating overpressure up to 1.15 technical atmospheres (atm). Clinically healthy animals kept under standard vivarium conditions served as controls. At the end of exposures, the blood was taken from rat abdominal aorta under sodium thiopental anesthesia (35 mg/kg) and stabilized with 3.8% sodium citrate (9:1). The rats were then sacrificed by narcotic overdose. Activated partial thromboplastin time (APTT) tests were performed with Solar SGL-2110 turbidimetric hemocoagulometer CGL according to the instructions of the manufacturer of reagent kits (NGO RENAM). Fibrinogen concentration, the content of soluble fibrin monomer complexes (SFMC), blood fibrinolytic activity (euglobulin method), and ADP-induced platelet aggregation were determined according to [1]. The obtained data were analyzed statistically using criteria of variation statistics [6] and χ^2 test.

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RESULTS

AH was accompanied by changes in the blood coagulation and fibrinolysis (Table 1). We detected a significant shortening of APTT and thrombin time, increased concentration of fibrinogen as well as appearance of SFMC in rats after 3-h AH, which indicated increased coagulation capacity of plasma associated with reduced fibrinolytic activity. Activation of blood coagulation after 1 and 3 days of AH did not significantly differ from that after short-term exposures, except for the more pronounced SFMC reaction and decrease in fibrinolysis. Platelet aggregation did not differ from the control at all time points.

Hyperbaric oxygenation for 3 h produced less pronounced effect on the hemostatic system (Table 1). No abnormalities in the extrinsic and intrinsic coagulation pathways were detected. At the same time, shortened thrombin time, increased fibrinogen concentration and elevated SFMC content were recorded against the background of reduced fibrinolytic activity of plasma euglobulin fraction.

Combined exposure to hyperoxia and hypokinesia (3 h) led to appresiable activation of both coagulation and platelet hemostasis. Shortened thrombin time and reduced blood fibrinogen concentration in experimental rats were associated with high level of SFMC, the earliest marker of latent thrombinemia.

Prothrombin time changed towards hypocoagulation. Blood fibrinolytic activity was reduced. Platelet aggregation induced by ADP in a concentration of 0.47 mg/ml increased by 1.2 times. These findings attest to aggravating effect of hyperoxia combined with AH on platelet hemostasis. Signs of hemostatic system dysfunction were also observed on day 3 after combined exposure to stress factors.

Plasma homeostasis in the blood of γ-irradiated animals (Table 2) responded to the impact by inhibition of the intrinsic coagulation pathway. Shortening of APTT and thrombin time, decrease in fibrinogen concentration, and appearance of SFMC were observed. Increased blood fibrinolytic activity against the background of coagulation homeostasis activation was recorded. ADP-induced platelet aggregation was not altered.

In irradiated rats subjected to 3-day AH, activation of the extrinsic pathway of blood coagulation was observed, as was seen from reduced prothrombin time. Activation of the final stage of blood coagulation manifested in accumulation of SFMC and reduced fibrinogen concentration. These hypercoagulation changes were accompanied by fibrinolysis depression. Platelet hemostasis was not impaired.

After exposure to ionizing radiation and more prolonged AH (3 days), hypercoagulation was accom-

TABLE 1. Parameters of the Hemostatic System in Wistar Rats after AH and Combined Impact of 3-h Hyperoxia and AH (*M*±*m*)

Parameter	Control	AH			3-h hyperoxia+AH			
		3 h	1 day	3 days	hyperoxia	3-h AH	1-day AH	3-day AH
Prothrombin time, sec	13.9±0.2	15.0±0.5	13.2±0.5	13.6±0.7	14.0±0.6	17.7±1.5*	14.0±0.3	14.3±0.3
APTT, sec	31.3±0.5	27.0±0.7*	31.7±0.7	34.4±1.3	31.6±1.0	33.2±1.4	29.5±0.7	34.7±1.9
Thrombin time, sec	16.9±0.2	14.2±0.2*	14.7±0.4*	13.7±0.4*	15.8±0.2*	16.1±0.2*	16.1±0.5	16.1±0.6
Fibrinogen concentration, g/liter	1.52±0.02	1.69±0.06*	2.09±0.1*	1.96±0.03*	1.70±0.05*	1.36±0.05*	1.70±0.04*	1.55±0.06
Fibrinolytic activity, min	303±6.1	338±10.8*	356±6.0*	365±10.6*	330±7.1*	397±7.1*	380±2.9*	366±7.3*
Positive SFMC test	3/21 (1)	8/9** (II)	7/12** (III)	4/5 ** (III)	3/7** (II); 4/7** (III)	6/6** (III)	7/7** (II)	7/7** (II)
ADP-induced platelet aggregation, %	34.4±0.5	35.2±0.5	35.0±0.8	35.4±1.5	33.7±1.2	41.9±1.1*	38.9±0.6*	36.6±0.6*

Note. Here and in Tables 2, 3: *$p<0.05$, **$p<0.01$ compared with intact controls. In parentheses: SFMC concentration (score). Groups comprise from 7 to 20 animals.

TABLE 2. Parameters of the Hemostatic System in Wistar Rats after Combination of γ-Irradiation and AH (*M*±*m*)

Parameter	Control	Type of exposure			
		γ-irradiation	γ-irradiation+ 3-h AH	γ-irradiation+ 1-day AH	γ-irradiation+ 3-day AH
Prothrombin time, sec	16.8±0.1	20.1±0.6*	13.8±0.4*	12.1±0.5*	13.9±0.5*
APTT, sec	31.9±0.6	29.0±0.6*	32.4±0.9	34.3±0.5*	26.7±0.6*
Thrombin time, sec	14.9±0.1	13.8±0.4*	14.6±0.3	12.9±0.3*	13.4±0.2*
Fibrinogen concentration, g/liter	1.38±0.01	1.26±0.06*	1.24±0.04*	1.51±0.04*	1.65±0.03*
Fibrinolytic activity, min	207.0±7.0	150.0±8.3*	244.0±12.1*	193.0±6.1	210.0±16.0
ADP-induced platelet aggregation, %	36.9±0.5	37.9±1.1	37.4±0.8	41.6±0.9*	44.0±1.8*
Positive SFMC test	1/7(I)	7/7 (II)	7/7**(II)	7/7** (II)	6/6** (II)

TABLE 3. Parameters of the Hemostatic System in Wistar Rats after Combination of γ-Irradiation, AH, and 3 Hours of Hyperoxia (*M*±*m*)

Parameter	Control	Type of exposure			
		γ-irradiation+ hyperoxia	γ-irradiation+ hyperoxia+ 3-h AH	γ-irradiation+ hyperoxia+ 1-day AH	γ-irradiation+ hyperoxia+ 3-day AH
Prothrombin time, sec	16.8±0.1	13.6±0.3*	19.0±0.2*	14.2±0.3*	15.6±0.4*
APTT, sec	31.9±0.6	29.1±1.3	33.9±1.0	32.2±0.5	37.7±1.1*
Thrombin time, sec	14.9±0.1*	13.4±0.3*	14.7±0.3	12.6±0.2*	12.1±0.5*
Fibrinogen concentration, g/liter	1.38±0.01	1.4±0.04	1.3±0.04	1.48±0.05*	1.88±0.05*
Fibrinolytic activity, min	207.0±7.0	226.0±1.5*	204.0±5.3	231.0±4.5*	234.0±5.3*
ADP-induced platelet aggregation, %	36.9±0.5	43.7±2.4*	46.7±2.6*	41.1±1.5*	43.7±1.2*
Positive SFMC test	1/7 (1)	7/7** (II)	5/7** (I)	3/7**(III); 4/7** (II)	4/7**(III); 3/7** (II)

panied by increased platelet aggregation, activation of factors of the intrinsic and extrinsic coagulation pathways, and increased SFMC level. Hemostatic parameters in irradiated animals exposed to 1-day hyperoxia changed in a similar way except APTT increase. At that time points, fibrinolytic activity approached control, but the revealed differences were not statistically significant.

Parameters of hemostatic system after irradiation and hyperoxia in animals (Table 3) were similar to those under irradiation and 3-h AH except for significantly shortened thrombin time. Increased platelet aggregation was a distinctive feature.

Plasma hemostasis after γ-irradiation, hyperoxia, and 3-h AH underwent no significant changes, except for inhibition of the extrinsic mechanism of blood clotting. In contrast, activation of the extrinsic coagulation pathway and accelerated formation of fibrin were revealed after 1- and 3-day AH. Positive SFMC test (grade 3) against the background of persistent increase in fibrinogen concentration was observed. It should be noted that significant increase in platelet aggregation recorded at all time points after combined exposure, also contributed to the thrombogenic potential, which is was exacerbated by reduced fibrinolytic activity in the blood.

These results suggest that isolated and combined exposures to γ-irradiation in a dose of 4.8 mGy, hyperoxia, and AH impaired the relative dynamic balance in the hemostatic system of rats within the timeframe of the experiment. Complex effects of the above stress factors were accompanied by a more pronounced hypercoagulation shift with signs of thrombinemia. Considering reduced blood fibrinolytic activity this may increase the risk for intravascular coagulation. The observed changes in hemostatic parameters in rats did

not suggest effective adaptation for at least 3 days after the end of exposures.

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