Antioxidant Effect of Cytochrome *c* Under Conditions of Prolonged Immobilization Stress

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Prolonged immobilization stress activates lipid peroxidation, causes ischemic damage to the myocardium, and promotes the development of some manifestations of atherosclerosis in rabbits. Intravenous infusion of cytochrome *c* (1 mg/kg) during 30 days of immobilization lowers the extent of lipid peroxidation and produces cardio- and endothelium-protective effects.

Key Words: *prolonged immobilization stress; cytochrome c; arteriosclerosis*

The development of poststress complications caused by prolonged influence of damaging factor is a consequence of exhaustion of compensatory mechanisms (phase III of stress according to Selye) leading to irreversible changes in the organism and diseases [6]. Therefore, prevention of stress and poststress changes is an important problem. It is known that hypoxia is a damaging factor in stress of any genesis. Consequently, antihypoxants are prospective candidates for stress-controlling drugs. In the present study we examined the effect of cytochrome *c* on adaptational processes, namely, lipid peroxidation (LPO) under conditions of prolonged immobilization stress.

MATERIALS AND METHODS

The study was carried out in summer. Twenty male Chinchilla rabbits weighing 2-3 kg were used. Chronic immobilization stress (30 days) was modeled by placing the animals in small cages. Control group consisted of 12 rabbits. Starting from the second day of immobilization, experimental rabbits (n=8) were administered cytochrome *c* (1 mg/kg, daily, into the marginal ear vein). Electrocardiogram (ECG) was recorded before and 30 days after stress. The contents of malonic dialdehyde (MDA) [4], catalase [5], glutathione peroxidase (GTP) [1], and superoxide dismutase (SOD) [7] were measured in plasma and erythrocytes on days 7, 14, and 30.

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RESULTS

Prolonged immobilization stress had a negative effect on general condition of the animals and caused 20% lethality. The rabbits died predominantly from acute cardiac insufficiency, pulmonary edema, and infarction and congestion pneumonia. Most rabbits (70%) died within 7-14 days of immobilization.

Severe hypodynamia led to activation of LPO, as evidenced by increased plasma content of MDA by 98.7, 142.2, and 127.7% on days 7, 14, and 30, respectively (Table 1). A similar dynamics of MDA content was observed in erythrocytes: an increase by 51.3 and 92.9% on days 7 and 14. By the 30th day, the MDA content dropped in erythrocytes but remained high in the plasma, which is probably due to the outflow of LPO products from damaged tissues. Activation of LPO in the blood led to myocardial damage. On day 30, 58% of the rabbits developed myocardial infarction (the presence of the *Q* wave) and 35% of them developed myocardial ischemia (displacement of the *R-ST* interval). Pathomorphological changes in the aorta were observed in all rabbits: in 44% of the rabbits they were severe and destructive (thinning of the aortic wall, aneurisms, ulcers, and multiple plaques), in 33% the changes

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TABLE 1. Dynamics of MDA and Antioxidant Enzymes During Immobilization (*M±m*)

Parameter	Original level	Hypodynamia, days		
		7	14	30
MDA, μmol/liter:				
plasma	1.8±0.08	3.57±0.8*	4.36±0.1*	4.1±0.35*
erythrocytes	2.4±0.3	3.63±0.4*	4.63±0.3*	2.81±0.4
GTP, mmol/min/liter:				
plasma	16±3.1	34.1±1.2*	32.3±2.9*	23.7±0.2
erythrocytes	7±0.5	27.25±2.5*	28.3±3.6*	18.3±3.2
Catalase, μcat/ml/sec:				
plasma	0.63±0.1	0.47±0.06	0.266±0.04*	0.39±0.09**
erythrocytes	3.4±0.3	2.62±0.73	2.45±0.6	1.22±0.3*
SOD of erythrocytes, arb. units	1.05±0.1	4.99±0.1*	1.82±0.3	1.88±0.1

Note. Here and in Table 2: *$p<0.001$, **$p<0.05$ compared with the initial level.

TABLE 2. Effect of Cytochrome *c* on MDA Content and Antioxidant Systems During Prolonged Immobilization (*M±m*)

Parameter	Initial level	Hypodynamia+cytochrome *c*, days		
		7	14	30
MDA, μmol/liter:				
plasma	1.8±0.08	3.3±0.85**	2.59±0.5+	4.1±0.4*
erythrocytes	2.4±0.32	2.63±0.4	2.5±0.4+	2.9±0.3
GTP, mmol/min/liter:				
plasma	16.0±3.1	15.5±2.5+	15.3±1.1+	18.62±1.8+
erythrocytes	7.0±0.5	13.1±0.43*,+	13.8±1.1*,+	10.4±0.9+
Catalase, μcat/ml/sec:				
plasma	0.638±0.1	0.792±0.1+	0.77±0.01+	1.2±0.1*,+
erythrocytes	3.48±0.3	3.9±0.95	5.39±0.5*,+	4.22±0.9+
SOD of erythrocytes, arb. units	1.05±0.1	4.16±0.4*,+	2.58±0.1*,+	5.38±0.6*,+

Note. +Statistically significant difference compared with the control.

were moderate (occasional plaques), and in 21% they were reversible (intimal thickening).

The antioxidant system responded to LPO intensification by increased plasma content of GTP (by 106, 101.2, and 46.7% on day 7, 14, and 30, respectively). A similar dynamics of the enzyme activity was observed in erythrocytes: their GTP content increased by 287, 224.3, and 160.2% on day 7, 14, and 30, respectively. After an increase on day 7, SOD activity decreased 3.7-fold compared with the original level. Catalase activity significantly decreased both in the plasma (by 58.4 and 37.9%) and in erythrocytes (by 29.6 and 61.2%) on days 14 and 30 of immobilization.

It is noteworthy that there was no positive correlation between the response of the antioxidant system and activation of LPO. Although the content of LPO products increased, the activity of antioxidant enzymes progressively lowered, which is indicative of exhaustion of the antioxidant defense system by the 30th day of immobilization.

Cytochrome *c* had a positive effect on general condition of the rabbits, judging from the dynamics of the studied parameters and absence of death. The preparation inhibited LPO activation in the plasma on days 7 and 14 of immobilization: the MDA content increased only by 83 and 43% compared with the original level (Table 2). However, this effect of cytochrome *c* was not observed on day 30 of the experiment.

A more stable inhibition of LPO was observed in erythrocytes: their MDA content remained unchanged throughout the experimental period. Cytochrome *c* increased catalase activity both in the plasma

and in erythrocytes. The preparation activated SOD by 294, 144, and 412% on days 7, 14, and 30, respectively, compared with the original activity and had cardioprotective effect: only minimal reversible changes (reduction of the *T* wave) were observed on ECG. The absence of destructive changes in all aortas testified to angioprotective activity of the preparation.

Our results indicate that prolonged hypodynamia causes LPO activation and inadequate reaction of the antioxidant system. A short-term activation of SOD (up to 7 days) is probably due to its inhibition at later stages by higher concentrations of MDA and hydroperoxides [3]. A low level of catalase, which neutralizes hydrogen peroxide, potentiates toxic effect of LPO at high MDA activity [2]. Cytochrome *c* produces stress-protective effect during immobilization by limiting LPO activation up to 14 days and optimizing the reaction of the antioxidant system. Increased catalase activity is a specific mechanism of action of the antioxidant system under conditions of chronic immobilization. The high SOD activity observed throughout the experiment indicates that this mechanism operates to limit the initiation of chain free-radical oxidation of lipids after administration of cytochrome *c*. Upon immobilization, disintegration of tissue respiration results from the "switching off" of the respiratory chain enzymes. It can be suggested that cytochrome *c* to a certain extent prevents the damage to the respiratory chain enzymes and produces antihypoxic effect, thus optimizing the function of antioxidant enzymes and inhibiting lipid peroxidation in immobilization stress.

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