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Effect of Selank on Functional State of Rat Hepatocytes under Conditions of Restraint Stress

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We studied the effect of Selank administered intraperitoneally in doses of 100, 300, and 1000 μg/kg to male Wistar rats 15 min prior to restraint stress on the content of aminotransferases and total protein concentration in blood serum and intensity of free radical oxidation in the liver. Under conditions of acute restraint stress, Selank in doses of 100 and 300 μg/kg decreased catalase and superoxide dismutase activities and malondialdehyde concentration and increased total antioxidant activity in the liver homogenate. Administration of Selank in a dose of 1000 μg/kg reduced the content of aminotransferases in blood serum, decreased superoxide dismutase activity in the liver, and increased total antioxidant activity. Under conditions of chronic stress, Selank in all doses produced similar effects: reduced superoxide dismutase activity and malondialdehyde concentration in the liver tissue and AST activity in the serum. The other parameters remained unchanged.

Key Words: *Selank; restraint stress; liver; free radical oxidation; serum aminotransferases*

Stress is an important factor of liver damage. Stress promotes the development of steatohepatitis and non-alcoholic fatty liver disease [9]; activation of cytolysis as a result of hepatocyte apoptosis due to increased expression of Fas-receptors and enhanced migration of NK cells into the liver was also demonstrated [7]. Stress-induced elevation of glucocorticoid and catecholamine levels leads to activation of Kupffer cells and increase in TNF production [6], which results in enhanced ROS generation in the liver and activation of lipid peroxidation in cell membranes [11] followed by imbalance of antioxidant system components in hepatocytes [5,8,14]. It should be noted that the severity of stress reaction is significantly influenced by negative psychological perception of stressful situation [4]. In this regard, the possibility of correction of stress-induced changes in the body with neurotropic drugs based on regulatory peptides, including Selank, seems to be a timely and promising line of research. In contrast to many anxiolytics and antidepressants, regulatory peptides exhibit a wide range of biological activities and produce no damaging effects on the body. Selank is characterized by pronounced anxiolytic and antidepressant effects and reduces the ulcerogenic effect of stress [1,3] and therefore was chosen for our experiments as a drug that potentially reduces the damaging effect of stress on the liver.

Our aim was to study functional state of hepatocytes in stressed rats after administration of Selank.

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MATERIALS AND METHODS

The experiments were performed on Wistar male rats (n=100) weighing 250-280 g that were kept under standard vivarium conditions at 12-h illumination regimen and received water and standard granulated food *ad libitum*. The animals were divided into groups (10 rats per group).

We used heptapeptide Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences. Selank was dissolved in physiological saline and administered intraperitoneally 15 min prior to the each stress exposure in doses of 100, 300, and 1000 μg/kg. Control animals received equivalent volumes of saline (1 ml/kg body weight).

Restraint stress was modeled by placing the rats in tight individual plastic boxes for 4 h (acute stress) or for 2 h daily for 5 days (chronic stress). After completion of stress exposure, the animals were sacrificed by exsanguination under ether anesthesia by taking blood from the right ventricle of the heart.

Total protein, ALT, AST in the serum were assayed on a Vitalit 1000 automatic biochemical analyzer using Vital reagents. The liver was homogenized in ice-cold saline and centrifuged; the resulting supernatant was used for measurement of LPO products and antioxidant enzymes. LPO intensity was evaluated by malondialdehyde (MDA) content measured on an Apel 330 PD spectrophotometer using TBK-Agat reagent kits. Superoxide dismutase (SOD) and catalase activities were also measured by the spectrophotometric method. The total antioxidant activity was determined by the degree of inhibition of ascorbate- and iron-induced oxidation of Tween-80 to MDA on a BTS-330 biochemical analyzer.

The results were statistically processed using one-way Student's t test and the Mann—Whitney U test.

RESULTS

Acute restraint stress significantly influenced the studied parameters (Table 1). In the control group, an increase in serum content of AST (by 32%, p<0.01) and total protein (by 8%, p<0.01) was observed in comparison with intact animals. The increase in ALT level (by 14%) did not reach significance. Stress increased catalase and SOD activities (by 13 and 14%, respectively, p<0.05) in liver homogenate; MDA content increased by 22% (p<0.01) and total antioxidant activity decreased by 7% (p<0.01). The effects of Selank were most pronounced in doses of 100 and 300 μg/kg. Administration of the peptide in a dose of 100 μg/kg was accompanied by an increase in the total protein content in the blood serum (by 9%, p<0.01), decrease in catalase and SOD activities (by 18 and 14%, p<0.05) and MDA (by 49%, p<0.01) in liver homogenate; total antioxidant activity increased by 4% (p<0.05). The administration of Selank in a dose of 300 μg/kg had a similar effect on the parameters of the liver antioxidant system, and also caused a significant decrease in the concentration of AST by 22% (p<0.05). Increasing the dose of the peptide to 1000 μg/kg was followed by a decrease in both AST (by 23%, p<0.05) and ALT (by 19%, p<0.05) and increase in total serum protein (by 10%, p<0.01). In liver homogenate, SOD activity decreased by 14% (p<0.01) and total antioxidant activity significantly increased (by 20%, p<0.01). In this case, Selank did not correct MDA content and catalase activity in comparison with the control.

Chronic stress produced more pronounced effect on the studied parameters than acute stress exposure. In stressed control animals, serum AST increased by 40% in comparison with intact animals (p<0.01). ALT and total protein did not change significantly. In the liver homogenate, an increase in SOD (by 17%, p<0.05) and MDA content (by 31%, p<0.05) was observed with unchanged catalase and total antioxidant activity. Selank corrected the studied parameters in all applied doses. For instance, peptide in doses of 100, 300, and 1000 μg/kg reduced serum AST by 23, 16, and 17%, respectively, MDA in the liver homogenate by 29, 26, and 32%, and inhibited SOD by 17, 16, and 19% (p<0.05).

These findings suggest that Selank profoundly affects peroxidation processes and liver antioxidant system; the changes in the studied parameters depended on stress duration. Acute stress increased the activity of antioxidant system enzymes (SOD and catalase) and content of LPO products (MDA) and decreased total antioxidant activity, while chronic exposure led to isolated increase in SOD and MDA without appreciable changes in catalase activity. Similar shifts were reported previously [8,14] and can be explained by activation of NF-κB signaling. In particular, neuroendocrine stimulation during acute stress leads to activation of Kupffer cells in the liver and secretion of TNF that activate free radical oxidation, while enhanced ROS generation induces NF-κB translocation into the nucleus, which enhances transcription of antioxidant enzymes [8] and thereby their activity. The corrective effect of Selank in doses of 100 and 300 μg/kg on these parameters can be explained by modulation of the content of inhibitory and excitatory amino acids in the hypothalamus [2], which produces an anxiolytic effect, reduces activity of the hypothalamic—pituitary axis, and compensates stress-induced changes to the level of non-stressed animals. Less pronounced effect of Selank in a dose of 1000 μg/kg can be caused by its stimulating effect on the nervous system at this dose [1].

TABLE 1. Effects of Selank in Acute and Chronic Restraint Stress (*M±SD*)

Parameters	Intact animals	Control	Selank dose, μg/kg		
			100	300	1000
Acute stress					
Blood serum					
ALT, U/liter	54.8±2.5	64.2±4.6	57.9±3.1	58.3±2.4	51.6±3.2*
AST, U/liter	116.8±3.8	171.5±16.7+	154.7±9.5	134.4±6.9*	131.1±6.7*
Total protein, g/liter	60.1±0.9	55.6±1.1+	60.4±0.8*	56.3±1.0	61.3±1.3*
Liver homogenate					
MDA, μmol/ml	1.8±0.1	2.3±0.1+	1.2±0.1*	1.8±0.1*	2.4±0.2
Catalase, μkat/liter	3.1±0.1	3.5±0.2+	3.0±0.1*	3.1±0.1*	4.0±0.2
SOD, arb. units	2.2±0.1	2.5±0.1+	2.1±0.1*	2.1±0.0*	2.2±0.1*
GAA, %	23.9±0.4	22.2±0.3+	23.2±0.3*	23.6±0.2*	26.7±0.4*
Restrain stress					
Blood serum					
ALT, U/liter	61.3±4.5	63.8±3.3	57.4±2.8	63.5±4.6	56.5±5.0
AST, U/liter	132.6±6.6	210.3±10.0+	161.7±10.5*	176.6±12.9*	174.8±7.1*
Total protein, g/liter	62.4±1.5	61.3±1.9	61.6±1.6	61.8±1.4	60.2±1.6
Liver homogenate					
MDA, μmol/ml	2.5±0.3	3.6±0.4+	2.6±0.2*	2.7±0.1*	2.5±0.2*
Catalase, μkat/liter	4.6±0.4	4.6±0.3	4.2±0.3	4.0±0.2	4.7±0.3
SOD, arb. units	2.4±0.1	2.9±0.2+	2.4±0.1*	2.4±0.1*	2.3±0.1*
GAA, %	25.6±0.7	25.5±0.6	25.6±0.6	25.0±0.4	25.5±0.6

Note. TAA is total antioxidant activity. $p<0.05$-0.001 in comparison *with control, +intact rats.

Opposite shifts in SOD and catalase activities and more pronounced MDA accumulation under conditions of chronic stress can be explained by gradual exhaustion of the antioxidant system and accumulation of LPO products, which in turn can lead to more pronounced cytolytic effect and activation of NF-κB. Gradual exhaustion of the adaptive reserves is accompanied by a decrease in glucocorticoid concentrations and weakening of the functional response of cells to these hormones [13], which can lead to inadequate suppression of proinflammatory NF-κB signals by anti-inflammatory signals of glucocorticoids [12]. Excessive activation of NF-κB enhances transcription and synthesis of SOD as first-line antioxidant defense enzyme [10]. Selank corrects shifts in the studied parameters caused by chronic stress by modulating the content of inhibitory and excitatory amino acids in the hypothalamus [2]. Selank at a dose of 1000 μg/kg also normalized the changed parameters, probably due to stimulating effect on animal behavior and the antidepressant effect [1], which prevents exhaustion of the adaptive capabilities of the nervous system.

Thus, the use of Selank under conditions of acute and chronic restraint stress in rats is accompanied by hepatoprotective effect due to inhibition of cytolytic processes and normalization of free radical oxidation.

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