DOI 10.1007/s10517-019-04512-1

Effect of Selank on Morphological Parameters of Rat Liver in Chronic Foot-Shock Stress

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 167, No. 2, pp. 257-260, February, 2019
Original article submitted June 21, 2018

We studied the effects of Selank on morphological parameters of the liver in Wistar male rats subjected to chronic foot-shock stress. Selank was injected intraperitoneally in doses of 100, 300 and 1000 μg/kg 15 min before each stress session. Morphological and morphometrical analysis showed that chronic foot-shock stress induced hydropic degeneration of hepatocytes, an increase of the nucleus/cytoplasm ratio due to an increase in the area of nuclei and reduction of the cytoplasm area, the appearance of focal necroses, and lymphohistiocyte infiltration. Injection of Selank in all doses reduced the intensity of stress-induced degenerative changes. Administration of Selank in doses of 300 and 1000 μg/kg restored the nucleus/cytoplasm ratio in hepatocytes. The maximum stress-limiting effect was attained after administration of 300 μg/kg Selank.

Key Words: *Selank; regulatory peptides; foot-shock stress; liver; nucleus/cytoplasm ratio*

Long-term exposure to stress factors leads to homeostasis disturbances and contributes to the development of liver pathologies, such as steatohepatitis and non-alcoholic fatty liver disease [12,14]. Pain stimuli accompanied by emotionally negative reaction often cause stress [9] and are used as an experimental model. Stress-induced increase in the blood concentrations of catecholamines and glucocorticoids affects microcirculation in the liver lobule and promotes LPO processes [10], which stimulates synthesis in proinflammatory cytokines in the liver [11], migration of natural killers, hepatocytes apoptosis [12], and could result in the development of hepatocyte ballooning and/or lipid degeneration [14]. Correction of these alterations could be implemented via activation of stress-limiting systems mediated by regulatory peptides characterized by high polyfunctional biological activity, including their effects on the liver parenchyma [1]. Selank is a regulatory peptide that exhibits adaptogenic [7], anxiolytic, and antidepressant activities [2], ameliorates stress-induced ulcer formation [6], and regulates inflammatory response [5]. In view of well-known spectrum of pharmacological effects of Selank, it was interesting to study the effects of this peptide on the liver under stress conditions.

Here we studied the effects of Selank on morphological state of the liver parenchyma in rats exposed to chronic foot-shock stress.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250-280 g (n=50) and divided into 5 groups (10 rats per group). The animals were kept under standard vivarium conditions at 12/12 h light/dark cycle, water and food were provided *ad libitum*.

Heptapeptide Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro; synthesized at the Institute of Molecular Genetics, Russian Academy of Science) was dissolved in saline and injected intraperitoneally in doses of 100, 300, or 1000 μg/kg 15 min before each stress sessions. Control animals received injections of 1 ml/kg saline.

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To model chronic foot-shock stress (CFSS), the rats were placed in pairs in a box with electrified stainless grid floor. Electric pulses (0.2-0.3 mA, 5 sec) were delivered through the floor grids with an interval of 15 sec; the sessions were performed over 30 min once a day over 5 consecutive days [15]. Immediately after the last stress session, the animals were sacrificed by exsanguination under ether anesthesia.

For morphological analysis of the liver parenchyma, the gallery of images of 5-7-μ paraffin sections stained with hematoxylin and eosin were created using a Mirax Desk scanner (Carl Zeiss Microimaging GMbH). In each section, areas of cells and their nuclei were measured in 100 hepatocytes in periportal and centrilobular zones using Pan-noramicViewer 1.15.4 software (3DHISTECH Ltd,) at ×400. Areas of hepatocyte cytoplasm and nucleus/cytoplasm ratio (NCR) were calculated using Microsoft Excel.

Statistical significance of differences between the means in the compared groups was evaluated by unpaired Student›s *t* test and nonparametric Mann—Whitney test.

RESULTS

Histological examination of the liver of stressed animal revealed increased number of vacuoles of irregular shape with blurred contours, morphoplasm displacement to the cell periphery, focal hepatocyte necrosis, histiocyte infiltration, and changes in the lobular architectonics; in central zones, hepatocytes with large eosinophil nuclei were seen; in intermediate zones, small round vacuoles were revealed (Fig. 1. *a*) CFSS modeling was associated with significant increase in the area of hepatocyte nuclei in both periportal and centrilobular zones (by 8.9 and 12.5%, respectively; $p<0.01$) and cytoplasm area reduction in periportal hepatocytes (by 7.4%, $p<0.05$; Table 1). These changes led to NCR increase in both periportal and centrolobular hepatocytes (by 15.8 and 17.1%, respectively; $p<0.01$),

Selank injection dose-dependently improved hepatocyte condition, which manifested in less pronounced inflammatory changes and vacuolation of hepatocytes. Administration of the peptide in a dose of 100 μg/kg led to reduction of cytoplasm granularity, primarily in the periportal hepatocytes (Fig. 1, *b*), but in central zones, no improvement was seen. Effect of Selank in a dose of 1000 μg/kg was more pronounced: only residual granules were seen in the hepatocyte cytoplasm in all zones of the lobule and lobule architectonics was restored (Fig. 1, *d*). After injection of the peptide in a dose of 300 μg/kg, practically no degenerative changes were seen (Fig. 1, *c*).

Peptide administration in doses of 300 and 1000 μg/kg led to a significant decrease in NCR in both periportal and centrilobular hepatocytes; this was a result of changes in either numerator (nucleus area) or denominator (cytoplasm area) (Table 1). In the group receiving Selank in a dose of 300 μg/kg, NCR reduction by 14.8% ($p<0.01$) in periportal zone and by 9.8% ($p<0.01$) in centrilobular zone occurred due to an in-

TABLE 1. Parameters of Hepatocyte Nucleus and Cytoplasm after CFSS Modeling against the Background of Selank Treatment (*M*±*m*)

Parameter	Intact animals	Stressed animals			
		control	Selank, μg/kg		
			100	300	1000
Hepatocytes of peripheral zones of the lobule					
Cell area, μ²	273.0±6.0	284.6±6.3	275.7±4.8	301.6±5.1*	273.1±5.6
Nucleus area, μ²	48.2±1.0	52.9±0.9⁺	52.5±0.9	52.4±1.0	48.5±1.1*
Cytoplasm area, μ²	236.3±5.8	220.1±5.4⁺	223.1±4.5	249.2±4.6*	224.6±5.1
NCR	0.215±0.005	0.255±0.005⁺	0.247±0.005	0.218±0.004*	0.229±0.006*
Hepatocytes of peripheral zones of the lobule					
Cell area, μ²	306.4±6.4	307.3±6.7	316.2±7.0	320.8±5.6	315.8±6.0
Nucleus area, μ²	50.3±1.2	57.4±1.1⁺	58.0±1.6	56.6±1.2	57.1±1.4
Cytoplasm area, μ²	257.0±5.9	248.9±5.8	258.2±6.0	264.2±5.0*	264.9±5.1*
NCR	0.202±0.004	0.245±0.005⁺	0.232±0.005	0.220±0.005*	0.223±0.005*

Note. $p<0.05$-0.001 in comparison with *stressed control, ⁺intact control.

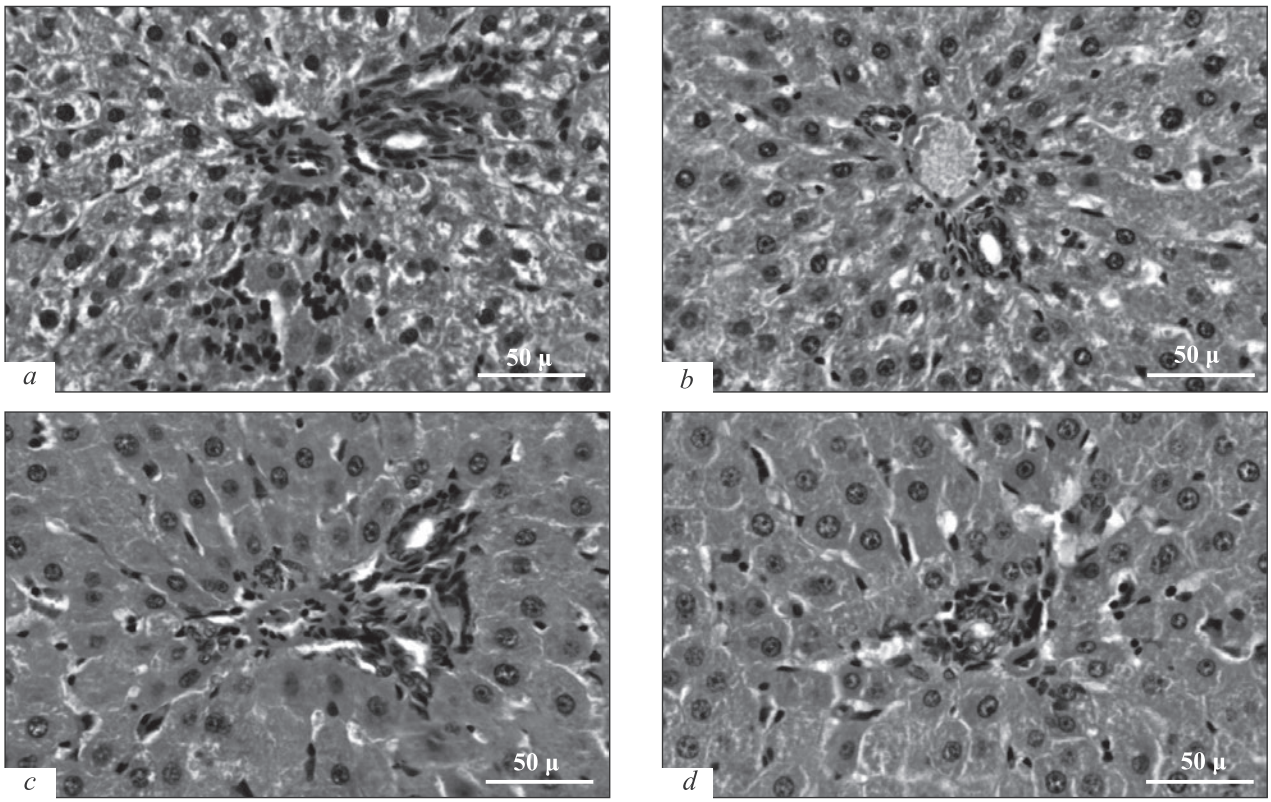

Fig. 1. Hepatocytes of peripheral zones of the lobule in rats subjected to CFSS (*a*) and receiving Selank in doses of 100 (*b*), 300 (*c*) and 1000 (*d*) μg/kg. Hematoxylin and eosin staining, ×400.

crease in hepatocyte cytoplasm area by 13% ($p<0.01$) and 6% ($p<0.05$), along with the growth of periportal hepatocytes area (10.5%, $p<0.01$). In the peripheral zones, the hepatocytes area also increased by 10.5% ($p<0.01$). In the group receiving Selank in a dose of 1000 μg/kg, NCR in periportal hepatocytes decreased by 10.2% ($p<0.01$) mainly due to reduction of nucleus area by 8.4% ($p<0.01$), whereas in centrilobular hepatocytes, NCR decreased by 8.9% ($p<0.01$) due to an increase in the cytoplasm area by 6% ($p<0.01$).

These findings suggest that CFSS induces cytoplasm vacuolation in hepatocytes, increase in the nucleus area, and reduction of hepatocyte cytoplasm area leading to an increase in NCR, these changes being more pronounced at the periphery of the lobule. The described changes could develop due to stress-induced increase in glucocorticoid concentration, stimulation of catabolic processes, and activation of transcription of signaling factors. Injection of Selank reduced the severity of hepatocyte vacuolation and normalized NCR; the maximum effect was observed at peptide dose of 300 μg/kg. The observed effects of Selank could be explained by its complex anxiolytic activity including stimulation of the inhibitory processes in the hippocampus [8] due to increased $GABA_A$ receptor density [2] and by inhibition of enkephalin degradation enzymes [4], which, in turn, can affect central mechanisms of the stress reaction. Considering the important role of 5-HT synthesis by hepatocytes in the steroid-mediated effect of chronic stress [13], along with the effect of Selank on monoamine metabolism [3], we cannot exclude local effects of the peptide on hepatocytes.

Thus, injection of Selank under conditions of CFSS in rats produced stress-limiting hepatoprotective effect on the morphofunctional state of hepatocytes, which manifected in less pronounced degenerative changes. The gradient of the stress-limiting effect of the peptide was directed from the periphery to the center of the liver lobule, which was seen from the increase in the intensity of stress-induced morphological changes.

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