PHARMACOLOGY AND TOXICOLOGY

Evaluation of Anti-Inflammatory Effects of Trombovazim in the Model of Liver Ischemia-Reperfusion

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Administration of enzyme preparation Trombovazim as a corrector of ischemic damage to animals with experimental liver ischemia-reperfusion reduces neutrophilic infiltration of liver parenchyma and decreases hyperfermentemia, which attests to a decrease in the intensity of destructive changes in hepatocytes.

Key Words: *liver; ischemia-reperfusion injury; neutrophils; Trombovazim*

Partial or global ischemia of the liver followed by blood flow resumption can occur during surgical interventions, transplantation, and traumas to this organ. Incomplete reperfusion can lead to severe complications, such as graft rejection, inflammation, and necrosis of hepatocytes [5]. An important role in the development of ischemic injury is played by neutrophilic granulocytes. Microcirculatory disturbances lead to the inflammatory response activating leukocytes and Kupffer cells. Activated neutrophils infiltrate the damaged liver, which is accompanied by enhanced expression of adhesion molecules on endothelial cells. Neutrophils release reactive oxygen and proteases thus causing hepatocytes injury [6]. Since many aspects of the pathogenesis of the ischemia–reperfusion injury to the liver have been elucidated, the search for ways to prevent the injury by affecting various stages of the process is required. In this context, Trombovasim is a promising thrombolytic, anti-inflammatory, and antithrombotic agent obtained by immobilization of *Bacillus subtilis* proteinases on a soluble polyethylene glycol. It was developed at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences [4].

Here we studied morphofunctional disturbances in the liver during the acute period after ischemia–reperfusion and under conditions of Trombovazim correction.

MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 200-250 g. The animals were divided into 5 groups: ischemia-reperfusion (control, group 1); ischemia–reperfusion+Trombovazin intragastrically once in a dose of 250 U/kg (group 2); ischemia-reperfusion+Trombovazin intraperitoneally once in a dose of 30 U/kg (group 3); sham-operated animals (group 4); intact animals (background, group 5).

Liver ischemia was modeled by clamping the hepatoduodenal ligament for 20 min [3]. After that, the liver was reperfused for 1, 3, and, 6 h. Trombovazim was administered 1 h before the experiment for preventing postischemic damage to hepatocytes. In sham-operated animals, all surgical manipulations were performed except clamping of the hepatoduodenal ligament.

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For histological assay, liver portions were fixed in 10% formalin, embedded in paraffin, and 5 μ-thick sections were cut. On sections stained with hematoxylin and eosin, the number of inflammatory infiltrate cells per 500 hepatocytes and the percentage of granulocytes were estimated [1]. ALT activity was measured in rat serum [2].

Statistical processing of the results was performed using the nonparametric Mann–Whitney test.

RESULTS

Morphological examination showed that ischemia–reperfusion led to hemodynamic disturbances in rat liver, fine droplet fatty degeneration, and monocellular necrosis of hepatocytes. Lymphoid macrophage infiltration of the parenchyma and portal tracts was detected. Extended hepatic sinusoids disturbed hepatic lobular architectonics.

Morphometric assay revealed that ischemia followed by 1- and 3-h reperfusion resulted in enhanced inflammatory infiltration of liver compared to intact animals (Table 1). The percentage of neutrophils in the infiltrate increased by almost 2.5 and 3.8 times, respectively, in comparison with background values. Cell infiltration of liver parenchyma slightly decreased by 6 h after reperfusion, but the number of neutrophilic granulocytes during this period 2.7-fold exceeded the background values.

Pretreatment with Trombovazim did not abolish the development of increased inflammatory infiltrate during ischemia, but significantly reduced the migration of neutrophils to the infiltrate during the first hours after blood flow resumption (Table 2). Thus, the corrector in a dose of 250 U/kg even 1-h reperfusion reduced the number of neutrophils in the inflammatory infiltrate to background values. After 3-h reperfusion, neutrophil infiltration of liver in groups 2 and 3 was decreased by 1.3 and 1.5 times in comparison with the control, respectively. After 6 h, there were no significant differences in the number of neutrophils in the compared groups.

Biochemical assay detected significant increase in plasma ALT activity. Even after 1-h reperfusion, ALT activity increased by more than 2 times, and after 3 and 6 h, by almost 3 times in comparison with

TABLE 1. Effect of Trombovazim Treatment on Total Number of Infiltrate Cells (absolute number per unit area; $X \pm m$)

Period of reperfusion	Background	Control	Ischemia+Trombovazim, 250 U/kg	Ischemia+Trombovazim, 30 U/kg
1 h	227.75±6.75	305.20±14.88^{++}	330.80±30.93^{++}	357.60±33.07^{++}
3 h	227.75±6.75	291.40±11.65^{++}	276.20±11.83^{+}	327.20±23.98^{++}
6 h	227.75±6.75	260.00±21.73	316.20±14.72*$^{++}$	259.80±23.25

Note. Here and in Tables 2, 3: *p_U<0.05, **p_U<0.01 versus control; $^{+}p_U$<0.05, $^{++}p_U$<0.01 versus background.

TABLE 2. Effect of Trombovazim Treatment on Neutrophil Content in Inflammatory Infiltrate (%; $X \pm m$)

Period of reperfusion	Background	Control	Ischemia+Trombovazim, 250 U/kg	Ischemia+Trombovazim, 30 U/kg
1 h	5.23±1.04	12.80±1.09^{++}	6.66±0.80**	15.76±0.67^{++}
3 h	5.23±1.04	20.10±1.30^{++}	15.46±1.24*$^{++}$	13.42±1.75**$^{+}$
6 h	5.23±1.04	14.14±1.59^{++}	14.42±1.08^{++}	12.58±1.33^{++}

TABLE 3. Effect of Trombovazim Treatment on ALT Activity of Rat Plasma (μcat/liter; $X \pm m$)

Period of reperfusion	Background	Control	Ischemia+Trombovazim, 250 U/kg	Ischemia+Trombovazim, 30 U/kg
1 h	0.49±0.02	1.02±0.10^{++}	1.13±0.11^{++}	1.31±0.05^{++}
3 h	0.49±0.02	1.32±0.02^{++}	0.99±0.1*$^{++}$	0.85±0.17*$^{++}$
6 h	0.49±0.02	1.35±0.16^{++}	1.15±0.08^{++}	1.36±0.16^{++}

the background values (Table 3). Postischemic hyperfermentemia practically did not decrease after Trombovazim administration. After 3-h reperfusion, ALT activity in the group with corrector was somewhat lower than in controls, but almost 2 times exceeded the background values. By 6 h, this parameter in animals treated with Trombovazim increased again and did not significantly differ from the control.

Liver structure and functional parameter in the group of sham-operated animals did not significantly differ from those in intact rats.

Thus, ischemia followed by reperfusion induced hemodynamic, structural, and functional disturbances in the liver. Trombovazim does not reduce the intensity of inflammatory infiltration, but prevents massive migration of neutrophils in the liver tissue during the first 3 h after blood flow resumption probably due to its anti-inflammatory action [4]. This effect is realized due to reduced expression of adhesion molecules, as well as decreased production of proinflammatory for neutrophils by the effector cells [5,6]. In the acute period of ischemia–reperfusion, inhibition of leukocyte migration to the liver parenchyma reduces hepatocytes damage, which is seen from decreased ALT activity in rat plasma.

Trombovazim in a dose of 250 U/kg provides earlier (from the first hours) inhibition of neutrophil migration into an inflammatory zone.

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