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DYNAMICS OF VIOLATIONS THE ABSORPTION FUNCTION OF THE SMALL INTESTINE IN CONDITIONS OF ACUTE BLOOD LOSS COMPLICATED BY ISCHEMIA-REPERFUSION OF THE LIMBS AND THEIR CORRECTION

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Abstract

Introduction. In the structure of modern injuries in wartime and peacetime the share of gunshot wounds increased significantly. The timeliness of applying a tourniquet is one of the main methods of saving lives on the battlefield. The patterns of formation of enteral insufficiency syndrome in conditions of acute blood loss complicated by ischemia-reperfusion of the limb have not been studied enough. There are no data on the features of violations of the absorption function of the small intestine in these conditions and the effectiveness of correction with Carbacetam, which is able to reduce the manifestations of acute hemic hypoxia and ischemic-reperfusion syndrome.

The objective of research: To establish the peculiarities of the absorption function of the small intestine in the pathogenesis of acute blood loss, ischemia-reperfusion of the limb and their combination. To evaluate the effectiveness of Carbacetam in the correction of identified abnormalities.

Materials and methods. The experimental studies were conducted on 108 white

nonlinear male rats weighing 200-220 g, which were housed in standard vivarium conditions. All animals were divided into five groups: control and four experimental. Under thiopental-sodium anesthesia in the first experimental group was simulated ischemia-reperfusion of the limb, in the second group – acute blood loss, and in the third group – these injuries were combined. In the fourth experimental group, animals with acute blood loss and ischemia-reperfusion of the limb were intraperitoneally administered Carbacetam at a dose of 5 mg per kilogram of animal weight. After 1 and 2 hours, as well as after 1, 7 and 14 days studied the absorption function of the intestines by D-xylose test.

The results and discussion. Simulation of limb ischemia-reperfusion compared with the control group causes a decrease in the content of D-xylose in the urine after 1 day of the reperfusion period. Also, acute blood loss is accompanied by a significant decrease in this indicator after 1 day. Until the end of the experiment the rate increases, but up to 14 days does not reach the level of control. The combination of acute blood loss and ischemia-reperfusion of the limb causes a decrease in the content of D-xylose in the urine starting from 1 hour of the experiment, which reaches a minimum after 1 day and remains at the same level until 14 days. In these terms the rate was the lowest compared to other studied groups. The use of Carbacetam for corrective purposes in rats with acute blood loss complicated by ischemia-reperfusion of the limb, compared with animals without correction significantly reduces the detected abnormalities after 7 days and more after 14 days of use.

Conclusions. Complications of acute blood loss by ischemia-reperfusion of the limb causes a significant decrease in the absorption function of the small intestine after 1 day of the experiment. The intensity of disorders was significantly higher compared to other experimental groups. The use of Carbacetam for 7-14 days in the reperfusion period in animals with acute blood loss complicated by limb ischemia-reperfusion, compared with animals without correction, causes a significant increase in the absorption function of the small intestine. This indicates the protective effect of the drug under the conditions of simulated pathology.

Key words: small intestine; blood loss; ischemia-reperfusion of the limb; antioxidant-absorption function; Carbacetam.

Introduction. In the structure of modern injuries in wartime and peacetime the share of gunshot wounds increased significantly. More than 60 % of cases are lesions of the extremities, which are accompanied by massive external blood loss.

The timeliness of first aid, especially the timeliness of applying a tourniquet is one of the main methods of saving lives on the battlefield [1, 2]. The safe period of complete ischemia of the limb is two hours.

According to some authors, acute limb ischemia during two hours and, especially, revascularization lead to the formation of active forms of oxygen, development of metabolic disorders, activation of lipid peroxidation processes, cell membrane damage with the release of endogenous toxins into the systemic bloodstream. The toxins deepen under conditions of acute blood loss and initiate systemic disorders in tissues and organs remote from the site of direct lesion [3, 4, 5]. The development of metabolic and functional disorders under conditions of acute blood loss complicated by ischemia-reperfusion of the limb in the liver, kidneys and lungs has been proved [6, 7, 8].

It is known that the leading role in the pathogenesis of the syndrome of endogenous intoxication and multiorgan failure belongs to the gastrointestinal tract. In the gastrointestinal tract under conditions of acute blood loss appear disorders due to the centralization of hemodynamics, disorders of microcirculation in the intestinal wall, hypoxic and reperfusion damage of enterocytes, the action of pro-inflammatory mediators, edema of the intestinal wall [9, 10].

In these conditions, arises a syndrome of enteral insufficiency, that manifested by violation of motor-evacuatory, barrier, secretory and absorption functions of the intestines, as well as parietal and intraluminal digestion [11]. Due to the translocation of the microflora and the mass entry into the bloodstream of a microbial toxin – lipopolysaccharide, another "false" pathological circle closes, which triggers a cascade of reactions that accelerates the development of multiorgan failure.

However, the patterns of formation of enteral insufficiency syndrome in conditions of acute blood loss complicated by ischemia-reperfusion of the limb have not been studied enough. There are no data on the features of violations of the absorption function of the small intestine in these conditions and the effectiveness of correction with Carbacetam, which is able to reduce the manifestations of acute hemic hypoxia and ischemic-reperfusion syndrome [12].

The objective of research: To establish the peculiarities of the absorption function of the small intestine in the pathogenesis of acute blood loss, ischemia-reperfusion of the limb and their combination. To evaluate the effectiveness of Carbacetam in the correction of identified abnormalities.

Materials and methods. In the experiments used 108 nonlinear males rats weighing 200-220 g. All animals were divided into five groups: control and four experimental (6 animals in each groups).

All experiments were performed under thiopental sodium anesthesia (40 mg kg⁻¹ body weight) dosed. In the first experimental group were simulated ischemia-reperfusion of the limb. For this purpose, a strip of elastic tourniquet "SWAT-T" (USA) width of 10 mm was proximally applied to the left foot, which completely stopped the blood flow for 120 minutes. In the second experimental group, acute blood loss (20 % of the circulating blood volume) was simulated by crossing the femoral vein. In the third experimental group, these injuries were combined. In the fourth experimental group, animals with acute blood loss and ischemia-reperfusion of the limb intraperitoneally administered Carbacetam, for corrective purposes. Administered at a dose of 5 mg per kilogram of animal weight (L.M. Litvinenko Institute of Physical-Organic Chemistry and Coal Chemistry of the National Academy of Sciences of Ukraine, Kyiv) [6]. In the control group, the animals were only anesthetized using an equivalent dose of sodium thiopental. After 1 and 2 hours, as well as after 1, 7 and 14 days under conditions of thiopental sodium anesthesia in experimental animals studied the absorption function of the intestine by D-xylose test. D-xylose is a chemically inert carbohydrate that is absorbed by passive diffusion, is not metabolized in the human body, excreted in the urine and adequately reflects the absorption function of the small intestine [13]. Experimental animals were intragastrically injected with 10% solution of D-xylose at the rate of 100 mg·kg⁻¹ body weight. Next, were collected urine during the 4 hours. The concentration of D-xylose was determined [14]. The test animals were subsequently euthanized through complete exsanguination from the heart.

All experimental procedures fulfilled the international standards for the humane treatment of animals in compliance with the regulations of «The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (European Convention, 1986)».

All obtained data were processed to the statistical analysis by the nonparametric The Mann–Whitney U test using STATISTICA 10.0 software («StatSoft Inc.», USA).

The results and discussion. As we can seen from table.1, under conditions of ischemia-reperfusion of the limbs the content of D-xylose in the urine compared with the control group decreased, however, the result was statistically significant after 1 day of the reperfusion period (20.1 %, p<0.05).

Table 1 – Dynamics of D-xylose content (mg·ml⁻¹) in urine after acute blood loss complicated by ischemia-reperfusion of the limb (Me (LQ; UQ) – median (lower and upper quartiles))

Experimental group	The term of the reperfusion period				
	1 h	2 h	1 day	7 days	14 days
Control = 51,70 (46,25; 57,75) (n=6)					
<i>Experimental group 1</i> Ischemia-reperfusion	50,50 47,35; 54,25 (n=6)	48,50 44,60; 51,65 (n=6)	41,30* 37,35; 44,65 (n=6)	46,40 ^{1д} 45,60; 50,05 (n=6)	51,40 ^{1д} 49,25; 52,35 (n=6)
<i>Experimental group 2</i> Blood loss	44,50 42,40; 51,25 (n=6)	41,20 37,60; 46,60 (n=6)	34,20* ^{1r} 30,45; 38,10 (n=6)	38,40* ^{1r} 37,75; 41,45 (n=6)	42,40* ^{1д} 41,00; 45,45 (n=6)
<i>Experimental group 3</i> Ischemia-reperfusion + blood loss	42,90* 42,25; 45,50 (n=6)	32,30* ^{1r} 30,85; 35,70 (n=6)	22,90* ^{1r,2r} 20,85; 24,35 (n=6)	26,60* ^{1r,2r} 23,70; 28,00 (n=6)	28,90* ^{1r,2r} 25,60; 29,95 (n=6)
p ₁₋₂	>0,05	>0,05	>0,05	<0,05	<0,05
p ₁₋₃	<0,05	<0,05	<0,05	<0,05	<0,05
p ₂₋₃	>0,05	<0,05	<0,05	<0,05	<0,05

Notes.

1. * - differences relative to the control group are statistically significant (p<0.05);
2. p₁₋₂ – the probability of differences between experimental groups 1 and 2;
3. p₁₋₃ – the probability of differences between experimental groups 1 and 3;
4. p₂₋₃ – the probability of differences between experimental groups 2 and 3.

Simulation of acute blood loss also caused a decrease in the content of D-xylose in the urine. The result was statistically significant compared with the control after 1, 7 and 14 days (33.8, 25.7 and 18.0%, p<0.05). After 1 and 7 days of the experiment, the indicator was also significantly lower than after 1 hour (on 23.1 and 13.7 %, p<0.05). After 14 days, the rate increased and became 24.0 % higher than after 1 day of the experiment (p<0.05).

Complications of acute blood loss by ischemia-reperfusion of the limb led to a decrease in the content of D-xylose compared with the control after 1 hour of the experiment (by 17.0 %, p<0,05). Then the rate decreased and reached a minimum after 1 day of the experiment (by 55.7 %, p<0.05 compared with the control), that was also significantly less than after 1 hour and 2 hours, respectively, at 46.6 and 29, 1 %, p<0.05). It is noteworthy that at the same level the content of D-xylose in the urine remained until the 14th day of the experiment (p>0.05).

Comparison of the experimental groups showed that after 1 hour of the experiment, the content of D-xylose in the urine was significantly lower in experimental group 3 compared with experimental group 1 (15.0 %, $p_{1-3}<0.05$). After 2 hours the result was similar. From the conditions of acute blood loss complicated by limb ischemia-reperfusion (experimental group 3), the indicator was 33.4 % lower than in the group where only limb ischemia-reperfusion was simulated (experimental group 1) ($p<0.05$).

After 1 day, the content of D-xylose in the urine continued to remain statistically significantly lower in experimental group 3 compared with experimental groups 1 and 2 (respectively by 44.6 %, $p_{1-3}<0.05$ and 33.0 %, $p_{2-3}<0.05$). Starting from 7 days, a pattern was established, according to which with increasing severity of the lesion decreased the content of D-xylose in the urine ($p_{1-2}<0.05$, $p_{1-3}<0.05$, $p_{2-3}<0.05$).

Under the influence of correction (Fig. 1) after 7 days of Carbacetam use, the content of D-xylose in the urine increased statistically significantly (by 18.6 %, $p<0.05$) compared with the experimental group without correction. After 14 days of Carbacetam use compared with animals without correction, the figure became even higher – by 39.8 % ($p<0.05$). However, in this period the indicator did not reach the level of the control group and remained 21.9 % lower ($p<0.05$).

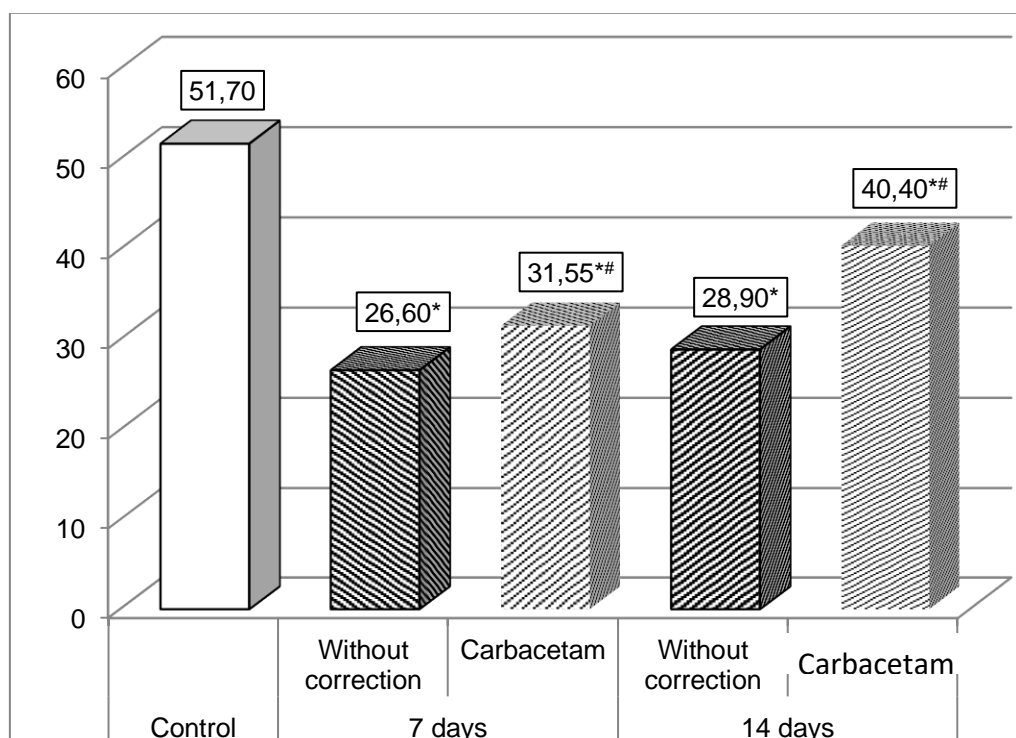


Figure 1 – The effect of Carbacetam on the content of D-xylose (mg ml⁻¹) in the urine after acute blood loss complicated by ischemia-reperfusion of the limb. (Note. Here and in Fig. 2: * – differences to the control group are statistically significant, $p<0.05$; # – differences to the group without correction are statistically significant, $p<0.05$).

The obtained results indicate that the simulation of ischemia-reperfusion of the limb, compared to the control, causes a decrease in the content of D-xylose in the urine only after 1 day of the reperfusion period. Also, acute blood loss accompanied of significant decrease in this indicator after 1 day. By the end of the experiment, the rate increases, but up to 14 days does not reach the level of control.

The combination of acute blood loss and ischemia-reperfusion of the limb causes a decrease in the content of D-xylose in the urine from 1 hour of the experiment, which reaches a minimum after 1 day and remains at the same level until 14 days. In these terms, the indicator is the lowest compared to other experimental groups. Therefore, with increasing severity of damage, the violation of absorption function of the small intestine occurs faster and more pronounced.

At the heart of the identified violations, obviously, nonspecific reaction of gastrointestinal tract to the centralization of blood circulation and hypoxemia that caused by acute blood loss, as well as the influence of endotoxins that enter the systemic bloodstream from the ischemic limb after reperfusion. All this creates the preconditions for the activation of prooxidant mechanisms in the wall of the small intestine, that was shown in our previous studies [15]. So, intensification of lipid peroxidation processes causes damage to cell membranes of structural components of the small intestinal wall, that leads to worsening of absorption function. We can assume, that under these conditions, other functions of the small intestine may suffer: motor, barrier, immune and others.

The use of Carbacetam for corrective purposes in rats with acute blood loss complicated by ischemia-reperfusion of the limb, compared with animals without correction significantly reduces the detected abnormalities after 7 days and more after 14 days of use. This positive action is obviously based on antioxidant, immunomodulatory, detoxifying, membrane-stabilizing and tissue-protective actions, as evidenced by the study of individual authors [12].

Thus, Carbacetam is accompanied by a systemic effect on the body and is able to significantly reduce the violation of the absorption function of the small intestine in conditions of acute blood loss complicated by ischemia-reperfusion of the limb, that requires further in-depth study.

Conclusions. 1. As a result of two-hour ischemia and reperfusion of the limb compared to the control in the small intestine after 1 day, significantly decreased of absorption function of the small intestine, that after 7 days normalizes and reaches the level of the control group. Under condition of acute blood loss, the studied indicator also decreases

significantly after 1 day, increases up to 14 days, but does not reach the level of control. Complications of acute blood loss by ischemia-reperfusion of the limb causes a significant decrease of absorption function of the small intestine after 1 day of the experiment. The intensity of disorders is statistically significantly higher compared to other experimental groups.

2. The use of Carbacetam for 7-14 days in the reperfusion period for animals with acute blood loss complicated by ischemia-reperfusion of the limb, compared with animals without correction, causes a significant increase in the absorption function of the small intestine, indicating a protective effect of the drug in simulated pathology.

Prospects for further research. In the future, it is advisable to study the effect of acute blood loss and ischemia-reperfusion of the limb on other functions of the small intestine and evaluate the effectiveness of Carbacetam in these conditions.

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