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VIOLATIONS OF ANTIOXIDANT-PROOXIDANT SPLEEN INDEX AND CERULOPLASMIN CONTENT IN SERUM DURING ACUTE BLOOD LOSS, COMPLICATED BY ISCHEMIA-REPERFUSION OF LIMB AND THE EFFECTIVENESS OF ITS CORRECTION WITH CARBACETAM

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Abstract

Antioxidant-prooxidant imbalance is one of the key mechanisms of acute blood loss and ischemic-reperfusion syndrome. Under these conditions, the level of ceruloplasmin (CP) plays an important role, enhancing antioxidant protection and promoting hematopoiesis, exhibiting ferrooxidase action. However, in this case, the state of antioxidant-prooxidant balance in the spleen and the content of CP in the serum is studied insufficiently, thus there is no data on the effectiveness of carbacetam, which revealed a pronounced protective effect on antioxidant protection of the spleen.

Objective of research: to determine the dynamics of the antioxidant-prooxidant index of the spleen and the content of CP in the serum in acute blood loss complicated by ischemia-reperfusion of the limb, and to evaluate the effectiveness of carbacetam in correction of the identified disorders.

Materials of the research and their discussion. 108 nonlinear male rats weighing 200–220 g were used in the experiments. All experiments were performed under thiopental-

sodium anesthesia. In animals, limb ischemia-reperfusion and acute blood loss were modeled separately and in one of the groups these injuries were combined. In a separate group, the detected disorders were corrected with carbacetam. After 1 and 2 hours, as well as after 1, 7 and 14 days in the spleen of experimental animals was determined content of reagents to thiobarbituric acid and catalase activity, and according to these results the antioxidant-prooxidant index (API) was calculated and was determined the content of CP in serum.

Results of the research and their discussion. Modeling of acute blood loss, complicated by ischemia-reperfusion of the limb, contributed to a decrease in the value of API in the spleen, which indicates a shift in antioxidant-prooxidation balance towards the dominance of prooxidant mechanisms. Under these conditions, the content of CP in the blood serum increases significantly, which on the one hand compensates the lack of antioxidant protection, on the other hand indicates on the activation of the mechanisms of the body's systemic response to inflammation. The use of carbacetam helped to shift the antioxidant-prooxidant balance of the spleen towards the dominance of antioxidant mechanisms and to reduction of the CP content in the serum, which indicates on a pronounced antioxidant and anti-inflammatory effect of the drug, as well as its prospects in cases connected with hemic hypoxia and ischemia-reperfusion syndrome.

Conclusions: Complication of acute blood loss by limb ischemia-reperfusion contributes to the shift of antioxidant-prooxidant balance of the spleen towards the dominance of prooxidant mechanisms with a simultaneous increase in the content of CP of serum. The use of carbacetam helps to increase the value of API and reduce the content of CP in the serum.

Key words: acute blood loss; ischemia-reperfusion of the limb; spleen; antioxidant-prooxidant balance; ceruloplasmin; carbacetam.

Introduction. Acute blood loss from extremities is one of the most serious complications of modern combat trauma. In response to the massive loss of blood in the body, with the need to maintain its viability, a lot of compensatory mechanisms are activated, including the restoration of the circulating blood volume and the maintenance of systemic blood pressure and the restoration of lost red blood cells. In these processes, an important role is played by the spleen, which during the period of embryogenesis is an organ of hematopoiesis [11]. The process of hematopoiesis in the spleen continues under conditions of acute blood loss [7] and other extreme conditions and pathological processes [13-15]. In addition, up to 15.5% of circulating blood volume, 30-50% of all platelets and a part of the

neutrophils that later can enter the peripheral blood stream during bleeding and compensate the volume and cell composition of lost blood are deposited in the vascular network of the spleen [16].

One of the key pathogenic mechanisms of acute blood loss is the development of hypoxia with increased formation of reactive oxygen species and the initiation of free radical oxidation, which leads to degradation of cell membranes and is a trigger for the development of multiple organ failure. However, the balance of pro- and antioxidant mechanisms in the spleen under conditions of acute blood loss has not been studied enough. There are no data on the dynamics of the content of ceruloplasmin (CP) in the blood serum, which is characterized by ferrooxidase action [12]. Trivalent iron, which is formed under the action of CP, is incorporated into the apotransferrin molecule and is used for heme synthesis.

It is known that from the battlefield to the next stages of evacuation, the wounded with massive bleeding from the extremities can be delivered with a hemostatic tourniquet, which causes complete ischemia of the extremity, the maximum safe period of which is 2 hours [18]. However, the role of two-hour ischemia-reperfusion of the limb in the pathogenesis of disorders in the spleen due to acute blood loss has not been studied. There are no data on the effectiveness of carbacetam under these conditions, which has an antioxidant effect in such cases [6].

The purpose of the work: to determine the dynamics of the antioxidant-prooxidant index of the spleen and the content of CP in the serum in conditions of acute blood loss complicated by ischemia-reperfusion of the limb, and to evaluate the effectiveness of carbacetam in the correction of identified disorders.

Materials and methods. 108 nonlinear male rats weighing 200-220 g were used in the experiments. All experiments were performed in accordance with the rules of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (European Convention, 1986).

The experimental animals were divided into five groups: control and four experimental (6 rats per group). All interventions were performed with sodium- thiopepntal anesthesia (40 mg/kg- per body weight). In the first experimental group, the animals were modeled ischemia-reperfusion of the limb by applying the tourniquet proximally on the left leg for 120 minutes A strip of elastic harness "SWAT-T" (USA) 10 mm wide was used. This tourniquet has minimal adverse effects on the underlying tissues and provides permanent complete ischemia during the experiment [19]. In the second experimental group was modeled acute blood loss of 20-22% of the circulating blood volume by crossing the femoral

vein. In the third experimental group, these injuries were combined. In the fourth experimental group, to animals with acute blood loss complicated by ischemia-reperfusion of the limb injected intraperitoneally carbacetam (LM Litvinenko Institute of Physical-Organic Chemistry and Coal Chemistry of the National Academy of Sciences of Ukraine, Kyiv) at a dose of 5 mg per kilogram of the animal weight. [6].

Under the influence of thiopental-sodium anesthesia, animals of the first, second and third experimental groups were recovered from the experiment after 1 and 2 hours, as well as after 1, 7 and 14 days, animals of the fourth experimental group - after 7 and 14 days. In the control group, the animals were only anesthetized using an equivalent dose of sodium thiopental, a tourniquet was applied without cessation of blood flow and subsequently recovered from the experiment after 2 hours.

The spleen was taken for additional investigation, its homogenate was used to determine the content of reagents for thiobarbituric acid (TBA-active LP(lipid peroxidation) products) - one of the main screening indicators of lipid peroxidation activity [3] and catalase activity - a key component of the enzymatic link of antioxidant protection [9]. Based on these data, the antioxidant-prooxidant index (API = catalase activity / content of TBA-active LP products) was calculated [5]. The content of CP in the blood serum was determined by the method described in [4].

The probability of differences between the control and experimental groups was assessed using the nonparametric Mann-Whitney test.

The results of the study and their discussion. The study showed that the value of API of the spleen (Table 1) under the conditions of modeling only ischemia-reperfusion of the limb increased while comparing with the control, that became statistically significant starting from 2 h of the experiment (41.4%, $p< 0.05$). The indicator reached its maximum after 1 day of the experiment (2.14 times, $p<0.05$), was decreasing till day 14, but remained statistically significantly higher than the control level (61.0%, $p<0.05$). Simulation of acute blood loss, in contrast, was accompanied by a significant decrease in the value of API in the spleen, also starting from 2 h of the experiment (by 35.5%, $p<0,05$). The indicator reached its minimum after 1 day and remained at the same level until day 7 (by 54.3 and 55.7%, respectively, $p<0.05$). By day 14, the value of API in this group increased, but continued to be 44.1% lower than the control level ($p<0.05$). Complications of acute blood loss by ischemia-reperfusion of the limb caused an even greater decrease in the value of API in the spleen compared with control. After day 1, the indicator became statistically significantly lower than the control level (41.4%, $p<0.05$). After 1 and 7 days, the indicator reached a minimum value (85.2 and

79.8%, p <0.05, respectively) and increased by day 14, but was 73.0% lower than the control level (p <0,05).

Table 1 - The value of API (AU(arbitrary units)) of the spleen after acute blood loss complicated by ischemia-reperfusion of the limb (Me (LQ; UQ) - median (lower and upper quartile))

Experimental group	Period of reperfusion				
	1 h	2 h	Day 1	Day 7	Day 14
Control = 57,45 (50,64; 58,89) (n=6)					
Group 1 Ischemia-reperfusion	57,6 (51,2;65,1) (n=6)	77,7* (67,1;82,0) (n=6)	117,4* (107,4;120,4) (n=6)	105,3* (101,4;115,9) (n=6)	88,5* (67,9;106,2) (n=6)
Group 2 Bleeding	55,8 (51,8;61,0) (n=6)	35,4* (34,1; 37,1) (n=6)	25,1* (19,3; 26,5) (n=6)	24,3* (20,7; 25,3) (n=6)	30,7* (28,0; 39,0) (n=6)
Group 3 Ischemia-reperfusion+ bleeding	32,7* (27,2; 37,5) (n=6)	15,2* (14,1; 17,5) (n=6)	8,1* (7,7; 11,3) (n=6)	11,1* (9,8;14,7) (n=6)	14,8* (12,8; 17,3) (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. Here i in table. 2:
1.* - differences with respect to the control group are statistically significant (p<0,05);
2. p_{1-2} - the probability of differences between experimental groups 1 and 2;
3. p_{1-3} - the probability of differences between experimental groups 1 and 3;
4. p_{2-3} - the probability of differences between experimental groups 2 and 3

Comparison of experimental groups showed that after 1 h the value of API was significantly lower in experimental group 3, in which we combined acute blood loss and ischemia-reperfusion of the limb while comparing with other experimental groups (56.8 and 41.4%, p_{1-3}<0.05, p_{2-3}<0.05 respectively). Subsequently, starting from the 2 h of the experiment, with increasing severity of the disorder, was observed slight decrease of API value (p_{1-2}<0,05, p_{1-3}<0,05, p_{2-3}<0,05).

Therefore, the content of CP in the serum (Table 2) under the conditions of modeling only the ischemia-reperfusion of the limb compared with the control group was statistically significantly increasing starting from 2 h of the experiment (24.4%, p<0,05), reaching a maximum after 1 and 7 days (40,0 and 35,0%, p<0,05 respectively) and returned to the control level (p>0,05) to the day 14. Blood loss was also accompanied by an even greater increase of the studied indicator, starting from the 2 h of the experiment (by 35,9%, p<0,05).

Table 2 - The content of CP (mg/l^{-1}) in blood serum after acute blood loss complicated by ischemia-reperfusion of the limb (Me (LQ; UQ) - median (lower and upper quartiles))

Experimental group	Period of reperfusion				
	1 h	2 h	Day 1	Day 7	Day 14
Control = 3,40 (3,19; 3,86) (n=6)					
Group 1 Ischemia-reperfusion	3,43 (3,19;3,54) (n=6)	4,23* (4,06;4,42) (n=6)	4,76* (4,69;5,16) (n=6)	4,59* (4,27;5,34) (n=6)	3,70 (3,46;3,84) (n=6)
Group 2 Bleeding	3,85 3,75;3,93) (n=6)	4,62* (4,55; 5,02) (n=6)	6,60* (5,73; 6,71) (n=6)	6,11* (5,67; 6,24) (n=6)	5,51* (5,28; 5,62) (n=6)
Group 3 Ischemia-reperfusion+ bleeding	4,68* (4,61; 4,91) (n=6)	5,49* (5,04; 5,66) (n=6)	7,77* (7,49; 8,16) (n=6)	6,64* (6,50; 6,71) (n=6)	6,16* (5,97; 6,23) (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

The indicator reached a maximum after 1 and 7 days (by 94,1 and 79,7%, $p<0,05$, respectively) and decreased till the day 14, continuing to remain 62,1% higher than the control level ($p<0,05$). The combination of acute blood loss and ischemia-reperfusion of the limb caused the greatest increase in serum CP, starting from 1 h of the experiment with a maximum after 1 day (in 2,28 times, $p<0,05$). Even after 14 days, the indicator continued to exceed the control level by 81,2% ($p<0,05$).

Comparison of experimental groups showed that after 1 h, 1, 7 and 14 days with increasing severity of the disorder, the content of CP in the serum was increasing ($p_{1-2}<0,05$, $p_{1-3}<0,05$, $p_{2-3}<0,05$).

The use of carbacetam was accompanied by a significant increase in the value of API compared with animals without correction (Fig. 1). After 7 days of medication usage, the rate increased by 45,9% ($p<0,05$), after 14 days - by 40,5% ($p<0,05$). The drug caused a decrease in the content of CP in the serum: after 7 days of use - by 23,0% ($p<0,05$), after 14 days - by 19,8% ($p<0,05$).

The obtained results indicate that after modeling only the ischemia-reperfusion of the limb, the antioxidant-prooxidant balance in the tissues of the spleen shifted towards the dominance of antioxidant mechanisms. Similar deviations were observed in works of other authors [8], which has compensatory nature in response to toxins coming from the ischemic limb after its reperfusion. Blood loss causes a significant decrease in the value of API in the

spleen, which indicates on a shift in antioxidant-prooxidant balance towards the dominance of prooxidant mechanisms and depletion of antioxidant protection and is a typical reaction to acute blood loss and was described by other scientists [2, 10]. At the same time, the combination of acute blood loss and ischemia-reperfusion of the limb causes the greatest decrease of the studied indicator, which, starting from 2 h of the experiment was statistically significantly lower than in other experimental groups. The obtained result indicates that complete ischemia of the limb for two hours significantly deepens the intensity of lipid peroxidation processes and contributes to greater depletion of antioxidant protection of the spleen in cases of acute blood loss, which may affect key functions of the spleen.

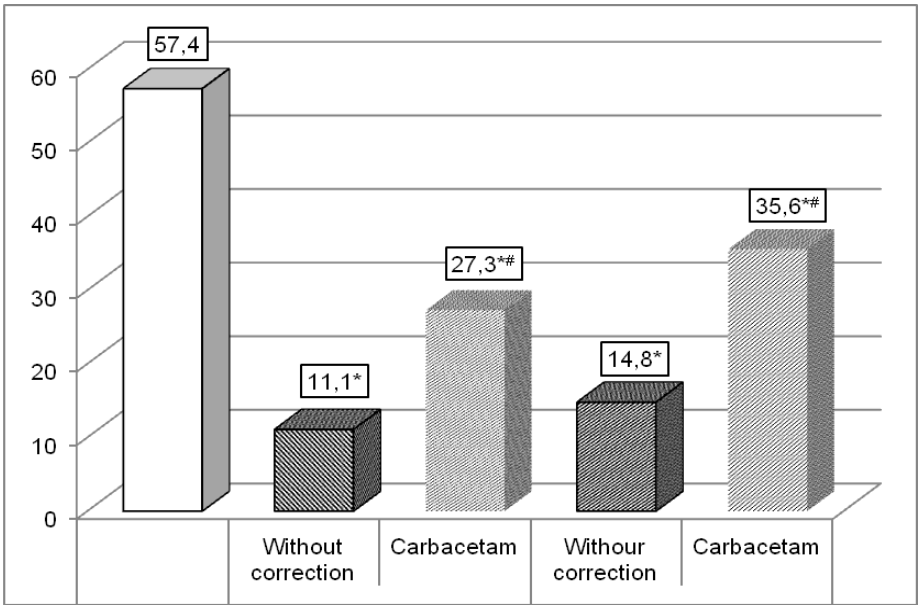

Figure 1 - The effect of carbacetam on the value of API of the spleen (AU) after acute blood loss complicated by ischemia-reperfusion of the limb

Note. Here and in Fig. 2: * - differences relative to the control group are statistically significant, $p<0,05$; # - differences relative to the group without correction are statistically significant, $p<0,05$

Whereas ischemia-reperfusion of the limb will increase the content of CP in the serum during the period from 2 hours to 7 days of the experiment. Taking into concideration the fact that CP is one of the main antioxidants of blood serum [1], the result is also an evidence of a systemic compensatory response to the prooxidant factors in the ischemic limb after its reperfusion. At the same time, after acute blood loss, this indicator increases even more with its maximum at day 1 and until day 14 it does not reach the level of control. In light of this, the increase of CP content on the one hand is a consequence of the activation of lipid peroxidation due to hypoxia and generation of reactive oxygen species. On the other hand,

damage of cell membranes affected by reactive oxygen species promotes migration and activation of leukocytes, that deepens oxidative stress and promotes formation of pro-inflammatory mediators. The latter are known to activate the transcription of the CP gene [20]. In addition, blood loss due to iron deficiency also promotes the activation of the transcription of the CP gene by hypoxia inducible factor 1 (HIF-1), which simultaneously activates the genes of erythropoietin, heme oxygenase-1, transferrin and its receptor [17].

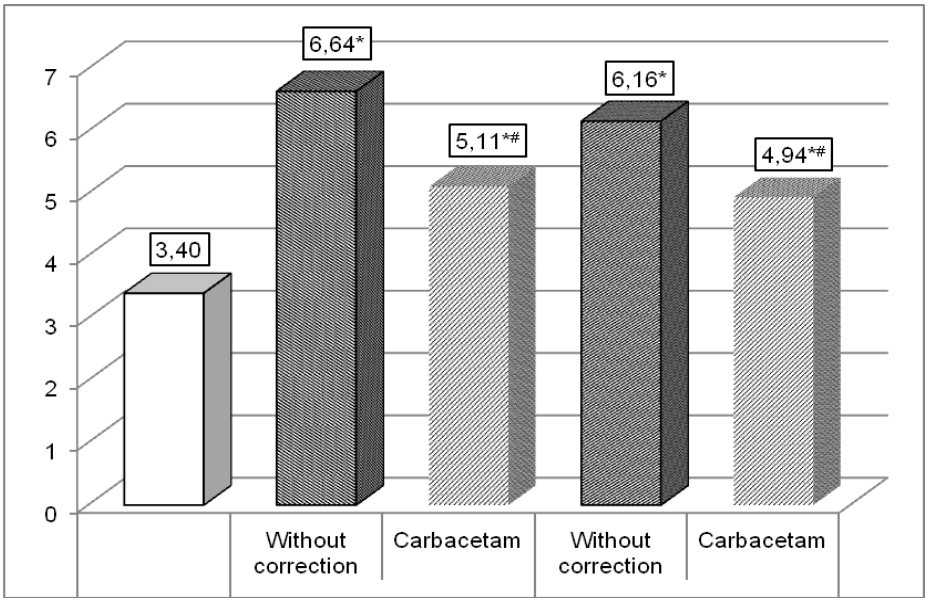

Figure 2 - The effect of carbacetam on the content of CP in the serum ($mg \cdot l^{-1}$) after acute blood loss complicated by ischemia-reperfusion of the limb.

After the complication of acute blood loss by ischemia-reperfusion of the limb, there is probably a layering of the mechanisms of CP synthesis due to these effects. As a result, the content of CP in the serum in this experimental group at all observation periods becomes significantly higher compared to other experimental groups.

Thus, acute blood loss complicated by ischemia-reperfusion of the limb contributes to a significant shift of the antioxidant-prooxidant ratio towards the dominance of prooxidant mechanisms and increases CP formation, which has a compensatory character and at the same time indicates on the development of systemic body response to inflammation.

The use of carbacetam promotes the shift of the antioxidant-prooxidant balance towards the dominance of antioxidant mechanisms and reduces the intensity of CP formation, which points on a pronounced antioxidant and anti-inflammatory effect of the drug and the prospects for its use in hemic hypoxia and ischemic reperfusion.

Conclusions 1. Complication of acute blood loss by ischemia-reperfusion of the limb contributes to the shift of antioxidant-prooxidant balance of the spleen towards the dominance

of prooxidant mechanisms, which is manifested by a significant decrease in the value of API, starting from second hour of the experiment. At the same time the content of CP in blood serum increases, which is compensatory in nature and indicates the activation of the body's systemic response to inflammation.

2. The use of carbacetam helps to shift the balance of antioxidant-prooxidant mechanisms of the spleen in the direction of strengthening of antioxidant protection and, at the same time, is accompanied by a decrease in the content of CP in the serum.

Prospects for further research. It is promising to deepen the study of markers of the body's systemic response to inflammation, their dynamics and means of correction in response to acute blood loss complicated by ischemia-reperfusion of the limb.

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