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## SIGNIFICANCE OF LIPID PEROXIDATION PROCESSES IN RENAL EXCRETORY DYSFUNCTION IN CASES OF ACUTE BLOOD LOSS COMPLICATED BY LIMB ISCHEMIA-REPERFUSION AND ITS CORRECTION

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### Abstract

**Introduction.** Activation of lipid peroxidation processes, which are exacerbated in cases of limb ischemia-reperfusion associated with temporary application of a tourniquet is a key mechanism of acute blood loss. However, its significance in the pathogenesis of renal dysfunction is understudied. The information on the effectiveness of carbacetam administration under these conditions, which has an antioxidant effect, is lacking.

**Objective.** The aim of the research was to investigate the significance of lipid peroxidation processes in renal excretory dysfunction in cases of acute blood loss complicated by limb ischemia-reperfusion, and to evaluate the effectiveness of carbacetam for correction of these disorders.

**Materials and methods.** The experiments were performed on 108 nonlinear male rats weighing 160-180 g. All animals were divided into five groups: a control group and four experimental groups (6 rats in each). The first experimental group comprised the animals anesthetized with thiopental sodium, in which a two-hour limb ischemia was simulated with further reperfusion. In the second experimental group the acute blood loss in the amount of

20-22% of a circulating blood volume was simulated to the anesthetized animals by femoral vein dissection. In the third experimental group these injuries were combined. In the fourth experimental group the animals with acute blood loss complicated by limb ischemia-reperfusion were administered with carbacetam intraperitoneally at a dose of 5 mg per kilogram of animal weight for correction. In the control group, the animals were anesthetized with equivalent dose of thiopental sodium; a tourniquet was applied for 2 hours without stopping the blood flow, and subsequently studied in 1 hour.

In 1 and 2 hours, as well as in 1, 7 and 14 days, the renal function was assessed in the experimental animals by means of the water loading test. Urine was collected for 2 hours and diuresis was determined. After urine collection, the rats were anesthetized with thiopental sodium and taken out of the experiment by total bloodletting from the heart. Concentrations of sodium in the urine and serum, as well as protein in the urine were determined. According to these data, sodium and protein excretion was assessed. Additionally, the content of reagents with regard to thiobarbituric acid –TBA-active products of lipid peroxidation, was determined in the renal medulla.

**Results and discussion.** In cases of limb ischemia-reperfusion, acute blood loss and their combination, the content of TBA-active products of lipid peroxidation in the renal medulla increased the most in 1 day. Up to the 14<sup>th</sup> day, the index decreased, but did not reach the level of the control group and was significantly higher in the group of animals with acute blood loss complicated by limb ischemia-reperfusion. In cases of the simulated injuries, increased sodium and protein excretion was evidenced compared to the control group.

The highest increase in these parameters corresponded to the maximum activation of lipid peroxidation in the renal medulla in relation to the terms, and according to the amplitude increased from the group with limb ischemia-reperfusion to the group with acute blood loss and then to the group with combination of these disorders. This pattern was manifested the most significantly in 7-14 days of the reperfusion period. In case of carbacetam administration, the content of TBA-active products of lipid peroxidation and sodium excretion in the renal medulla significantly decreased in 7-14 days, compared to the animals without correction.

Protein excretion decreased in 14 days of carbacetam administration. Taking into account the pronounced antioxidant properties of carbacetam, the attained result proved the significance of activation of lipid peroxidation processes in cases of kidney damage after limb ischemia-reperfusion, acute blood loss and their combination.

**Conclusions.** With underlying limb ischemia-reperfusion, acute blood loss, and their

combination, the sodium and protein excretion increase compare to the control group. The highest increase of these parameterstakes placein 1 day of the reperfusion period thatcorresponds to the maximal accumulation of TBA-active products of lipid peroxidation in the renal medulla in relation to the terms.

The amplitude of changes in the studied parameters increases from the group of animals with limb ischemia-reperfusion to that with acute blood loss, and then to the group with their combination that is the most pronounced in 7-14 days of the reperfusion period.

The carbacetam administration (at a dose of 5 mg per kilogram of animal body weight) causes a significant nephroprotective effect and up to the 14<sup>th</sup> day leads to a significant decrease in the content of TBA-active products of lipid peroxidation, reduced sodium and protein excretion in the renal medulla.

**Keywords: blood loss; limb ischemia-reperfusion; kidney; lipid peroxidation; sodium and protein excretion.**

### **Introduction**

The frequency of terrorist attacks and local armed conflicts has significantly increased recently [14]. The firearms and explosive devices employment causes limb damage predominantly, which is often complicated by massive blood loss. In 80-90% of cases, blood loss is the main cause of death and is one of the key causes of preventable death [9]. Applying of haemostatic tourniquets that completely stop the arterial blood flow is established to be an effective means for limb external bleeding [13].

However, according to literature, a complete exsanguination of the limb within two hours can cause nerves and blood vessels damage with the tourniquet, as well as myonecrosis, rhabdomyolysis, deep vein thrombosis [5, 10]. However, after its reperfusion even more damages of the limb soft tissues may take place. Revascularization of the ischemic limb causes a number of systemic problems characterized by metabolic acidosis, hyperkalemia and myoglobinemia [15]. The content of superoxide radical increases [12] and the total antioxidant defence decreases [11] in the homogenates of muscles, internal organs and blood plasma. All this has a negative effect on the functional state of internal organs, the kidneys in particular [7]. Moreover, it was proved that limb reperfusion after two-hour ischemia with underlying closed abdominal injury and blood loss mayexacerbate the manifestations of multisystem failure [3, 6].

The key mechanism of acute blood loss is the activation of lipid peroxidation processes. The latter worsens in cases of limb ischemia-reperfusion associated with temporal

tourniquet. However, its role in the pathogenesis of renal dysfunction has not been studied sufficiently. The information on the carbacetam efficacy under these conditions, which has antioxidant, immunomodulatory, detoxifying, membrane-stabilizing and tissue-protective effects, is lacking [2].

### **Objective**

The aim of the research was to investigate the significance of lipid peroxidation processes in renal excretory dysfunction in cases of acute blood loss complicated by limb ischemia-reperfusion, and to evaluate the effectiveness of carbacetam in the correction of these disorders.

### **Materials and methods**

The experiments were performed on 108 nonlinear male rats weighing 160-180 g following the rules of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (European Convention, 1984).

All animals were divided into five groups: a control group and four experimental groups (6 rats in each). The first experimental group comprised the animals, for which limb ischemia-reperfusion was simulated. The animals were anesthetized with thiopental sodium (40 mg·kg<sup>-1</sup> of body weight) and a tourniquet was applied proximally to the left paw for 120 min. A 10 mm strip of elastic tourniquet “SWAT-T” (USA) was used, which corresponded to the width of the tourniquet applied to an adult’s thigh. The tourniquet was tightened according to the pressure indicator fixed on it, which stops the blood flow, that was additionally confirmed by the rheographic method. For the anesthetized animals of the second experimental group acute blood loss was simulated by femoral vein dissection. After the volume of blood loss amounted to 20-22% of the circulating blood volume, haemostasis was simulated for the animals. In the third experimental group, these injuries were combined. In the fourth experimental group the animals with acute blood loss complicated by limb ischemia-reperfusion were administered with carbacetam (L. M. Litvinenko Institute of Physical-Organic Chemistry and Coal Chemistry of the NAS of Ukraine, Donetsk) intraperitoneally at a dose of 5 mg per kilogram of animal body weight for correction of the disorders.

For the control group of animals anesthetized with the equivalent dose of thiopental sodium a tourniquet was applied for 2 hours without stopping the blood flow, and subsequently studied in 1 hour. In 1 and 2 hours, as well as in 1, 7 and 14 days, the renal function was assessed in the experimental animals by means of the water loading test [4]. Heated to 30 °C tap water in the amount of 5% of the animal’s body weight was introduced

into the stomach via a metal tube. Urine was collected for 2 hours and diuresis was determined. After urine collection, the rats were anesthetized with thiopental sodium and taken out of the experiment by total bloodletting from the heart that at the early period of the experiment was equal to 3 and 4 hours of reperfusion. The concentrations of sodium in the urine and blood serum, as well as protein in the urine were determined by the unified method using the biochemical analyser "Humalazer 2000".

According to the attained results, sodium and protein excretion was assessed. Moreover, the content of reagents with regard to thiobarbituric acid – TBA-active products of lipid peroxidation relating to screening intensification parameters of lipid peroxidation, was determined in the renal medulla [1].

The statistical significance of differences between the control and experimental groups was evaluated using the non-parametric Mann-Whitney test. The differences were true at statistical significance of the null hypothesis less than 5% ( $p < 0.05$ ).

### **Results and Discussion**

Already in 1 hour of reperfusion after two-hour limb ischemia, the content of TBA-active products of lipid peroxidation increased in the renal medulla (by 63.6%,  $p < 0.05$ ) compare to the control (Table 1, Fig. 1). Later, in 1 hour the index reached its maximal level (209.9% of the control level,  $p < 0.05$ ) and decreased up to the 14<sup>th</sup> day, not reaching the control, but was statistically significantly lower compare to the previous follow-up period ( $p < 0.05$ ).

In cases of acute blood loss, the content of TBA-active products of lipid peroxidation in the renal medulla increased even more with a maximal level in 1 day of the post-traumatic period (in 3.6 times compare to the control,  $p < 0.05$ ). Up to the 14<sup>th</sup> day, the parameter decreased, but still was in 2.31 times higher than the control ( $p < 0.05$ ). At all times of the reperfusion period, the content of TBA-active products of lipid peroxidation in the renal medulla was statistically significantly higher ( $p_{1-2} < 0.05$ ).

The complication of acute blood loss by limb ischemia-reperfusion increased the intensity of lipid peroxidation in the renal medulla more than that in case of acute blood loss: in 1 hour, 7 and 14 days, the content of TBA-active products of lipid peroxidation was significantly higher ( $p_{2-3} < 0.05$ ). The maximal level of the studied parameter in this group was in 3.57 times higher than the control ( $p < 0.05$ ) in 1 day of reperfusion; in 14 days it slightly decreased, but was still higher than the control in 2.52 times ( $p < 0.05$ ).

**Table 1.** Content of TBA-active products of lipid peroxidation (mcmol·kg<sup>-1</sup>) in the renal medulla after acute blood loss complicated by limb ischemia-reperfusion ((Me (LQ; UQ) – median (lower and upper quartile))

Experimental group	Reperfusion period				
	3 hours	4 hours	1 day	7 days	14 days
Control = 1.21 (1.14; 1.24) (n=6)					
<i>Group 1</i> Ischemia-reperfusion	1.98* (1.90;2.13) (n=6)	2.54* (2.33;2.62) (n=6)	2.20* (2.08;2.27) (n=6)	1.59* (1.55;1.70) (n=6)	1.45* (1.43;1.53) (n=6)
<i>Group 2</i> Blood loss	2.85* (2.63;2.93) (n=6)	3.55* (3.35; 3.62) (n=6)	4.35* (4.08; 4.70) (n=6)	3.38* (3.29; 3.46) (n=6)	2.80* (2.66; 2.94) (n=6)
<i>Group 3</i> Ischemia-reperfusion+ blood loss	3.28* (3.09; 3.46) (n=6)	3.78* (3.52; 3.91) (n=6)	4.32* (4.25; 4.51) (n=6)	3.90* (3.82; 4.00) (n=6)	3.05* (2.98; 3.11) (n=6)
p1-2	<0.05	<0.05	<0.05	<0.05	<0.05
p1-3	<0.05	<0.05	<0.05	<0.05	<0.05
p2-3	<0.05	>0.05	>0.05	<0.05	<0.05

Notes. Herein and in Tables 2, 3:

\* – statistically significant differences concerning the control group (p<0.05);

p<sub>1-2</sub> – statistically significant differences between the study groups 1 and 2;

p<sub>1-3</sub> – statistically significant differences between the study groups 1 and 3;

p<sub>2-3</sub> – statistically significant differences between the study groups 2 and 3;

Thus, in cases of limb ischemia-reperfusion, acute blood loss and their combination, the intensification of the processes of lipid peroxidation in the renal medulla takes place. Taking into account a high sensitivity of epithelium of the descending and ascending nephron tubules, which are mainly located in the renal medulla, to the influence of reactive oxygen intermediates and free radicals, the detected damages do affect the excretion of sodium and protein ions. Sodium is the most active participant of osmotic dilution and urine concentration, and its reabsorption is the most significant process by volume and energy costs that absorbs about 80% of the energy produced in the kidney cells. Therefore, its increased excretion is the evidence of reabsorption disturbances in the proximal and distal tubules. Normally, the protein excretion is minimal. At the same time, it can increase mainly in cases of increased permeability of the glomerular filter, decreased tubular reabsorption, and intensified tissue dissolution [8].

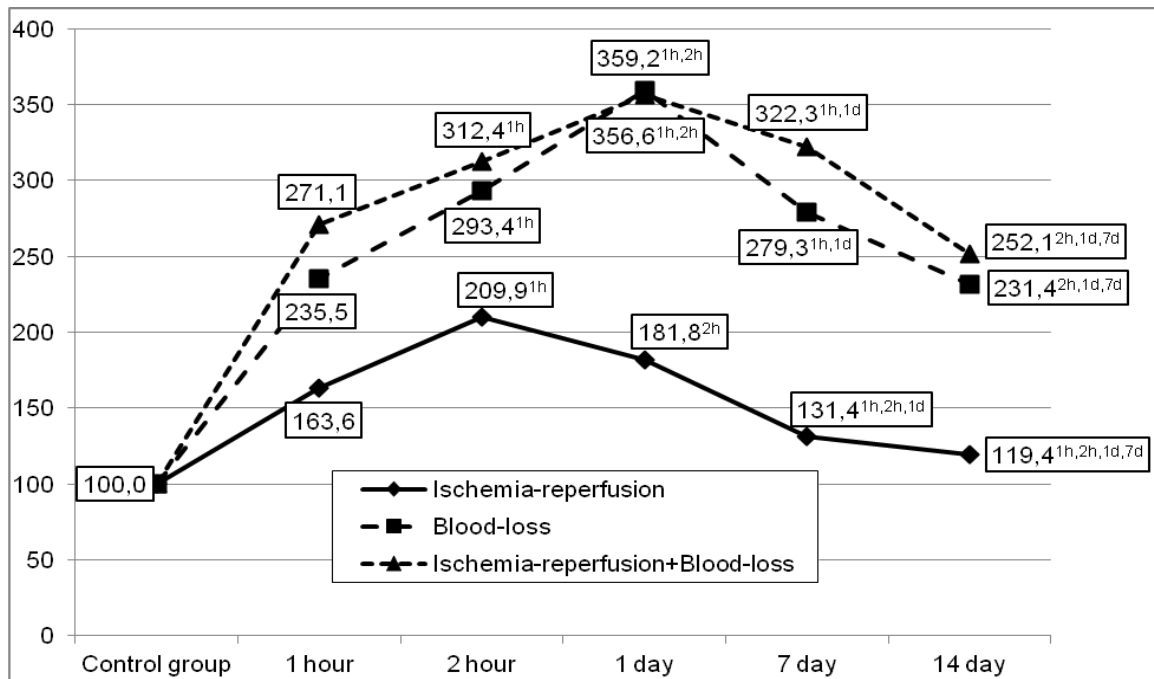


Fig. 1. Dynamics of TBA-active products of lipid peroxidation in the renal medulla (in percentage regarding the control) after acute blood loss complicated by limb ischemia-reperfusion

Note. Herein and on Fig. 2, 3:  
<sup>1h,2h,1d,7d</sup> – statistically significant differences concerning 1 hour, 2 hours, 1 day, 7 days ( $p < 0.05$ ).

The studies showed that the intensification of lipid peroxidation processes affected the intensity of sodium and protein excretion. In cases of limb ischemia-reperfusion, sodium excretion statistically significantly increased in 2 hours, 1 and 3 days of the reperfusion period (58.1, 137.7, and 26.0% respectively,  $p < 0.05$ ) compare to the control (Table 2, Fig. 2). The index was normal up to the 14<sup>th</sup> day. After acute blood loss simulation, the sodium excretion was significantly higher than the control at all times of the reperfusion period ( $p < 0.05$ ) with a maximal level in 2.61 times ( $p < 0.05$ ) in 1 day. The index was significantly higher in 1 hour, 7 and 14 days compare to the group of animals with limb ischemia-reperfusion ( $p_{1-2} < 0.05$ ).

Similarly, sodium excretion was statistically significantly higher than the control after simulation of acute blood loss complicated by limb ischemia-reperfusion ( $p < 0.05$ ) as well. The maximal disorders severity took place also in 1 day of the reperfusion period. At all times of the follow-up, the parameters did not differ from those in the group of animals with acute blood loss, except for the 14<sup>th</sup> day, when the sodium excretion was significantly higher – by 25.5% ( $p_{2-3} < 0.05$ ).

**Table 2.** Urinary excretion of sodium ions ( $\text{mcmol}\cdot\text{min}^{-1}$ ) per 100 g of animal body weight after acute blood loss complicated by limb ischemia-reperfusion ((Me (LQ; UQ) – median (lower and upper quartile))

Experimental group	Reperfusion period				
	1 hour	2 hours	1 day	7 days	14 days
Control = 0.0277 (0.0276; 0.0283) (n=6)					
<i>Group 1</i> Ischemia-reperfusion	0.031 (0.030;0.032) (n=6)	0.044* (0.043;0.045) (n=6)	0.067* (0.063;0.068) (n=6)	0.035* (0.034;0.038) (n=6)	0.030 (0.029;0.031) (n=6)
<i>Group 2</i> Blood loss	0.039* (0.037;0.044) (n=6)	0.045* (0.040; 0.047) (n=6)	0.073* (0.071; 0.077) (n=6)	0.059* (0.056; 0.061) (n=6)	0.047* (0.042; 0.048) (n=6)
<i>Group 3</i> Ischemia-reperfusion+ blood loss	0.036* (0.034; 0.039) (n=6)	0.040* (0.038; 0.043) (n=6)	0.069* (0.067; 0.079) (n=6)	0.058* (0.057; 0.063) (n=6)	0.059* (0.058; 0.060) (n=6)
p1-2	<0.05	>0.05	<0.05	<0.05	<0.05
p1-3	<0.05	<0.05	>0.05	<0.05	<0.05
p2-3	<0.05	>0.05	>0.05	>0.05	<0.05

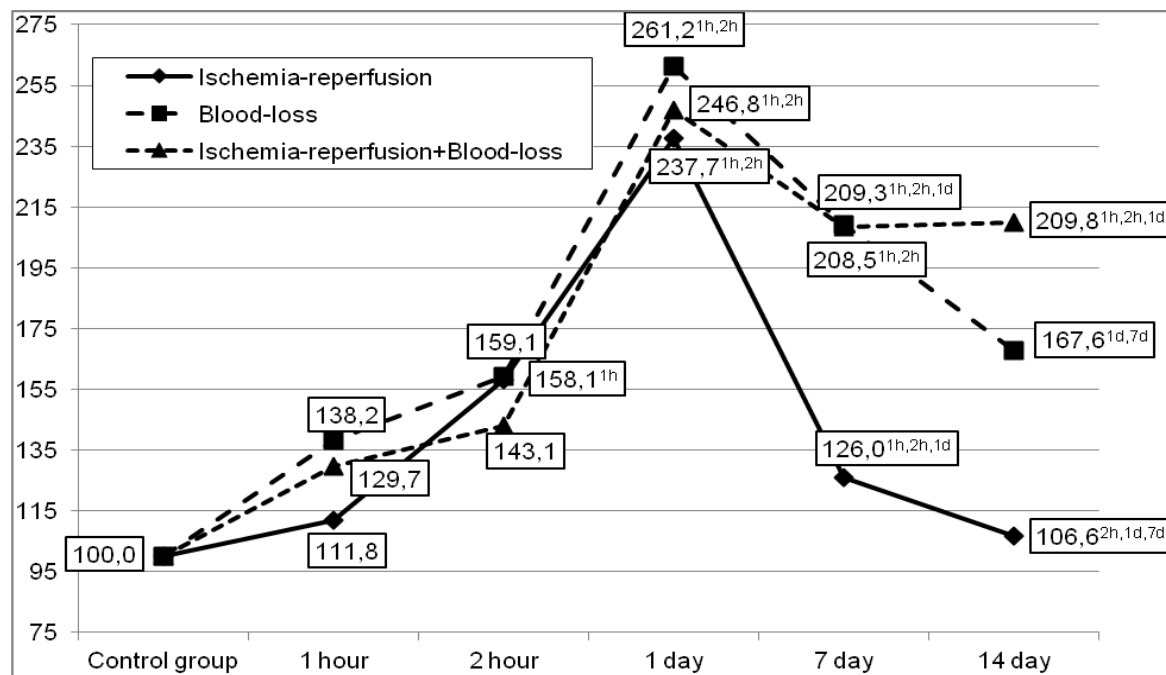


Fig. 2. Dynamics of sodium excretion (in percentage regarding the control) after acute blood loss complicated by limb ischemia-reperfusion

After the limb ischemia-reperfusion simulation, protein excretion increased up to 1 day compare to the control, and then decreased up to 14 days and reached the control level



( $p > 0.05$ ) (Table 3, Fig. 3). The result was statistically significant in 2 hours, 1 and 7 days of reperfusion (by 75.0, 161.5 and 190.1%, respectively,  $p < 0.05$ ). In cases of acute blood loss, protein excretion also increased up to 1 day, and then – decreased. The index at all times of reperfusion was statistically significantly higher compare to the control ( $p < 0.05$ ). In the group of animals with limb ischemia-reperfusion, protein excretion was by 26.5% lower ( $p_{1-2} < 0.05$ ) in 2 hours, but increased by 70.6% in 14 days ( $p_{1-2} < 0.05$ ).

**Table 3.** Urinary protein excretion ( $\text{mg} \cdot 2 \text{ h}^{-1}$ ) per 100 g of animal weight after acute blood loss complicated by limb ischemia-reperfusion ((Me (LQ; UQ) – median (lower and upper quartile))

Experimental group					
	1 hour	2 hours	1 day	7 days	14 days
Control = 0.013(0.010; 0.016) (n=6)					
<i>Group 1</i> Ischemia-reperfusion	0.023* (0.017;0.029) (n=6)	0.034* (0.031;0.039) (n=6)	0.038* (0.034;0.044) (n=6)	0.027* (0.022;0.032) (n=6)	0.014 (0.016;0.018) (n=6)
<i>Group 2</i> Blood loss	0.027* (0.024;0.029) (n=6)	0.025* (0.022; 0.027) (n=6)	0.042* (0.038; 0.048) (n=6)	0.032* (0.027; 0.035) (n=6)	0.027* (0.024; 0.032) (n=6)
<i>Group 3</i> Ischemia-reperfusion+ blood loss	0.026* (0.025; 0.030) (n=6)	0.030* (0.029; 0.033) (n=6)	0.038* (0.034; 0.041) (n=6)	0.030* (0.026; 0.033) (n=6)	0.032* (0.029; 0.034) (n=6)
p <sub>1-2</sub>	>0.05	<0.05	>0.05	>0.05	<0.05
p <sub>1-3</sub>	>0.05	>0.05	>0.05	>0.05	<0.05
p <sub>2-3</sub>	>0.05	>0.05	>0.05	>0.05	>0.05

In cases of a combination of acute blood loss and limb ischemia-reperfusion, protein excretion was also higher compare to the control at all follow-up period ( $p < 0.05$ ), reaching a maximal level in 1 day (292.5% of the control,  $p < 0.05$ ) and decreased afterwards. In the group of animals with limb ischemia-reperfusion, the index was statistically significantly higher in 14 days of the reperfusion period (by 88.2%,  $p_{1-3} < 0.05$ ) compare to the group of animals with acute blood loss, the differences were not statistically significant ( $p_{2-3} > 0.05$ ).

Thus, both limb ischemia-reperfusion and acute blood loss, as well as their combination were accompanied by increased sodium and protein excretion compare to the control group. Taking into account the underlying mechanism of the increase in these parameters, it can be assumed that in the experimental groups under the influence of

pathogens of limb ischemia-reperfusion (penetration of toxins from the ischemic muscles into the systemic circulation) and acute blood loss (renal hypoperfusion, hypoxia) the damage of glomerular and tubular apparatus of kidney takes place that leads to activation of lipid peroxidation processes and intensification of sodium and protein excretion. It should be noted that the highest increase in these parameters in relation to the terms corresponds to the maximal activation of lipid peroxidation in the renal medulla, and according to the amplitude it increases from the group of animals with limb ischemia-reperfusion to the group of animals with acute blood loss, and then to the group of animals with their combination. This pattern was evidenced the most in 7-14 days of the reperfusion period indicating a significant duration of the detected disorders as well as significance of intensification of lipid peroxidation in their manifestations.

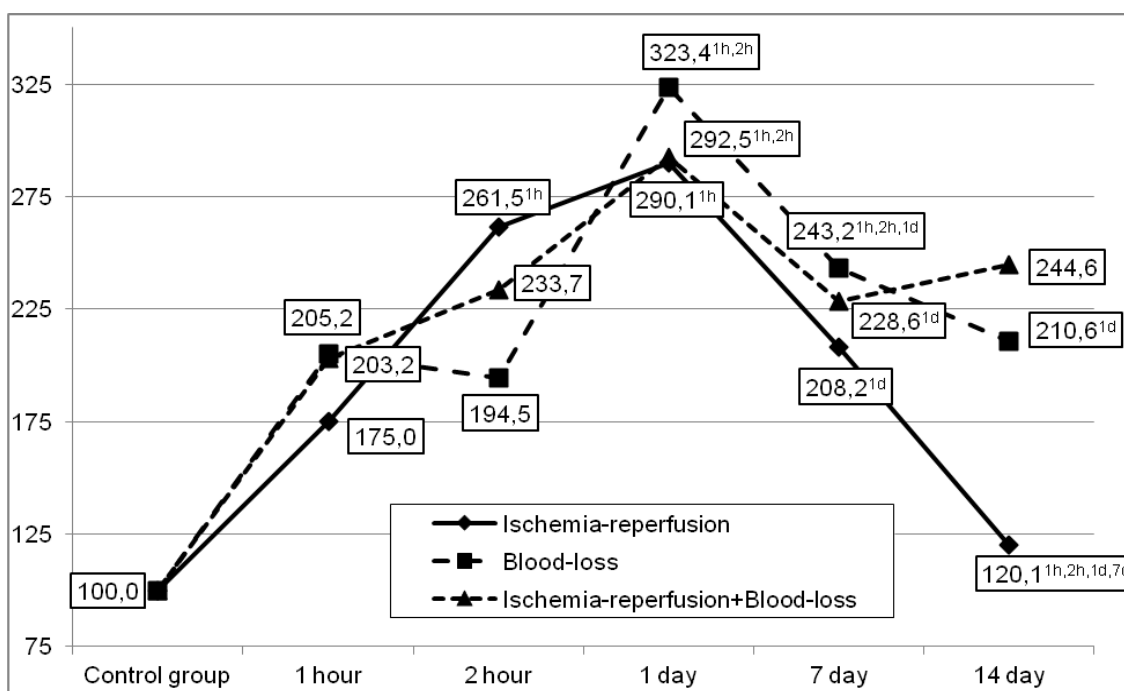


Fig. 3. Dynamics of urinary protein excretion (in percentage regarding the control) after acute blood loss complicated by limb ischemia-reperfusion

The attained results necessitated the use of medications with antioxidant properties for correction of the disorders. So, carbacetam was used. The studies proved (Fig. 4) that administration of carbacetam in to the renal medulla for 7 days significantly decreased the content of TBA-active products of lipid peroxidation (by 24.4%,  $p < 0.05$ ), and by 29.8% ( $p < 0.05$ ) in 14 days of administration, compare to the animals without correction.

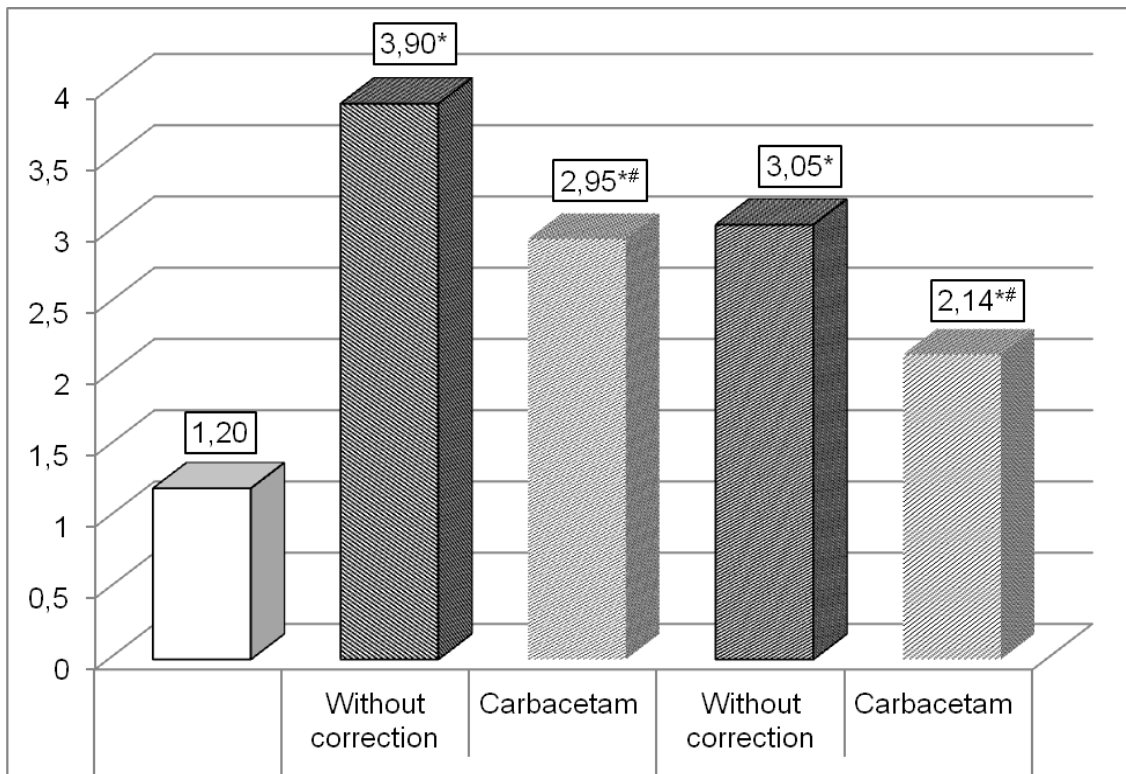


Fig. 4. Carbacetam effect on the content of TBA-active products of lipid peroxidation in the renal medulla (mcmol·kg<sup>-1</sup>) after acute blood loss complicated by ischemia-reperfusion of the limb.

Note. Herein and on Fig. 5, 6:

\* – statistically significant differences concerning the control group,  $p < 0.05$ ;

# – statistically significant differences concerning the group of animals without correction,  $p < 0.05$ .

In administration of carbacetam during the follow-up period, sodium excretion significantly decreased (Fig. 5) by 13.8% and 16.9% respectively,  $p < 0.05$ . The protein excretion significantly decreased in 14 days of carbacetam administration (by 25.0 %,  $p < 0.05$ ). Taking into account the pronounced antioxidant properties of carbacetam, the attained results proved the significance of activation of lipid peroxidation processes in cases of kidney damage with underlying limb ischemia-reperfusion, acute blood loss and their combination. Thus, administration of carbacetam is a promising nephroprotective agent for correction of post-haemorrhagic and ischemic-reperfusion kidney damages.

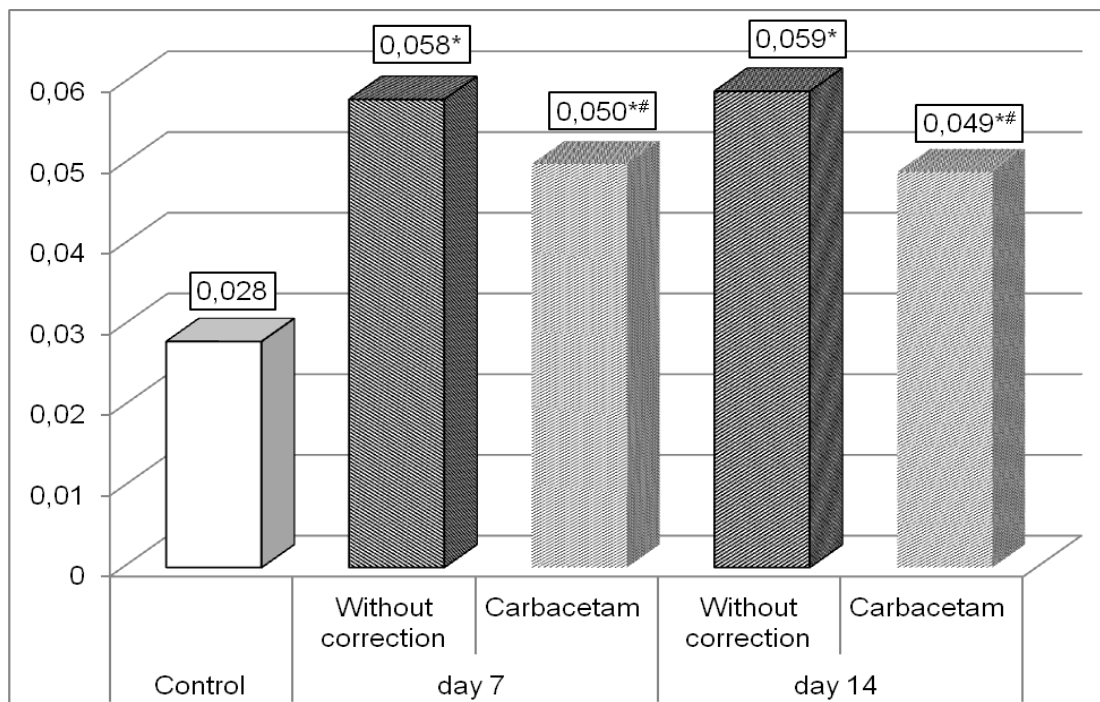


Fig. 5. Carbacetam effect on sodium excretion (mcmol·kg·100<sup>-1</sup>) after acute blood loss complicated by limb ischemia-reperfusion

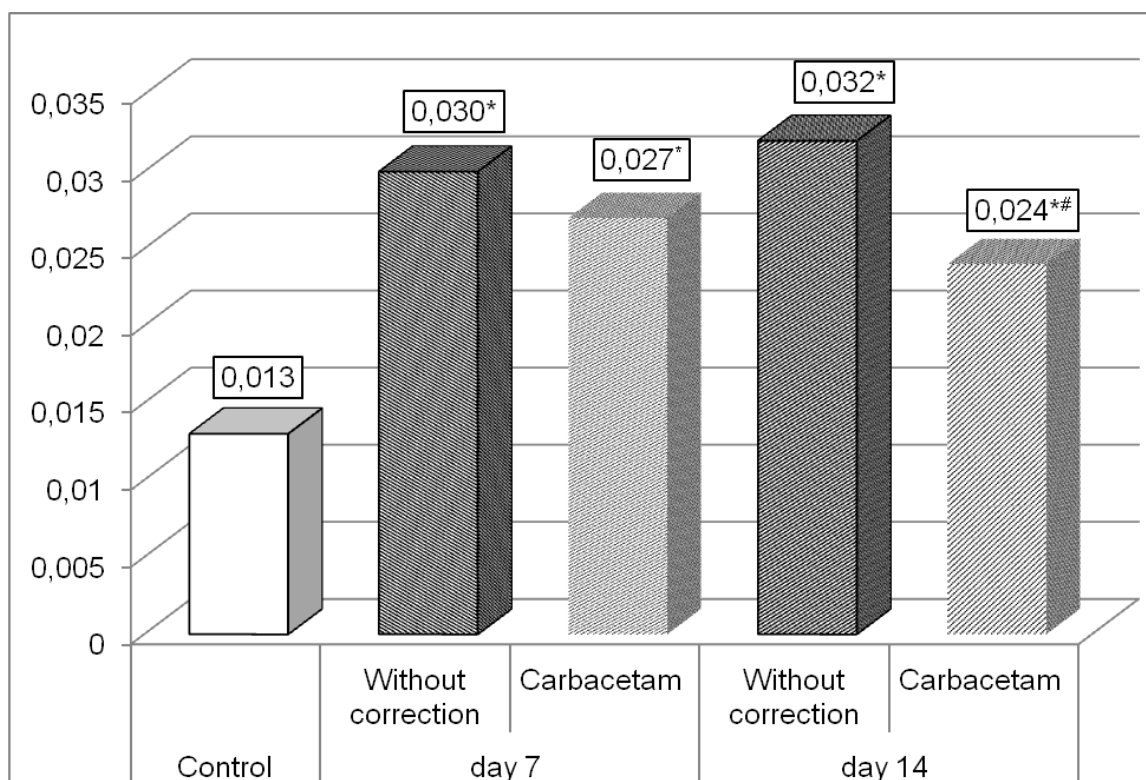


Fig. 6. Carbacetam effect on sodium excretion (mg·2 h<sup>-1</sup>·100<sup>-1</sup>) after acute blood loss complicated by limb ischemia-reperfusion

## Conclusions

With underlying limb ischemia-reperfusion, acute blood loss, and their combination, the processes of lipid peroxidation are intensified in the renal medulla, the sodium and protein excretion are increased compare to the control group. The highest increase of sodium and protein excretion takes place in 1 day of the reperfusion period, which in relation to the terms corresponds to the maximal accumulation of TBA-active products of lipid peroxidation in the renal medulla.

The amplitude of changes of the studied parameters increases from the group of animals with limb ischemia-reperfusion to the group of animals with acute blood loss, and then to the group of animals with their combination. The pattern is manifested the most in 7-14 days of the reperfusion period that is the evidence of summation of their negative effect.

The carbacetam administration at a dose of 5 mg per kilogram of animal body weight causes a significant nephroprotective effect and leads to a significant decrease in the content of TBA-active products of lipid peroxidation, sodium and protein excretion in the renal medulla up to 14 days. Taking into account pronounced antioxidant properties of carbacetam, the attained results have proved the significance of activation of lipid peroxidation processes in renal damage after limb ischemia-reperfusion, acute blood loss and their combinations.

## Further Research Prospects

A more profound study of the mechanisms of organ failure in cases of acute blood loss complicated by limb ischemia-reperfusion, as models of massive blood loss from a limb and a tourniquet applied for 2 hours, is perspective.

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