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PECULIARITIES OF IMPAIRMENTS OF ABSORPTIVE-EXCRETORY AND GLYCOGEN SYNTHESIS FUNCTIONS OF THE LIVER IN THE PRESENCE OF ACUTE BLOOD LOSS COMPLICATED BY LIMB ISCHEMIA-REPERFUSION AND THEIR CORRECTION BY CARBACETAM

I. I. Horban, A. A. Hudyma, R. V. Maksymiv, N. A. Dzhavadova

I. Ya. Horbachevsky Ternopil National Medical University

Abstract

Introduction. Under conditions of massive bleeding from the extremities, both in combat and in peacetime, the only means for salvation is applying tourniquet, which completely stops the arterial blood flow. It is believed that the maximum safe time for bleeding of the limb due to the use of the tourniquet is up to two hours. The systemic effect of acute blood loss complicated by ischemia-reperfusion of the limb on the liver, which is the main organ of detoxification of the body, remains almost unexplored.

Objective of research: to determine the effect of acute blood loss complicated by two-hour limb ischemia and its reperfusion on the absorption-excretory and glycogen-synthesizing function of the liver.

Materials of the research and their discussion. The experiments were performed on 108 nonlinear male rats weighing 200-220 g. All animals were divided into four groups: control and three experimental. All interventions were performed under thiopental-sodium

anesthesia. In the first experimental group, the animals were simulated ischemia-reperfusion of the limb by applying a strip of elastic harness "SWAT-T" (USA) 10 mm wide for 120 minutes proximal to the left paw. In the second experimental group, acute blood loss (20% of the circulating blood volume) was simulated by cutting the femoral vein. In the third experimental group, these injuries were combined. In the control group, the animals were anesthetized, and then taken for research after 1 hour.

After 2 and 3 hours, as well as after 1, 7 and 14 days in experimental animals were determined by the absorption-excretory and glycogen-synthesizing functions of the liver.

Result of the research and their discussion. It was found that the simulation of two-hour limb ischemia after 3 h and 1 day of reperfusion is accompanied by a significant decrease in hepatic excretory function, accompanied by a slowing of bile bromosulfalein excretion and its normalization starting from 7 days of the experiment. Under conditions of acute blood loss, the disorders deepen at all times of observation. Complications of acute blood loss by ischemia-reperfusion of the limb, contributes to a greater slowing of the absorption and excretory function of the liver compared to the simulation of only acute blood loss. The result was statistically significant after 7 and 14 days of the experiment.

With regard to the content of glycogen in the liver, it was found that the simulation of only ischemia-reperfusion of the limb was accompanied by a significant decrease in the content of glycogen in the liver after 3 h of the experiment. Under conditions of acute blood loss, the rate was lower than the control level for seven days of the experiment. Additional ischemia-reperfusion of the limb on the background of acute blood loss caused a more pronounced decrease in glycogen content in the liver after 1 and 7 days of the experiment, which did not return to normal by 14 days of the experiment.

The use of carbacetam showed that compared with animals without correction of the drug for 14 days significantly accelerated the purification of blood from bromosulfalein and normalized glycogen content. Thus, carbacetam is a promising means of correcting the functional state of the liver in conditions of acute blood loss complicated by ischemia-reperfusion of the limb, which requires further preclinical study.

Conclusions. Complications of acute blood loss by two-hour limb ischemia in the reperfusion period lead to liver dysfunction, accompanied by a greater slowing of liver blood clearance from bromosulfalein compared to the model of isolated blood loss after 7 and 14 days of the experiment and a greater decrease in glycogen in the liver.

The use of carbacetam for 14 days in the reperfusion period in animals with acute blood loss complicated by limb ischemia-reperfusion, compared with animals without

correction, significantly accelerates hepatic clearance of bromosulfalein and normalizes glycogen content in the liver which indicates the prospects of carbacetam as a means of systemic correction in the simulated pathology. It could be the theoretical basis for its use in the clinic.

Key words: blood loss; ischemia-reperfusion of the limb; liver; carbacetam.

Introduction. Today it is recognized that under conditions of massive bleeding from the extremities, both in combat and in peacetime, the only means of salvation is the imposition of a tourniquet, which completely stops the arterial blood flow. It is believed that the maximum safe time for bleeding of the limb due to the use of the tourniquet is up to two hours [1, 2].

Recently, in the literature has increasingly raised questions about the safety of two-hour limb ischemia. It was found that complete cessation of arterial blood flow within two hours can cause the need for amputation of the limb, damage to nerves and vessels under the tourniquet, myonecrosis, compartment syndrome of the limb requiring fasciotomy, rhabdomyolysis with acute renal failure, deep thrombosis and thrombosis. damage to the skin under the tourniquet with the abscess development [3]. The basis of the detected disorders is the direct compression of the tissues under the tourniquet, the metabolic rearrangement of the tissues of the limb due to ischemia and its deepening after reperfusion. Under these conditions, toxic metabolites enter the bloodstream, exacerbate endotoxicosis and have a systemic effect on the body. According to some authors, reperfusion damage to the soft tissues of the limb is possible as early as 60 min after local ischemia [3, 4].

In the scientific literature there is a large number of studies of systemic disorders in the body with both massive blood loss and the use of tourniquet in civilian [5] and in combat [1]. Greater renal dysfunction was experimentally proven under conditions of acute blood loss, which was complicated by two-hour ischemia of the limb and did not reach normal within 14 days of the reperfusion period [6]. There are data of some authors that two-hour ischemia-reperfusion of both extremities on the background of combined trauma of the abdominal organs can deepen the development of multiorgan dysfunction [7–9].

However, the systemic effect of acute blood loss complicated by limb ischemia-reperfusion on the functional state of the liver, which is the main organ of detoxification of the body, remains virtually unexplored. There are no data on the effectiveness of carbacetam under these conditions, one of the mechanisms of action of which is the tissue-protective effect of [10].

Objective: to determine the effect of acute blood loss complicated by two-hour ischemia of the limb and its reperfusion, on the absorption-excretory and glycogen-synthesizing function of the liver.

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

All animals were divided into five groups: the control one and four experimental (6 rats per each group). All interventions were performed under thiopental-sodium anesthesia. In the first experimental group, the animals underwent simulated ischemia-reperfusion of the limb by applying a strip of elastic band "SWAT-T" (USA) 10 mm wide for 120 minutes proximal to the left foot. The tourniquet was tightened in accordance with the indicator of effective pressure – absence of the blood flow [11]. In the second experimental group, acute blood loss (20% of the circulating blood volume) was simulated by cutting 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 were administered intraperitoneally for corrective purposes with carbacetam (Institute of Physical-Organic Chemistry and Coal Chemistry of the NAS of Ukraine, Donetsk) at a dose of 5 mg per kilogram of animal [10]. In the control group, the animals were anesthetized using an equivalent dose of sodium thiopental, a tourniquet was applied for 2 h without cessation of blood flow and then the animal was taken for examination after 1 h.

After 2 and 3 hours, as well as after 1, 7 and 14 days in experimental animals was determined by the absorption and excretory function of the liver. The method is based on the ability of hepatocytes to capture bromosulfalein and, by binding it to glutathione, excrete it in bile. The bile acquires a purple color [12]. In rats, the common bile duct was catheterized. Then a 0.6% aqueous solution of bromosulfalein was injected into the femoral vein at a dose of 5 mg per kilogram of animal weight. The duration of bromosulfalein release from the moment of dye appearance in bile to the moment of its complete purification was established. Next, the animals were removed from the experiment by total bloodletting from the heart. According to the method described in [13], the glycogen content in the liver was determined. Liver portions weighing 25 mg were homogenized with ethanol for glucose extraction. Subsequently, the proteins were precipitated with a 5% solution of acetic acid. The glycogen-containing supernatant was heated with concentrated sulfuric acid. Under these conditions, glycogen was hydrolyzed to glucose, which reacted with concentrated sulfuric acid. The color

intensity was evaluated on a photoelectrocolorimeter with a green light filter.

Evaluation of the probability of differences between the experimental groups was performed using the nonparametric Mann-Whitney test.

Research results and their discussion. As can be seen from table 1, due to the simulation of ischemia-reperfusion of the limb, the duration of bromosulfalein release increased. Starting from 3 h of observation, the differences in the control group became statistically significant (by 21.4%, $p<0.05$). The indicator was at the same level in 1 day. Subsequently, the duration of bromsulfalein release decreased and reached the control level by 14 days ($p<0.05$). During this period, the rate became significantly lower than after 2 and 3 hours, as well as 1 day of observation ($p<0.05$).

Table 1 – The duration of excretion of bromosulfalein with bile (min) after acute blood loss complicated by ischemia-reperfusion of the limb (Me (LQ;UQ)) – median (lower and upper quartile)

Experimental group	Duration of the reperfusion period				
	2 hours	3 hours	1 day	7 days	14 days
Control group = 42,00 (38,50; 45,50) (n=6)					
Group 1 Ischemia-reperfusion	45,00 (43,25; 46,75) (n=6)	51,00^{*2h} (50,25; 52,50) (n=6)	52,00^{*2h} (50,25; 54,50) (n=6)	44,00^{3h,1d} (42,50; 47,00) (n=6)	39,00^{2h,3h,1d} (36,50; 43,00) (n=6)
Group 2 Blood loss	53,00* (52,00; 55,50) (n=6)	57,00* (55,25; 59,50) (n=6)	62,00^{*2h,3h} (60,50; 63,50) (n=6)	56,00^{*1d} (53,00; 57,50) (n=6)	53,00^{*3h,1d} (49,00; 54,00) (n=6)
Group 3 Ischemia-reperfusion+blood loss	57,00* (56,00; 61,00) (n=6)	62,50* (60,50; 63,75) (n=6)	67,00^{*2h,3h} (64,50; 71,00) (n=6)	60,00^{*1d} (57,00; 61,50) (n=6)	54,00^{*2h,3h,1d} (53,25; 57,00) (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 and in the Table 2:

1. * – differences concerning the control group are statistically significant ($p<0,05$);
2. p_{1-2} – the significance of differences between the experimental groups 1 and 2;;
3. p_{1-3} – the significance of differences between the experimental groups 1 and 3;;
4. p_{2-3} – - the significance of differences between experimental groups 2 and 3;
5. ^{2h,3h,1d,7d} – differences concerning 2 h and 3 h as well as 1 day and 7 days respectively are statistically significant ($p<0,05$).

After modeling of acute blood loss, the duration of bromsulfalein secretion was

statistically significantly longer compared to the control at all follow-up periods: after 3 h - by 26.2%, after 3 h - by 35.7%, after 1 day - by 47.6%, after 7 days - by 33.3%, after 14 days - by 26.2% ($p<0.05$). As can be seen, the maximum of bromosulfalein secretion disorders occurred after 1 day, which was statistically significantly higher than the previous observation time ($p<0.05$). After 14 days, the rate decreased and became significantly smaller than after 3 h and 1 day of observation ($p<0.05$).

After simulation of acute blood loss complicated by ischemia-reperfusion of the limb, the violations compared with the control were even greater with a maximum of 1 day (59.5%, $p<0.05$), which also significantly exceeded 2 and 3 h of observation ($p<0.05$). By day 14, the indicator decreased, continued to exceed the control group by 28.6% ($p<0.05$) and was statistically significantly lower than after 2 and 3 h and 1 day of observation ($p<0.05$).

Comparison of experimental groups showed that after modeling acute blood loss (experimental group 2), the duration of bromsulfalein secretion at all observation times was statistically significantly longer than after modeling only limb ischemia-reperfusion (experimental group 2) ($p_{1-2}<0.05$). Under the conditions of a combination of acute blood loss and ischemia-reperfusion of the limb (experimental group 3), the indicator was also higher in all terms than after modeling only ischemia-reperfusion of the limb ($p_{1-3}<0.05$). Compared with experimental group 2, the duration of bromsulfalein release was significantly longer after 7 and 14 days of observation ($p_{2-3}<0.05$).

Analysis of glycogen content in the liver showed (Table 2) that under the influence of ischemia-reperfusion of the limb, the rate decreased to 3 h of the experiment and was 9.1% lower than in the control, which was statistically significant ($p<0.05$). Subsequently, the indicator increased and did not differ significantly from the level of control ($p>0.05$).

Under conditions of acute blood loss, the violations were greater. The glycogen content after 2 h of the experiment became significantly lower than in the control (15.6%, $p<0.05$). By one day, the indicator reached a minimum value and was 22.1% lower than in the control ($p<0.05$) and was comparable with 2 hours of observation ($p>0.05$). Subsequently, the indicator increased and reached the level of control by 14 days ($p>0.05$).

After modeling of acute blood loss and ischemia-reperfusion of the limb, the glycogen content in the liver at all follow-up was significantly lower than in the control: after 2 h - by 18.9%, after 3 h - by 25.7%, after 1 day - by 27.7%, after 7 days - by 22.1%, after 14 days - by 12.3% ($p<0.05$). The rate reached a minimum after 1 day and was significantly less than after 2 hours of the experiment. By day 14, the rate increased and became statistically significantly greater than after 3 h, 1 and 7 days of the experiment ($p<0.05$).

Table 2 – Glycogen content in the liver (g kg^{-1}) after acute blood loss complicated by limb ischemia-reperfusion (Me (LQ;UQ)) – median (lower and upper quartile)

Experimental group	Duration of the reperfusion period				
	2 hours	3 hours	1 day	7 days	14 days
Control group = 24,8 (24,1; 25,5) (n=6)					
Group 1 Ischemia-reperfusion	22,9 (21,4; 24,8) (n=6)	22,5* (22,2; 23,0) (n=6)	23,6 (22,9; 24,0) (n=6)	24,2 (23,7; 25,8) (n=6)	24,3 (23,5; 24,9) (n=6)
Group 2 Blood loss	20,9* (20,4; 21,2) (n=6)	20,0* (19,0; 20,6) (n=6)	19,3*2г (18,4; 19,7) (n=6)	20,5*1д (20,3; 20,9) (n=6)	22,4*3г,1д (21,0; 23,0) (n=6)
Group 3 Ischemia-reperfusion+blood loss	20,1* (19,5; 21,5) (n=6)	18,4*2г (17,8; 19,1) (n=6)	17,9*2г (17,8; 18,1) (n=6)	19,3*3г,1д (18,8; 20,1) (n=6)	21,7*3г,1д,7д (21,3; 22,8) (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 comparison of experimental groups showed that after modeling of acute blood loss (experimental group 2) the glycogen content in the liver became significantly lower than after modeling only ischemia-reperfusion of the limb (experimental group 1) at all times of observation (p_{1-2}, $p<0.05$). Under conditions of acute blood loss complicated by limb ischemia-reperfusion (experimental group 3), the rate was also significantly lower compared to experimental group 1 after 3 h, 1, 7 and 14 days ($p_{1-3}<0.05$), and compared with the experimental group 2 after 1 and 7 days of the experiment ($p_{2-3}<0.05$).

The use of carbacetam for correction (Fig. 1) compared with animals without correction for 7 days was accompanied by a decrease in the duration of bromsulfalein release, but the result was not statistically significant ($p>0.05$). The use of carbacetam for 14 days caused a significant decrease in this indicator - by 11.1% ($p<0.05$). With regard to the content of glycogen in the liver, after 7 days after the use of carbacetam, the rate was statistically significantly increased compared with animals without correction ($p<0.05$). After 14 days of drug use, the glycogen content in the liver reached the level of the control group ($p>0.05$).

The obtained results indicate that the simulation of two-hour limb ischemia after 3 h and 1 day of reperfusion is accompanied by a significant decrease in the absorption and excretory function of the liver, accompanied by a slowing of bile bromosulfalein excretion

and its normalization starting from 7 days of the experiment. Under conditions of acute blood loss, the disorders deepen during all period of observation.

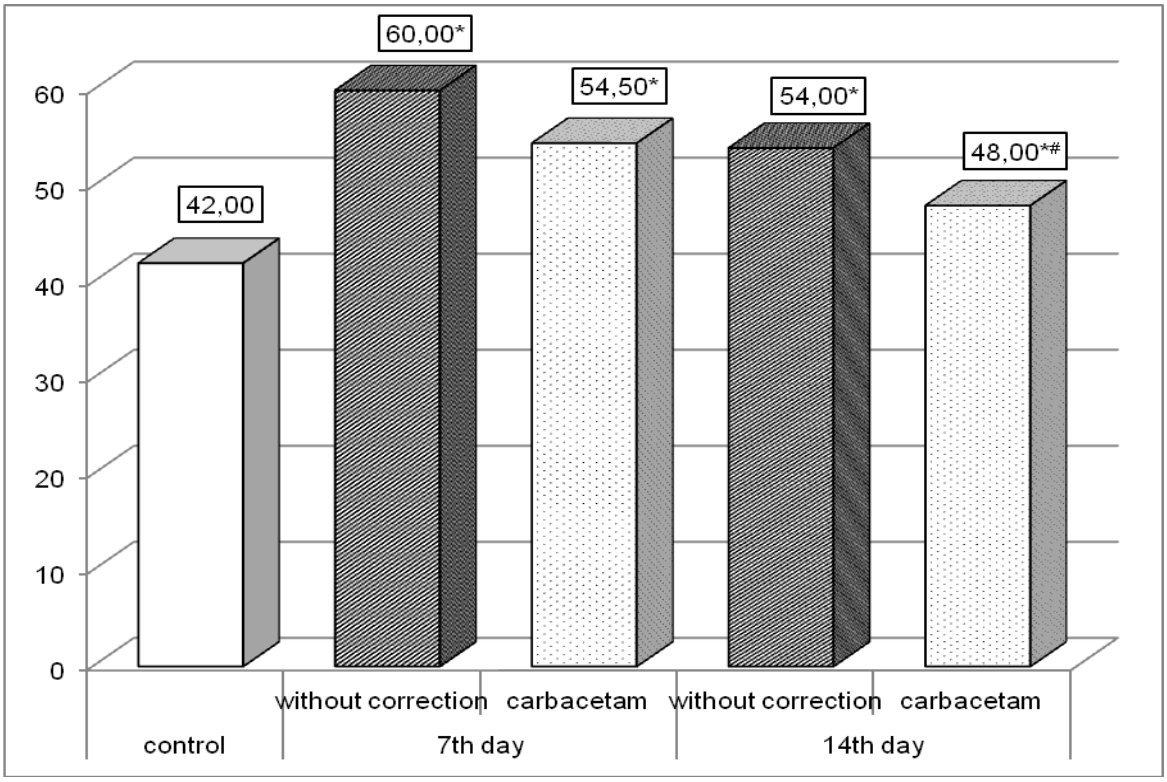

Figure 1 - The effect of carbacetam on the duration of bromsulfalein (min) after acute blood loss complicated by ischemia-reperfusion of the limb. (Note. Here and in Figure 2: * - differences in control are statistically significant, $p<0.05$; # - differences in the group without correction are statistically significant, $p<0.05$).

However, we found for the first time that the complication of acute blood loss by ischemia-reperfusion of the limb contributes to a greater slowing of the absorption and excretory function of the liver compared to the simulation of only acute blood loss. The result was statistically significant after 7 and 14 days of the experiment. It is known that Kupffer liver cells completely absorb bromosulfalein from the blood. Subsequently, its glucuronidation occurs in the endoplasmic reticulum of the liver and the release into the bile capillary at the biliary pole of hepatocytes. Therefore, the bromosulfalein test characterizes the functional state of endotheliocytes and endoplasmic reticulum of the liver and is one of the most sensitive tests in assessing the functional state of the liver [12]. Thus, additional ischemia-reperfusion of the limb on the background of acute blood loss, causes greater impairment of the functional state of the liver than modeling only acute blood loss. The basis

of the detected disorders is probably an increase in toxic effects on the liver due to the intake of non-oxidized products, reactive oxygen species and their toxic metabolites from the ischemic limb and indicates the development of mutual burden syndrome [14].

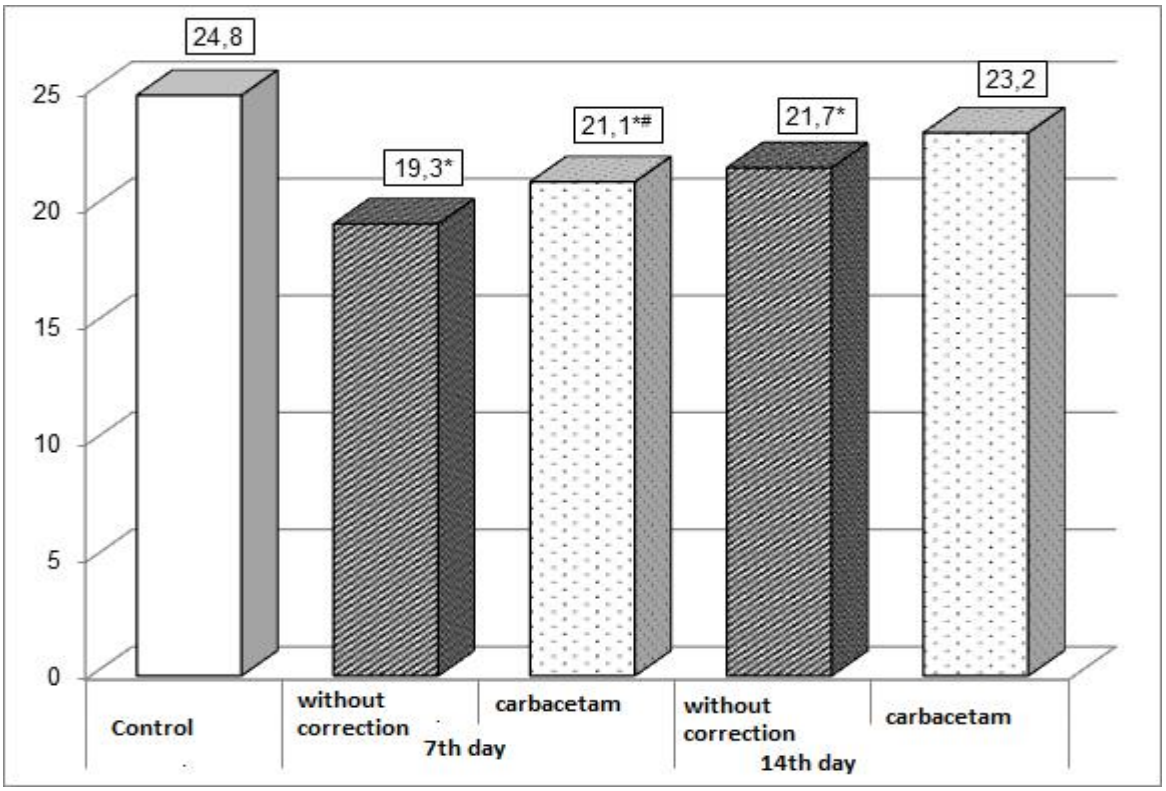

Figure 2 - The effect of carbacetam on the glycogen content in the liver (g l^{-1}) after acute blood loss complicated by ischemia-reperfusion of the limb.

Decreased levels of glycogen in the liver under the influence of ischemia-reperfusion of the limb, acute blood loss and their combination indicates the activation of the sympatho-adrenal system and disorder of the mechanisms of glycogen resynthesis [15]. Our results indicate that the simulation of only limb ischemia-reperfusion after 3 h of the experiment is a pronounced stressor for the body of experimental animals, which is accompanied by a decrease in glycogen content in the liver. Under conditions of acute blood loss, the rate is lower than the control level for seven days of the experiment. Its further normalization is a sign of the development of adaptation - the predominance of glucocorticoids over catecholamines, which help maintain adequate metabolism under stress and, in particular, the accumulation of glycogen in the liver. However, additional ischemia-reperfusion of the limb causes a more pronounced decrease in glycogen content in the liver on the background of acute blood loss after 1 and 7 days of the experiment, which is not normalized to 14 days of

the experiment. The obtained result indicates that additional ischemia-reperfusion of the limb deepens the development of energy deficiency in tissues and processes of cellular intoxication and can cause secondary functional and structural changes [16].

In order to correct the detected disorders, we used carbacetam, which according to some authors proved to be effective in conditions of acute blood loss complicated by ischemia-reperfusion of the limb [17]. Studies have shown that compared with animals without correction, the use of the drug for 14 days significantly accelerates the purification of blood from bromosulfalein and normalizes glycogen content. Its positive effect is obviously based on antioxidant, immunomodulatory, detoxifying, membrane-stabilizing and tissue-protective action, which was proved by some authors [10]. Thus, carbacetam is a promising means of correcting the functional state of the liver in conditions of acute blood loss complicated by ischemia-reperfusion of the limb, which requires further preclinical study.

Conclusion. 1. Complication of acute blood loss by two-hour limb ischemia in the reperfusion period causes liver dysfunction, accompanied by a greater slowing of hepatic blood purification from bromosulfalein compared to modeling only blood loss after 7 and 14 days of the experiment and a greater decrease in glycogen content in the liver.

2. The use of carbacetam for 14 days in the reperfusion period in animals with acute blood loss complicated by ischemia-reperfusion of the limb, compared with animals without correction, significantly accelerates hepatic clearance of bromosulfalein and normalizes glycogen content in the liver, which indicates the prospects of carbacetam as a means of systemic correction in the simulated pathology and approves the theoretical basis for its use in the clinic.

Perspectives of further research. In the future, it is advisable to deepen the study of molecular mechanisms of liver dysfunction on the background of acute blood loss under conditions of ischemia-reperfusion of the limb and the effectiveness of carbacetam.

References:

1. Efficacy of prehospital application of tourniquets and hemostatic dressings to control traumatic external hemorrhage. – Access mode: https://www.ems.gov/pdf/research/Studies-and-Reports.pdf

2. Lee C. Tourniquet use in the civilian prehospital setting / C. Lee, K. M. Porter, T. J. Hodgetts // Emerg. Med. J. – 2007. – Vol. 24 (8). – P. 584–587.

3. Clasper J. C. Limb complications following pre-hospital tourniquet use / J. C. Clasper, K. V. Brown, P. Hill // J. R. Army Med. Corps. – 2009. – Vol. 155 (3). – P. 200–202.

4. Постернак Г. И. Место кровоостанавливающего жгута в современном алгоритме временной остановки кровотечения на догоспитальном этапе / Г. И. Постернак, В. В. Лесной // Медицина неотложных состояний. – 2017. – Т. 4, – № 83. – С. 57–60.

5. Development of personalized tourniquet systems using a new technique for measuring limb occlusion pressure: World Congress on Medical Physics and Biomedical Engineering, (June 7-12, 2015). Toronto, Canada /J.A. McEwen, B.A. Masri, B. Day, A. S. Younger; Jaffray D. (Eds.). Springer International Publishing, 2015.- Vol. 51.- P. 1325–1328.

6. Шацький В. В. Вплив експериментальної гострої крововтрати, ускладненої ішемією-реперфузією кінцівки, на діурез і швидкість клубочкової фільтрації / В. В. Шацький, А. А. Гудима, Р. В. Близнюк // Шпитальна хірургія. Журнал імені Л. Я. Ковальчука. – 2019. – № 4. – С. 50–58.

7. Кузьмінський І. В. Особливості жовчовидільної функції печінки в умовах ішемічно-реперфузійного синдрому кінцівок, закритої травми органів черевної порожнини, ускладненої масивною крововтратою / І. В. Кузьмінський // Актуальні проблеми транспортної медицини. – 2018. – № 4 (54). – С. 148–158.

8. Цимбалюк Г. Ю. Особливості транспорту іонів натрію в умовах ішемії-реперфузії нижніх кінцівок, травами органів черевної порожнини та гіповолемічного шоку / Г. Ю. Цимбалюк // Актуальні проблеми транспортної медицини. – 2018. – № 4 (54). – С. 156–167.

9. Волотовська Н. В. Зміни активності глутатіонової системи внутрішніх органів у перші години експериментального ішемічно-реперфузійного синдрому кінцівки, поєднаного з крововтратою та механічною травмою / Н. В. Волотовська, Т. Кліфф Нхоквара, І. В. Жулкевич // Здобутки клінічної і експериментальної медицини. – 2019. – № 1. – С. 23–27.

10. Козак Д. В. Структурні зміни деяких внутрішніх органів тварин із модельованою політравмою за умов корекції карбацетамом / Д. В. Козак, К. С. Волков // Науковий вісник Ужгородського університету, серія «Медицина». – 2014. – Вип. 2 (50). – С. 3–6.

11. Point of injury tourniquet application during Operation Protective Edge-What do we learn? / A. Shlaifer, A. Yitzhak, E. N. Baruch [et al.] // J. Trauma Acute Care Surg. – 2017. – Vol. 83, № 2. – P. 278–283.

12. Доклінічні дослідження лікарських засобів : метод. рекоменд. ; за ред. чл.-кор. АМН України О. В. Стефанова. – К. : Авіценна, 2001. – 528 с.

13. Прохорова М. И. Большой практикум по углеводному обмену и липидному обмену /М. И. Прохорова, З. Н. Тупикова. – Л.: Изд-во Ленингр. ун-та, 1995. – С. 53–65.

14. Телевяк А. Т. Динаміка показників перекисного окислення ліпідів та антиоксидантного захисту в м'язовій тканині задніх кінцівок щурів при розвитку ішемічно-реперфузійного синдрому (експериментальне дослідження) / А. Т. Телевяк // Здобутки клінічної і експериментальної медицини. – 2018. – № 3. – С. 132–139.

15. Влияние мексидола и его структурных компонентов на содержание углеводов и перекисное окисление липидов при остром стрессе [Электронный ресурс] / Т. А. Девяткина, Р. В. Луценко, Е. М. Важничая, Л. Д. Смирнов // Вопросы медицинской химии. − 1999. − № 3. – Режим доступа: http://medi.ru/pbmc/8890309.htm.

16. Ельский В. Н. Моделирование черепно-мозговой травмы / В. Н. Ельский, С. В. Зяблицев. – Донецк : Изд-во «Новый мир», 2008. – 140 с.

17. Шацький В. В. Динаміка антиоксидантно-прооксидантного балансу кіркового і мозкового шарів нирки після гострої крововтрати, ускладненої ішемією-реперфузією кінцівки, та його корекція карбацетамом / В. В. Шацький, А. А. Гудима, Л. Я. Федонюк // Здобутки клінічної і експериментальної медицини. – 2019. – № 4. – С. 144–153.

References

1. Efficacy of prehospital application of tourniquets and hemostatic dressings to control traumatic external hemorrhage. Access mode: https://www.ems.gov/pdf/research/Studies-and-Reports.pdf

2. Lee, C., Porter, K.M., & Hodgetts, T.J. (2007). Tourniquet use in the civilian prehospital setting. *Emerg. Med. J.*, 24 (8), 584-587.

3. Clasper, J.C., Brown, K.V., & Hill, P. (2009). Limb complications following pre-hospital tourniquet use. *J. R. Army Med. Corps.*, 155 (3), 200-202.

4. Posternak, G.I., & Lesnoy, V.V. (2017). Mesto krovoostanavlivayushchego zhguta v sovremennom algoritme vremennoy ostanovki krovotecheniya na dogospitalnom etape [Place of a hemostatic tourniquet in the modern algorithm for temporarily stopping bleeding at the pre-hospital stage]. *Meditsina neotlozhnykh sostoyaniy – Medicine of emergency conditions,* 4, 83, 57-60 [in Russian].

5. McEwen, J.A., Masri, B.A., Day, B., & Younger, A.S. (2015). Development of personalized tourniquet systems using a new technique for measuring limb occlusion pressure. Jaffray, D. (Eds). Proceedings of the *World Congress on Medical Physics and Biomedical Engineering,* (June 7-12, 2015). Toronto, Canada. IFMBE Proceedings. Springer, Cham.

6. Shatskyi, V.V., Hudyma, A.A., & Blyzniuk, R.V. (2019). Vplyv eksperymentalnoi hostroi krovovtraty, uskladnenoi ishemiieiu-reperfuziieiu kintsivky, na diurez i shvydkist klubochkovoi filtratsii [Influence of experimental acute blood loss complicated by ischemia-reperfusion of the extremity on the size of diuresis and the glomerular filtration rate]. *Shpytalna khirurhiia. Zhurnal imeni L. Ya. Kovalchuka – Hospital Surgery. Journal Named by L.Ya. Kovalchuk,* (4), 50-58. Retrieved from: https://doi.org/10.11603/2414-4533.2019.4.10710.

7. Kuzminskyi, I.V. (2018). Osoblyvosti zhovchovydilnoi funktsii pechinky v umovakh ishemichno-reperfuziinoho syndromu kintsivok, zakrytoi travmy orhaniv cherevnoi porozhnyny, uskladnenoi masyvnoiu krovovtratoiu [Peculiarities of biliary function of the liver in the conditions of ischemic-reperfusion syndrome of the extremities, closed trauma of the abdominal cavity, complicated by massive blood loss]. *Aktualni problemy transportnoi medytsyny – Actual problems of transport medicine,* 4 (54), 148-158 [in Ukrainian].

8. Tsymbalyuk, H.Yu. (2018). Osoblyvosti transportu ioniv natriyu v umovakh ishemii-reperfuzii nyzhnikh kintsivok, travamy orhaniv cherevnoyi porozhnyny ta hipovolemichnoho shoku [Peculiarities of sodium ion transport in the conditions of ischemia-reperfusion of lower extremities, herbs of abdominal organs and hypovolemic shock]. *Aktualni problemy transportnoi medytsyny – Actual problems of transport medicine,* 4 (54), 156-167.

9. Volotovska, N.V., Nhokwara, T.C., & Zhulkevych, I.V. (2019). Zminy aktyvnosti hlutationovoi systemy vnutrishnikh orhaniv u pershi hodyny eksperymentalnoho ishemichnoreperfuzinoho syndromu kintsivky, poiednanoho z krovovtratoiu ta mekhanichnoiu travmoiu [Changes in the glutathione system's activity of internal organs in the first hours of experimental limb ischemia-reperfusion syndrome, combined with blood loss and mechanical injury]. Zdobutky klinichnoi i eksperymentalnoi medytsyny – Achievements of Clinical and Experimental Medicine, 1, 23-27. DOI 10.11603/1811-2471.2019.v0.i1.10043

10. Kozak, D.V., & Volkov, K.S. (2014). Strukturni zminy deiakykh vnutrishnikh orhaniv tvaryn iz modelovanoiu politravmoiu za umov korektsii karbatsetamom [Structural changes of some internal organs of animals with simulated polytrauma under conditions of

correction with carbacetam]. *Naukovyy visnyk Uzhhorodskoho universytetu, seriya «Medytsyna» – Scientific herald of the Uzhhorod University, series "Medicine"*, 2 (50), 3-6 [in Ukrainian].

11. Shlaifer, A., Yitzhak, A., Baruch, E.N., Shina, A., Satanovsky, A., Shovali, A., & Glassberg, E. (2017). Point of injury tourniquet application during Operation Protective Edge-What do we learn? *J. Trauma Acute Care Surg.*, 83, 2, 278-283.

12. Stefanova, O.V. (Ed.). (2001). *Doklinichni doslidzhennia likarskykh zasobiv: metod. rekomend. [Preclinical drug research: Guidelines]*. Kyiv: Avitsenna. 528 p. [in Ukrainian].

13. Prokhorova, M.I., & Tupikova, Z.N. (1995). *Bolshoy praktikum po vuglevodnomu obmenu i lipidnomu obmenu [Big workshop on carbohydrate metabolism and lipid metabolism]*. Leningrad : Izd-vo Leningr. un-ta. 5365 p. [in Russian].

14. Televiak, A.T. (2018). Dynamika pokaznykiv perekysnoho okyslennia lipidiv ta antyoksydantnoho zakhystu v miazovii tkanyni zadnikh kintsivok shchuriv pry rozvytku ishemichno-reperfuziinoho syndromu (eksperymentalne doslidzhennia) [The dynamic of indicators of lipid peroxidation and antioxidant protection in muscle tissue of the hind limbs of the rats in development of the ischemic-reperfusion syndrome (experimental study)]. *Zdobutky klinichnoi i eksperymentalnoi medytsyny – Achievements of clinical and experimental medicine,* 3, 132-139 [in Ukrainian]. Retrieved from: https://doi.org/10.11603/1811-2471.2018.v0.i3.9318

15. Devyatkina, T.A., Lutsenko, R.V., Vazhnichaya, E.M., & Smirnov, L.D. (1999). The influence of mexidol and its structural components on carbohydrate content and lipid peroxidation during acute stress. *Voprosy meditsinskoi khimii – Questions of medicinal chemistry,* 3. Retrieved from: http://medi.ru/pbmc/8890309.htm.

16. Yelskiy, V.N. & Zyablitsev, S.V. (2008). *Modelirovaniye cherepno-mozgovoy travmy [Modeling of traumatic brain injury]*. Donetsk : Izd-vo «Novyy mir». 140 p. [in Russian].

17. Shatskyy, V.V., Hudyma, A.A., & Fedonyuk, L.Ya. (2019). Dynamika antyoksydantno-prooksydantnoho balansu kirkovoho i mozkovoho shariv nyrky pislia hostroi krovovtraty, uskladnenoi ishemiieiu-reperfuziieiu kintsivky, ta yoho korektsiia karbatsetamom [Dynamics of antioxidant-prooxidant balance of renal cortex and medulla after acute blood loss complicated by ischemia-reperfusion of the extremity, and its correction with carbacetam]. *Zdobutky klinichnoi i eksperymentalnoi medytsyny – Achievements of clinical and experimental medicine,* 4, 144-153 [in Ukrainian].