

Therapeutic effect of thiocetam on the manifestations of ischemia-reperfusion complicated by massive blood loss and mechanical trauma

Nataliya Volotovska

I. Ya. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine

Department of Physiology with the Basics of Bioethics and Biosafety

Maidan Voli, 1; Ternopil town, post code: 46000, Ukraine

Volotovska Nataliya: <https://orcid.org/0000-0003-4073-3148>
volotovskanv@tdmu.edu.ua

Abstract

Among the current medical and social problems, injuries and blood loss occupy a prominent place, causing stress on the antioxidant defenses. Hypoxia, which underlies the pathogenesis of the post-traumatic period of both diseases, leads to a significant imbalance in the work of internal organs. Scientists are increasingly attracted by the need to use a tourniquet or intraoperative ligatures, as reperfusion local and systemic damage develops. Antioxidants are considered a promising means of correction.

The aim of the study was to investigate the features of metabolic disorders in the liver in the early post-traumatic period on the background of the use of a tourniquet and the effectiveness of thiocetam correction.

Materials and methods. The experiment was performed on 130 white male rats (200-250 g), which were divided into 4 groups: control – the CG, the EG-1 – combination of limb ischemia-reperfusion (IR) with blood loss, the EG-2 – combination of limb IR with blood loss

and mechanical trauma of the thigh; the EG-3 combination of limb IR, blood loss, mechanical injury and thiocetam administration. The Malonic dialdehyde level catalase activity were estimated in the liver.

Results. The use of thiocetam, which is able to struggle against of ischemia and lipid peroxidation by reactivating antiradical enzymes: superoxide dismutase, catalase and glutathione peroxidase, had a positive effect on the state of antioxidant and prooxidant units in the organ, located far from the place of primary ischemia-reperfusion. If in the group of untrated animals (the EG-2, where massive blood loss was combined with a thigh fracture and the use of hemostatic tourniquet) in the early period, the MDA level exceeded the CG data in 5,4 times, and on the 7th and 14th days remained high – being higher on 2,1 times and on 2,7 times, then in the EG-3 (group of treated animals) on the 1st day the level of MDA exceeded the CG data in 4,3 times, but on the 7th and 14th days was higher by 90,5 % and 64 % respectively. The supportive effect of thiocetam on the activity of catalase in the liver was also noted. Thus, in EG-2 the level of antioxidant enzyme on the 1st day decreased by 71,7 %, and remained almost at this level throughout the all post-experimental period. As for the group of treated animals, the level of activity on the 1st day after the intervention decreased by 44,7%, and was so for almost the entire period. On the 14th day, it remained reduced compared to the CG by 35,1 %, while in EG-2 this index was lower compared to the CG by 70,5 %.

Conclusion. Having the positive effect of the introduction of thiocetam in the ischemic area, we can eventually add new complex, given the world experience, which would affect the development of the inflammatory response and the rheological properties of blood.

Key words: Ischemia reperfusion injury; oxidative stress; catalase; skeletal trauma; blood loss; liver; hemostatic tourniquet; treatment; antioxidants.

Introduction

Ischemic-reperfused syndrome in itself is a significant factor that can trigger the processes of lipid peroxidation in tissues that are pressed by a tourniquet or a ligature [1]. The pathogenesis of this process is based not only on the oxygen lack, but also on the nutrients deficiency carried by the blood. Protease-dependent conversion of the enzyme xanthine hydrogenase to xanthioxidase (which is usually absent in the cell) is activated under conditions of tourniquet-induced hypoxia, [2, 3]. Cellular macroergs, releasing energy, broken down into hypoxanthine; under the influence of xanthioxidase, xanthine and the active form of oxygen – superoxidanion – are formed. However, the cell's energy reserves are rapidly

depleted, and this process is combined with the accumulation of metabolites, in particular lactate. Changes of the structure of lipid and protein fractions in the cell membrane, depolarization of the cell membrane leads not only to disruption of transport functions of the corresponding elements, but to a decrease in the efficiency of enzyme systems. These processes follow by ionic imbalance in cellular and extracellular spaces [4]. Over time, the violations only deepen: the antioxidant system is exhausted, the production of physiologically important biologically active substances – nitric oxide and carbon dioxide are decreased on the background of activating the synthesis of proinflammatory factors, which is again accompanied by a new portion of free radical synthesis [5, 6]. The increase in these changes further exacerbates the situation, contributing to the development of endothelial dysfunction [7].

In turn, restoring the blood supply to the bleeding area only worsen muscle damage [8]. Thus, the paradoxical effect of reoxygenation deepens the existing local imbalance [9, 10]. The moment of arising of systemic manifestations are not easy to distinguish from local ones, because they are based on the same pathophysiological mechanisms – hypoxia, inflammation, microcirculation disorders.

If we are talking about a combination of local exposure with the use of a tourniquet, then even a 2-hour ischemia of the limb can have ambiguous effects on the affected organism. This is especially important to consider when there is a combined defeat of the body, in particular, on the battlefield, when there is little time, and all actions must be clearly spelled out and coordinated. So now there is an active search for ways to minimize the probable pathogenic effects of the tourniquet.

The aim of the study was to investigate the features of metabolic disorders – catalase and malonic dialdehyde levels – in the liver in the early post-traumatic period on the background of the use of a tourniquet and the effectiveness of thiocetam correction

Materials and methods of research. To study the effect of the combination of ischemia-reperfusion of the limb with trauma and the therapeutic effect of thiocetam for the experiment were selected 130 animals - white male rats (200-250 g) were used in the experimental study. Some of them died in the postoperative period. All animals were divided into 4 groups: the EG-1 included animals that were modified with simulated ischemia-reperfusion of the limb, combined with acute volumetric blood loss (up to 40% of volume of circulating blood – it was modeled by puncture of the femoral vein with farther hem stasis). Under thiopental-sodium anesthesia ($40 \text{ mg} \cdot \text{kg}^{-1}$ body weight intraperitoneally), SWAT-T (US) tourniquet with width 10 mm was applied to the thigh of an animal and adequately

corresponds pressure of the tourniquet when applied to the thigh of an adult human. According to the literature, such a tourniquet is characterized by minimal negative traumatic effects on the underlying tissues due to its width and long-term pain threshold [11]. The tourniquet was tightened according to the applied effective pressure marking, which is able to stop the blood flow. In the EG2 under conditions of anesthesia, two injuries (heavy blood loss and mechanical fracture of the opposite thigh – were combined with ischemia-reperfusion of limb. In the EG-3 thiocetam according to body mass proportion was administrated to the animals intramuscularly within period of ischemia. Treatment was given distally comparably to the tourniquet, which was realized after 2 hours of ischemia.

Animals were eliminated from experiment at the 1st hour after intervention, and on the 1st, 3rd, 7th and 14th days after trauma on the base of thiopental-sodium anaesthesia by total bleeding from the heart. In the case of the CG animals, they were anesthetized with an equivalent dose of sodium thiopental and investigated materials were collected for the study, as from the experimental groups.

To detect TBA-active products (metabolic products that react with thiobarbituric acid), 10% homogenate was prepared from liver tissue. It is known that the secondary products of lipid peroxidation (LPO), namely malonyldialdehyde (MDA), when interacting with thiobarbituric acid (TBA) under conditions of high temperature and acidotic pH have the ability to form a colored complex with optical density, possible for registration, at length waves of 532 nm. The amount of MDA was calculated based on the molar extinction coefficient of the stained complex and expressed in $\mu\text{mol} / \text{kg}$ of tissue [12].

In the homogenate of the liver an activity of the key component of the enzymatic link of antioxidant protection and antioxidant mechanisms – katalase –was established. [13].

The study design was considered with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (European Convention, 1984) and Law of the Ministry of Health of Ukraine № 690 [14], considered by the commission at a meeting of the commission on bioethics of I. Ya. Gorbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine № 61 by 11.01.2021

A statistical analysis on obtained data was performed by Excel (Microsoft, USA). In addition to the absolute values, which are presented in the tables in the form of median (Me), lower and upper quartiles (LQ; UQ), the deviation of each indicator as a percentage to the control level (100,0%) was calculated. To rate the probability of differences we defined the peculiarities of the variational grouping of indicators in the compared groups. Due to the lack of a normal

grouping, the nonparametric Mann-Whitney test was used. The differences were considered as a true when the probability of the null hypothesis was not more than 5 % ($p < 0.05$).

Results and discussion

For the treatment of systemic consequences of critical ischemia of the lower extremity, various modifications of efferent methods of treatment are used, aimed at removing toxic and pro-inflammatory metabolites from the systemic bloodstream, which contribute to the development of multiorgan failure during reperfusion.

The liver, like the kidneys, is one of the first important organs that face with increasing of endogenous intoxication, which is indisputable on the background of massive blood loss. Considering our previous studies, which showed an increase in the concentration of malonic dialdehyde on the background of massive blood loss and the fact that these disorders were more expressed on the background of its combination with a tourniquet by experimental methods [15], the idea of early use of antihypoxants was natural to research [16].

As can be seen from the table 1, when comparing the level of LPO in the liver in the post-traumatic period relative to the data of the CG the following peculiarities were found: on the 1st day after intervention the index in the EG-1 increased in 3,8 times, on the 3rd day continued growing, exceeding the CG data in 4,9 times. On the 7th and 14th days, despite the decrease in activity, it exceeded the CG data in 3,6 times and 2,7 times, appropriately. In the EG-2, the adding of mechanical trauma led to even more deep changes, when the rate on the 1st and 3rd days exceeded the CG data in 5,4 times and in 4,6 times, appropriately. On the 7th and 14th days, the rate was in 2,1 times higher and in 2,7 times higher than the CG data times higher, appropriately. The use of thiocetam in animals with the most severe trauma in EG-3 reduced the manifestations of peroxidation in liver tissue, namely – despite the fact that on the 1st and on the 3rd days the rate was very high, exceeding the CG data in 4,3 times and 3,8 times respectively, but on the 7th and 14th days it decreased significantly – exceeding the CG data by 90,5 % and by 64 %, accordingly.

When comparing the severity of LPO activity between groups with different severity of injury and treated, it was found that the lowest level of LPO in liver tissue was in the EG-3. Thus, when comparing the data of the EG-1 and the EG-2, the level of LPO was higher in EG-2 by 41,3 %. Also on the 7th day, the EG-2 index was statistically significantly higher by 41,6 % than in the EG-1 data.

Table 1 - The content of TBA-active derivatives of lipid peroxidation in the 10 % liver homogenate after limb ischemia-reperfusion, blood loss, skeletal trauma and its correction (Me (LQ; UQ)) - median (lower and upper quartiles)

Experimental group	Reperfusion period			
	1 st day	3 rd day	7 th day	14 th day
<i>KG</i> Control Group = 1,89 (1,69; 2,05) (n=10) $\mu\text{mol} / \text{kg}$				
<i>EG-1</i> ischemia-reperfusion, blood loss	7,22* (7,05; 7,31) (n=6)	9,22* (8,74; 9,59) (n=6)	6,73* (6,46; 6,99) (n=6)	5,15* (5,10; 5,21) (n=5)
<i>EG-2</i> ischemia-reperfusion, blood loss, skeletal trauma	10,2* (9,73; 10,58) (n=7)	8,73* (8,10; 9,16) (n=6)	3,94* (3,70; 4,08) (n=7)	5,15* (4,91; 5,64) (n=7)
<i>EG-3</i> ischemia-reperfusion, blood loss, skeletal trauma+treatment	8,16* (8,09; 8,99) (n=6)	7,17* (6,98; 7,50)* (n=6)	3,60* (3,30; 3,68) (n=6)	3,10* (3,10; 3,15) (n=5)
p ₁₋₂	<0,05	>0,05	<0,05	>0,05
p ₁₋₃	<0,05	<0,05	<0,05	<0,05
p ₂₋₃	<0,05	<0,05	<0,05	<0,05

Notes: 1.* – differences in relation to the control group are statistically significant (p<0,05);

2. p₁₋₂ – the probability of differences in relation to experimental groups 1 and 2;

3. p₁₋₃ – the probability of differences in relation to experimental groups 1 and 3;

4. p₂₋₃ – the probability of differences in relation to experimental groups 2 and 3.

When comparing the data of the EG-1 and of the EG-3, it was found that on the 1st day after the intervention, the activity in the EG-3 was even higher than in the EG-1 – by 13%. However, in the next period the results of thiocetam action were obvious: in the EG-3 on the 3rd, 7th and 14th days, compared with the EG-1, the rate was lower by 22,2 %, by 46,5 % and by 39,8 %, respectively. When comparing the data of the EG-2 and the EG-3, a positive effect from the use of this drug was also present. Thus, on the 1st and 3rd days, the index rate in the EG-3 was lower than in the EG-2, by 20 %, and on the 3rd day – by 17,9 %. On the 7th day, which apparently followed the critical period of the 3rd day the rate of LPO in the EG-3 was still lower than in EG-2 by 8,6 %, and on the 14th day – by 9,8 % .

As it could be seen from the table 2, the dynamics of catalase activity had its own distinct features in all study groups. Thus, in the EG-1 on the 1st day after the intervention, its rate decreased compared to CG by 51,9 %, and continued to decline until the end of the whole investigated period – has been lower than the CG data on the 3rd, 7th and 14th days by 60,1 % , by 70,1 % and 63,3 % accordingly. In the EG-2, the rate of catalase activity in the liver tissue

remained consistently low in all study periods – on the 1st day it was lower by 71,7 %, compared with the CG, on the 3rd and 7th days it was lower by 79,4 % and by 79,1 %, accordingly, and on the 14th day remained lower than in the CG data by 70,5 %. With regard to the group of treated animals (EG-3), the rate on the 1st day after the intervention was lower than the CG data by 44,7 %, and on the 3rd, 7th and 14th days it was lower than the by 41,5 %, by 43,1 % and by 35,1 % respectively.

Table 2 - The content of catalase level (mkkat • kg-1) in the 10 % liver homogenate after limb ischemia-reperfusion, blood loss, skeletal trauma and its correction (Me (LQ; UQ)) - median (lower and upper quartiles)

Experimental group	Reperfusion period			
	1 st day	3 rd day	7 th day	14 th day
<i>KG Control Group</i> = 4,41 (4,16; 4,74) (n=10) $\mu\text{mol} / \text{kg}$				
<i>EG-1</i> ischemia-reperfusion, blood loss	2,16* (1,97; 2,32) (n=6)	1,76* (1,68; 1,85) (n=6)	1,32* (1,24; 1,44) (n=6)	1,62* (1,60; 1,75) (n=5)
<i>EG-2</i> ischemia-reperfusion, blood loss, skeletal trauma	1,25* (1,22; 1,30) (n=7)	0,91* (0,86; 0,94)* (n=6)	0,92* (0,91; 1,02) (n=7)	1,30* (1,21; 1,35) (n=7)
<i>EG-3</i> ischemia-reperfusion, blood loss, skeletal trauma+treatment	2,44* (1,38; 1,52) (n=6)	2,58* (1,45; 1,65) (n=6)	2,51* (1,43; 1,59) (n=6)	2,86* (1,70; 1,92) (n=5)
p ₁₋₂	<0,05	<0,05	<0,05	<0,05
p ₁₋₃	<0,05	<0,05	>0,05	>0,05
p ₂₋₃	<0,05	<0,05	<0,05	<0,05

Notes: 1.* – differences in relation to the control group are statistically significant (p<0,05);

2. p₁₋₂ – the probability of differences in relation to experimental groups 1 and 2;

3. p₁₋₃ – the probability of differences in relation to experimental groups 1 and 3;

4. p₂₋₃ – the probability of differences in relation to experimental groups 2 and 3.

When comparing the indicators of catalase activity between groups with different severity of intervention and treated, it was found that: on the 1st day after the intervention, the level of catalase in the EG-2 was by 42,1 % lower compared to the EG-1. On the 3rd, 7th and 14th days catalase levels remained lower in the EG-2 by 48,3 %, by 30,3 %, and by 19,6 %, respectively. When comparing the results between the EG-1 and the EG-3, it was found that on the 1st and on the 3rd days the level of catalase in the EG-3 was higher compared to the EG-1 by 13 % and by 46,6 %, but on the 7th and 14th days, apparently due to the sanogenic effect

of Thiocetam, the rate has been constantly higher compared to the EG-1 by 90,2 % and by the 76,5 %. Also, a significant increasing of the catalase level in the liver homogenate was found in the EG-3 comparably to the EG-2, when the index in the EG-3 was higher than the data of the EG-2 by 95,2 % on the 1st day after intervention, and then on the 3rd, on the 7th and on the 14th days it was higher than in the EG-2 in 2,8 times, in 2,7 times and in 2,2 times respectively.

Currently, there is an active search for ways and substances that would counteract the onset of ischemia-reperfusion syndrome or reduce its local and systemic manifestations. Thus, given the fact that in conditions of ischemia-reperfusion a systemic inflammatory response develops, a group of scientists found a positive effect of Iloprost on the lungs in these conditions [17]. At the same time, the results of catalase administration are of interest, claiming that oxidation-mediated lung damage is likely to be potentiated as a result [18]. However, it is impossible to exclude the local growth of free radical influence [19], which even before the development of the systemic inflammatory response carries hidden risks. Therefore, the establishment of the effect of an antioxidant introduced directly into the center of future oxidative formation should theoretically suppress these reactions. Considering the fact that peroxide damage [20] develops locally in the ischemic and then reinfused muscle, this idea has been developed to some extent by another group of scientists who have witnessed the remote damage to other vital organs [21].

The experimental results of a researchers group who investigated the introduction of pentoxifylline before, during and after the ischemic period are noteworthy. In the muscles of both study groups (treated and un treated) there were expressed necrotic changes, but they were less under the conditions of pentoxifylline. The main idea of this experiment was that the effect of pentoxifylline is similar to the therapeutic effect of hypothermia previously simulated by group of scientists [22].

Group of researchers studied the level of SOD, katalase and MDA and nitric oxide in the serum on the background of the administration of acetylcysteine, beta glucan and coenzyme in order to reduce systemic manifestations of limb ischemia-reperfusion. It was found decreasing of LPO compared to the untreated control group. N-acetylcysteine, beta-glucan, and coenzyme Q(10) were shown to have antioxidant and anti-inflammatory effects on reperfusion injury. similar positive results were obtained with the use of lycopene [24, 25].

Undoubtedly, the use of antithrombotic agents and correction (stabilization of ionic balance by exposure to biological membranes) with cellostazol and levosimendan are of interest in the study of modeling infrarenal aortic occlusion for modeling ischemia-

reperfusion of the lower extremity, followed by a study of the level of MDA, SOD, and glutathione in lung tissue. This group of scientists was able to achieve better results in simulated trauma languages by prior ischemia-reperfusion of the introduction of therapeutic substances [26].

Conclusions

1. The use of thiocetam, which is able to struggle against of ischemia and lipid peroxidation by reactivating antiradical enzymes: superoxide dismutase, catalase and glutathione peroxidase, had a positive effect on the state of antioxidant and prooxidant units in the organ, located far from the place of primary ischemia-reperfusion;

2. If in the group of untrated animals (the EG-2, where massive blood loss was combined with a thigh fracture and the use of hemostatic tourniquet) in the early period, the MDA level exceeded the CG data in 5,4 times, and on the 7th and 14th days remained high – being higher on 2,1 times and on 2,7 times, then in the EG-3 (group of treated animals) on the 1st day the level of MDA exceeded the CG data in 4,3 times, but on the 7th and 14th days was higher by 90,5 % and 64 % respectively;

3. The supportive effect of thiocetam on the activity of catalase in the liver was also noted. Thus, in EG-2 the level of antioxidant enzyme on the 1st day decreased by 71,7 %, and remained almost at this level throughout the all post-experimental period. As for the group of treated animals, the level of activity on the 1st day after the intervention decreased by 44,7%, and was so for almost the entire period. On the 14th day, it remained reduced compared to the CG by 35,1 %, while in EG-2 this index was lower compared to the CG by 70,5 %.

The prospects for futher research Thus, in our case seeing the positive effect of the introduction of thiocetam in the ischemic area, we can eventually add new complex, given the world experience, which would affect the development of the inflammatory response and the rheological properties of blood.

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