CATALASE ACTIVITY AS SIGNAL OF ANTIOXYDANT SYSTEM AFFECTION UNDER INFLUENCE OF LIMB ISCHEMIA-REPERFUSION

Nataliya Volotovska
Department of Physiology, Bioethics and Biosafety
I. Horbachevskyi Ternopil National Medical University
1 Vofi ave., Ternopil, Ukraine, 46001
volotovskanv@tdmu.edu.ua

Abstract
The use of hemostatic tourniquet is a proved means of primary care. However, systemic disorders, as well as ultrastructural, in the area of compression can significantly worsen the condition of the injured organism.

The aim. Estimation of catalase level in rats’ liver on the background of modifications of ischemic-reperfusion syndrome to know the severest pathogenic combination for organism.

Materials and methods. 260 white adult male rats were divided into 5 groups: control (KG), EG1 – simulation of isolated ischemia-reperfusion syndrome (IRS) of the limb, EG2 – simulation of isolated volumetric blood loss, EG3 – combination of IRS of the limb with blood loss, EG4 – simulation of isolated mechanical injury of the thigh, EG5 – combination of IRS of the limb and mechanical injury. The variability of catalase level in liver was analyzed.

Results. It was found that each of the experimental interventions has led to changes of catalase activity in the liver. The most expressed pathological expressions were observed on the 3rd after interventions, when the studied index in EG3 was lower than in EG1 and EG2 in 6.2 times and by 33.1 %. On the 7th day catalase activity in EG3 was in 9.4 times and by 44.5 % times lower than in EG1 and in EG2 data concordantly. The combination of limb ischemia-reperfusion with blood loss in EG3 led to exhausting of liver antioxydant enzyme catalase in the most critical posttraumatic period (day 3). The same, but less significant effect was registered in the group of combination of mechanical trauma with ischemia-reperfusion in EG5. This proved the role of the tourniquet as a factor that complicated the course of traumatic disease due to ischemic reperfusion.

Conclusions. In this experiment, founded risk factors of combination of ischemia-reperfusion with heavy blood loss emphasized the importance and particular attention on such widespread method of bleeding tratment, as the imposition of a tourniquet, as in our experiment it triggered risk factors of ischemia-reperfusion. It was shown katalase activity depression respectively to the periods of increasing of lipid peroxydation. There was peculiarity, that on the base of isolated IRS catalase activity was increased in 2.5 times comparely to control group, whereas the hardest depression of it was found on the background of IRS, combined with blood loss – catalase activity was lower, comparely to KG – in 2.5 times. The importance of understanding the suppression of hepatocytes’ antyoxydants is great, as it might help in prevention the development of liver failure or hepatorenal syndrome on the background of limb ischemia-reperfusion.

Keywords: ischemia-reperfusion, blood loss, trauma, tourniquet, catalase, experiment, liver, lipid peroxidation.

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1. Introduction
Although it is known that oxidative stress is the predominant cause of aging [1], but blood loss of various origin can trigger it as well and activate lipid peroxidation [2, 3]. One of the first enzymes to fight against reactive oxygen is catalase [4]. According to the newest data, catalase recognized as a key factor that can inhibit the growth of H2O2, especially when long term course of pathological process is observed [5].

It is known that the optimal pH for efficient catalase activity is 6–8 [6], and various pathological conditions, including massive blood loss or skeletal injury, trigger increasing of acidity due to increased concentration of hydrogen ions. It is resulted with development of metabolic acidosis and also reducing of reserve alkalinity. Such conditions significantly block catalase power, enzyme will not be able to protect cells membranes at such a shift in pH. It must be mentioned, that one of the serious factors that triggers a heavy release of free radicals into the systemic circulation is ischemia-reperfusion [7].

Peroxidation is activated due to hypoxia in the tightened area, and its derivatives has been accumulated as a result of compression and cessation of blood supply locally. After repairing the
blood circulation in affected limb these dangerous biologically active substances are released to
the blood. Rhabdomyolysis products also reach the systemic bloodstream [8]. Also among the ini-
tial factors that trigger systemic ischemic-reperfusion injury is an immediate nonspecific inflam-
matory response [9].

Thus, the newest literary sources promulgate that the use of hemostatic tourniquet, includ-
ing its application on the battlefield, by activating pathological chains leads to intensification of
excretory function of the kidneys and overwork of the liver with next exhausting of its detoxifica-
tion functions [10, 11]. In available literature there is not enough information about the systemic
manifestations of limb ischemia-reperfusion. Therefore, that fact shows necessity of studying of
future methods of prevention of IRS complications.

**The aim** of the work – to estimate catalase level in rats with modifications of ischemic-
reperfusion syndrome to know the severest pathogenic combination for organism.

2. Materials and methods

Experiments were performed in the vivarium of I. Ya. Gorbachevsky Ternopil National
Medical University of the Ministry of Health of Ukraine in the morning. Special room had stable
temperature (18–22 °C), relative humidity (40–60 %) and illumination 250 lux in the period of sum-
ter and autumn of 2016. 260 nonlinear male rats (200–250 g) were used in experiments and all ani-
imals were divided into 5 groups: control and 5 experimental (10 animals each). In EG1 animal were
simulated with ischemia-reperfusion of the limb. Under thiopental-sodium anesthesia (40 mg·kg –1
body weight intraperitoneal), 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 [12]. The
tourniquet was tightened according to the applied effective pressure marking, which is able to stop
the blood flow. In EG2 under conditions of anesthesia, acute heavy blood loss (up to 40 % of vo-
lume of circulating blood) was modelled by puncture of the femoral vein with further hemostasis.
In EG3, these two injuries were combined. In EG4 animals were done with mechanical trauma of
the femur with aim of closed fracture simulation. The combination of ischemia-reperfusion of the
thigh with mechanical trauma was modelled in EG5. Animals were eliminated from experiment
at the 1st hour after intervention, and on the 1st, 3rd, 7-th and 14-th days after trauma on the base
of thiopental-sodium anaesthesia by total bleeding from the heart. In the case of KG animals, they
were anesthetized with an equivalent dose of sodium thiopental and material were collected for the
study, as from the experimental groups.

The study design was considered with the rules of the «European Convention for the Pro-
tection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (European
Convention, 1984) and Law of the Ministry of Health of Ukraine No. 690 [13], 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 No. 61 by 11.01.2021.

In the homogenate of the liver [14] an activity of the key component of the enzymatic link of
antioxidant protection and antioxidant mechanisms – catalase – was established.

A statistical analysis on obtained data was performed by Excel (Microsoft, USA). In ad-
dition 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 group-
ing, 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 $).

3. Results

As could be seen from the data of Table 1 on the background of ischemia-reperfusion,
combined with various additional injuries modelling, affection of the catalase activity was am-
biguously, but in accordance to the severity of the intervention. Thus, after 1 hour in EG1 (on the
background of isolated ischemia-reperfusion) the indicator exceeded the data of the control group (KG) by 12.6 % (0.56 μcat·kg⁻¹) [4.41–4.97=0.56], after which a rapid increase in the concentration of the enzyme in the liver tissue was noticed. On the 1st day after the intervention, it was higher than in the KG data by 17.9 % (0.79 μcat·kg⁻¹), and on the 3rd and 7th days exceeded the KG by 2.5 times (6.51 μcat·kg⁻¹) and 2.8 times (7.95 μcat·kg⁻¹), concordantly.

Table 1
Liver catalase activity (μcat·kg⁻¹) after ischemia reperfusion of the limb and blood loss (Me (LQ; UQ)) – median (lower and upper quartiles)

| Experimental group                      | Reperfusion period |
|-----------------------------------------|--------------------|
|                                         | 1st hour | 1st day | 3rd day | 7th day | 14th day |
| KG Control = 4.41 (4.16; 4.74) (n=10)   |          |         |         |         |          |
| EG1 isolated ischemia reperfusion (n=10)| 4.97 (4.60; 5.30) | 5.20* (4.94; 5.40) | 10.92* (10.44; 11.16) | 12.36* (11.59; 12.91) | 8.38* (8.17; 8.90) |
| EG2 Blood loss (n=7)                     | 3.00* (2.97; 3.22) | 2.27* (2.21; 2.72) | 2.63* (2.52; 2.75) | 2.38* (2.20; 2.45) | 2.47* (2.42; 2.50) |
| EG3 ischemia-reperfusion + Blood loss (n=6) | 2.83* (2.70; 2.95) | 2.16* (1.97; 2.32) | 1.76* (1.68; 1.85) | 1.32* (1.24; 1.44) | 1.62* (1.60; 1.75) |

Note: * – differences in relation to the control group are statistically significant (p<0.05); p₁–₃ – the probability of differences in relation to experimental groups 1 і 3; p₂–₃ – the probability of differences in relation to experimental groups 2 і 3

On the 14th day, the indicator remained significantly higher than in the KG – by 90.2 % (3.97 μcat·kg⁻¹).

On the background of isolated volumetric blood loss after 1 hour in the EG2 the activity decreased by 32 % (1.41 μcat·kg⁻¹), compared with KG. In subsequent periods, as a result of hemic hypoxia, the activity of the indicator remained suppressed – on the 1st, 3rd, 7th days it was lower by 49.2 % (2.14 μcat·kg⁻¹), by 40.4 % (1.78 μcat·kg⁻¹) and by 46 % (2.03 μcat·kg⁻¹), concordantly. On the 14th day statistically significance was 44 % (1.94 μcat·kg⁻¹) lower than in KG.

On the background of IR combined with blood loss in the EG3, after the 1st hour after the intervention, catalase activity decreased by 36.9 % (1.58 μcat·kg⁻¹) compared with KG. And on 1st, 3rd, 7th and 14th days, it remained reduced, compared with KG by 27.3 % (1.22 μcat·kg⁻¹), 35.4 % (1.56 μcat·kg⁻¹), by 46.2 % (2.04 μcat·kg⁻¹) and 39.9 % (1.76 μcat·kg⁻¹), respectively.

As could be seen from Table 2, on the background of an isolated injury in EG4 after 1st hour, the rate decreased slightly – by 7.9 % (0.35 μcat·kg⁻¹) compared with KG. On the 1st, 3rd, 7th and 14th days it was lower than in KG by 14.7 % (0.65 μcat·kg⁻¹), 16.8 % (0.74 μcat·kg⁻¹), 17.5 % (0.77 μcat·kg⁻¹) and 8.4 % (0.37 μcat·kg⁻¹), respectively.

Decrease in catalase activity on the background of mechanical trauma combined with IR in EG5 was slightly more expressed, compared with EG4. After the 1st hour the indicator was lower than in the KG level by 12.9 % (0.57 μcat·kg⁻¹). On the 1st, 3rd, 7th and 14th days, it remained reduced, compared with KG by 27.3 % (1.22 μcat·kg⁻¹), 35.4 % (1.56 μcat·kg⁻¹), by 46.2 % (2.04 μcat·kg⁻¹) and 39.9 % (1.76 μcat·kg⁻¹), respectively.

The dynamics of the catalase activity on the background of each intervention had its own characteristics, but in general it was found that isolated IR led to an adequate increase in catalase activity, while other types of intervention were excessive for its possible compensatory properties. Thus, it was found that in the EG1 the indicator exceeded the data of the 1st hour by 4.6 % (0.23 μcat·kg⁻¹) [5.20–4.97=0.23], after that the activity increased and on the
3rd day was higher than the data of the 1st hour and 1st day by 2.2 times (5.95 μcat·kg\(^{-1}\)) and by 2.1 times (5.75 μcat·kg\(^{-1}\)), respectively. On the 7th day, the indicator increased slightly, compared with the 3rd day by 13.2 % (1.44 μcat·kg\(^{-1}\)), but remained significantly increased, compared with the 1st hour in 2.5 times (7.39 μcat·kg\(^{-1}\)), compared to the 1st day it was increased in 2.4 times (7.16 μcat·kg\(^{-1}\)). On the 14th day, despite a certain decrease in activity compared to the 3rd and 7th days by 23.3 % (2.54 μcat·kg\(^{-1}\)) and 32.2 % (3.98 μcat·kg\(^{-1}\)), respectively, it unfortunately remained increased compared to the 1st hour by 68.6 % (3.41 μcat·kg\(^{-1}\)) and compared to the data of the 1st day by 61.2 % (3.18 μcat·kg\(^{-1}\)), respectively. Such changes in catalase activity can probably be explained by the depletion of the compensatory capacity of this enzyme, because for so long the liver was able to maintain its protective function. Similar dynamics is established in [15].

The activity of catalase in muscle tissue was also studied: it was found that it gradually increased to 7th day, after which it sharply decreased by 14th days. Such dynamics is evaluated as the absence of uncompensated antioxidant system violation.

**Table 2**
Liver catalase activity (μcat·kg\(^{-1}\)) after ischemia reperfusion of the limb and skeletal trauma (Me (LQ; UQ)) – median (lower and upper quartiles)

| Experimental group | Reperfusion period | 1st hour | 1st hour | 1st hour | 1st hour | 1st hour |
|--------------------|-------------------|----------|----------|----------|----------|----------|
| **EG4** Trauma (n=10) | 4.06 (3.57; 4.23) | 3.76 (3.63; 4.17) | 3.67* (3.56; 3.77) | 3.64* (3.51; 3.78) | 4.04 (3.81; 4.18) |
| **EG5** ischemia-reperfusion + trauma (n=9) | 3.84 (3.58; 4.04) | 3.19* (3.10; 3.35) | 2.85* (2.77; 3.13) | 2.37* (2.10; 2.40) | 2.65 (2.50; 2.82) |

Note: \( p_{1-5} \) – the probability of differences in relation to experimental groups 1 and 5 (\( p < 0.05 \)); \( p_{4-5} \) – the probability of differences with respect to experimental groups 4 and 5

In the EG2, the indicator remained stable at a quite low level: on the 1st day it was lower than the data of the 1st hour by 24.3 % (0.73 μcat·kg\(^{-1}\)), and the indicator of the 3rd day, although it was lower than the data of the 1st hour by 12.3 % (0.37 μcat·kg\(^{-1}\)), was statistically significantly increased, compared with 1st hour by 15.9 % (0.36 μcat·kg\(^{-1}\)). On the 7th catalase activity decreased again, and relatively to the 3rd day it was less by 9.5 % (0.25 μcat·kg\(^{-1}\)), and was lower relatively to the 1st hour by 20.7 % (0.62 μcat·kg\(^{-1}\)). On the 14th day the indicator remained statistically lower comparably to the 1st hour by 17.7 % (0.53 μcat·kg\(^{-1}\)).

In the EG3 the expressed suppression of activity of catalase is fixed. On the 1st day the indicator decreased compared to the 1st hour by 23.7 % (0.67 μcat·kg\(^{-1}\)). On the 3rd day the activity was lower, compared with the 1st hour by 37.8 % (μcat·kg\(^{-1}\)), and compared with the 1st day by 18.5 % (μcat·kg\(^{-1}\)). On the 7th day the indicator decreased even more and was lower, compared to the 3rd day, by 25 % (0.44 μcat·kg\(^{-1}\)), as well as less compared to the data of the 1st hour by 53.4 % (1.51 μcat·kg\(^{-1}\)) and 1st day – by 38.9 % (0.84 μcat·kg\(^{-1}\)), respectively. On the 14th day a slight increase in catalase activity was fixed, compared to the data of the 7th day by 22.7 % (0.3 μcat·kg\(^{-1}\)), but it still remained below compared to the level of 1st hour and 1st day by 42.8 % (1.21 μcat·kg\(^{-1}\)), by 25 % (0.54 μcat·kg\(^{-1}\)), respectively.

In EG4 the dynamics was as follows, but in all investigated period of time \( p \) was higher than 0.05: on the 1st day the catalase activity decreased compared to the 1st hour by 3.8 % (0.3 μcat·kg\(^{-1}\)). On the 3rd day it was decreased and lower compared to the 1st hour – by 9.6 % (0.39 μcat·kg\(^{-1}\)). On the 7th day, the indicator remained at the level of 3rd day and was lower than the level of the
1st hour by 10.3 % (0.42 μcat·kg\(^{-1}\)). On the 14th day, the indicator returned to the level of the 1st hour, exceeding the data of 7th day and 11 % (0.4 μcat·kg\(^{-1}\)).

In EG5 on the 1st day the indicator was lower than the level of the 1st hour by 16.9 % (0.65 μcat·kg\(^{-1}\)), on the 3rd day decreased by 10.7 % (0.34 μcat·kg\(^{-1}\)) compared to the 1st day and remained reduced compared to the 1st hour by 25.8 % (0.99 μcat·kg\(^{-1}\)). At 7th day the decline in activity has been continuing when the rate was lower than the level of the 1st hour and the 1st day by 38.3 % (1.47 μcat·kg\(^{-1}\)) and 25.7 % (0.82 μcat·kg\(^{-1}\)). On the 14th day, the activity began to recover slightly, compared with the data of the 7th day, the indicator increased by 11.8 % (0.28 μcat·kg\(^{-1}\)), but still remained lower than the data of the 1st hour and 1st day by 31 % (1.19 μcat·kg\(^{-1}\)), 16.9 % (0.54 μcat·kg\(^{-1}\)), respectively.

Analyzing the change in the studied indicator, the correlation between the severity of severe manifestations of inhibition of catalase activity was established, which testified to the most destructive effect of the combination of IRS with massive blood loss. Thus, after the 1st hour the indicator in EG3 was lower than the data of EG1 by 43.1 % (2.14 μcat·kg\(^{-1}\)). Also, indicator in EG5 was lower than the data of EG1 by 22.7 % (1.13 μcat·kg\(^{-1}\)). On the 1st day, the index in EG3 was lower than the data of EG1 by 58.5 % (3.4 μcat·kg\(^{-1}\)). Herewith, indicator of EG5 was lower comparably to data of EG1 by 38.7 % (2.01 μcat·kg\(^{-1}\)) and lower than data of EG4 by 15.2 % (0.57 μcat·kg\(^{-1}\)). On the 3rd day the studied index was lower than in EG1 and EG2 in 6.2 times (9.16 μcat·kg\(^{-1}\)) and by 33.1 % (0.87 μcat·kg\(^{-1}\)); also it was lower in EG5 comparably to data of EG1 and EG4 in 3.8 (8.07 μcat·kg\(^{-1}\)) times and by 22.3 % (0.82 μcat·kg\(^{-1}\)), respectively. On the 7th day catalase activity in EG3 was in 9.4 times (11.4 μcat·kg\(^{-1}\)) and by 44.5 % times (1.06 μcat·kg\(^{-1}\)) lower than in EG1 and in EG2 data, respectively and in EG 5 the indicator was lower comparably to data of EG1 and EG4 in 3.2 times (5.73 μcat·kg\(^{-1}\)) and by 34.4 % (1.39 μcat·kg\(^{-1}\)) respectively. As for 14th day the rate in EG3 was lower compared to EG1 and EG2 in 5.2 times (6.76 μcat·kg\(^{-1}\)) and by 34.4 % (0.85 μcat·kg\(^{-1}\)), and indicator in EG5 was lower comparably to data of EG1 and EG4 in 3.2 times (5.73 μcat·kg\(^{-1}\)) and by 34.4 % (1.39 μcat·kg\(^{-1}\)) respectively.

4. Discussion

In general, analyzing the dynamics of the activity catalase – the enzyme of the antioxidant system – we can identify the following pathogenetic factors that underlie similar changes. Previously we have found that each of these types of experimental interventions contributed to the growth of endogenous intoxication (EI) and stress impact for excretory renal function and the detoxifying role of the liver [16, 17]. In this case, the more significant the level of EI, the more expressed suppression of antioxidant protection in almost all internal organs [18], and of course, in the liver.

Thus, it was possible to show that in the structure of the degrees of expression of the suppression of AOS the most critical was the combination of IR with heavy blood loss. Among the reasons that suppress the concentration of antioxidants are reactive oxygen species due to increased lipid peroxidation of cells membranes, oxidative stress [19]; in the structure of factors that can trigger previously mentioned processes are both hemic hypoxia [20] and the systemic inflammatory response syndrome [21, 22]. In case of our experiments the realization of toxic substances that have accumulated as a result of 2-hour application of the tourniquet leads to the further release into the blood of products of damaged muscle tissue [23–25] on the background of endothelial dysfunction of the microcirculatory tract [26].

Researchers also point to the special insidiousness of the development of the syndrome of multiorgan damage and the subsequent mutual reinforcement of existing manifestations. The results of the studies highlighted the role of ischemic reperfusion of the limb on the systemic manifestations of mechanical trauma and volumetric blood loss.

Study limitations. A limitation of our study is the investigation of effect of 2-hour imposition of tourniquet into the tie and its effect to antioxidant activity of liver, while the reactive forms of oxygen were neglected. Thus, obtained information was in accordance with previously elucidated data of lipid peroxidation activity in this organ.

Prospects for further research. Further studies of the problem of postischemic complications, mainly associated with hypoxic disorders on the background of heavy blood loss, required further
investigation of changes in other internal organs and describing of systemic expressions of this pathology. At the same time, the establishment of critical periods when oxidative stress is in its peak and the greatest inhibition of AOS appears can help in avoiding of complications after reinfusion therapy. The following data will help in optimisation of treatment regimen, in particular actions in the first hours after bleeding, when additionally drug administration, antioxidants, could be recommended.

5. Conclusions

In this experiment, founded risk factors of combination of ischemia-reperfusion with heavy blood loss emphasized the importance and particular attention on such widespread method of bleeding treatment, as the imposition of a tourniquet, as in our experiment it triggered risk factors of ischemia-reperfusion. It was shown catalase activity depression respectively to the periods of increasing of lipid peroxidation. There was peculiarity, that on the base of isolated IRS catalase activity was increased in 2.5 times comparatively to control group, whereas the hardest depression of it was found on the background of IRS, combined with blood loss – catalase activity was lower, compare to KG – in 2.5 times. The importance of understanding the suppression of hepatocytes’ antioxidants is great, as it might help in prevention the development of liver failure or hepatorenal syndrome on the background of limb ischemia-reperfusion.

Conflict of interests

The authors declare that they have no conflicts of interest.

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