ANTIOXIDANT ENZYMES ACTIVITY IN EXPERIMENTAL ISCHEMIA-REPERFUSION INJURY

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Background. *Blood loss during civil and military limb trauma is the most common cause of preventable death. Complications due to the use of a hemostatic tourniquet are widely investigated nowadays. Therefore, the standards of the past have to be improved.*

Objective. *The aim of the research is to study the reaction of the enzyme chain of the liver antioxidant system in the presence of modifications of ischemia-reperfusion injury (IRI).*

Methods. *210 white male-rats, aged 5-5.5 months, were used in the research. The dynamics of antioxidant enzymes activity catalase (Cat) and superoxide dismutase (SOD) in liver tissue in cases of modifications of ischemia-reperfusion injury (IRI) were studied. The period of investigation was in 24 hours, 3, 7, 14 days after the injury.*

Results. *In cases of simulated IRI the catalase level mainly decreased at each period of the experiment. The peak of SOD activity was evidenced on the 1st, 3rd or 7th days after the experimental IRI according to the degree of trauma severity. Thus, IRI combined with severe blood loss and mechanical trauma caused the severest affection of the antioxidant system. Even a single application of hemostatic tourniquet caused similar wavelike reactions at different times.*

Conclusions. *The development of IRI is accompanied by a significant depression of the liver antioxidant system. The most significant changes were evidenced in cases of IRI combined with blood loss and mechanical trauma, but even a single application of a tourniquet caused active response of the antioxidant enzymes.*

KEY WORDS: **ischemia-reperfusion injury; trauma; blood loss; hemostatic tourniquet; catalase; superoxide dismutase.**

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Introduction

The damage to both skeletal muscles and internal organs caused by local ischemia-reperfusion injury (IRI) is mostly present as a result of intraoperative use of tourniquet for temporary stop of blood circulation in internal organs or for stop of blood loss from the limb [1-10]. Primary damage leads to mechanical pressure, as for secondary mechanisms – reactive oxygen intermediates and lipid peroxidation are responsible for it [11]. Nowadays, in state of affairs of Ukrainian hostilities, gunshot wounds are widespread, and use of hemostatic tourniquet is one of efficient methods. But complications accompanying it are more serious than it was though previously.

In the case of the hemostatic tourniquet use it is important to know about period of depression of antioxidant system in vitally important internal organs, for preventing exhaustion in further period consequences.

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Oxidative stress is crucial in development of local and systemic damage and progress of ischemia-reperfusion injury in cases of oxygen insufficiency of the tissues [12]. Overproduction of reactive oxygen species (ROS) is combined with the violation of oxidative-reduction systems activity, damage of DNA, membrane receptors, dysfunction of ion channels and changes in composition of membrane phospholipids, as well as activation of caspase mechanism of apoptosis [13, 14, 15]. All this is leading to activation of endogenous antioxidant defense [13, 16-22].

Cellular systems of antioxidant protection are classified as enzymatic and non-enzymatic. The first one includes superoxide dismutase (SOD), glutathione peroxidase (GP), and catalase (Cat), which provide the first line of defense against the action of ROS in that way, when the product of the first reaction becomes a substrate for the next one [23].

Overexpression of SOD1 prevents neuronal death in the area of hippocampus [24]. However, the extremely short period of half-life of SOD1 in circulating blood makes it difficult to use enzyme therapy for brain damage. In

some studies GP provides even greater defense against oxidative stress than SOD – its expression, as well as SOD provides cytoprotective effect [16, 18]. Cat is a very important element in maintaining the intracellular concentration of reduced glutathione and is crucial in neutralization of free radicals [24, 25].

Various methods are used nowadays in therapeutic treatment: from lowering the temperature of the limb to the use of various drugs. Thus, the protective effect of curcumin on the myocardium, kidneys, nervous tissue and lung has been proved in cases of this pathology [26-29]; montelucast reduces the level of local and systemic manifestations [29], legalon, thiotriazolin, emoxipine, silymarin have antioxidant effect [3-9, 25-30]. Studying of changes in the activity of antioxidant enzymes is needed for prediction of the effect of a specific antioxidant corrector. This is essential to avoid exhaustion of the impaired body systems.

Objective. The aim of the research was to study the reaction of enzyme chain of the liver antioxidant system in the presence of modifications of IRI.

Methods

The experiments were performed on 210 non-linear white male rats, 250-270 g in weight and 5-5.5 months of age. They were divided into 5 groups, each of them contained 10 animals: the control group involved rats, which were administered thiopental-sodium anesthesia (40 mg/kg of body weight intramuscularly) only, the **1st experimental group** (tourniquet was applied to the upper thigh 1/3 for 2 hours, reperfusion for 1 hour; the **2nd experimental group** (blood loos in amount of 40% of circulating blood volume was simulated; the **3rd experimental group** (a tourniquet on a thigh was combined with 40% blood loss from femoral vein of another lower limb), the **4th experimental group** (a tourniquet on a thigh was combined with femoral bone fracture of another lower limb), the **5th experimental group** (a tourniquet on a thigh was combined with 40% blood loss and femoral bone fracture of another lower limb).

The experiments were performed in the vivarium of I. Horbachevsky TNMU in the morning. Special room had stable temperature (18-22 °C), relative humidity (40-60%) and illumination 250 lux. Animals were sacrificed on the 1st, 3rd, 7th and 14th days after the trauma by thiopental-sodium anesthesia (40 mg/kg of body weight intraperitoneally by total blood-letting from the heart. An activity of catalase (mckat/kg) and superoxide dismutase (U/mg) of 10% liver homogenate samples were determined by means of Koroliuk MA, et al. (1988) and Chevari S, et al. (1985) methods respectively.

All experimental stages of the research were performed following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Order of the Ministry of Health of Ukraine No. 690, dated September 23, 2009.

A statistical analysis of the attained data was performed by Excel (Microsoft, USA). Statistically significant differences between the independent indices were estimated by the Student t-test at normal distribution and by nonparametric methods in other cases. The results were presented as (M±m), where M means value, m – standard error. Correlation analysis was performed for the attained data. Linear correlation coefficient (r) and its significance (b) were evaluated as well. The link was considered to be lost if the r index was 0, the link evidenced of a week correlation when the range was 0-0.03. In the case of the range 0.3-0.7 – a medium link was established, the range of 0.7-1.0 proved a strong interaction correlation. The correlation coefficient was significant at $p<0.05$.

Results

The total content of the investigated enzymes of the liver antioxidant system is presented in Table 1.

On the 1st day, compare to the control, an increase of SOD in 3.1 times (p1<0.01), by 80% (p2<0.01), by 13% (p3<0.002), in 70% (p4<0.003), in 4.6 times (p5<0.006) was evidenced. As for Cat, its level decreased the most. Thus, on the 1st day its decrease in 24.1 times (p1<0.01), in 13.5 times (p2<0.01), in 17.8 times (p5<0.004) took place as well as an increase by 16% (p3<0.004) and 38.6% (p4<0.002).

On the 3rd day, compare to the control, in all experimental groups there was an total increase of SOD activity in 3 times (p1<0.003), in 5.4 times (p2<0.007), in 3.6 times (p3<0.03), in 2.48 times (p4<0.01), by 86% (p5<0.007). As for Cat, its dynamic was variable. A decrease in its level in 24 times (p1<0.003), in 11.9 times (p2<0.005) was evidenced, but in cases of other traumas – an increase of its level in 1.8 times

(p3<0.3), by 77.6% (p4<0.05), in 1.8 times (p5<0.08).

On the 7th day, compare to the control, there was an evident increase if SOD activity in all experimental groups: in 13 times (p1<0.02), in 3.1 times (p2<0.006), in 12.8 times (p3<0.02), in 4.2 times (p4<0.009), in 5.3 times (p5<0.003). In all groups, except for the 3rd one, a decrease of catalase activity was evidenced: in 3.5 times (p1<0.02), in 8.3 times (p2<0.005), in 15.2 times (p4<0.005), in 21.8% (p5<0.02). So, in the 3rd group an increase in 12.8 times (p3<0.02) took place.

The similar dynamic of SOD, compare to the control, was evidenced on the 14th day. An increase in its level in 2.2 times (p1<0.01), in 2.4 times (p2<0.004), in 5.8 times (p<0.006), in 2.36 times (p4<0.007), in 5.4 times (p5<0.01) took place.

As for Cat mostly depression was evidenced: a decrease of activity, compare to the control, in 10.3 times (p2<0.005), in 13.2 times (p3<0.004), in 1.67 times (p4<0.05), in 21.4% (p5<0.04), and a very slight increase of it in cases of isolated tourniquet – by 17% (p1<0.05).

In cases of activation of lipid peroxidation and oxidative stress, according to the literature, different levels of activity of the antioxidant system in all organs of the affected organism take place. The attained results proved that in 1 day in liver homogenate, the SOD activity increased in all experimental groups, but the most– in cases of a tourniquet alone and IRI combined with blood loss and mechanical trauma, when its level, compare to the control, increased in 3.1 and 4.6 times respectively.

In all groups, a wavelike reaction of enzyme activity was revealed. Thus, in cases of isolated IRI on the 1st and 3rd days it increased in 3-3.1 times; it suddenly increased (including the steady rates, compared with the previous periods) in 13 times, compare to the control, on the 7th day – in 13 times; it did not reached the norm on the 14th day. Blood loss only, associated with primarily hypoxia of the ischemic genesis, triggered activation with peak of its activity on the 3rd day; it was still high. The combination of classic IRI with blood loss contained elements of both abovementioned manifestations: a mild increase in the activity (apparently due to the fight against hypoxia) in the presence of blood loss only as well as in cases of IRI only; the peak of increased SOD activity on the 7th day reached 12.8-fold rate compare to the control.

The activity of SOD in cases of IRI combined with mechanical trauma increased and decreased very smoothly with its peak on the 7th day, then it did not reached a normal level on the 14th day, being higher than the initial level in 2.36 times, and lower than the previous period index by 44%.

In cases of IRI combined with blood loss and mechanical trauma, two peaks of the SOD activity increase were evidenced: on the 1st day (in 4.6 times, compare to the control) and in 5.3 times on the 7th day, compare to the control. On the 3rd day, which was obviously life-threatening for this type of injury, the index

Table 1. Changes in superoxide dismutase (U/mg) and catalase activity (mckat/kg) in 10% liver homogenates of the studied rats in cases of modifications of ischemic reperfusion injury and isolated blood loss, (M±m).

Group			1	2	3	4	5
Post traumatic period	Control	Indices	Tourniquet T	Blood loss BL	T+BL	T+F	T+BL+F
In 24 hours after trauma	SOD 0.15±0.09	SOD	0.47±0.06*	0.27±0.04*	0.17±0.01	0.26±0.02*	0.70±0.02*
		Cat	0.18±0.08*	0.33±0.07*	5.17±0.02*	6.15±0.01*	0.25±0.02*
In 3 days after trauma		SOD	0.45±0.02	0.82±0.03*,**	0.54±0.16*,**	0.37±0.01*,**	0.28±0.03*,**
		Cat	0.18±0.01*	0.37±0.02*	8.01±1.3*,**	7.89±0.3*,**	8.09±0.36*,**
In 7 days after trauma	Cat 4.44±0.36	SOD	1.94±0.05*,**	0.47±0.03*,**	1.93±0.06*,**	0.29±0.02*,**	0.80±0.02*,**
		Cat	1.28±0.16*,**	0.54±0.02*,**	6.63±0.27*,**	0.63±0.04*,**	3.47±0.05*,**
In 14 days after trauma		SOD	0.32±0.06*,**	0.36±0.03*,**	0.88±0.02*,**	0.35±0.03*,**	0.83±0.06*
		Cat	5.20±0.36*,**	0.43±0.03*	0.34±0.02*,**	2.65±0.20*,**	3.49±0.20*

Notes: * – statistical significance compare to the control, ** – statistical significance compare to the previous period of the study.

slightly exceeded the initial control level, but was statistically significantly lower than on the 1st day (in 2.5 times). On the 14th day it was still increased and the same as on the 7th day.

In cases of IRI only, the dynamics of Cat activity was depressed with a peak of decrease on the 3rd day (in 24 times). In the presence of blood loss only in all study periods, the activity of catalase was statistically significantly lower compare to the control.

The peculiar features of the IRI course combined with blood loss was an increase in the activity of this enzyme on the 3rd day in 1.8 times compare to the control; on the 14th day the index decreased in 13.2 times compare to the control, and in 23.6 times compare to that on the 3rd day.

A somewhat similar dynamics of catalase activity was evidenced in cases of IRI combined with mechanical trauma, when after a gradual increase till the 3rd day, it suddenly decreased in 15.2 times compare to the control on the 7th day. Although, the index was still decreased on the 14th day being lower than the initial level in 1.67 times.

The peculiar features of the Cat dynamics in cases of IRI combined with blood loss and mechanical trauma, was a sudden decrease of its activity on the 1st day compare to the control; then the Cat activity suddenly increased on the 3rd day in 1.8 times compare to the control and was of the initial level up to the end of the experimental period, which obviously proved the reduction of enzymatic component on the 14th day.

Discussion
The correlative analysis proved that, in development of ischemia-reperfusion syndrome caused by application of hemostatic tourniquet, liver failure is significant. This is the focus of the further research on morphological changes of liver tissue. Hepatic active response is a predictable reaction, which belongs to the multiply organ failure syndrome. Association of IRI as the cause of such pathological effect in the organism was proved by a couple of researchers [32-36].

Probably, the depression of SOD is caused by overproduction of malonic dialdehyde and other derivates of peroxidation. Detection of reactive oxygen intermediates accumulation, enzymes of cytolysis and oxidative modification of proteins might be studied in our future experiments, as these indexes are typical in such course of pathological external affection [37, 38]. Moreover, the data on changes in the activity of serum and organ catalase in cases of trauma and blood loss vary. Both decrease and increase of Cat level were presented in different experimental studies [13, 21, 22]. And in our research such fluctuations of this enzyme activity were proved.

In our case active response of the liver antioxidant system was evidenced by an increased activity of antioxidant enzymes. Our studies coincide with those of other researches. E. Orlova *et al*. proved that SOD activity was the highest in liver and they advised correction of its insufficiency with Vin-Vita [23]. Affection of liver tissue caused by application of tourniquet led to possible development of multiple organ failure due to the ischemic-reperfusion limb syndrome [37].

Besides, we consider that IRI combined with mechanical trauma may cause development of abrupt affecting of the liver antioxidant system that was proved by higher level of SOD activity than in other groups and which was still increased till the end of experiment. Such dynamics of SOD and Cat activity is a result of lipid peroxidation, i.e. increase in malonic dialdehyde level [2, 11]. It has been established that reperfusion syndrome affects the development of systemic changes in cases of combined trauma complicated by bleeding that is manifested by a significant activation of lipid peroxidation.

Conclusions
Even a single use of a hemostatic tourniquet leads to significant changes in the activity of enzymatic level of antioxidant defense. In present hostilities, blood loss is often combined with skeletal injuries, so it is advisable to limit the time of use of a hemostatic tourniquet or to take measures that counteract the development of lipid peroxidation caused by an injury in the need of bleeding stopping with a tourniquet.

Conflict of interest
The authors declare no conflict of interest.

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ЗМІНА АКТИВНОСТІ ФЕРМЕНТІВ АНТИОКСИДНОГО ЗАХИСТУ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ІШЕМІЧНО-РЕПЕРФУЗІЙНОМУ СИНДРОМІ

Н.В. Волотовська, Т.В. Кащак

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Крововтрата при цивільній та бойовій травмі кінцівок є найпоширенішою причиною т.з. «превентивних смертей». Оскільки стандарти минулого щодо надання невідкладної медичної допомоги для зупинки кровотеч потребують поліпшення, сьогодні широко досліджуються ускладнення при накладанні гемостатичного джгута.*

Мета дослідження. *Вивчити особливості реакції ферментативної ланки антиоксидантної системи печінки на тлі модифікацій ішемічно-реперфузійного синдрому (IPC).*

Методи дослідження. *У експерименті використано 210 білих щурів-самців віком 5-5,5 місяців. Досліджено динаміку активності антиоксидантних ферментів каталази (Кат) та супероксиддисмутази (СОД) в тканині печінки при розвитку модифікацій IPC. Забір зразків тканини здійснювали через 24 години, на 3, 7 та 14 доби після травми.*

Результати. *У наших моделях IPC рівень Кат в основному зменшувався в кожному періоді експерименту. Пік активності СОД спостерігався на 1, 3 або 7 добу після експериментального IPC – згідно модифікацій ступенів тяжкості травми. Таким чином, застосування кровоспинного джгута в поєднанні з втратою крові та механічною травмою, викликали найважчі ураження антиоксидної системи. При цьому, навіть одноразове застосування джгута викликало подібні хвилеподібні реакції.*

Висновки. *Розвиток IPC супроводжується сильним пригніченням антиоксидної системи печінки. Найбільш значні зміни спостерігалися на тлі застосування кровоспинного джгута, поєднаного з втратою крові та механічною травмою, але навіть ізольоване застосування джгута викликало суттєву зміну активності антиоксидних ферментів.*

КЛЮЧОВІ СЛОВА: **ішемічно-реперфузійний синдром; травма; втрата крові; гемостатичний джгут; каталаза; супероксиддисмутаза.**

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