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WHY THE LUNGS BECAME A TARGET ORGAN DUE TO ISCHEMIC-REPERFUSION SYNDROME OF THE LIMB, CAUSED BY THE USE OF HAEMOSTATIC TOURNIQUET

N. V. Volotovska

I. Ya. Horbachevsky Ternopil National Medical University

Department of Physiology, Bioethics and Biosafety

B. Lepkyi streat, 6, f. 12, Ternopil town, 46024

e-mail: volotovskanv@tdmu.edu.ua

ORCID Волотовська Н. В.: https://orcid.org/0000-0003-4073-3148

Abstract. Much attention has been paid to the ambiguous effects of the tourniquets and hemostatic bandages - namely the local and systemic consequences of planned operations, as well as the changes that occur after the cessation of bleeding from a wounded limb on the battlefield. However, there is still no consensus on the ischemic-reperfusion syndrome (IRS). Aim. Detect changes in the activity of lipid peroxidation in lung tissue on the background of experimental modifications of IRS. For this goal 260 male white rats aged 5-5.5 months were divided into 5 experimental groups: 1) EG1 - imposition of the tourniquet on the thigh for 2 h; 2) EG2 - modeling of venous blood loss in the amount of 40% of the volume of circulating blood; 3) EG3 - a combination of hemostatic tourniquet and blood loss 4) EG4 - mechanical injury to the thigh bone 5) a combination of hemostatic tourniquet and mechanical trauma. The biochemical study in 10 % lung homogenate was performed by reacting of peroxidation derivatives with thiobarbituric acid. Conclusions. It was found that

each of these types of intervention caused the activation of lipid peroxidation in the lungs. The peculiarities of the reaction were such increase of this rate, which was the highest on the background of blood loss combined with the use of a tourniquet. However, the concentration of malonic dialdehyde was higher in the group where the imposition of the tourniquet was combined with mechanical trauma, compared with isolated mechanical trauma of the thigh. This has shown the role of the tourniquet as a factor that complicated the course of traumatic disease due to ischemic reperfusion.

Key words: ischemia-reperfusion syndrome; lungs; trauma; blood loss; hemostatic tourniquet; lipid peroxidation

Introduction. An inevitable side effect of technical progress is an increase of the level of trauma caused by various factors. The development of traumatic illness due to mechanical impact is characterized by a conditional division of it into periods manifested by acute disorders of regulation and functioning of body systems, and then – periods of adaptation and rehabilitation [1-4]. We have focused on a combination of pathogenic factors – blood loss with ischemic-reperfusion syndrome (IRS) due to the use of a tourniquet on the limb and mechanical trauma combined with IRS. Such combinations have become not accidental, because today, in the conditions of hybrid war, gunshot wounds are often combined with bone injuries.

In the structure of combat trauma gunshot wounds of large vessels take near 2.8-8 % [5-7], and among all vascular injuries the cases, localized in the lower extremities, were found in 90-95% of events [8]. Thus, according to the last data collected in the conditions of rendering of help to victims in anti-terrorist operation in Ukraine damage of the main blood vessels of extremities is found in the 1,6 % of victims, from them injury of vessels of the lower extremities were in 56,4 %, and in structure of a combat trauma in general damage of extremities is found at 62,5 % of injured (of which 38 % accounted for the damage of upper extremities, and 62 % of the lower) [9]. One of the main causes of prehospital mortality in the structure of combat trauma and the second place in the structure of civilian trauma is occupied by uncontrolled bleeding [10]. The use of hemostatic tourniquet is one of the effective and rapid methods of first aid in combat, the time and features of the application of which is regulated by ambient temperature and general recommendations for use [11-13].

The first places among the shortcomings in providing surgical care to the wounded and injured are the development of compartment syndrome and improper application of a tourniquet on the limb (at a considerable distance from the wound, for a long time), and the

shortcomings of surgical treatment of injuries and chest injuries according to [9] there is «no use of reinfusion». There are also many sources of information that indicate the emergence of various complications due to the use of hemostatic bandages, including violations on the systemic level. [14, 15]. In addition, there are data from clinical studies that give examples of systemic disorders in the conditions of intraoperative use of the tourniquet (for operations on the heart, liver, joints) [16-22], as well as due to the use of a typical hemostatic tourniquet [23-27]. This indicates the particular relevance of research in this area. Thus, complications on a local region are the occurrence of paresthesias and pain due to compression of peripheral nerves and muscles, as well as due to the accumulation of intercellular fluid in the perifascial spaces.

Due to the direct pressure of the tourniquet on the skin and muscles, there is a local violation of microcirculation in the skin and muscles, followed by reinfusion of hypoxic tissues with degeneration of axons, [28]. Though, as a result of a significant increase in the concentration of rhabdomyolysis products and the triggering of local oxidative stress, lipid peroxidation products enter the systemic bloodstream, cytokines are activated [29-31]. In particular, in these cases, the use of a tourniquet is already a recognized factor that causes changes in the activity of neurophiles, activation of monocytes, stimulates neutrophil transendothelial migration, followed by the likelihood of tissue damage [32].

The results of a series of experimental studies have identified the effects of IRS on internal organs [33-36]. It should be noted that although the information base on the pathogenesis of IRS is updated, but data on the systemic affections and methods of its correction are insufficient. Thereby, the aim of this experimental interventions was to study the features of the lung response to the use of hemostatic tourniquet on the limb.

Materials and methods of research. The experiment was performed on 260 male nonlinear white rats aged 5-5.5 months, which were on the standard mode of keeping the vivarium. Such amount was based on the necessity to reach statistically authentic data in each group the same as in every time point – to see stages of development of posttraumatic disease, in particular lipid peroxidation activity, that in our case was cased with haemostatic tourniquet. Besides this, the most complicated course of pathology on the background of EG3 and EG5 caused higher mortality compared to other experimental groups. Animals were divided into 5 groups every of which contained 10 personages: control (**KG**), where rats were only entered into thiopental-sodium anaesthesia (40 mg·kg of body weight intraperitoneally), **1 experimental group EG1** (rubber tourniquet was applied to the upper 1/3 of the thigh for 2 hours, reperfusion lasted for 1 hour – simulated isolated ischemia-reperfusion; **2nd**

experimental group (blood loos in volume of 40 % from volume of circulating blood from femoral vein was simulated – EG2 ; **3rd experimental group** (tourniquet on thigh was combined with 40 % blood loss from femoral vein on another lower limb) – EG3; **4th experimental group** (mechanical trauma that caused fracture of femoral bone using the device «ЩП-1»); **5th experimental group** (tourniquet on thigh was combined with fracture of femoral bone of another lower limb).

The ischemic-reperfusion syndrome was modeled by applying a 1 cm wide strip of rubber tourniquet to the upper 1/3 of the thigh, calculating the force of pressure under the control of the marking applied to the tourniquet. It was left for 2 hours, after that the animal were eliminated after 1 hour of in another necessary time point. To simulate blood loss, 40% of the circulating blood volume was taken from the femoral vein. The fracture of the femur occurred due to a dosed blow with a metal bar, the strength of which was established empirically, resulting in a closed bone fracture. To avoid the pronounced effects of painful shock on the day of surgery in EG4 and EG5 injection of solution of the Lidocaine 2 % has been injected within 7 days of posttraumatic period. Animals of another groups were done with analgesics two times – on the day of intervention and on the next day.

Animals were eliminated from the experiment after the 1 h, on the 1, 3, 7 and 14 days after injury by total bleeding from the heart on the base of thiopental-sodium anaesthesia (40 mg·kg of body weight intraperitoneally) by total bleeding from the heart.

Experiments were performed in the vivarium of I. Horbachevsky TNMU in the morning. Special room had stable temperature (18-22 º C), relative humidity (40-60 %) and illumination 250 lux. An activity of thiobarbituric acid active derivatives in 10 % lung homogenate samples were determined by the method based on ability of secondary products of LPO, especially malonic dialdehyde, during reaction with thiobarbituric acid in the high temperature conditions and acidic PH to form coloured complex with optic density possible for the registration on waves 532 nm [37].

All experimental stages of work were executed in accordance to 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 № 690 from September 23, 2009.

A statistical analysis on obtained data was performed by Excel (Microsoft, USA). A statistical significance of the differences between independent indices was estimated by

Student t-test at normal distribution and by nonparametric methods in other cases. The correlation coefficient was significant at $p<0,05$.

Results and discussion

As it could be seen from the data of the table 1 and the of Figures 1 and 2, in the conditions of isolated use of the tourniquet the content of TBA-active derivatives in the lungs was increased. Thus, significant changes are recorded after the 1 hour next to the tourniquet deflation – in this period the index was greater than the same in the CG by 55.6 % ($p <0.05$).

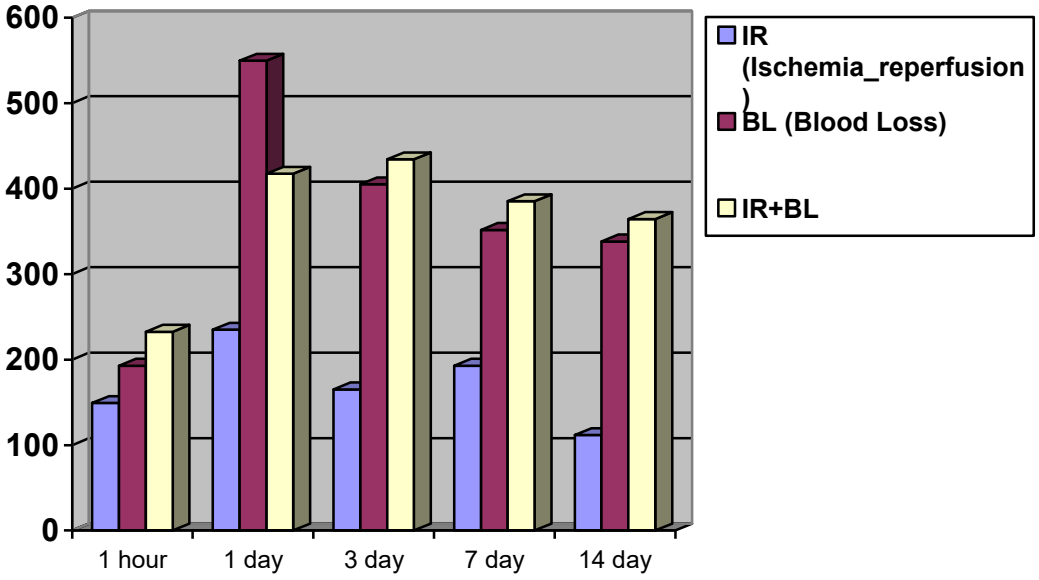
Table 1. Changes in Lipid Peroxydative Activity in 10 % rat's lungs homogenates on the base of modifications of ischemia-reperfusion syndrome, isolated blood loss and mechanical trauma

Group	Reperfusion period				
	1 hour	1 day	3 day	7 day	14 day
Control = 1,89 (n=10)					
Group *1* isolated ischemia-reperfusion	2,94* (n=10)	4,63* (n=10)	3,25* (n=10)	3,80* (n=10)	2,20 (n=10)
Group *2* Blood loss	3,80* (n=7)	8,63* (n=7)	7,98* (n=6)	6,93* (n=7)	6,66* (n=7)
Group *3* ischemia-reperfusion + Blood loss	4,58* (n=6)	8,23* (n=6)	8,56* (n=6)	7,59* (n=6)	7,18* (n=5)
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
Group *4* Trauma	2,85* (n=10)	4,18* (n=10)	4,22* (n=10)	3,20* (n=10)	2,86 (n=10)
Group *5* ischemia-reperfusion + trauma	3,40* (n=9)	5,36* (n=9)	5,76* (n=8)	4,32* (n=9)	2,86* (n=9)
p_{1-5}	<0,05	<0,05	<0,05	<0,05	<0,05
p_{4-5}	<0,05	<0,05	<0,05	<0,05	>0,05

Notes:1.* – differences in relation to the control group are statistically significant ($p<0,05$);
2. p_{1-3} – the probability of differences in relation to experimental groups 1 i 3;
3. p_{2-3} – the probability of differences in relation to experimental groups 2 i 3;
4 p_{1-5} – the probability of differences in relation to experimental groups 1 i 5;
5. p_{4-5} – the probability of differences in relation to experimental groups 4 i 5.

Our data about the activation of LPO in the lung homogenate at such an early period are consistent with a data of the LPO activity in serum and changes in the lungs on the background of the tourniquet on the limb [16, 17]. Thus, a group of scientists proved that in the first hours after removal of the tourniquet there is a violation of pulmonary gas exchange,

where the enhancement of lipid peroxidation and systemic inflammatory response may be involved.



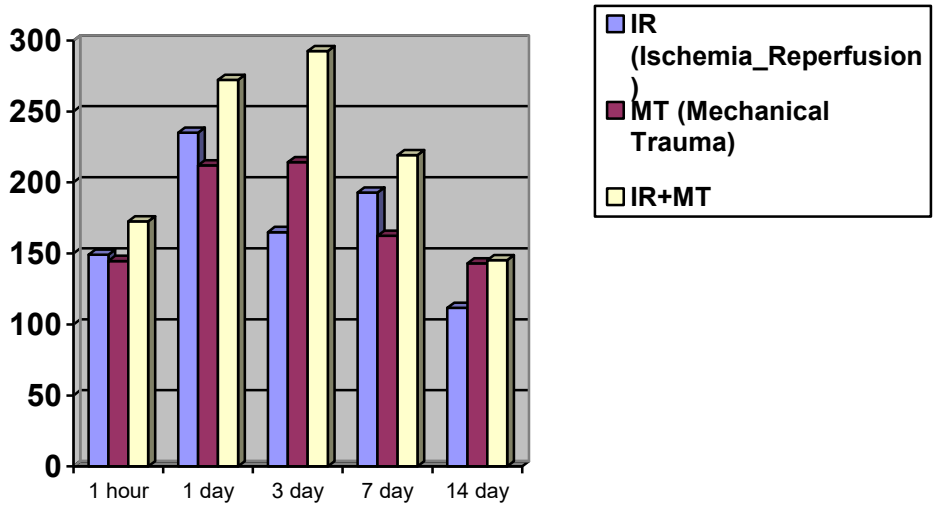
Picture 1 – the Dynamics of TBA-active derivatives of LPO in the rat's lungs (comparing to Control level) after IRS of limb and Blood Loss

Notes: statistical differences between EG1, EG2, EG3 are significant, p<0,05)

On the 1, 3, 7 and 14 days this index exceeded the CG value in 2.4 times ($p < 0.05$), by 72 % ($p < 0.05$), in 2.0 times ($p < 0.05$) and by 16.4 % ($p < 0.05$), respectively.

On the background of isolated blood loss, the content of TBA-active products of LPO after the 1 h following the intervention was 2 times higher ($p < 0.05$) than this index in the CG, on the 1 day – in 4.6 times ($p < 0.05$), on the 3 day – in 4.2 times ($p < 0.05$), on the 7 and 14 days – in 3.7 and 3.5 times respectively ($p < 0.05$). In the conditions of the blood loss combined with a plait, increase of the investigated indicator was the greatest in all terms of supervision. Thus, after the 1 h the level of TBA-active derivatives of LPO exceeded the CG in 2.4 times, on the 1, 3 days was higher in 4.3 times and in 4.5 times ($p < 0.05$). At the same time, even on the 7 and 14 days, it was significantly increased in 4.0 and in 3.8 times ($p < 0.05$).

On the background of mechanical trauma (EG4), the activity of peroxidative processes was as follows: after the 1 h exceeded the level of CG by 50.8 % ($p < 0.05$), on the 1, 3 days – in 2.2 times ($p < 0.05$), 7 day – by 63 % ($p < 0.05$). Though the activity decreased on the 14 day, it exceeded the initial level by 49.2 % ($p < 0.05$).



Picture 2 – the Dynamics of TBA-active derivatives of LPO in the 10 % rat's lungs (comparing to Control level) after IRS of limb and Mechanical trauma

Notes: statistical differences between EG3, EG4, EG5 are significant, p<0,05)

With regard to mechanical trauma combined with ischemia-reprefusion (EG5), after the 1 hour, on the 1, 3, 7 and 14 days the rate exceeded the CG indices by 80 % ($p < 0.05$), in 2.8 times ($p < 0.05$), in 3 times ($p < 0.05$) , in 2.3 times and by 51.3 %, respectively ($p < 0.05$).

The dynamics of the studied index had certain patterns regardless of the severity of the injury: up to the 3 days the it has been increased increased in most cases, than has been decreased by 7, 14 days. Thus, on the background of isolated IRS (EG1), the content of TB-active LPO derivatives in the lung homogenate reached the highest level for the 1 day, exceeding the 1 h level by 57.5 %. The rate of the 3 day was statistically significantly higher than the rate of the 1 hour by 10.5 % and lower than the rate of the 1 day by 29.8 %. On the 7th day, the index exceeded the level of the 1 hour and 3 day by 29.3 % and 16.9 % respectively, has being lower than the index of the 1 day by 17.9 %($p < 0.05$). Thus, the stepwise dynamics of LPO activity on the background of isolated IRS was manifested.

On the background of isolated blood loss (EG2) on 1 day the index exceeded the threshold of the 1 hour significantly – in 2.3 times ($p < 0.05$). On the 3 day activity decreased compared to 1 day by 7.5 % ($p < 0.05$), but still remained higher than the 1 h – in 2.1 times ($p < 0.05$). On the 7 day the value of the index continued decreasing, has being lower than the 1 day by 19.7 % ($p < 0.05$), comparably to the 3 day – by 13.6 % ($p < 0.05$), but exceeded the index of the 1 hour by 82,4 % ($p < 0.05$). On the 14 day the index decreased slightly, remaining lower than the 1 day by 22.8 % ($p < 0.05$), comparably to the 3 day – by 16.5 % (p

<0.05), 7 day – by 3.9 % (p <0.05) and, herewith, being higher than the 1 hour – by 75.2 % (p <0.05).

On the background of IRS combined with blood loss (EG5), the rate on the 1 day exceeded the rate of the 1 h by 79.7 % (p <0.05), on the 3 day there was a statistically significant increase compared to the 1 h by 86.9% (p <0,05) – which turned out to be a peak period. On the 7 day the activity of LPO in the lungs decreased, has being lower than the 3 day by 11.3 % (p <0.05), but still exceeding the 1 hour level by 65.7 % (p <0.05). This index remains elevated on the 14 day, when, compared to the 1 h remained higher by 56.8 % (p <0.05) and has been lower than the rate of the 1 day by 12.8 % (p <0.05), 3 day – by 16.1 % (p <0.05), 7 day – by 5.4 % (p <0.05)) respectively. On the background of an isolated injury (EG4), the dynamics of the index activity was characterized as a "plateau", after which, from the 7 day – the value of the LPO began to decline. Thus, on the 1 and 3 days the index was higher than the index of the 1 h by 46.7 % (p <0.05) and by 48.1 (p <0.05) % respectively, on the 7 day it remained higher than the index of the 1 h by 12.3 % (p <0.05) and decreased compared to the 1 and 3 days by 23.4 % and 24.2 % (p <0.05). On the 14 day the index was lower than the value of the 1 and 3 days by 31.6 % and 32.2 % (p <0.05) respectively.

As for the dynamics of the index on the background of IRS, combined with trauma (EG5) the peak of peroxidation occurred on the 3rd day. Thus, on the 1 and 3 days the figure was higher than the same after the 1 hour next to the intervention by 57.6 % and 69.4%, respectively (p <0.05). On the 7 day it was significantly higher than the value of the 1 hour by 27.1 % (p <0.05) and decreased compared with days 1 and 3 by 19.4 % and 25 % (p <0.05) respectively. On the 14 day the index decreased compared to the 1 hour, 1, 3 and 7 days by 15.9 %, 46.6 %, 50.3 % and 33. 8%, respectively (p <0.05).

Comparison of the severity of LPO between EG1 and EG3 found that the combination of IRS with blood loss already after the 1 h following the intervention was higher than the isolated IRS by 35.8 % (p <0.05) and higher than on the background of the isolated blood loss – by 17.3% (p <0.05). On the 1 day and subsequent time points of the experimental injury, the EG3 index remained higher than the same in EG1 – by 43.7 %, 62 %, 49.9 % and 69.3 %, respectively (p <0.05). Also, the index of EG3 remained higher than the similar in EG2 – by 3, 7 and 14 days – by 6.8 %, 8.7 % and 7.2 % (p <0.05), respectively.

The exception was the 1 day, when the level of hemic hypoxia caused a condition in which the EG2 index was higher than the EG3 index by 4.9% (p <0.05).

Comparison between the indexes of the EG1 and EG5 found that after the 1 h in EG5 the studied index was higher than similar in EG1 by 13.5 % ($p < 0.05$) and higher than in EG4 – by 16.2 % ($p < 0.05$). On the 1 day EG5 index exceeded EG1 data by 13.6 % ($p < 0.05$), and EG4 data – by 22 % ($p < 0.05$). On the 3 day the index of LPO in EG5 continued to grow and also exceeded the increased EG1 and EG4 by 43.6 % and 26.7 %, respectively ($p < 0.05$). After that, even decreasing in activity, the LPO in EG5 exceeded the indices of similar data in EG1 and EG4 by 12 % and 25.9 % on the 7 day ($p < 0.05$). However, on the 14 day the index exceeded only the data of EG1 – by 23.1 % ($p < 0.05$).

Ischemic-reperfusion syndrome, according to information sources, has numerous pathogenic local and systemic effects, including on the lungs. Despite conflicting sources of information about the risk of pulmonary thromboembolism after total knee arthroplasty using a tourniquet, a number of scientists are still inclined to the existence of such a connection, in particular, proving the informative value of the signal molecule plasma D-dimer [38].

Conclusion. Therefore, the effect of the tourniquet on the animal's body led to an increase in the content of TBA-active derivatives of LPO in the internal organs, in particular in the lungs, as evidenced by relatively lower levels of LPO caused by isolated blood loss and isolated mechanical trauma. Consequently, in the lungs in response to the modification of IRS significantly increased the level of concentration of TBA-active products of LPO, reaching a maximum on day 3, with such a further decrease to 14 days, which did not return to baseline.

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