Liuta O. O. Trends in the indicators of lipid peroxidation and antioxidant protection of rat blood serum and heart under acute experimental lung injury. Journal of Education, Health and Sport. 2018;8(1):320-329. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1341905
http://ojs.ukw.edu.pl/index.php/johs/article/view/5777

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 23.01.2018. Revised: 26.01.2018. Accepted: 31.01.2018.

UDC 616.12-091.8-02:616.24-001-036.11-085]-092.9

TRENDS IN THE INDICATORS OF LIPID PEROXIDATION AND ANTIOXIDANT PROTECTION OF RAT BLOOD SERUM AND HEART UNDER ACUTE EXPERIMENTAL LUNG INJURY

O. O. Liuta

SHEI I.Ya. Horbachevsky Ternopil State Medical University,
Ministry of Public Health, Ukraine

Abstract

Dynamically, acute experimental lung injury (ALI), caused by intratracheal injection of chloride acid, revealed one-direction disorder of free radical oxidation processes in the blood serum and heart homogenate, the intensity of the processes increasing within 12 and 24 experimental hour. However, 48 hours after ALI simulation the growing concentration of TBA-active products was found in the blood serum only, while in the heart homogenate the index was found to be decreasing. In 72 hours, the blood serum revealed insignificant decrease in TBA-active products' concentration. However, the index was about 3 times the control value. In the heart homogenate the index was found to decrease 72 hours after experiment start and was reaching the level of the control group.

Owing to ALI simulation, the catalase activity in the blood serum exceeds the control level throughout the experiment, peak values found in 24 hours, whereas in the heart homogenate the index was found to be gradually decreasing till the 48th hour, remaining significantly lower as compared to the control level. Nevertheless, in 72 hours the catalase

activity in the heart homogenate grows and exceeds the control level notably.

Analysis of the antioxidant-pro-oxidant balance that was evaluated in the blood serum and heart homogenate by the API value revealed significant growth of the index in the blood serum within 12 and 24 hours as compared to the controls. However, by 72 hour the index was found to be decreasing reliably below the control level. Meanwhile, in the heart homogenate the API value is below the control level throughout 48 hours since ALI simulation, growing rapidly further and exceeding the control level notably in 72 hours.

Key words: acute lung injury, lipid peroxidation, catalase, heart.

Introduction. Syndrome of acute lung injury (ALI) as a type of acute respiratory deficiency, occurring owing to primary or mediated damage to alveolar-capillary membrane by exogenous factors, is characterized by non-cardiogenic pulmonary oedema, disturbed external respiration, and progressive hypoxia, resistant to oxygen therapy. It is not infrequent that ALI is a constituent of multiple organ insufficiency [1]. The severest version of ALI course is acute respiratory distress syndrome.

ALI and acute respiratory distress syndrome most often aggravate the course of sepsis, gastric content aspiration, thoracic trauma, prolonged shock, burns, fat embolism, drowning, pancreatitis, oxygen intoxication, massive hemotransfusions, and extracorporal perfusions [2].

Today, diffuse lung inflammation and damaged microcirculatory bed of the pulmonary circulation are known to be essential in the pathogenesis of ALI. This results in impaired transmembrane gas transport, prolonged pulmonary hypoxia and ischemia, disturbed microcirculation owing to the disorders of rheological blood properties, as well as in long-lasting arterial hypertension, inhibition of synthesis and destruction of surfactant. Inflammatory process in the lungs is associated with the activation of neutrophils and endotheliocytes, increased levels of pro-inflammatory cytokines and chemokines in the lungs, accumulation of lipid peroxidation toxic products [3]. Hypoxemia dynamics, as well as ingress of inflammation mediators and endotoxins into systemic blood flow adversely affect the organs and tissues by impairing both structure and function and causing multiple organ insufficiency [4].

A balance between lipid peroxidation and antioxidant system is essential in the pathogenesis of local and systemic ALI effect. Insufficient antioxidant protection leads to enhanced destruction of parenchymatous organs' cellular membranes with the loss of their functions, thus closing another "false" pathologic circle and aggravating the course of a major pathologic process [5].

However, the trend regularities of lipid peroxidation processes in the blood serum and heart at the background of ALI have not been studied properly so far, thus restricting the evidence-based approach to the development of efficient cardioprotective agents.

The aim of the work is to find out the trends in the indicators of lipid peroxidation and antioxidant protection of the blood serum and heart in experimental ALD.

Materials and methods. 86 medium-resistant to hypoxia pubertal non-linear male rats (b.w. 200-220 g) kept in standard vivarium conditions were used. V. Ya. Berezovsky's methodology was used for determining resistance to hypoxia. [6]. ALI was simulated in the experimental group (68 animals) in the G. Matute-Bello method [7]. For this purpose, cervicotomy was performed under thiopental sodium anaesthesia (40 mg. kg^{-1}) and the trachea was found. The animals were positioned at an angle of 45° and chloride acid was injected (pH=1.2) with an insulin syringe on inhale, based on 1 $ml.kg^{-1}$ of rat body weight. The animals of the control group (18 altogether) were injected with saline solution at an equivalent dose.

The animals were withdrawn from the experiment under thiopental sodium anaesthesia by the method of total bloodletting from the heart: experimental group – 12, 24, 48, and 72 hours after chloride acid had been injected into the trachea; control group – 12 hours after the injection of saline solution. The blood samples were drawn to make serum, and heart – to make 10% homogenate.

Concentration of reagents to the thiobarbituric acid (TBA-active lipid peroxidation products) was determined in the blood serum, heart homogenate [8] and catalase [9]. Antioxidant-prooxidant index (API) was calculated according to the formula: API=catalase activity/content of TBA-active lipid peroxidation products [10.].

The research was performed with strict adherence to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 18.03.1986) and the decision of the 1st National Congress on Bioethics (Kyiv, 2001).

The digital data obtained were processed statistically. Difference reliability between experimental and control groups was evaluated on the basis of non-parametric Mann–Whitney test using STATISTICA («StatSoft, Inc.», USA).

Results and discussion. As can be seen in Tables 1, 2 and Fig. 1, changes in the concentration of TBA-active products in the blood serum and heart homogenate were of one-direction type, tending to increase in 12 and 24 hours of the experiment that is indicative of lipid peroxidation initiation in this pathology.

Table 1. Indices of lipid peroxidation and antioxidant protection activity in the blood serum 12, 24, 48, and 72 hours after ALI simulation (M±m)

Index	Control (n=9)	ALD term			
		12 hours (n=10/0)	24 hours (n=11/1)	48 hours (n=10/2)	72 hours (n=11/3)
TBA-active products lipid peroxidation, mcmol.l^{-1}	0.810± 0.019	1.671± 0.015*	1.904± 0.019*	2.557± 0.005*	2.437± 0.018*
Catalase, Mccat.l^{-1}	0.095± 0.002	0.313± 0.002*	0.348± 0.002*	0.291± 0.006*	0.222± 0.010*
API, standard units	0.118± 0.004	0.187± 0.003*	0.183± 0.002*	0.114± 0.05	0.091± 0.004*

Notes: here and in Table 2:

1. * – differences against control group statistically reliable ($p<0.05$);

2. n – numerator – the total number of animals in the group; denominator – the number of animals that died in the course of the experiment.

Table 2. Indices of lipid peroxidation and antioxidant protection activity in the heart homogenate 12, 24, 48, and 72 hours after ALI simulation (M±m)

Index	Control (n=9)	ALD term			
		12 hours (n=10/0)	24 hours (n=11/1)	48 hours (n=10/2)	72 hours (n=11/3)
TBA-active products lipid peroxidation, mcmol.l^{-1}	1.572± 0.039	2.631± 0.021*	2.746± 0.004*	2.004± 0.015*	1.506± 0.086
Catalase, Mccat.l^{-1}	0.877± 0.007	0.522± 0.05*	0.594± 0.003*	0.286± 0.004*	1.434± 0.011*
API, standard units	0.560± 0.012	0.199± 0.05*	0.216± 0.05*	0.143± 0.003*	0.985± 0.083*

In the course of the experiment, increased concentration of TBA-active products was found in the blood serum of the rats with ALI, the peak value being observed in 48 hours (Fig. 1). In 12 hours, the content of TBA-active products increased 2.06 times ($p<0.05$) in comparison with the control group. 24 hours after ALI simulation, the concentration of TBA-active products was 2.35 times the control group ($p<0.05$) and 13.9% ($p<0.05$) higher as compared to the 12th experimental hour. In 48 hours, the value of the index studied was 3.16 times the control group ($p<0.05$) and 53.0% higher as compared to the 12th experimental hour ($p<0.05$) and 34.3% higher in comparison with the 24th experimental hour ($p<0.05$). 72 hours

after ALI simulation, it decreased by 4.7% ($p<0.05$) against 48th hour, but was 3.01 times and 45.8 and 28.0% higher against the control group of the 12th and 24th experimental hour, respectively ($p<0.05$).

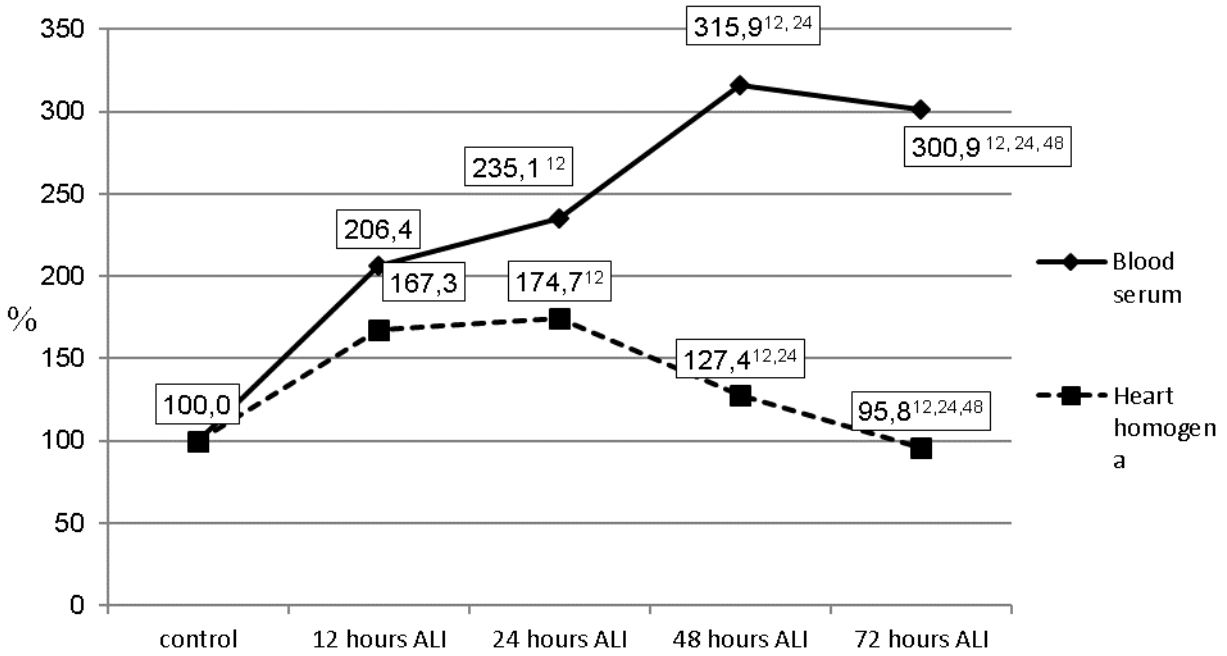

Figure 1. Trends in the concentration change of TBA-active products of lipid peroxidation in the blood serum and heart homogenate in ALI in 12, 24, 48, and 72 hours (in % against the controls).

Note: here and in the other figures: ^{12,24,48} – differences regarding 12, 24, and 48 hours after ALI simulation are statistically reliable ($p<0.05$)

In the heart homogenate the concentration of TBA-active products increased in 12 hours by 67.3% ($p<0.05$) as compared to the control group. 24 hours after ALI simulation, the level of TBA-active products increased by 74.7% against the control group ($p<0.05$), and by 4.4% – of the 12th hour after ALI simulation ($p<0.05$). The sharp decline in the concentration of TBA-active products was found 48 hours after ALI simulation as compared to 12 and 24 hours (by 23.5 and 27.0%, respectively, $p<0.05$), but the index was higher by 27.4% ($p<0.05$) in comparison with the control group. 72 hours after ALI simulation, decreased concentration of TBA-active products was found, the index being lower against 12, 24, and 48 hours by 4.2, 42.8, 45.2, and 24.8 %, respectively ($p<0.05$).

After ALI simulation, the indices of antioxidant protection in the blood serum and heart homogenate changed ambiguously.

12 hours after ALI simulation, the catalase activity in the blood serum (Table 1, Fig. 1)

increased considerably as compared to the control group (3.29 times, $p<0.05$). The highest catalase activity was found in 24 hours – 3.66 times and 11.2% ($p<0.05$) higher than the index in the control group and the one 12 hours after ALI simulation, respectively. On the 48th experimental hour, the catalase activity declined 7.2 times and by 16.3% ($p<0.05$) as compared to 12 and 24 hours, respectively, though exceeding 3.06 times the index of the control group ($p<0.05$). 72 hours after ALI simulation, the catalase activity declined against 12, 24, and 48 hours by 29.1, 36.2, and 23.7 %, respectively ($p<0.05$), but was 2.34 times the index of the control group ($p<0.05$).

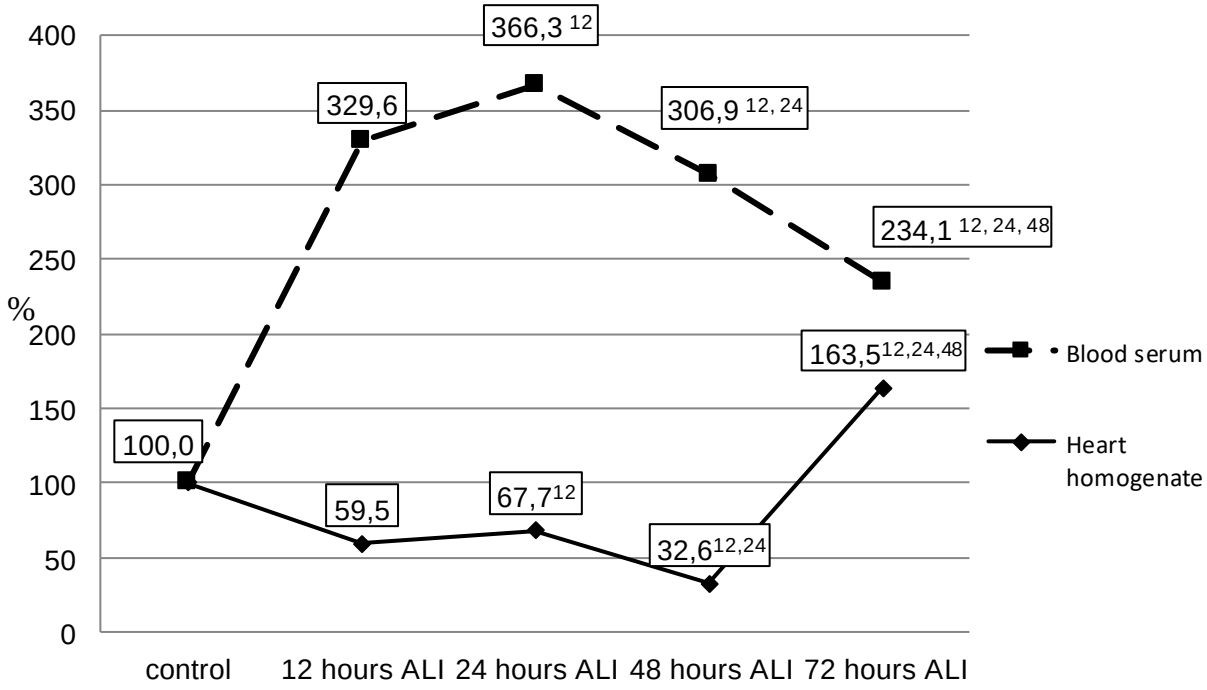

Figure 2. Dynamics of the catalase activity in the blood serum and heart homogenate 12, 24, 48, and 72 hours after ALI simulation (in % against the controls)

12 hours after ALI simulation, the catalase activity in the heart homogenate (Table 2 and Fig. 2) decreased by 45% ($p<0.05$) in comparison with the control group. In 24 hours, the index increased by 13.8% ($p<0.05$) as compared to 12 hours after ALI simulation, but remained 32.3% lower ($p<0.05$) than in the control group. The peak value in the decline of the catalase activity was found on the 48th experimental hour. The index was lower as compared to the control group, 12, and 24 hours by 67.4, 45.2, and 51.9% ($p<0.05$), respectively. In 72 hours, the sharp increase in the catalase activity was found: 5.01 times ($p<0.05$) as compared to 48 hours, by 63.5% – against controls ($p<0.05$), 2.74 times – in comparison with 12 hours ($p<0.05$), and 2.41 times – against 24 hours after ALI simulation ($p<0.05$).

Antioxidant-pro-oxidant balance is essential for the evaluation of antioxidant protection mechanisms. The value of blood serum API was found to have grown by 59.1% 12 hours after ALI simulation as compared to the control group ($p<0.05$), (Table 1 and Fig. 3). On the contrary, in the heart homogenate the API value decreased by 64.4% against the controls ($p<0.05$), (Table 2 and Fig. 3). 24 hours after ALI simulation, the value of API was found to decline as compared to 12 hours. However, the result was unreliable statistically. In comparison with the controls, the index was 55.2% higher ($p<0.05$). In the heart homogenate the growth of API value was 8.5% ($p<0.05$) against 12 hours, but the index remained significantly lower than in the control group (by 61.4%, $p<0.05$).

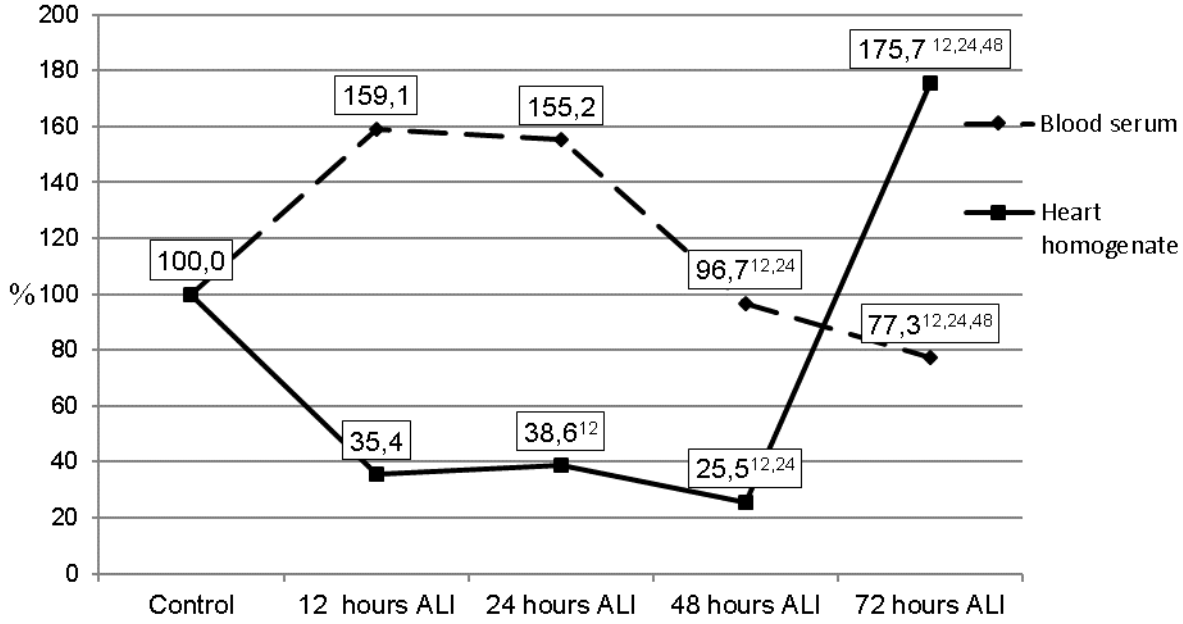

Figure 3. Dynamics of the API value in the blood serum and heart homogenate 12, 24, 48, and 72 hours after ALI simulation (in % against the controls)

In 48 hours, decreased value of API in the blood serum and heart homogenates was found. In the blood serum the index was 39.0 and 37.7% lower ($p<0.05$) in comparison with 12 and 24 hours. In 48 hours, the API value in the heart homogenate decreased in comparison with the controls, 12, and 24 hours of ALI by 74.5, 28.1, and 33.8%, respectively ($p<0.05$). In 72 hours, the sharp decline in the API value in the blood serum was found: against controls – by 22.8% ($p<0.05$), of 12 hours – by 51.3% ($p<0.05$), of 24 hours – by 50.3% ($p<0.05$), of 48 hours – by 20.2% ($p<0.05$). However, the sharp increase in the API value in the heart homogenate was found on the 72nd experimental hour, the index against the controls, 12, 24, and 48 hours of ALI being by 75.9%, 4.81, 4.56, and 6.89 times, respectively ($p<0.05$).

Thus, the dynamics of acute experimental lung injury (ALI), caused by intratracheal injection of chloride acid, revealed one-direction disorder of free radical oxidation processes in the blood serum and heart homogenate, the intensity of the processes increasing by the 24th experimental hour. The data obtained match with the current concepts of progressive free radical oxidation processes at the systemic level during the first day of ALI simulation [5].

However, we were the first to detect the activation of lipid peroxidation in the heart. To our mind, underlying them are three major causes: hypoxemia as a background for the formation of hydrogen peroxide or superoxide radical, initiating the reactions of free radical oxidation; ingress of endotoxins and activated neutrophils from damaged lungs through the pulmonary circulation into the heart, aggravating the severity of hypoxic myocardial injury; developing insufficiency of the utilisation system of active oxygen forms and free radicals – antioxidant system – through its depletion owing to massive free radicals' ingress or rehabilitation slowdown [11].

In addition, our research was the first to show that 48 hours after ALI simulation the increase of TBA-active products' concentration occurs, though in the blood serum only. Meanwhile, in the heart homogenate the index was found to decline. This fact indicates that the whole set of systemic disorders, resulting in the increased concentration of lipid peroxidation secondary products in 48 hours, appears at the background of ALI. Obviously, the systems of antioxidant protection, decreasing the intensity of free radical processes, are activated at that time in the heart.

In 72 hours, a slight decrease in the concentration of TBA-active products was found, the index exceeding the control value three times. Meanwhile, in the heart homogenate the index declined to the level of the control group, thus confirming the assumption about the activation of antioxidant protection systems in the myocardium owing to ALI.

Analysis of catalase activity dynamics revealed the index in the blood serum significantly exceeding the control level at the background of ALI, the maximum value being observed in 24 hours, whereas in the heart homogenate the index was found to be gradually declining by the 48th hour, remaining far below the control level. However, in 72 hours the catalase activity in the heart homogenate increases sharply, exceeding the control level significantly that is indicative of activated myocardium antioxidant protection at the background of ALI. The balance between pro-and antioxidant mechanisms, estimated by ALI value, is the striking evidence. According to our findings, in the blood serum under ALI this index grows significantly in 12 and 24 hours against the control one, however declining till the 72nd hour to the level, reliably below the control. Meanwhile, in the heart homogenate the

API value is lower than the control one throughout 48 hours of ALI simulation.

Our findings indicate that antioxidant mechanisms intensify in the blood serum till the 48th hour due to the extracellular catalase release. Since catalase release into the blood is caused by the increased permeability of cellular membranes, it can be assumed that this mechanism is compensatory and aimed at the utilisation of hydrogen peroxide, circulating in the blood. However, the mechanism depletes till the 72nd hour.

At the same time, domination of pro-oxidant mechanisms is clearly traced in the heart throughout 48 hours. However, in 72 hours antioxidant mechanisms intensify. Probably, this is the period when long-term adaptation mechanisms develop, with the stimulation of genetic apparatus and formation of adaptive structural trace.

Conclusions. 1. Under ALI, activation of lipid peroxidation is a pathogenic mechanism of systemic heart injury and damage that reveals itself in the significant growth of the content of reagents to thiobarbituric acid with the maximum in 24 experimental hours, as compared to the control group. In the blood serum the index is high throughout the experiment, whereas in the heart it declines by the 72nd hour, reaching the control level ($p>0.05$).

2. Activation of lipid peroxidation in the blood serum after the intratracheal injection of 0.1 H chloride acid solution occurs by the 48th hour at the background of the catalase activation, followed by depletion in 72 hours. Meanwhile, insufficient antioxidant protection is noted in the heart by the 48th hour that is followed by its intensification by the 72nd hour. This is evidenced by increased catalase activity in response to decreasing API value.

Bibliography

1. Johnson E. R., Matthay M. A. Acute lung injury: epidemiology, pathogenesis, and treatment. *J. Aerosol. Med. Pulm. Drug Deliv*. 2010. Vol. 23(4). P. 243–252.

2. Beasley M.B. The Pathologist's Approach to Acute Lung Injury. *Arch Pathol Lab Med*. 2010. Vol. 134. P. 719–727.

3. Pathogenic role of neutrophil granulocytes in the development of acute lung injury / A. A. Gudyma, M.I. Maruschak, G.G. Gabor, M.I. Kalitskaya. *Bukovinsky medical bulletin*.2011. № 3. P. 82–84.

4. Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury / Liu K. D., Glidden D. V., Eisner M. D. [et al.]. *Crit. Care Med*. 2007. Vol. 35. P. 2755–2761.

5. Gryshchuk L.A., Maruschak M.I. Dynamics of lipid peroxidation and antioxidant defense in rats with acute lung injury. *Tuberculosis, pulmonary diseases and HIV-infection.* 2011. № 1. P. 16–20.

6. Berezovsky VA Hypoxia and individual peculiarities of reactivity. K .: Naukova dumka, 1978. - 216 p.

7. Animal models of acute lung injury / G. Matute-Bello, C. W. Frevert, T. R. Martin. *American Journal Phisiology*. 2008. Vol. 295, Issue 3. P. 379–399. doi: 10.1152/ajplung.00010.2008

8. Korobechnikova E. N. Modification of the determination of LPO products in reaction with tiobarbituric acid . *Laboratory business*. 1989. № 7. P. 8–10.

9. Method for determining the activity of catalase / M.A. Korolyuk, L.I. Ivanova, I.G. Mayorov[et all]. *Laboratory business*. 1988.№ 1. P. 16–19.

10. Experimental methods for the study of osteogenesis stimulators. Methodical recommendations / A.P. Levitsky, O.A. Makarenko, O.V. Denga [and others]. Kyiv: State Pharmacological Center of Ministry of Health of Ukraine "Avicenna". 2005. P. 31–38.

11. Changayeva E.A, Ayzman R.I., Gerasev A.D. Contemporary notions about the antioxidant system of the human body. *Ecology of a person*. 2013. №. 7. P. 50–58.