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**METABOLIC PECULIARITIES AT
EXPERIMENTAL GENERALIZED PROCESS
CAUSED BY *PSEUDOMONAS AERUGINOSA***

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Introduction

Currently great attention is paid to the definition of biological mechanisms, in particular to the state of metabolic processes at various types of pathology. It is well known that the integrity of reparative processes in organism is depended on intensity and coherence of the development of main biochemical reactions of the organism [1]. It was currently proved that also numerous enzymes are activated on the background of complex biochemical processes taking place under violation of tissues and cells of the organism, which is one of the protective adaptive mechanisms of the organism in response to tissue lesion. Several dozen of the most important enzymes taking part in inflammatory processes are found in dynamics of the pathological process. Alkaline phosphatase (AP) is one of the most important enzymes taking an active part in cells proliferation. It is a polyfunctional enzyme which is closely connected with the formation of protein matrix and metabolites transport through the cell membrane. It is well known that enzymatic systems are connected with membranes by structural-cooperative interaction while many enzymes are lipid-dependent. The change of lipids composition leads to rearrangement of protein molecule structure affecting its catalytic activity through the change of lipid-protein state. At liposomal membranes damage the excessive activity of acid phosphatase taking an active part in lytic processes at phagocytosis and synthetic reactions of granulation tissue formation is marked. In this connection penetrating ability of liposomal membranes is changed which results in cellular hydrolases yield into cell cytoplasm, these phenomena are an important pathogenic link at various extreme states. A great role in the development of cell membranes lesions as a result of inflammatory process is connected with the state of antioxidant system (AOS) which is in pathogenic connection with enzymatic one [2].

System of free radical oxidation is a non-specific link of most of pathologic processes formation in organism. AOS includes antioxidant as well as antiradical enzyme superoxide dismutase enzyme (SOD) and antiperoxide enzyme catalase. Enzymological studies allowing the definition of not only organospecific violations but also accession of the state of biological membranes are of great interest in complex estimation of structural and metabolic peculiarities of organism in conditions of inflammatory pathology. In this regard the study of the activity of such enzymes as aspartate aminotransferase (AsAT), alanine aminotransferase

(AlAT), γ – glutamyl transpeptidase would be relevant [3]. Therefore the following indices were studied and analyzed for the estimation of antioxidant and lipids peroxidation systems at experimental generalized process caused by *Pseudomonas aeruginosa* in liver, kidneys, heart: alkaline phosphatase (AP) activity, laktatedehydrogenase (LDG) activity, AlAT activity, AsAT activity, γ – glutamin transpeptidase (γ - GTP) activity, malonic dialdehyde (MDA) content, diene conjugates (DC) content, sulfhydryl groups (SH-groups) content, molecules of average weight level (MAW), catalase activity.

The aim of the given **study** was the definition of the peculiarities of metabolic state at experimental generalized process caused by *Pseudomonas aeruginosa*.

Materials and methods

The experimental research was carried out on 40 inbred lines mice C57Bl/6JSto [4]. The studies were carried out according to national «General ethnic principles of the experiments on animals» (Ukraine, 2001), which are approved with the statements of «European Convention for the protection of vertebrate animals used for experimental and other scientific purposes» (Strasburg, 18.03.1986). [5].

The division of laboratory animals (20 mice in each group) was done in such way: 1 group – intact mice; 2 group – infected. The definition of LDG was done with the used of reagents of “Lactate dehydrogenase (LD)” set, Lachema, Czechia. Activity of indicator enzymes AsAT, AlAT, γ -glutamine transpeptidase (γ -GTP) were defined by unified colometric methods with the help of reagent sets “Lachema” according to the instructions for the definition of each corresponding enzyme. Catalase activity and DC were defined by spectrometric method. The MDA definition was done according to the reaction with thiobarbituric acid. The definition of MAW content in blood plasma was done by the express-test with the help of trichlorosucrose acid [6]. The Excel program for personal computer was used for statistic processing of the results [7, 8].

Results and discussion

According to the results of the conducted study the activity of the processes of lipids peroxidation increases in the myocardium of the infected animals: the MDA content - 3,4 times higher and DC –2 times in comparison with intact animals. Besides the SH-groups content (the component of the non-enzymatic link of antioxidant system) is lowered – 3,7 and 2,3 times consequently, i.e. the oxidative stress leading to energy-deficient state development takes place in the myocardium of the infected animals, which testifies for DF-enzyme activity increase, this enzyme implements the hydrolysis of monophosphoric esters and LDG-enzyme of anaerobic glycoside 13,7 and 2,2 times consequently (table 1). Activity of AsAT, AlAT and γ -GTP is reliably higher (in comparison with intact animals), which testifies for protein biosynthesis activation into tissues. Protein synthesis activation is probably connected with the acceleration of the enzymes by synthesis under the influence of mediators of inflammation, i.e. the compensatory reaction activation takes place.

Table 1. LPO level and AOS activity indices in tissues of experimental animals

№	Indices	LPO level and AOS activity		
		myocardium	kidney	liver
1	AF (intact)	0,16±0,01	0,42±0,03	0,14±0,02
2	AF (infected)	2,19±0,12*	6,39±0,28*	1,78±0,16*
3	LDG (intact)	27,4±1,8	48,2±1,9	18,3±1,5
4	LDG (infected)	59,6±2,4*	79,8±2,6*	48,2±2,3*
5	AsAT (intract)	0,24±0,02	0,36±0,08	0,48±0,02
6	AsAT (infected)	0,69±0,03*	0,92±0,06	0,88±0,06*
7	γ-GTP(intact)	134,55±3,6	269,8±3,2	34,6±1,4
8	γ-GTP (infected)	198,4±4,7*	435,3±4,3*	72,6±3,2*
9	AlAT (intact)	0,16±0,04	0,24±0,02	0,32±0,03
10	AlAT (infected)	0,42±0,06*	0,63±0,05*	0,78±0,08*
11	MDA(intact)	14,5±0,9	21,1±1,2	23,6±1,8
12	MDA (infected)	49,4±1,8*	62,9±2,6*	84,2±2,8*
13	RC (intact)	84,6±2,3	125,1±4,3	238,3±2,5
14	RC (infected)	168,4±3,2*	281,7±4,2*	479,5±4,6*
15	SH-groups (intact)	84,8±2,1	68,4±2,2	79,2±2,7
16	SH-groups (infected)	22,9±1,6*	18,6±1,3*	19,7±1,2*
17	catalase (intact)	264,1±2,5	382,7±2,8	425,6±3,1
18	catalase (infected)	112,4±1,5*	167,3±1,6*	182,8±1,9*

Note: * - p<0,01 in comparison with intact animals.

The similar picture is found in kidneys: LPO activation under AOS insufficiency, energy-deficient state. The growth of AsAT, AlAT and γ-GTP is stated which testifies for proteins metabolism activation with predominance of catabolic processes. LPO also activates in liver under AOS insufficiency, i.e. the oxidative stress is developing. LDG, AF, AlAT, γ - GTP, AsAT activities reliably increase.

At experimental generalized process caused by *Pseudomonas aeruginosa* the content of MAW in mice blood grows 9,4 times – an integral index of intoxication (fig. 1), products of LPO: MDA –12,3 times and DC –3,8 times (fig. 2) which testifies for a high level of inflammatory process and organism intoxication. The increase of acute-phase protein concentration – haptoglobin – 27,6 times testifies for a high level of inflammatory process.

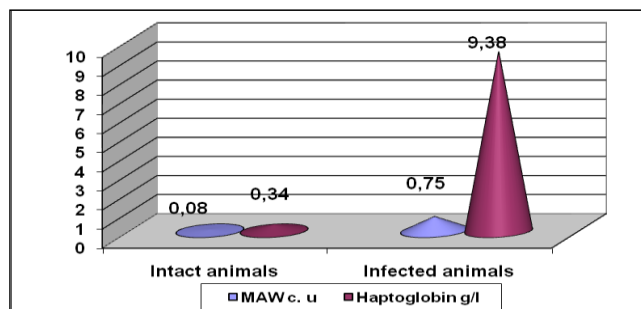


Fig 1. MAW and haptoglobin content in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*.

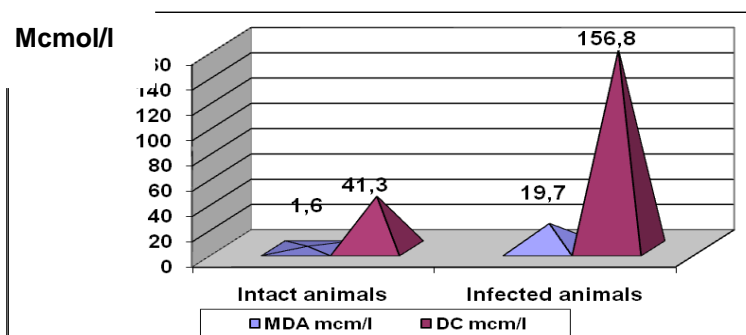


Fig 2. LPO products content in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*.

In mice at experimental generalized process caused by *Pseudomonas aeruginosa*, the AF activity in

blood grows 4,2 times (fig.3) and transaminase activity increases (fig.4).

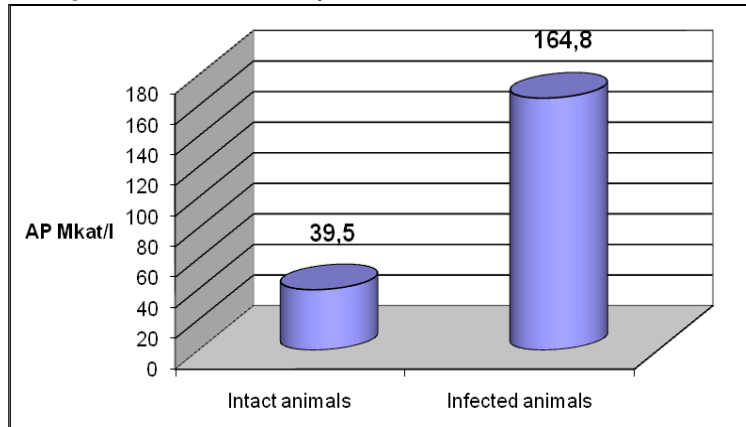


Fig 3. AP activity in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*

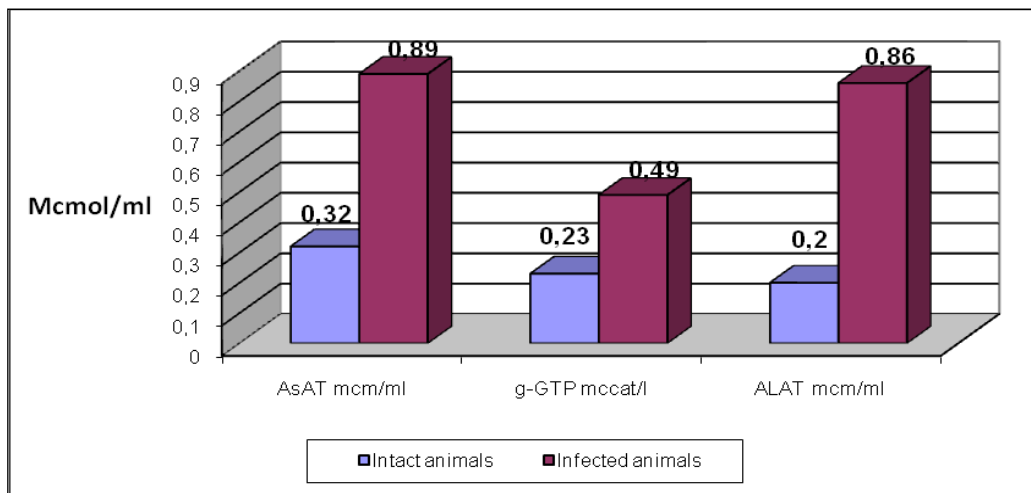


Fig 4. Transaminase activity in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*

The increase of LDG (general) activity is experienced – cytoplasmic enzyme, the increase of the content of which in blood serum (2,2 times) testifies for

cytoplasmic membranes injury. While LDG (heart fraction) increases 3 times (fig.5).

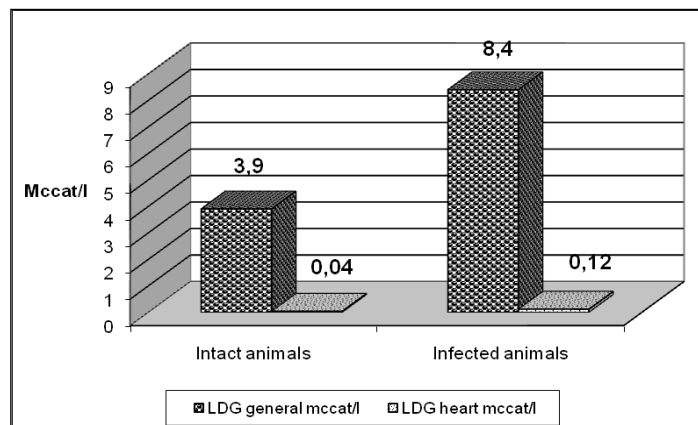


Fig 5. LDG level of general and LDG of heart fraction in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*.

The decrease of catalase activity and SH-groups of blood level are experienced in the organism of experimental animals (fig. 6) which testifies for lowering

of thiol compounds (first of all low-molecular) in the organism due to oxidizing modification into disulfide one.

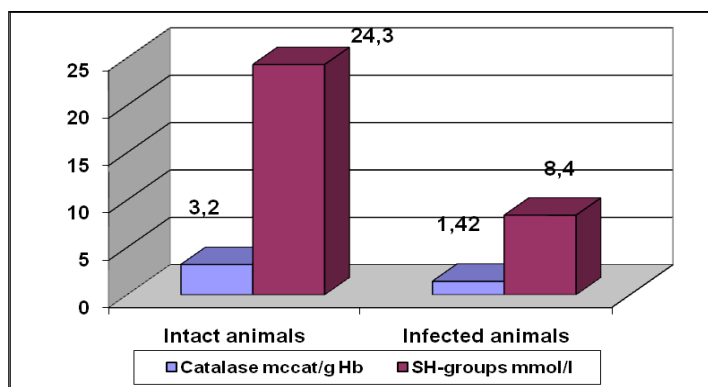


Fig 6. Level of AOS indices in mice blood at experimental generalized process caused by *Pseudomonas aeruginosa*

Thus the AOS enzymatic link of mice at experimental generalized process caused by *Pseudomonas aeruginosa* appears to be insufficient which leads to the development of the oxidizing process which is harmful for tissues.

Conclusions. The results of the metabolic processes study at experimental generalized process caused by *Pseudomonas aeruginosa*, allowed the installation of LPO activation, processes injuring the biological membranes structure simulating the development of free-radical pathology in the organism. Lingering stimulation of LPO leads to the exhaustion of AOS, inhibition of the processes of bioenergetics and biosynthesis which are specifying in the formation of biologic mechanisms of AOS suppression under the influence of endotoxiosis, acute phase proteins and the misbalance in fermentative link work.

References

1. Baraboy V.A. Oxidative-antioxidant homeostasis in norm and pathology / Baraboy V.A., Sutkovoy D.A. – K.: Scientific thinking, 1997. – 420 p.
2. Vladimirov Yu.A. The estimation of antioxidative and antiradical activities of the substances and biological objects with the help of iron initiated chemiluminescence / Vladimirov Yu.A., Sherstnev M.P., Azimbayev T.K. //Biophysics. – 1992. – V. 37, Вып. 6. – P. 1041-1047.
3. Shapoval G.S. Mechanisms of antioxidant protection of the organism under the influence of oxygen active forms / Shapoval G.S., Gromovaya V.F. // Ukr. biochem. journal. – 2003. – V. 75, № 2. – P. 5-13.
4. Peshin G.N. Methods of experimental chemotherapy: Practical guide / Pershin G.N. – M.: Medicine, 1971. – 539 p.
5. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes // Strasbourg. Council Treatu Series, 1987. – №123. – 52 p.
6. Methodological aspects of the study of the organism antioxidant system and the level of lipids peroxidation /Scherban N.G., Gorbach T.V., Mishura A.I. [and oth.] Methodical recommendations for doctoral students, graduates, ,asters, SRW executers. - Kharkov KhSMU, 2004.- 39p.
7. Lapach S.N. Statistical methods in medico-biological research with the use of Excel / Lapach S.N., Chubenko A.V., Babich P.N. – K.: MORION, 2000. – 320p.

8. Methods of statistical treatment of information in scientific research /V.P. Osipov,H.M. Lukyanova, Yu.G. Antipkin [and all.] – K.: Planet of people, 2002. – 200 p.

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System of free radical oxidation is a non-specific link of most of pathologic processes formation in organism. Enzimological studies allowing the definition of both organospecific violations and the state of biological membranes are of great interest in complex approach to the estimation of structural and metabolic peculiarities of organism in conditions of inflammatory pathology. Thus the purpose of the given study is the definition of metabolic state peculiarities at experimental generalized process caused by *Pseudomonas aeruginosa*. According to the results of the carried out studies the activity of the processes of lipids peroxidation in myocardium of infected animals rises: the content of MDA and DC is increased in comparison with intact animals while SH-groups content and catalase activity are decreased, i.e. the oxidative stress takes place in myocardium of infected animals which leads to energy-hungry state process which is also proved by AF – enzyme activity increase which implements hydrolysis of monophosphoric esteris and LDH – enzyme of anaerobic glycolysis. Activity of AsAT, AIAT and γ -GTP is reliably higher which proves about the activation of protein biosynthesis into tissues which is connected with accelerated enzyme synthesis under the influence of inflammation mediators, i.e. compensatory reaction activation takes place. The similar picture is found in kidneys and liver: LPO under insufficiency of AOS, power-hungry state. The level of MCB – integrated indicator of intoxication as well as LPO products grows in blood of infected experimental animals which proves about high level of inflammatory process and organism intoxication. Increasing of protein concentration of acute phase – haptoglobin – also proves about high level of inflammatory process. High activity of LDG (cytoplasmatic enzyme) proves about cytoplasmic membranes injury. The decrease of catalase activity and level of SH-groups of blood are found in organisms of experimented animals which proves that the content of thiol compounds in organism decreases due to oxidative modification to disulfide. Thus AOS enzymatic

link becomes insufficient, as a result of which the oxidative stress is developing which harms tissues. The results of metabolic processes study at experimental generalized process caused by *Pseudomonas aeruginosa* allowed determining the activation of LPO processes which destroyed the structure of biological membranes modulating the development of free radical pathology in organism. Lingering stimulation of LPO leads to the exhaustion of AOS, inhibition of the processes of bioenergetics and biosynthesis which are specifying in the formation of biologic mechanisms of AOS suppression under the influence of MSM, acute phase proteins and the misbalance in fermentative link work.

Key words: metabolic state, experimental study, *Pseudomonas aeruginosa*, LPO level and AOS activity indices.