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OXIDATIVE STRESS IN CASE OF ACUTE PANCREATITIS AND UNDER CONDITIONS OF DEXAMETHASONE CORRECTION

Abstract. *The study was performed on 82 white male rats of Wistar line weighing 180-220 g, with modeled acute pancreatitis (AP) and dexamethasone correction. The following samples for analysis were taken: the blood serum on 12, 24 and 48 hours to determine the level of malondialdehyde, diene conjugates and catalase in order to evaluate the intensity of oxidative stress.*

The main pathophysiological link of AP was found to be a release of pancreatic enzymes to the blood. General inflammatory response occurs with formation of free radicals accumulating in many tissues and organs, and may cause irreversible changes.

The introduction of dexamethasone in the early stages of AP is evidenced to slow down the process of lipid peroxidation, whereas injection in the later stages of pancreatitis is not accompanied by a significant decrease of oxidative processes in the blood serum of animals.

Key words: *acute pancreatitis, lipid peroxidation, diene conjugates, catalase, rats.*

Introduction. One of the main issues of abdominal surgery is acute pancreatitis (AP), especially its destructive form. In spite of introduction of new methods of treatment lethal outcome in case of AP remains stably high and reaches 85% in severe cases. The highest mortality rate is associated with various complications of pancreatitis, such as purulent-septic ones and multiple organ failure. In case of AP a great number of pro-inflammatory mediators are released and systemic inflammatory response syndrome develops (SIRS) [11].

Release of numerous inflammatory mediators is closely connected with the processes of lipid peroxide oxidation (LPO). The pancreas possesses the lowest level of antioxidants in the body. Under conditions of inflammation and ischemia peroxide reactions are imbalanced, anaerobic way of glycolysis is triggered, a number of under-oxidized metabolites and concentration of free oxygen radicals (FOR) increase, and antioxidant systems (AOS) become quickly depleted (catalase, superoxide dismutase, glutathione system) [2]. In case of AOS insufficiency peroxidation of membrane lipids occurs resulting in an increased passive permeability of the membranes at the expense of formation of through polar canals, changes of the flow and charge of the lipid layer, and eventually – damage of exocrine pancreatic cells and disorders of intercellular contacts [15]. Damaged exocrine pancreatic cells release FOR and LPO products able to activate neutrophils with further intensification of SIRS and microcirculatory disorders [14].

Severe course of acute pancreatitis is often associated with an increased permeability of micro-vessels resulting in great loss of intravascular fluid into the tissues, and thus decreasing perfusion of the lungs, kidneys and other organs [16].

Excessive production of LPO products leads to cytotoxic action manifested by damage of phospholipids of the cellular membranes of many organs. At the same time, the structure of the lipid biological layer changes even to its rupture, cytochromoxidase activity is inhibited, stable toxic waste products are released, such as Malone dialdehyde (MDA), able to increase cellular damage of those acting as chemoattractants in case of SIRS with participation of a cascade of complements, various cytokines and other substances of an acute phase [9]. At the same time, MDA inhibits prostacyclin promoting aggregation of platelets and clot formation. Therefore, intensity of LPO processes can be determined by MDA level [7].

Thus, highly toxic compounds such as diene conjugates (DC) and active products of thiobarbituric acid (TBA-AP) formed in case of POL activation result in damage of the membranes and

cellular structures, and together with energy deficiency and metabolic acidosis promote affliction of the majority of parenchymal organs, and the lung tissue in particular [3].

One of the most effective medicines inhibiting inflammatory response is glucocorticosteroids (GCS). The drug Dexamethasone is a synthetic GCS widely used in clinical practice. This group of medicines possesses a powerful anti-inflammatory, immunosuppressive and anti-allergic action. These steroid hormones affect all the stages of inflammatory process. Dexamethasone effect promotes reduced permeability of the blood vessels, inhibition leukocyte migration as a result of inhibited expression of adhesion molecules, and blockage of arachidonic acid products formation. Immunosuppressive action is manifested in reduced formation of NFκβ, which is a transcription factor for the main stimulators of inflammation, such as TNF-α, IFN-γ and numerous interleukins. Due to extended inhibiting action of the drug on neutrophils and macrophages, where oxygen explosion occurs, dexamethasone is able to decrease oxidative stress [8].

Objective of the study was to find regularities of LPO development in case of acute L-arginine induced pancreatitis and under conditions of dexamethasone correction.

Materials and methods. The study was performed on 82 white male rats of Wistar line weighing 180-220 g, kept on a standard diet with free access to water. The animals were distributed into 4 groups: I – intact group of animals (n = 10); II – control group (n = 10) receiving physiological solution in the dosage of 1 ml per 100 g of the body weight; III – rats with experimental acute pancreatitis (n = 32), IV – animals with experimental acute pancreatitis and correction by means of dexamethasone (“Darnytsia”, Kyiv, Ukraine) (n = 30). All the experiments were conducted under general anaesthesia using ketamine (40 mg/kg). The animals were kept and all the manipulations performed according to the regulations of the Law of Ukraine “On protection of Animals against Cruel Treatment” (N 1759-VI dated 15.12.2009). After the experiment was over, all the animals were euthanized.

Experimental pancreatitis was modeled by means of two intraabdominal injections with 20% L-arginine solution in the total dose of 5 g/kg with one hour interval. Dexamethasone solution was injected i/m in the dose of 1 ml per 1 kg. The drug was injected to animals with modeled AP 1 hour before the material for examination was taken (on 11, 23 and 47 hours). The blood was taken for biochemical analysis in 12, 24 and 48 hours after the experiment was initiated.

LPO processes in the blood serum were studied by means of diene conjugates detection method of unsaturated higher fatty acids (V.G. Gavrylov et al., 1998). The method is based on the following principles: in the process of LPO related double bonds are formed in the molecules of oxidized substrates. At the same time, the maximum appears in the spectrum of optic radiation absorption with the wave length of 232 nm [5].

The method to detect TBA-active products (E.N. Korobeynikova, 1989) consists in the following: with heating a part of LPO products, which belong to the class of endoperoxides, in acid medium are hydrolyzed with formation of Malone aldehyde. Interaction of its molecules with two molecules of thiobarbituric acid results in the formation of a stained complex [6].

The method of detection of catalase activity (A.N. Bakh, I.S. Zubkova, 1968) is based on the following: a certain amount of hydrogen peroxide is added to a sample containing an enzyme, and after a certain period of time by means of titration with potassium permanganate the amount of undamaged peroxide is detected [1].

The data obtained were statistically processed by means of non-parametric criteria on a personal computer and the program «Statistica 7» («Statsoft, Inc.» – USA). The reliability was determined by Wolcoxon’s criterion and Sign-test. Differences were considered reliable in case the value of P was 95% and more ($p<0,05$).

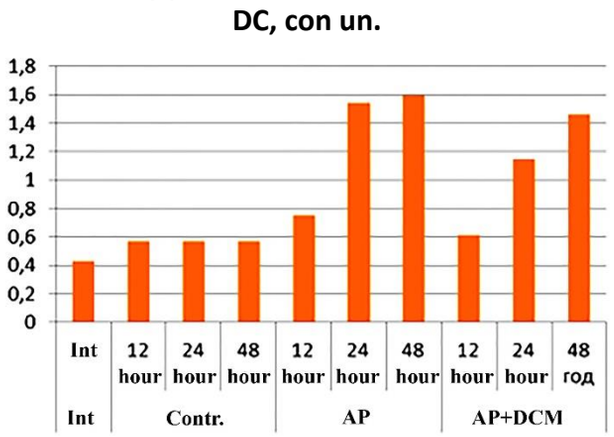
Results and discussion. With the aim to characterize oxidative-antioxidative imbalance in the organism of animals with experimental AP to determine LPO intensity, the concentrations of diene conjugates (Fig. 1), TBA-AP (Fig. 2), and catalase (Fig. 3) in the blood serum were examined. The biochemical analyses conducted are indicative of intensification of LPO processes in the blood serum even during the first 12 hours, demonstrating reliable increase of the levels of diene conjugates, TBA-AP and catalase in comparison with the control values in all the experimental groups. Thus, in III group of rats

diene conjugates became 31,6% as much, and TBA-AP – 78,3% as much. Catalase level also increased and became 40,3% as much (p<0,05), as with increased formation of hydrogen peroxide this antioxidant is the most active for its neutralization.

In IV group 12 hours later after the beginning of the experiment inconsiderable growth of oxidative reactions occurred (p>0,05) as compared to that of the control. It should be noted that with dexamethasone correction lower values were found as compared to the experimental rats with AP: diene conjugates – 18,7% lower, TBA-AP – 32,5% lower (p<0,05), and catalase – 22,5% lower (p>0,05). Due to inhibition of neutrophil activation by means of dexamethasone there was no need to considerable increase of antioxidant level such as catalase.

It should be noted that at the following stages of the experiment LPO values continued to increase which was indicative of imbalance between the rate of the processes of active oxygen formation and antioxidant system. In its turn, it promoted activation of peroxide oxidation processes and resulted in a complete breaking down of unsaturated lipids.

The most intensive changes concerning the content of LPO products occurred 24 hours later in animals with modeled AP. Their reliable increase was found in the blood serum, thus TBA-AP and diene conjugates – by 1,98 and 2,7 times, and catalase – by 2,2 times respectively. As compared to the previous stage of the experiment 24 hours after the beginning of AC development the values of oxidative reactions increased very rapidly. Since with this experimental model 24 hours later exocrine pancreatic cells are necrotized it results in a powerful activation of inflammatory processes [12].



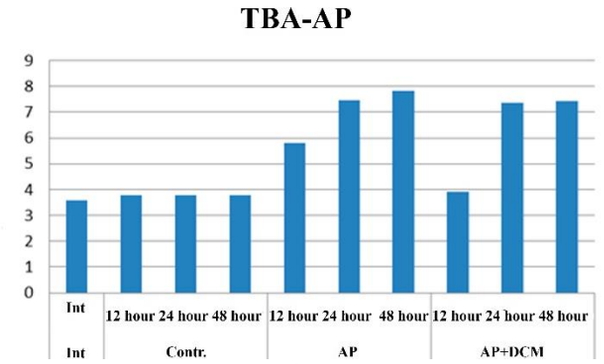


Fig. 1. Graphic analysis of diene conjugates level in rats in case of experimental acute pancreatitis

Fig. 2. Graphic analysis of thiobarbituric acid active products (TBA-AP) in rats with experimental acute pancreatitis

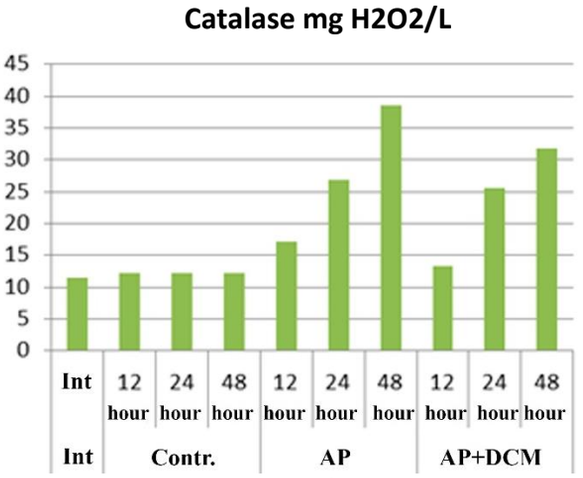


Fig. 3. Graphic analysis of catalase level in rats in experimental acute pancreatitis

In rats with dexamethasone correction a considerable intensification of the processes of free radical oxidation was found at this stage of the experiment. As compared to the control IV group of animals demonstrated a reliable increase of oxidative processes and antioxidants respectibely: TBA-AP – 95,2% as much (p<0,05), DC – twice as much (p<0,05), catalase – by 2,1 times (p<0,05). These results can be indicative of the fact that with administration of GCS 24 hours later when pancreonecrosis develops the drug is not able to inhibit SIRS considerably. Thus, changes occurred in case of AP are irreversible, uncontrollable potentiation of oxidative reactions is progressing.

While comparing rats in the groups with dexamethasone correction on the 12th and 24th hour lipid oxidation is clearly found to occur much more intensively with progressing of pancreatitis. Thus, TBA-AP becomes 28,4% as much (p<0,05), DC increase twice as much (p<0,05), catalase –

56,9% as much (p<0,05). The results of LPO products presented similar variants of a reliable growth: TBA-AP – 87,8% as much (p<0,05), DC – 88,5% as much (p<0,05), catalase – 91,7% as much (p<0,05) as compared to glucocorticoid therapy on the 12th hour of acute pancreatitis. It can be suggested that administration of dexamethasone on the 24th hour does not produce any considerable correcting effect on progressing of AP, and prevention of complications becomes less possible [13].

The next stage of the experiment was accompanied by slow rates of LPO growth evidenced by the following values. Thus, on the 48th hour of the experiment in III group the following values were found: TBA-AP – 5,1% as much (p>0,05), DC – 3,3% as much (p>0,05), catalase level – 43,4% as much (p<0,05), as compared to the results on the 24th hour. Similar tendency of unreliable elevation of biochemical parameters was found under conditions dexamethasone correction in IV group. Although, the results obtained in the blood increased reliably as compared to the control: TBA-AP – twice as much, diene conjugates – by 2,6 times, catalase – same value (2,6 times). Thus, on the second day of the experiment the level of antioxidant system activity remains high, which is indicative of the body attempt to inhibit impetuous reactions with formation of FOR.

Intensification of the processes of free radical lipid oxidation plays an important role in pathogenesis of complications of acute pancreatitis. A cascade of complicated pathobiochemical reactions forms the basis for the mechanisms of multiple organ failure. Reduced supply of oxygen molecules stimulates production of superoxide-anion in the respiratory chain of mitochondria with further formation of free radicals. Under conditions of neutrophil activation the lungs become the first line affected organ due to a developed flow of microcirculation, where in case of hypoxia sequestration of activated neutrophils occurs. Metabolic activity of neutrophil granulocytes in the blood results in respiratory explosion and generation of free radicals, which harmful action is directed to proteins and lipids of the basal membranes [10].

Blockage of SIRS by means of dexamethasone is an effective prophylactic measure to prevent auto-destructive processes in the tissues of target organs. Protection of macrophages, lymphocytes, neutrophils, endothelial cells against their hyperactivity and exhaustion is the main pathogenic link of the drug action [4].

Conclusions. The results obtained demonstrated that acute L-arginine induced pancreatitis is associated with intensive processes of LPO able to potentiate the development of multiple organ failure in case of pancreas inflammation.

Investigation of the parameters of oxidative stress occurring due to imbalance between LPO and antioxidant protection enables to detect pathogenesis of pathological processes, evaluate the degree of risk of their occurrence, and prognosticate peculiarities of complications of AP, which substantiate the topicality of this study.

Experimental AP during the whole period of the investigation is accompanied by intensification of lipoperoxidation processes which is manifested by increased LPO production, especially pronounced on the 24th hour of the experiment.

Administration of dexamethasone at the initial stages of AP development are proved to inhibit LPO processes, while untimely administration of the drug is not accompanied by a reliable decrease of oxidative processes in the blood of animals.

Prospects of further studies. Further investigations can reveal still unknown mechanisms of damaging target organs in case of AP and give the possibility to use glucocorticoids as an early prevention of severe complications in clinical practice.

References

1. Babenko GO. Viznachennja mikroelementiv i metalo fermentiv u klinichnih laboratorijah. K: 1968. – 138 p.

2. Bernik OO, Savchuk OM, Dvorshhenko KO, Beregova TV, Ostapchenko LI. Peroksidne okisnennja bilkiv pechinki za umov gipoacidnogo stanu. Fizika zhivogo. 2010;18(3):89–92.

3. Bes'kij VO, Grishhuk LA, Marushhak MI. Pokazniki vil'noradikal'nogo okisnennja krovi ta bronhoal'veoljarnogo lavazhu pri sindromi gostrogo ushkodzhennja legen'. Suchasni medichni tehnologiï. 2014;(2):48-54.

4. Blagovestnov DA, Hvatov VB, Upyrev AV. Kompleksnoe lechenie ostrogo pankreatita i ego oslozhnenij. Hirurgija. 2004;(5):68-75.

5. Kamyshnikov VS. Spravochnik po kliniko-biohimicheskim issledovanijam i laboratornoj

diagnostike. 3-rd ed. – M: MEDpress-inform; 2009. 896 p.

6. Korobejnikova EN. Modifikacija izmerenija produktov perekisnogo okislenija lipidov v reakcii s tiobarbiturovoj kislotoj. Lab Delo. 1989;(7):8-10.

7. Chuklin SM, Bigal's'kij IJu, Perejaslov AA. Markeri oksidacijnogo stresu jak pokazniki tjazhkosti gostrogo pankreatitu Ukraïns'kij zhurnal hirurgiï. 2011;15(6):159-163.

8. Bertorelli G, Pesci A, Peveri S, Mergoni M, Corradi A. Alpha glucocorticoid receptor expression in different experimental rat models of acute lung injury. Pulmonary Pharmacological Therapy. 2008;21:214–21.

9. Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med. 1995 Apr;23(4):646-51.

10. Di Filippo A, Ciapetti M, Prencipe D, Tini L, Casucci A, Ciuti R, Messeri D, Falchi S, Dani C. Experimentally-induced acute lung injury: the protective effect of hydroxyethyl starch. Ann Clin Lab Sci. 2006 Summer;36(3):345-52.

11. Granger J, Remick D. Acute pancreatitis: models, markers, and mediators. Shock. 2005;(24):45-51.

12. Gyorgy Biczo, Peter Hegyi, Sandor Berczi. Inhibition of arginase activity ameliorates L - arginine induced acute pancreatitis in rats. Pancreas. 2010;(6):868–874.

13. Hegyi P, Rakonczay ZJr, Sári R, Góg C, Lonovics J, Takács T, Czakó L. L-arginine-induced experimental pancreatitis. World J Gastroenterol. 2004 Jul 15;10(14):2003-9.

14. Anna Dziurkowska-Marek, Tomasz A. Marek, Andrzej Nowak, Andrzej Nowak, Teresa Kacperek-Hartleb, Ewa Sierka, Ewa Nowakowska-Dulawa. The dynamics of the oxidant-antioxidant balance in the early phase of human acute biliary pancreatitis Pancreatology. 2004;.(4):215-22.

15. Shi C, Andersson R, Zhao X. Potential role of reactive oxygen species in pancreatitis associated multiple organ dysfunction. Pancreatology. 2005;(5):492-500.

16. Wilmer A. ICU management of severe acute pancreatitis. European Journal of Internal Medicine. 2004;15:274–80.

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