Changes of Prooxidant-Antioxidant Systems in Experimental Acute Pancreatitis

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Abstract
Mortality in acute destructive pancreatitis, despite the development and introduction of new methods of treatment, remains stable high and in severe forms reaches 25-85%. Activation of neutrophils and macrophages in acute pancreatitis leads to an "oxygen burst", which is closely linked with the activation of lipid peroxidation.

Goals. The purpose is to establish dynamic changes in the indexes of prooxidant-antioxidant systems in acute L-arginine-induced pancreatitis.

Materials and methods. The study was performed on 62 white male rats of Wistar line weighing 180-220g, with modeled acute pancreatitis. Blood for analysis have been taken: the blood serum on 12, 24, 48 and 72 hours of experiment to determine the activity level of thiobarbituric acid products, diene conjugates, catalase and lactate for assessment of the intensity of oxidative stress and antioxidant systems.

Results. The obtained results of the study showed that acute L-arginine-induced pancreatitis is accompanied by an intensification of lipid peroxidation processes (LPO). Revealed that the most pronounced increase in all blood parameters is observed 24 hours after the beginning of the study. A significant increase in the active products of tiobarbituric acid (TBA-AP) and diene conjugates (DC) was detected - 1.98 and 2.7 times, respectively, and 2.2 times the growth of catalase (CT). At the next stage of the experiment there is a slowdown in the rate of LPO, as evidenced by the following values. Thus, for 48 years in the 3rd group: TBA-AP - they increased by 5.1% (p > 0.05), DC - by 3.3% (p > 0.05), and the level of CT - by 43.4% (P < 0.05), compared with data for 24 hours. It is important to note that at 72 hours, the CT level decreased by 23.3% (p > 0.05), which may indicate an exhaustion of antioxidant systems. Indicators of LPO on 72 hours compared with 48 hours in group III: TBA-AP - increased by 1.7% (p > 0.05), DC - by 5.7% (p > 0.05).

Conclusions. Acute L-arginine-induced pancreatitis is accompanied by an intensification of lipid peroxidation-oxidation processes that can potentiate the development of multiple organ failure in pancreatic inflammation. The most pronounced changes in lipid peroxidation-oxidation rates are observed for 24 hours of study.

Keywords
acute pancreatitis; lipid peroxidation; products of thiobarbituric acid; diene conjugates; catalase

Problem statement and analysis of the recent research

Today, acute pancreatitis (AP) is a significant issue in modern medicine. The disease is about 50 cases per 100,000 population, characterized by heterogeneity of clinical manifestations and has a steady tendency to increase. There is also an increase in the frequency of pancreatic necrosis from 11.5% to 64%. [7]. Mortality rate in acute destructive pancreatitis, despite the development and introduction of new methods of treatment, remains stably high and in severe forms reaches 25-85%. The largest number of fatal outcomes is associated with purulent-septic complications and polyorganic insufficiency, which in approximately 80% of cases is the direct cause of death [5].

An important role in the pathogenesis of damage in the AP is the release of a large number of pro-inflammatory mediators and the development of the syndrome of the systemic inflammatory response (SIR) [11]. Activation of neutrophils and macrophages leads to “oxygen burst”, and it is closely related to the activation of lipid peroxidation (LPO), which is the process of oxidative degradation of lipids with unsaturated double bonds and occur in a way of chain reaction. The initial stage of free radical oxidation is the formation of active forms of oxygen. This includes: superoxide anion radical, hydroxyl radicals and hydrogen peroxide [8]. In addition to products which reduce oxygen to active forms, oxygen metabolites also include activated products of thiobarbituric acid (TBA-AP), diene conjugates (DC), and this can serve as a marker for the intensity of LPO.

In the conditions of SIR in case of AP, there is a violation of the balance between peroxide reactions, the number of reduced metal metabolites, concentration of free radicals of oxygen, and depletion of antioxidant systems [2].

Hence, highly toxic compounds such as DC and TBA-AP, which are formed during the activation of LPO, lead to
damage to membranes and other cell components. Together with energy deficiency and metabolic acidosis, they can alter the majority of parenchymal organs, and in particular, the pulmonary tissue [3].

Objective. The purpose of the work was to determine the dynamics of changes in levels of the prooxidant-antioxidant systems in acute L-arginine-induced pancreatitis.

1. Materials and methods
The research was performed using 62 white male rats of the Wistar line weighing 180-220 g, which were kept in a standard diet with free access to water. Animals were divided into 4 groups: I - intact group of animals (n=10); II - control (n=10) - intraperitoneally injected saline solution, at the rate of 1 ml per 100 g of rat mass; III - with a model of acute pancreatitis (n=42). All studies were carried out under general anesthesia, using ketamine (40 mg/kg). Animal holding and manipulations were carried out in accordance with the provisions of Ukranian Law "Protection of Animals from Cruelty" (#1759-VI, 15.12.2009). After the experiment, all animals were made euthanasia.

Experimental pancreatitis was carried out by two intraperitoneal injections of 20% solution of L-arginine in a total dose of 5 g/kg, with one-hour interval. Blood collection for biochemical examination was performed at 12, 24, 48 and 72 hours after the beginning of the experiment.

The content of DC was determined using the method [4], TBA-AP - with the help of the method [6], CT - by the method [1] respectively.

The obtained data were statistically processed using non-parametric criteria on a personal computer using the program "Statistica 7" ("Statsoft, Inc." - USA). Reliability was evaluated according to the Wilcoxon criterion and the Sign-test. Differences were considered reliable if the value of P was 95% or more (p < 0.05).

2. Results and discussion of the research
The performed biochemical investigations revealed that during 12 hour of AP there was a significant increase of level of DC - by 31.6% (Fig. 1), TBA-AP - by 53.9% (Fig. 2), and simultaneous activation of antioxidant systems, in particular, CT level was increased at 40.3% (Fig. 3) (p < 0.05), compared with control in all experimental groups.

At the next stage of the experiment there is a slowdown of the growth rate of the LPO as evidenced by the following values. Thus, in 48 hours in the group III: TBA-AP - they increased at 5.1% (p > 0.05), DC - at 3.3% (p > 0.05), the level of CT - at 43.4% (p < 0.05), compared with data per 24 hours. We can assume that at 48 hour of the experiment, the level of activity remains high, which suggests an attempt to suppress the body of violent reactions with the formation of active forms of oxygen (AFO).

It is known that AFO can damage all cell components, including membrane lipids, structural and regulatory proteins and carbohydrates. Peroxidic damage of the membrane structures violates their fluidity, ion transport and eventually leads to cellular loss of functional activity. Oxidative damage to lipids in membranes induces cell death through apoptosis or necrosis, which is one of the mechanisms of damage to many organs, such as lungs in acute destructive pancreatitis [10].

Studies of the animal blood at the last stage of the experiment showed a slight increase in LPO indeces compared to 24 and 48 hours in group III. It is important to note that during 72 hour of the experiment, the CT level (see Fig. 3) decreased at 23.3% (p > 0.05), which may indicate the exhaustion of antiox-
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Figure 3. Graphical analysis of catalase level (CT) in rats in experimental acute pancreatitis

idant systems. Indicators of LPO at 72 hour compared with 48 hours in group III: TBK-AP - increased at 1.7% (p > 0.05), DC - at 5.7% (p > 0.05) respectively.

One of the peculiarities of the AP is the ability to change quickly from mild to severe, life-threatening form due to the release of a large number of pro-inflammatory mediators and the development of SIR, which largely determines the severity of the AP [9]. In the conditions of inflammation processes and ischemia, the peroxidic reactions are increased, the number of oxidized metabolites increases, and the concentration of AFO rose significantly, however antioxidant systems are depleted. In insufficient amount of antioxidant systems, such as CT, there is an uncontrolled peroxidation of membrane lipids, which leads to an increase in permeability of the cell membranes due to the formation of transverse polar channels and as a result, destruction of acinar cells of pancreatic parenchyme [12]. Damaged acinar cells release AFO, LPO products that are able to activate neutrophils with subsequent potentiation of SIR and microcirculatory disorders. Unsatisfactory results of treatment and a high mortality rate in severe forms of AP are largely determined by complications and inadequately studied pathogenesis, as well as prediction of the course of pancreatic inflammation [13].

Thus, acute destructive pancreatitis provokes and deepens the formation of LPO and leads to significant violations of the balance of the prooxidative-antioxidative systems, as it was pointed out by the number of certain authors [10, 12, 13].

3. Prospects for further research

The investigation of the prooxidative-antioxidative changes in the pulmonary tissue in acute experimental pancreatitis are planned to be done in further studies.

4. Conclusions

1. Acute L-arginine-induced pancreatitis is accompanied by the intensification of the processes of LPO, which can potentiate the development of multiple organ failure in inflammation of the pancreas.

2. The most pronounced changes in LPO are observed during the 24 hour of the performed experiment.

References


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