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GLUTATHIONE METABOLISM AND ITS STATE IN ACUTE PANCREATITIS DEPENDING ON THE BODY'S ANTIOXIDANT STATUS

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SUMMARY: The article analyzes the exchange of glutathione and its relationship with the processes of detoxification and lipoperoxidation in acute experimental pancreatitis, depending on the antioxidant state of the body.

KEY WORDS: Pancreatitis, intracellular processes, glutathione homeostasis, detoxification, antioxidant status.

INTRODUKTION

Acute pancreatitis, being one of the most severe acute diseases of the abdominal cavity, is accompanied by a high mortality rate (3.6-23.5%), which in total destructive forms reaches 80-100%.

Endogenous toxemia of the body that occurs in acute pancreatitis disrupts the functions of parenchymal organs, in particular the liver and kidneys. The liver, being a kind of collector of venous blood of the gastrointestinal tract, is one of the first to take on the "blows" of highly active pancreatic enzymes, which in large quantities enter the vascular bed from the damaged pancreas. At the same time, various combinations of functional and structural damages of different depth and severity occur.

Acute pancreatitis is characterized by a violation of one of the fundamental indicators of cellular homeostasis - intracellular processes. There is not an isolated lesion of any organ, but systemic membrane disorders that cause dysfunction of various organs and systems. The mechanism of these phenomena is quite complex, but one of its elements can be considered the accumulation of lipid peroxidation products (POL) in the lipids of membranes. Uncompensated amplification of POL can lead to disruption of membrane structures as the most sensitive to the action of reactive oxygen species.

Mechanisms of natural detoxification include the processes of biotransformation of lipophilic xenobiotics with the participation of the cytochrome P-450-dependent monooxygenase system(MOS), the processes of conjugation of hydrophilic compounds and their excretion from the body, anti-radical and anti-peroxide protection.[1,2,3,4] The coordinated and interrelated work of these processes ensures chemical homeostasis in the cell and prevents them from damage. In all these three links, the binding system is glutathione.[5,6,7] Despite the progress made in studying the role of glutathione in the mechanisms of natural detoxification, many aspects of its violations in acute pancreatitis remain undisclosed.

Taking into account the leading role of strengthening the processes of POL in the development of acute pancreatitis and liver damage, it is interesting to use a natural antioxidant - α - tocopherol in the prevention of liver lesions in acute pancreatitis, namely its effect on the mechanisms of natural detoxification in the liver in acute experimental pancreatitis.

The aim of the research work is to study glutathione metabolism and its relationship with detoxification and lipoperoxidation processes in acute experimental pancreatitis, depending on the antioxidant state of the body.

The following tasks were defined.

1) to study the preventive effect of α - tocopherol on the development of acute experimental pancreatitis;
2) investigate the effect of α - tocopherol on the state of POL processes and the activity of antioxidant defense enzymes;
3) to study the effect of α- tocopherol on the state of microsomal liver oxidation in rats with acute pancreatitis;
4) to study the effect of α - tocopherol on glutathione metabolism in the liver and blood in the dynamics of acute pancreatitis;
5) establish the relationship of the glutathione system with detoxification and lipoperoxidation processes in the development of acute experimental pancreatitis, depending on the antioxidant status of the body.

Scientific novelty: For the first time in acute experimental pancreatitis, a violation of the activity of

glutathione status enzymes in the liver and blood and the possibility of its recovery with the preliminary administration of α-tocopherol are shown. Alpha-tocopherol prevents the accumulation of toxic metabolites of lipoperoxidation, significantly activates superoxide dismutase (SOD) and catalase (CAT); preserves microsomal hemoproteins from inhibition, thereby contributing to the activation of detoxifying liver function and reducing endotoxemia. It is shown that the preliminary administration of α - tocopherol to experimental animals reduced the mortality rate in OP by 80%, reduced the activity of amylase by 2 times, which allows us to conclude that the important role of providing the body with α - tocopherol in acute experimental pancreatitis.

Experiments were carried out on 158 sexually Mature mongrel male rats with an initial body weight of 140-180 g, contained in the usual laboratory diet. 2 series of experiments were carried out. In series 1, the state of the MOS of the liver and the glutathione system was studied depending on the antioxidant status of the organism in animals with experimental pancreatitis. Animals of this series were divided into three groups (24 in each group): 1st-intact; 2nd - control; 3rd-experienced with acute pancreatitis.

Acute experimental pancreatitis was caused in rats by The method of p. S. Simovaryan[8]: local freezing of the surface of the pancreas with ethyl chloride.

The operation was performed under ether-mask anesthesia under aseptic conditions. They opened the abdominal cavity, extracted the stomach and duodenum along with the pancreas. The selected part of the intestine with the gland was carefully separated with napkins from the rest of the abdominal organs. Then, both surfaces of the spleen section of the pancreas were irrigated with chloroethyl from standard ampoules. 1.5-2 ml of chloroethyl was used for each irrigation. Irrigation of each adjacent section of the gland was continued until a light layer of frost appeared. During the irrigation process, the cooled tissue compacted and became lighter in comparison to the intact gland tissue. Approximately 4-5 minutes after irrigation, when the gland assumed a bright red color, the organs were set into the abdominal cavity and the latter was sewn in layers tightly. Only laporatomy was performed on control animals. The duration of the operation was on average 5-6 minutes.

Control animals underwent exactly the same operation (laporatomy), but without freezing the pancreas. To determine the degree of damage to the pancreas, the amylase content was determined in the blood. Studies were conducted on the 7th, 10th day and 1 month after the operation. At the same time, blood amylase in experimental animals increased by 3.5-4 times (7-day) compared to the control.

In the second series of experiments (86 rats), the prophylactic effect of the natural antioxidant α–tocopherol on the state of the studied parameters in the liver and the development of experimental acute pancreatitis was studied. For this purpose, animals of the control and experimental groups received tocopherol 0.5 mg per 100 g of body weight daily for 14 days inside. On day 15 of the experiment, the animals underwent a false operation and reproduced acute pancreatitis. Animals of this series were also slaughtered on the 7 -, 10-day and 1 month after the operation.

In accordance with the set tasks, we investigated:
1. State of the liver MOS (determination of cytochrome P-450).
2. State of POL and AOS processes (determination of MDA content, activity of SOD and CAT enzymes).
3. Determination of the glutathione pool and the activity of its exchange enzymes (total, reduced and oxidized glutathione content, activity of the GR and γ-GT enzymes).

Method of research
In the same timeframe of the study animals were euthanized simultaneously by decapitation in the cold room at a temperature of 0°C -+2°C. the Dissected abdominal cavity of the animals was quickly removed from her liver, weighed portion of liver (approximately 50-70 mg) to determine the pool of glutathione, the remainder was washed with cold solution environment selection, consisting of 0.05 M KCL, 0.25 M sucrose in a solution of 0.05 M Tris-HCL, pH 7,2, was crushed with scissors, and then homogenized using a Teflon pestle. Sequential centrifugation of the homogenate at 9000 g for 20 min. microsomal-cytosolic and microsomal fractions of rat liver were isolated on the PC-6 centrifuge and at 105000g for 1 hour on the VAC-601 centrifuge. The resulting microsome precipitate was carefully washed with a cold release medium and resuspended in a solution of 0.15 M CSL in a 0.05 M Tris-HCL buffer, pH -7.2. In the microsomal-cytosolic fraction, the activity of antioxidant defense enzymes was determined: SOD, CAT, and glutathione metabolism: GR, γ - GT. The content of cytochrome P-450, a product of MDA peroxidation, was studied in the microsome fraction.

Quantitative determination of the content of cytochromes in the microsomal fraction of the liver. The state of the liver MOS was evaluated by determining cytochromes P-450.

Quantitative determination of cytochrome P-450. Determination of the content of cytochrome P-450 in the microsomal fraction of the liver was performed on the recording spectrophotometer "Spekord-M" (154). For this purpose, 0.1-0.2 ml of microsome suspension was added to cuvettes containing 3 ml of 0.05 M Tris-HCL solution of the pH-7.4 buffer. Then the prototype was purged with carbon monoxide for 20-30 seconds and restored by adding dithionite. After 30 seconds, the

differential spectrum was recorded, which is the difference between the experimental and control cells. The calculation was based on the absorption difference between 450 nm and 490 nm using the molar extinction coefficient of 91 mm-1cm-1. The content of cytochrome P-450 was expressed in nm / mg of protein.

Methods for determining the state of Pol and activity of antioxidant defense enzymes. The state of POL in the membranes of the endoplasmic reticulum was judged by the content of MDA and AGP. The principle of the method is based on the interaction of thiobarbituric acid with malonic dialdehyde, which is formed during the re-oxidation of unsaturated fatty acids with 2-3 diene bonds. The content of products reacting with thiobarbituric acid was calculated taking into account the molar extinction of Malondialdehyde equal to 1.56 ×106 mol cm-1 and expressed in nm MDA/mg of protein.

Acyl hydroperoxides of lipids in biological materials were determined by the spectrophotometric method of B. G. Gavrilova and M. I. Mishkorudnaya[9], based on the extraction of acyl hydroperoxides with a mixture of heptane-propane in an acidic medium, followed by measurement of the optical density at 233 nm on SF-46. The content of lipid hydroperoxides in relative units was converted to mg of protein.

The main enzymes that characterize the activity of the antioxidant system are SOD and CAT. Determination of SOD activity is based on the ability of the enzyme to inhibit the reduction reaction of nitrotetrazolium blue in an alkaline environment.[10]

Determination of catalase activity is based on the ability of H2O2 to form a persistent yellow staining with molybdenum salts.[11] The enzyme activity was calculated from the absorption difference between the blank and experimental samples using a molar extinction coefficient of 22.2×103 mol-1cm-1 and expressed in mm H2O2/min * mg of protein.

Methods of studying the metabolism of glutathione. To characterize the exchange of glutathione in the liver of experimental animals, the pool of glutathione (total, reduced, and oxidized forms), the activity of GH enzymes involved in the reduction of the oxidized form, and □-GT involved in the breakdown of glutathione were studied.

The content of the reduced form of glutathione (GSH) was determined by the titrometric method of Woodvor and Frey in the modification of M. S. Chulkova. Glutathione is determined by potassium Iodate, which oxidizes the cysteine that is part of glutathione. The total glutathione content was determined after complete reduction of all glutathione with zinc. The calculation of the amount of glutathione is carried out using the formula:

$$Xmg\% = \frac{1(a - к)\ 1000\ /\text{linkage of the liver}}{1{,}63}$$

Where: 1 - ml of the sample taken (filtrate)

a - the number of ml of 0.001 N KIO_3 solution spent on titration of the test sample:
К - number of ml of the same solution spent for titrating the control
1,63-number of ml of KIO_3 corresponding to 1 mg of glutathione
1000-presetting coefficient per 100 g of raw fabric weight

The difference between total and reduced glutathione corresponds to oxidized glutathione (GSSG).
The determination of glutathione reductase activity is based on its ability to reduce glutathione oxidation using the reduced equivalent $NADFH_2$
GSSG + NADFH2 → 2 GSH + NADF

The determination of enzyme activity is based on measuring the rate of NADPH2 oxidation in the incubation medium at saturating concentrations of substrates and cofactors, the optimal pH value, and the optimal concentration of enzymatic protein (100F). The calculation of enzyme activity is based on the coefficient of millimolar absorption of NADPH2, which is equal to = 6.22 Mm * cm-1. The activity of the enzyme in n. moles of NADPH2 oxidized in 1 min. per 1 ml of protein was calculated using the formula:
A = 3E× 1000/ 6,22 × Q,
where: 3 is the volume of the reaction mixture
E-change in the optical density of the solution within 1 minute
1000 - conversion factor of microns to nm
Q - protein content in the test sample

γ-GT transfers the glutamyl residue from gamma-L - (+)-glutamyl-N-nitroanilide to the dipeptide acceptor, which is glycyl-glycine, which serves as a buffer at the same time. The concentration of the released 4-nitroanil is measured photometrically after stopping the enzymatic reaction by acidification (LachemaBiotest). the calculation is based on the difference between the optical densities of the sample and the control solution. The activity of the enzyme was expressed in mk.cat./ mg of protein.

The obtained data was processed using the variational statistics method on an IBM personal computer using a special software package.

CONCLUSIONS

1. The development of acute experimental pancreatitis against the background of the introduction of vitamin E in experimental animals reduces the degree of destruction, promotes a more favorable course, increases their resistance, and reduces the mortality rate from 15 to 3.8%.

2. Acute pancreatitis is characterized by a change in the glutathione status in the liver and blood tissue, the accumulation of its oxidized form due to a decrease in the activity of the enzymes γ-GT and, especially, GSSGred. In acute experimental pancreatitis, glutathione homeostasis is somewhat preserved against the background of pre-administration of vitamin E, the accumulation of GSSG decreases by 1.5-1.8 times, and GSSGred increases by 1.3-1.7 times.
3. In acute pancreatitis, the content of cytochrome P-450, a microsomal protein, is reduced, especially on the 7th or 10th day. Prior administration of vitamin E reduces the deficiency of microsomal hemoproteins. Its index increases by 1.6-2 times, but does not reach the norm.
4. In acute experimental pancreatitis, the content of POL products (especially AGP) in the microsomal fraction of the liver and blood of rats increases significantly. Pre-administration of vitamin E can reduce these indicators by 1.6-1.9 times and bring them closer to the values of the norm.
5. In the microsomal-cytosolic fraction of the liver and blood of rats with acute experimental pancreatitis, the activity of SOD and, especially, catalase is reduced. In contrast to the liver, the activity of SOD in the blood of experimental animals increases slightly, and catalase decreases sharply. Vitamin E increases the activity of enzymes by 1.6-2.3 times, and if the activity of SOD slightly exceeds the control values, the activity of CAT is still low.
6. Correlations between groups of experimental animals and at different times of the experiment are different and change their nature with the preliminary administration of vitamin E.

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