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GLUTATHIONE EXCHANGE AND ITS STATE IN ACUTE PANCREATITIS

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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.

KEYWORDS: Pancreatitis, intracellular processes, glutathione homeostasis, detoxification, antioxidant status.

INTRODUKTION

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.

Research material

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

Acute experimental pancreatitis was caused in rats by the method of p. S. Simovarian,[2] 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 isolated section of the intestine with the gland was carefully separated with napkins from the rest of the abdominal organs. Then both surfaces of the splenic pancreas were irrigated with chloroethyl from standard ampoules. 1.5-2 ml of chloroethyl was used for each irrigation. Irrigation of each adjacent area of the gland was continued until a light layer of frost appeared. During the irrigation process, the cooled tissue was compacted and became lighter compared to the intact gland tissue. Approximately 4-5 minutes after irrigation, when the gland assumed a bright red color, the organs were set in the abdominal cavity and the latter was sewn in layers tightly. Only laporatomy was performed on control animals. The average duration of the operation was 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 performed 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), we studied the preventive effect of the natural antioxidant α – tocopherol on the state of the studied parameters in the liver and the development of experimental acute pancreatitis. 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.[3] On the 15th day of the experiment, the animals were performed a false operation and reproduced acute pancreatitis. Animals of this series were also slaughtered on 7 -, 10-day and 1 month after the operation.

In accordance with the set tasks, we studied

1. State of MOS liver (determination of the content of cytochrome P-450).
2. State of POL and aoz processes (determination of MDA content, activity of SOD and CAT enzymes).
3. Determination of the glutathione pool and the activity of its exchange enzymes (the content of total, reduced and oxidized glutathione, the activity of GR and γ-GT enzymes)

Method of research

In the above-mentioned periods of research, animals were slaughtered by simultaneous decapitation in a cold room at a temperature of 0°C -+2°C. Opened the 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 isolation medium and resuspended in a solution of 0.15 M CSL in a 0.05 M Tris-NSL buffer, pH -7.2.the activity of antioxidant protection enzymes was determined in the microsomal-cytosolic fraction: SOD, CAT, glutathione exchange: 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 assessed by determining cytochromes P-450.

Quantitative determination of cytochrome P-450. The content of cytochrome P-450 in the microsomal fraction of the liver was determined using a recording spectrophotometer "Spekord-M".[4] For this purpose, 0.1-0.2 ml of microsome suspension was added to the cuvettes containing 3 ml of 0.05 M solution of Tris-HCL buffer pH-7.4. 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 cuvettes. The calculation was based on the absorption difference between 450 nm and 490 nm using a 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 protection enzymes. The state of POL in the endoplasmic reticulum membranes 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.

Acylhydrazines of lipids in biological materials were determined by spectrophotometry B. G. Gavrilov and M. I. Mishkorudny based on ekstraktsii of acylhydrazines with a mixture of heptane-propane in acidic medium with subsequent measurement of optical density at 233 nm on SF-46. The content of lipid hydroperoxides in relative units was converted to mg of protein.[5]

The main enzymes that characterize the reactivity of the antioxidant system are SOD and CAT. The determination of SOD activity is based on the enzyme's ability to inhibit the reduction reaction of nitrotetrazolium blue in an alkaline medium.[6] The calculation was based on the percentage of inhibition (T% reduction of nitrotetrazolium blue):

$$T\% = \frac{Ek - Eo}{Ek} \times 100\%$$

Where Ek is the control group spectrophotometer reading

EO - spectrophotometer readings of experimental samples.

SOD activity was calculated using the formula:

$$\text{Аусл.ед./мг белка} = \frac{T\%}{100\% - T\%} \times 0{,}2 \times N\text{:белок}$$

A-activity of the enzyme (usl. units / min x mg of protein)
T% - the percentage of braking
0.2 - quantity of the taken supernatant
N-breeding of the bioprobe

Determination of catalase activity is based on the ability of H2O2 to form a persistent yellow staining with molybdenum salts.[7] The enzyme activity was calculated from the absorption difference between the blank and experimental samples using the molar extinction coefficient of $22.2x10^3$ $mol^{-1}sm^{-1}$ and expressed in mm H_2O_2 /min * mg of protein.

Methods of studying the metabolism of glutathione. To characterize the exchange of glutathione in the liver

of experimental animals, we studied the pool of glutathione (total, reduced and oxidized forms), the activity of the enzymes GR, involved in the reduction of the oxidized form, and γ-GT, involved in the breakdown of glutathione.

The content of the reduced form of glutathione (GTH) was determined by The Woodward and Frey titrometric method in the modification of M. S. Chulkova.[8] Glutathione is determined by potassium iodide, which oxidizes cysteine, which 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:

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

Where: 1-ml of the sample taken (filtrate)
a-the number of ml of 0.001 H KCLO3 solution spent on titration of the experimental sample:
K - the number of ml of the same solution spent for titration control
1.63-the number of ml of KCLO3 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 restore glutathione oxidation using the reduced equivalent of $NADPH_2$

$$GSSG + NADPH_2 \rightarrow 2\ GSH + NADP$$

The determination of enzyme activity is based on measuring the rate of $NADPH_2$ oxidation in the incubation medium at saturating concentrations of substrates and cofactors, the optimal pH value, and the optimal concentration of the enzymatic protein (100F). The enzyme activity is calculated based on the coefficient of millimolar absorption of $NADPH_2$, which is equal to = 6.22 Mm•cm-1. The activity of the enzyme in n. mol of $NADPH_2$ oxidized in 1 min. per 1 ml of protein was calculated by the formula:

$$A = 3\ E \times 1000 / 6{,}22 \times Q,$$

Where: 3 - volume of the reaction mixture.

E-change in the optical density of the solution within 1 minute.

1000-conversion coefficient 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 also serves as a buffer. The concentration of the released 4-nitroanyl is measured photometrically after stopping the enzymatic reaction by acidification (Lachema Biotest). 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 by the method of variation statistics on an IBM personal computer using a special software package.

CONCLUSIONS

1. 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.
2. 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.
3. 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.

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