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Features of biochemical parameters of blood serum and antioxidant protective control of rats with extrahepatic cholestasis

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Annotation: This article presents the results of an experimental cholestasis conducted on 69 white outbred male rats of a mixed population with an initial weight of 180-200 g. In 37 rats, extrahepatic cholestasis was reproduced by ligation of the common bile duct. The overall mortality rate in this group was 30.3%. False-operated animals (24 rats) served only as laparatomy under aseptic conditions. In these groups, mortality was not observed. The intact group consisted of 8 rats. Studies were carried out 1, 3, 7 and 15 days after the reproduction of models. The timing of the study was associated with the development of significant morphological and functional changes in the liver during experimental cholestasis. The activity of aminotransferases, alkaline phosphatase, cholesterol, bilirubin was determined in blood serum. According to the results of the experiment, it was revealed that cholestasis causes hyperfermentemia, hyperbilirubinemia, hypercholesterolemia. Extrahepatic cholestasis changes the activity of AOD enzymes in different directions, more pronounced changes in the activity of SOD were noted in the early stages. It is inhibited in the homogenate of the digestive system, amplified by the kidneys in the early days of the study and inhibited after 7 days. Catalase activity in homogenates of the liver, kidneys, pancreas and small intestine showed less variability.

Keywords: extrahepatic cholestasis, antioxidant protection, serum biochemical parameters

Relevance of the problem. An increase in the prevalence of liver and biliary tract lesions, the severity of the course, the complexity of diagnosis, and a violation of the functional state of the liver and its morphological structure that occurs during obturation and largely determines the outcome of treatment require an accurate assessment before surgery of not only the state of the biliary tract, but also the degree of liver damage [4, 5, 9].

End-stage chronic liver diseases are among the top ten most common causes of death Worldwide, and the mortality rate in the development of liver failure reaches 90 %, despite modern achievements in intensive care. at the present stage, up to 20,000 liver transplants are performed annually in the world. At the same time, for most developed transplant centers, the survival rate of patients with transplanted liver is 85-90% one year after surgery, 75-85% five years after surgery, and up to 70% 10 years after surgery [12].

Treatment of patients with jaundice is one of the most urgent problems of modern Hepatology. This is due to the fact that prolonged cholestasis and hypertension in the bile ducts cause deep morphological and functional changes in the liver, which lead to such serious complications as acute liver failure [1,2,3].

The consequence of cytolysis and damage to hepatocyte organelles is an increase in the serum of patients with cholestasis syndrome of intracellular enzymes-aminotransferases, arginase, aldolase, glutamadehydrogenase, sorbitdehydrogenase, RNA-Aza, DNA-Aza [12,14].

According To K. Z. Minina [5], hyperbilirubnemia of more than 200 mmol/l corresponds to an increase in ammonia in obturation jaundice to an average of 434±14.6 mcg/l, in cholestatic jaundice - to 673±14.2 mcg/l, and in tumor Genesis - to 603.7±12.4 mcg / g. In parallel, there is an increase in the concentration of lactic and pyruvic acids in the blood. Some amino acids, without undergoing metabolic transformations in the liver, acquire toxic properties (phenylalanine, tryptophan, aparagine, arginine). With prolonged violation of bile outflow, the plasma content of lysine and histidine - the main sources of ammonia formation-significantly increases.

Violation of the secretory function of hepatocytes leads to the development of hypoalbuminemia, hypoprothrombinemia, and a decrease in the activity of pseudocholinesterase. Among the toxic products whose concentration increases in patients with hepatic insufficiency, the greatest importance is currently attached to free bilirubin, ammonia, fatty acids, mercaptan, phenols, gamma-aminobutaric acid and peptides of medium molecular weight [,7, 9, 15]. Metabolites concentrated in the blood also have a secondary hepatotropic effect, blocking liver enzyme systems-succinate dehydrogenase, monoamine oxidase, glucokinase, hexokinase, pyruvate kinase, phosphofructokinase, and ATPase [16,18,20]. In parallel, there is a decrease in the activity of serum lecithin cholesterol acyltransferase (its inhibition may be associated with the development of hyperlipoproteidemia). Symptoms associated with lipid retention in the body (xanthelasma, xanthomas on the skin, in internal organs, including on the membranes of nerve trunks with the development of polyneuropathy), observed with hypercholesterolemia of more than 450 mg/DL, persisting for at least 3 months. Elevated levels of cholestanol, X-lipoproteins, and bile acids are quite specific for cholestasis, although they do not allow us to distinguish between obstructive and non-obstructive variants [16, 18, 19, 20].

Purpose of research. To study some biochemical parameters of blood serum and enzymatic AOS control of rats in the dynamics of extrahepatic cholestasis.

Material and methods of research. Experiments were carried out on 69 white mongrel male rats of a mixed population with an initial weight of 180-200 g. contained in a laboratory diet in a vivarium. Extrahepatic cholestasis was reproduced in 37 rats by ligation of the common bile duct [20]. The overall mortality rate in this group was 30.3%. The control was false-operated animals (24 rats), which underwent only laparatomy under aseptic conditions. No mortality was observed in these groups. The intact group consisted of 8 rats. Studies were performed 1, 3, 7, and 15 days after the models were reproduced. The timing of the study is associated with the development of significant morpho-functional changes in the liver during experimental cholestasis. Method of research. At the specified time, the animals were slaughtered by decapitation, the liver, kidneys, pancreas and small intestine were quickly extracted, weighed and washed in an isolation medium consisting of 0.05 M Tris-HC1 buffer pH 7.2, and homogenized. In the blood serum was determined the activity aminotransferase, alkaline phosphatase, cholesterol, bilirubin.

The activity of Alat, ASAT, alkaline phosphatase, cholesterol, total, direct and indirect bilirubin in blood serum was determined using a German-made Aytohumalyrer Human biochemical analyzer.

The obtained data were subjected to statistical processing using the Excel-2000 statistical analysis software package with calculation of the arithmetic mean (M), mean square deviation, standard error (m), relative values (frequency, %), student's criterion (t) with calculation of the error probability (P). At the same time, we followed the existing guidelines for statistical processing of data from clinical and laboratory studies.

Determination of superoxide dismutase activity. The intensity of LPO, which is one of the dominant mechanisms for the development of damage to parenchymal organs by intestinal toxins, is determined not only by the formation of free radicals, but also by the function of the

antioxidant system of cells. One of the most significant links in the EPA system is SOD. Superoxide dismutase undergoes enzymatic dismutation of the superoxide anion-the O2 radical-to form hydrogen peroxide, which is subsequently cleaved by another AOS enzyme, catalase, to H2O and inactive triplet oxygen. Determination of SOD activity in our work was performed using the method of V. G. Mkhitaryan and G. E. Badalyan [3]. The principle of the method is based on the ability of SOD to inhibit the reduction reaction of nitrotetrazole blue in an alkaline medium. The enzyme activity was calculated using the percentage of reduction of nitrotetrazole blue, which was determined from the ratio:

$$T\% = \frac{(E_к - E_0) * 100\%}{E_к},$$

E_K-extinction index of the control sample,
E_O – the value of the extinction test sample;
T% - percentage of recovery of nitrotetrazole blue.
SOD activity was calculated using the formula:
A – enzyme activity in conventional units / min. mg. protein,
n-dilution of the bioassay;
0,2 – volume of the sample taken.

At the same time, in rats with extrahepatic cholestasis, the activity of enzymes changed in different directions in the studied organs. Thus, the study of SOD activity in liver homogenate of experimental animals showed its decrease by 40.5% ($P<0.001$) as early as 1 day after reproducing the model of extrahepatic cholestasis relative to the indicators of control rats. However, in the future (after 3 days), the activity of SOD increased statistically significantly by 53.4% compared to the values of the previous period and approached the parameters of the control and intact groups of rats. Apparently, this was a compensatory reaction of the body to the development of cholestasis, since in the future we observed a gradual decrease in the activity of SOD in the liver homogenate of experimental animals. By the end of the experiment (after 15 days), the studied indicator was statistically significantly lower than the standard parameters by 32.1%. One of the mechanisms of its reduction can be assumed to be the development of fibrotic changes in the liver as a result of long-term preservation of cholestasis.

In contrast to the liver, changes in the activity of SOD in the kidney homogenate were manifested to a greater extent by activation. Thus, 1 day after the model of extrahepatic cholestasis was reproduced, the extrahepatic activity of SOD in kidney homogenate significantly increased by 23.5% relative to the indicators of intact rats, and did not differ from the values of the control group of animals. This activation was even more pronounced after 3 days, when its values exceeded the parameters of the previous period by 22.9%, control and intact animals by 38 and 51.8%, respectively. However, this activation was replaced by inhibition after 7 days, since the studied indicator significantly decreased by 57.6% compared to the previous period and by 36.4% in relation to the values of control rats. It should be noted that the compensatory capabilities of the kidneys are apparently significantly higher than in the liver, since the activity of SOD by the end of the experiment significantly increased by 79.5% compared to the values of the previous period and by 15.8% exceeded the control parameters.

The study of SOD activity in the pancreas showed its inhibition only in the early stages of the experiment. Thus, the studied indicator was statistically significantly lower than the standard parameters of the control group by 48.7% of animals 1 day after the reproduction of the model of extrahepatic cholestasis. In the future, its activity increased slightly (by 1.31 times) in relation to the values of the previous period, but still remained below the values of the control group of rats. Gradual activation of SOD was maintained in the future, as the studied indicator increased relative to the values of the previous period 1.35 times and reached the parameters of the control intact group of animals. Only by the end of the experiment (after 15 days) , we detected a

tendency to decrease the activity of the enzyme in the pancreatic tissue: a decrease of 14.5% relative to the control values.

The study of the activity of SOD in the small intestinal mucosa showed the undulation of its changes. So, 1 day after reproducing the model of bile duct obturation, we found a statistically significant decrease in the activity of the enzyme by 37.4 % relative to the values of control animals. In the future (after 3 days), this inhibition was replaced by an increase in its activity by 37.2% relative to the values of the previous period and approaching the parameters of intact rats. After 7 days, the activity of SOD again decreased by 16.5% and 18.1%, respectively, to the values of the previous period and control rats. On day 15 of the experiment, we again observed an increase in SOD activity by 26.1% and its approximation to the values of the control group of animals.

Consequently, the dynamics of changes in the activity of SOD in the studied organs is different and, apparently, is more related to their involvement in the pathological process.

The study of the activity of another AOS enzyme, catalase, in a control group of animals showed a tendency to increase its activity in all the studied tissues on day 1-3 of the experiment.

1. Conclusion:

1. Extrahepatic cholestasis causes hyperfermentemia, hyperbilirubinemia, hypercholesterolemia. If the activity of Alat increases significantly after 3-15 days, ASAT - at all times, then the activity of alkaline phosphatase, cholesterol and bilirubin levels - after 3 days from the beginning of the experiment.
2. Extrahepatic cholestasis changes the activity of AOS enzymes in different directions, more pronounced changes in SOD activity were noted in the early stages. It is inhibited in the homogenate of the digestive system, increases in the kidneys in the first days of the study and is suppressed after 7 days.
3. Catalase Activity in liver, kidney, pancreas, and small intestine homogenates showed less variability.

2. References:

1. Alianov AL, Mamoshin AV, Borsukov AV, Abolmasov AV, Muradyan VF, Rubanik DS. Minimally invasive procedures in the diagnosis and treatment of patients with obstructive jaundice. Bulletin of Surgical Gastroenterology. 2016; (3): 43. Russia (Альянов А.Л., Мамошин А.В., Борсуков А.В., Аболмасов А.В., Мурадян В.Ф., Рубаник Д.С. Минимально-инвазивные вмешательства в диагностике и лечении больных с синдромом механической желтухи // Вестн. хирургической гастроэнтерологии. 2016. № 3. С. 43).
2. Alyanov AL, Mamoshin AV, Borsukov AV, Muradyan VF. Efficacy of minimally invasive technologies in the treatment of patients with the syndrome of obstructive jaundice. Orel State University Research Notes. Natural, Technical, and Medical Sciences. 2015; (4): 280-284. Russian (Альянов А.Л., Мамошин А.В., Борсуков А.В., Мурадян В.Ф. Эффективность применения минимально инвазивных технологий в лечении больных с синдромом механической желтухи // Ученые записки Орловского государственного университета. Серия: Естественные, технические и медицинские науки. 2015. №4. С. 280-284).
3. Avdosyev YuV, Boyko VV, Gryshina TA, Lavrentyeva OYu. Roentgen-surgical interventions in complex treatment of patients with obstructive jaundice of tumor genesis. Science and Health. 2015; (5): 26-35. Russian (Авдосьев Ю.В., Бойко В.В., Гришина Т.А., Лаврентьева О.Ю. Рентгенохирургические вмешательства в комплексном лечении больных с механической желтухой опухолевого генеза // Наука и здравоохранение. 2015. № 5. С. 26-35)

4. Chandrashekhara SH, Gamanagatti S, Singh A, Bhatnagar S. Current Status of Percutaneous Transhepatic Biliary Drainage in Palliation of Malignant Obstructive Jaundice: A Review. Indian J Palliat Care. 2016; 22 (4): 378-387.
5. D.A. Abildaev et al. Morphology of the mesenteric node after the liver is turned off // Materials of the II International Morphological Scientific and Practical Competition and Conference of Students and Young Scientists "Morphological Sciences - the Fundamental Basis of Medicine" dedicated to the memory of Professor M. A. Samoteikina December 14, 2017 (Д.А. Абильдаев соав. Морфология брыжеечного узла после «выключения» печени // Материалы II Международной морфологической научно-практической конкурс-конференции студентов и молодых ученых «Морфологические науки — фундаментальная основа медицины», посвященной памяти профессора М.А. Самотейкина 14 декабря 2017 г.)
6. El Nakeeb A, Salem A, Mahdy Y, El Dosoky M, Said R, Ellatif MA, et al. Value of preoperative biliary drainage on postoperative outcome after pancreaticoduodenectomy: A case-control study. Asian J Surg. 2018; 41 (2): 155-162.
7. Kadirov DM, Vosiev AS. Percutaneous transhepatic preoperative biliary drainage in the treatment of the obstructive jaundice. Proceedings of the Tajikistan Academy of Sciences. Biological and Medical Sciences. 2014; 186 (2): 63-70. Russian (Кадыров Д.М., Восиев А.С. Чрескожное чреспеченочное билиарное дренирование в лечении больных механической желтухой // Известия Академии наук Республики Таджикистан. Отд-е биологических и медицинских наук. 2014. Т. 186, № 2. С. 63-70).
8. Kalakhanova BH, Chechenin GM, Lebedev SS, Barinov YuV, Seregin AA, Melkonyan GG, et al. Modern approaches to the timing of endobiliary replacement in patients with obstructive jaundice of tumor genesis. Modern Technologies in Medicine. 2014; 6 (4): 97-101. Russian (Калаханова Б.Х., Чеченин Г.М., Лебедев С.С., Баринов Ю.В., Серегин А.А., Мелконян Г.Г. и др. Современный подход к выбору сроков эндобилиарного протезирования у больных механической желтухой опухолевого генеза // Современные технологии в медицине. 2014. Т. 6, № 4. С. 97-101).
9. Korolyuk M.A., Ivanova L.I., Mayorova I.T., Tokareva V.E., Method for determining the activity of catalase // Laboratory work.- 1988.- No. 1.- P.16-19. (Королюк М.А., Иванова Л.И., Майорова И.Т., Токарева В.Е., Метод определения активности каталазы // Лабораторное дело.- 1988.- №1.- С.16-19.)
10. Liu C, Lu JW, Du ZQ, Liu XM, Lv Y, Zhang XF. Association of Preoperative Biliary Drainage with Postoperative Morbidity after Pancreaticoduodenectomy. Gastroenterology Res Pract. 2015; 2015: 796893.