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Research Article

Catalase Activity in the Homogenates of Internal Organs in the Dynamics of Extrahepatic Experimental Cholestasis

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Abstract

Relevance of the study: From 15 to 30% of people on Earth suffer from liver disease. The leading place is occupied by cholelithiasis, which will account for 10-15% of cases in the adult population of Europe and America [3]. One of the most difficult problems in abdominal surgery is the diagnosis and treatment of cholestasis. The relevance of the problem lies in the still high lethality, among patients with mechanical jaundice of non-tumor genesis it is 5.6-6.3%.

Objective of the study: To assess the activity of catalase (AOZ) in homogenates of internal organs in the dynamics of extrahepatic ecxperimental cholestasis.

Material and Methods: The experiments were conducted on 69 white mongrel rats-males of a mixed population with an initial mass of 180-200 grams, contained in the laboratory diet in the conditions of the vivarium.

Outcomes: A study of catalase activity in a control group of animals showed a tendency to increase its activity in all studied tissues for 1-3 days of the experiment. At the same time, in rats with extrahepatic cholestasis, it changed more pronouncedly.

Conclusion: Consequently, changes in catalase activity in homogenates of the liver, kidneys, pancreas, and small intestine showed less variability.

Keywords: Cholestasis; Catalase Activity; Experiment; Obturation of Bile Ducts

Introduction

The formation of bile is a vital function of the body, its violation leads to cholestasis syndrome. According to modern concepts, cholestasis is understood as a violation of the synthesis, secretion and outflow of bile [3], starting from the hepatocyte, the primary bile tubules and ending with its postuptake through the nonhepatic bile ducts into the duodenum [7]. Functionally, cholestasis is manifested by a decrease in the tubular outflow of bile, hepatic excretion of water and organic anions (bilirubin, bile acids), and morphologically - by the accumulation of bile in the hepatocytes and biliary tract.

Clinically and patogenetically, it is accompanied by a delay and accumulation in the blood of bile components: bilirubin, bile acids and alkaline phosphatase. Cholestasis syndrome occurs in various conditions that can be combined into two large groups: 1) impaired bile formation (intrahepatic cholestasis), arising from viral, alcoholic, drug and toxic lesions, cholestasis of pregnant women, cirrhosis, bacterial infections, genetic defects; 2) impaired bile flow (extrahepatic cholestasis) - with obturation of the common bile duct with a stone, tumor, choledoch stricture [4], primary biliary cirrhosis, Caroli disease, primary sclerosing cholangitis, tuberculosis, graft rejection reactions [2]. Despite the optimization of surgical treatment of obstructive cholestasis, the high mortality rate is mainly due to the development of acute liver failure [9] due to damage to hepatocytes by both endotoxicosis products and an increased level of free radicals that cause a state of oxidative stress [8]. One of the powerful inducers of lipid peroxidation is endogenous intoxication (EI). Violation of the function of the destruction of liver cells leads to the accumulation in the patient's blood of a wide range of toxic metabolites, blockade of oxidative enzymes [10]. Therefore, an important measure to prevent the development of acute liver failure in mechanical jaundice is the correction of endogenous intoxication through the use of pathogenetic methods of treating acute liver damage [1].

Objective

To determine the activity of antioxidant protection enzymes (AOZ) in the homogenates of the liver, kidneys, pancreas and small intestine of experimental animals in the dynamics of extrahepatic cholestasis.

Material and Methods

Experiments were conducted on 69 white mongrel rats-males of a mixed population with an initial mass of 180-200 grams, contained in the laboratory diet in vivarium conditions. In 37 rats, extrahepatic cholestasis was reproduced by ligation of the common bile duct [5]. The overall mortality rate in this group was 32.4%. Control was falsely operated animals (2,4 rats), which underwent only laparatomy under aseptic conditions. No mortality was observed in these groups. The intact group consisted of 8 rats. Studies were conducted 1, 3, 7 and 15 days after the reproduction of the models. The choice of study timing is associated with the development of significant morpho-functional changes in the liver during experimental cholestasis [5].

A breakdown of the experience is presented in table 1.

Determination of the activity of the enzyme catalase

An important enzyme of AOS protection of cells from POL is catalase, which catalyzes the breakdown of hydrogen peroxide by decomposing hydrogen peroxide into oxygen and water (catalase reaction) or by participating in the oxidation of hydrogen peroxide of any hydrogen donor (peroxidase reaction). Determination of catalase activity was carried out by the method of M.A. Koralyuk., *et al*. [6]. The principle of the method is based on the ability of hydrogen peroxide to form a stable color complex with molybdenum salts, recorded spectrophotometrically. The activity of the enzyme was determined on the difference in optical density between the prototype and the comparative sample using the molar extinction coefficient E = 22.2 · 103 $^{m-1} \cdot cm^{-1}$ and expressed in mm $_{H2O2}$/min. mg. protein. m is the amount of protein in the bioassay.

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

Research Results

According to the literature, the processes of free radical and lipid peroxidation are under the control of the AOZ system, which consists of an enzymatic and non-enzymatic link. Catalase plays an important role in enzymatic AOZ. A study of catalase activity in a control group of animals showed a tendency to increase its activity in all studied tissues for 1-3 days of the experiment (Table 2).

At the same time, in rats with extrahepatic cholestasis, it changed more pronouncedly. 1 day after the model was reproduced, the activity of catalase in the liver homogenate increases by 13.3%, relative to the indicators of the control group of rats. Then it decreases somewhat, approaching the values of the control group of rats. This decrease persists in the future (after 7 days), and during this period the activity of the enzyme reaches normal values. In the future, we observed again an increase in the activity of the enzyme (an increase of 22.3%) in relation to the values of the control group of rats.

Series of experiments	Terms of the experiment, day				Altogether	Mortality, %
	1	3	7	15		
Intact	2	2	2	2	8	-
Control	6/6	6/6	6/6	6/6	24/24	-
Extrahepatic cholestasis	9/6	9/7	9/6	10/6	37/25	32,4

Table 1: Scheme of the experiment.

Note: the numerator contains the initial number of animals in groups; in the denominator - the number of animals taken for research, taking into account mortality.

Groups and terms of research (day)	Liver	Kidneys	Pancreas	Small intestine
Control	**0,149 ± 0,005**	**0,177 ± 0,003**	**0,208 ± 0,004**	**0,177 ± 0,0001**
Obturation, through:				
1	0,171 ± 0,005[a, b]	0,185 ± 0,002	0,200 ± 0,003b	0.189 ± 0.003a
	0,151 ± 0,005	0,192 ± 0,003	0.221 ± 0.003a	0.192 ± 0.005a
3	0.162 ± 0.003A	0,141 ± 0,002[a, b]	0,217 ± 0,009	0,173 ± 0,003b
	0,157 ± 0,005	0,176 ± 0,004	0,211 ± 0,007	0,188 ± 0,005
7	0,148 ± 0,005	0,177 ± 0,002	0,202 ± 0,008	0,179 ± 0,003
	0,146 ± 0,004	0,175 ± 0,002	0.208 ± 0.005A	0,175 ± 0,005
15	0,181 ± 0,005[a, b]	0,098 ± 0,001[a, b]	0,209 ± 0,002[a, b]	0,162 ± 0,006
	0,148 ± 0,004	0,168 ± 0,005	0.198 ± 0.004a	0,172 ± 0,008

Table 2: Catalase activity (μmol $_{H2O2}$/min.mg protein) in the studied tissues of experimental animals (Mm ±).

Note: 1. The indicators of the experimental group are placed in the numerator, and the control group is in the denominator.

2. Reliable difference (R < 0.05); a - from the intact group, b - from the control group.

Changes in catalase activity in the homogenate of the kidneys were manifested by a tendency to activate 1 day after the reproduction of cholestasis. However, these values did not differ significantly from the indicators of the control group of rats. After 3 days, the activity of the enzyme decreased by 23.8 and 19.9% compared with the indicators of the previous term and the values of the control group of animals, approached the control and norm values after 7 days and was statistically significantly inhibited by 41.7% by the end of the experiment.

We did not identify any special changes in the activity of catalase in the homogenate of the mucous membrane of the pancreas and small intestine, since the studied indicator in all periods of the experiment did not differ significantly from the control values and regulatory parameters.

Conclusion

Consequently, changes in catalase activity in homogenates of the liver, kidneys, pancreas, and small intestine showed less variability.

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