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THE ROLE OF SULFIDE ANION IN THE DEVELOPMENT OF OXIDATIVE STRESS IN THE LIVER UNDER CONDITIONS OF CHRONIC ALCOHOLIC HEPATITIS

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The pathogenesis of alcoholic hepatitis is multifactorial and is the final result of a complex interaction of ethanol metabolism, inflammation and immunological reactions. The molecular mechanisms of H_2S action in alcoholic hepatitis have not been sufficiently studied, and its protective role has not been proven. The experiments were carried out on 30 white, sexually mature outbred male rats, weighing 180–220 g. The animals were divided into 2 groups: I – control (n=6); II group – animals on which we simulated alcoholic hepatitis. In the homogenate of the liver of rats, the activity of superoxide dismutase and catalase, the concentration of malondialdehyde and oxidatively modified proteins, sulfide anion concentration and the production of superoxide anion were determined. A decrease in the concentration of sulfide anion against the background of an increase in the activity of superoxide dismutase and the presence of an inversely proportional strong correlation between them indicates the effect of alcohol on the thioredoxin system, namely, on the increase of expression of a protein that interacts with thioredoxin, stimulating the reaction of the latter with reactive oxygen species. Chronic alcohol intoxication leads to the development of oxidative stress in the liver of rats due to an increase in the production of reactive oxygen species. The decrease in the concentration of sulfide anion on the 28th day of the experiment is connected with its usage as an antioxidant.

Key words: liver, alcoholic hepatitis, rats, oxidative stress, sulfide anion.

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РОЛЬ СУЛЬФІДНОГО АНІОНУ У РОЗВИТКУ ОКСИДАТИВНОГО СТРЕСУ ПЕЧІНКИ ЗА УМОВ ХРОНІЧНОГО АЛКОГОЛЬНОГО ГЕПАТИТУ

Патогенез алкогольного гепатиту багатофакторний і є кінцевим результатом складної взаємодії метаболізму етанолу, запалення та імунологічних реакцій. Молекулярні механізми H_2S у алкогольному гепатиті не достатньо вивчені, а його захисна роль не доведена. Експерименти виконані на 30 білих статевозрілих безпородних щурах-самцях, вагою 180–220 г. Тварини були розділені на 2 групи: I – контрольна (n=6); II група – тварини, яким моделювали алкогольний гепатит. В гомогенаті печінки щурів визначали активність супероксиддисмутази та каталази, концентрацію малонового диальдегіду, окисно-модифікованих білків та сульфід-аніону, продукцію супероксид-аніону. Зниження концентрації сульфідного аніону на тлі збільшення активності супероксиддисмутази та наявність між ними обернено-пропорційного сильного кореляційного зв'язку свідчить про вплив алкоголю на тіоредоксинову систему, а саме на збільшення експресії білку, що взаємодіє з тіоредоксином стимулюючи реакцію останнього із активними формами кисню. Хронічна алкогольна інтоксикація призводить до розвитку оксидативного стресу в печінці щурів внаслідок збільшення продукції активних форм кисню. Зниження концентрації сульфідного аніону на 28 добу експерименту пов'язано із його використанням в якості антиоксиданта.

Ключові слова: печінка, алкогольний гепатит, щури, оксидативний стрес, сульфідний аніон.

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Alcohol consumption is a global health problem. Alcohol is the leading cause of liver disease, and alcoholic hepatitis is the leading chronic alcohol-related disease. Worldwide, per capita alcohol consumption is strongly correlated with mortality from cirrhosis of the liver. It is estimated that alcohol abuse is a causative factor in 60 types of diseases and injuries and a concurrent cause of at least 200 others [7]. Chronic alcohol consumption causes a wide range of liver lesions, the most characteristic of which are steatosis, hepatitis, fibrosis and cirrhosis [4].

Current research data indicate that the pathogenesis of alcoholic hepatitis is multifactorial and is the final result of a complex interaction of ethanol metabolism, inflammation, and immunological reactions. Despite the large number of data from experimental and clinical studies, the pathogenesis of alcoholic hepatitis has not been definitively clarified. Therefore, the search for new links of its pathogenesis is an urgent and important problem today [6]. Hidrogen sulfide (H_2S) is a gas transmitter that exhibits anti-inflammatory, antioxidant, anti-apoptotic and anti-proliferative properties. In addition to these effects, a number of studies demonstrate that low doses of exogenous and endogenous H_2S can delay and prevent the onset and progression of liver fibrosis. Several studies show that plasma H_2S levels decrease dramatically during the progression of liver fibrosis, and endogenous H_2S is significantly reduced in patients with portal hypertension caused by cirrhosis. These reports suggest that inhibition of endogenous H_2S production may be associated with the development of human liver fibrosis [3]. Conversely, one recent study demonstrates that exogenous and endogenous H_2S can increase the proliferation and activation of

hepatic stellate cells, and H_2S scavengers can reduce the proliferation and fibrotic features of these hepatic cells, mediated by cellular bioenergetics. [1]. These paradoxical and controversial reports suggest that the protective effect and molecular mechanisms of H_2S in liver fibrosis need to be proven in further studies.

The purpose of the study was to establish of the role of sulfide anion in the development of oxidative stress in the liver of rats under conditions of chronic alcohol intoxication.

Materials and methods. The experiments were carried out on 30 white, sexually mature outbred male rats, weighing 180–220 g. The animals were divided into 2 groups: I – control (n=6); II group – animals on which we simulated alcoholic hepatitis (n=24) by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal administration of a 16.5 % ethanol solution in a 5 % glucose solution, at the rate of 4 ml/kg of body weight [6]. The control group included animals that were subjected to similar manipulations throughout the study period, but were injected with a physiological solution (0.9 % sodium chloride). The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on 10th, 14th, 21st and 28th day by taking blood from the right ventricle of the heart under thiopental anesthesia. The object of research was blood serum and liver. During the experiments, we followed the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) in accordance with the "General Principles of Experiments on Animals" approved by the First National Congress on Bioethics, and the requirements of the "Procedure for Conducting Experiments and Experiments on Animals by Scientific Institutions" (2012).

In the homogenate of the liver of rats, the activity of superoxide dismutase (SOD) was determined by the method of Brusov O.S. (1976) and catalase by the method of Korolyuk M.A. (1988), the concentration of malondialdehyde (MDA) by the method of Gérard-Monnier D. (1998), oxidatively modified proteins (OMP) by the method of Dubinina E.E. (1995) and sulfide anion by the method of Sugahara S. (2016), production of superoxide anion by method of Kostenko V.O. (2000).

Statistical processing of the results of biochemical studies was carried out using a pairwise comparison by non-parametric Mann-Whitney method. All statistical calculations were performed in the Microsoft office Excel program and its extension Real Statistics 2019. The difference was considered statistically significant at $p<0.05$. Correlation coefficients between the studied parameters were calculated by the Spearman method. Correlation coefficients were considered statistically significant at $p<0.05$

Results of the study and their discussion. During biochemical studies of rat livers, it was established that catalase activity on the 14th day of the experiment was increased by 1.16 times, and on the 21st and 28th days, it decreased by 1.52 and 1.81 times, respectively, compared to the control group. On the 14th day of the experiment, catalase activity in the liver of rats increased by 1.22 times compared to the 10th day of the experiment. On the 21st day, catalase activity in the liver of rats decreased by 1.76 times compared to the 14th day of the experiment. On the 28th day of the experiment, catalase activity in the liver of rats decreased by 2.1 times compared to the 21st day of the experiment.

The activity of SOD in the liver of rats on the 10th day of the experiment decreased by 1.77 times, and on the 14th and 21st days, it increased by 1.71 and 1.21 times, respectively, compared to the control. On the 14th day of the experiment, SOD activity in the liver of rats increased by 3.02 times compared to the 10th day of the experiment. On the 21st day, SOD activity in the liver of rats decreased by 1.41 times compared to the 14th day of the experiment.

The production of the superoxide anion radical in the liver of rats on the 14th, 21st and 28th day of the experiment was increased by 1.17, 2.15 and 2.33 times, respectively, compared to the control. On the 14th day of the experiment, the production of the superoxide anion radical in the liver of rats increased by 1.34 times compared to the 10th day of the experiment. On the 21st day, the production of the superoxide anion radical in the liver of rats increased by 1.83 times compared to the 14th day of the experiment. On the 28th day of the experiment, the production of the superoxide anion radical in the liver of rats increased by 1.08 times compared to the 21st day of the experiment.

The concentration of MDA in the liver of rats on the 10th, 14th, 21st, and 28th day of the experiment was increased by 1.85, 2.14, 2.39, and 2.1 times, respectively, compared to the control. On the 14th day of the experiment, the concentration of MDA in the liver of rats increased by 1.16 times compared to the 10th day of the experiment. On the 21st day, the concentration of MDA in the liver of rats increased by 1.12 times compared to the 14th day of the experiment. On the 28th day of the experiment, the concentration of MDA in the liver of rats decreased by 1.14 times compared to the 21st day of the experiment (Table 1).

The concentration of OMP in the liver of rats on the 10th, 14th, 21st and 28th day of the experiment was increased by 9.25, 8.75, 7.0 and 7.25 times, respectively, compared to the control. On the 21st day, the concentration of OMP in the liver of rats decreased by 1.25 times compared to the 14th day of the

experiment. On the 28th day of the experiment, the concentration of OMP in the liver of rats increased by 1.04 times compared to the 21st day of the experiment.

Table 1

Biochemical indices in the liver of rats under the conditions of simulation of chronic alcoholic hepatitis, M±m

Biochemical parameters	Groups				
	Control, n=6	10th day, n=6	14th day, n=6	21st day, n=6	28th day, n=6
Catalase, μkat / g	0.38±0.008	0.36±0.005	0.44±0.01*^	0.25±0.001*^	0.21±0.0007*^
Superoxide dismutase, c.u.	12.34±0.55	6.99±0.26*	21.12±2.76*^	14.93±0.78*^	14.27±0.47
Superoxide anion radical, nmol / s per g	1.84±0.004	1.61±0.17	2.16±0.03*^	3.95±0.05*^	4.28±0.02*^
Malonic dialdehyde, μmol / g	12.32±0.11	22.75±0.36*	26.35±1.13*^	29.42±0.21*^	25.84±0.87*^
Oxidation-modified proteins, c.u.	0.04±0.002	0.37±0.02*	0.35±0.002*	0.28±0.003*^	0.29±0.001*^
Sulfide anion, μmol / g	7.23±0.17	2.17±0.04*	2.78±0.04*^	1.49±0.05*^	5.42±0.05*^

* - $p<0.05$ compared to a control group of rats; ^ - $p<0.05$ compared to the previous term of the experiment.

The concentration of sulfide anion in the liver of rats on the 10th, 14th, 21st, and 28th day of the experiment decreased by 3.33, 2.6, 4.85, and 1.33 times, respectively, compared to the control. On the 14th day of the experiment, the concentration of sulfide anion in the liver of rats increased by 1.28 times compared to the 10th day of the experiment. On the 21st day, the concentration of sulfide anion in the liver of rats decreased by 1.87 times compared to the 14th day of the experiment. On the 28th day of the experiment, the concentration of sulfide anion in the liver of rats increased by 3.64 times compared to the 21st day of the experiment.

During analysis the correlations of sulfide anion concentration with catalase activity, MDA and OMP concentration and superoxide anion radical production, it was established that there were no statistically significant correlations in the control group of animals and on the 10th, 14th, 21st, and 28th day of the experiment (table 2).

Table 2

Correlation analysis of biochemical indicators of the liver of rats under the conditions of simulation of chronic alcoholic hepatitis

Studied pairs biochemical parameters	Groups									
	Control		10th day		14th day		21st day		28th day	
	rho	P	rho	P	rho	P	rho	P	rho	P
Sulfide anion / Catalase	0.739	0.094	-0.794	0.059	0.353	0.492	0.145	0.784	0.058	0.913
Sulfide anion / Superoxide dismutase	-0.739	0.094	0.533	0.276	0.182	0.73	0.424	0.402	-0.97	0.001
Sulfide anion / Superoxide anion radical	0	1	0.185	0.73	-0.369	0.471	0.424	0.402	0.485	0.329
Sulfide anion / Malonic dialdehyde	-0.134	0.7	-0.537	0.272	0.353	0.492	-0.132	0.803	0.464	0.354
Sulfide anion / Oxidation-modified proteins	0.618	0.191	0.177	0.738	-0.177	0.738	0.464	0.354	0.406	0.425

The analysis of the correlation ratios of the concentration of sulfide anion with the activity of SOD established that on the 28th day of the experiment, there was a statistically significant strong inversely proportional relationship, however, there were no statistically significant correlations on 10th, 14th, 21st day and in the control group.

The increase in the concentration of MDA and OMP on the 10th, 14th, 21st and 28th days of simulation of chronic alcohol intoxication indicates the development of oxidative damage to hepatocytes, the cause of which is an increase in the production of the superoxide anion radical and a malfunction of the superoxide dismutase-catalase link of antioxidant system.

Ethyl alcohol, under conditions of its chronic consumption, has several pathogenetic ways of increasing the production of reactive oxygen species in the liver. The most studied and canonical is considered to be an increase in superoxide-anion radical production from the cytochrome P-450 system during biotransformation of ethanol, as well as from the microsomal ethanol-oxidizing system (MEOS) [8]. Another way that leads to excessive production of reactive oxygen species in the liver is the formation of acetaldehyde from ethyl alcohol, which disrupts the functioning of mitochondria due to the excessive accumulation of reduced $NADPH+H^+$ and $NADH+H^+$ [8]. The third pathogenetic mechanism of excessive production of reactive oxygen species in liver during chronic alcohol intoxication is the activation of Kupffer cells due to the translocation of bacteria from the intestine through the portal vein to the liver [10].

In parallel with the activation of Kupffer cells, activation of transcription factor κB in hepatocytes is possible, as a reaction to bacterial lipopolysaccharides [9].

A decrease in catalase activity on the 21st and 28th day may indicate a significant increase in the substrate and depletion of this enzymatic system, since under conditions of chronic alcohol intoxication, in addition to hydrogen peroxide, catalase neutralizes ethyl alcohol [13]. At the same time, SOD activity increases on the 21st and 28th days of the experiment due to an increase in the production of the superoxide anion radical.

A decrease in the concentration of sulfide anion against the background of an increase in SOD activity and the presence of an inversely proportional strong correlation between them can be explained by the effect of alcohol on the thioredoxin system, namely, on the increase of expression of a protein that interacts with thioredoxin, stimulating the reaction of the latter with reactive oxygen species [5]. Therefore, under conditions of chronic alcohol intoxication, thioredoxins can take on the role of antioxidants, which reduces the burden on SOD. Heo M.J. and others proved that alcohol induces pyroptosis of hepatocytes through overexpression of thioredoxin-interacting protein, which induces inflammation and contributes to the development of alcoholic hepatitis [3].

The presence of such a correlation on the 28th day of the experiment may indicate the cumulative accumulation of a sufficient dose of alcohol to activate the thioredoxin antioxidant mechanism.

Conclusion

Chronic alcohol intoxication leads to the development of oxidative stress in the liver of rats due to an increase in the production of reactive oxygen species. The decrease in the concentration of sulfide anion on the 28th day of the experiment may be related to its use as an antioxidant, which requires further research.

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