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BIOCHEMICAL CHANGES IN THE EXTRACELLULAR MATRIX OF RAT LIVER DURING CHRONIC ALCOHOL INTOXICATION

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Excessive alcohol consumption is a global health problem worldwide, resulting in more than 3 million deaths each year. The purpose of this work was to study the biochemical markers of intercellular matrix metabolism of rat liver under the conditions of chronic alcohol intoxication modeling. The experiments were performed on 30 male Wistar rats, weighing 180-220 g. The animals were divided into 2 groups: I – control; II – chronic alcohol intoxication. Animals were removed from the experiment on days 10, 14, 21 and 28. We studied concentration of glycosaminoglycans, oxyproline and sialic acids in rat liver. We established that the greatest depolymerization of proteoglycans was on the 21st day, the highest intensity of collagenolysis was on the 14th day. Glycoproteins underwent the greatest catabolism on the 28th day. Chronic alcohol intoxication on the 28th day led to increased breakdown of glycoproteins and proteoglycans of the extracellular matrix of the liver, and increased the intensity of collagenolysis.

Key words: alcohol, liver, glycosaminoglycans, sialic acids, oxyproline, extracellular matrix.

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БІОХІМІЧНІ ЗМІНИ У ЕКСТРАЦЕЛЮЛЯРНОМУ МАТРИКСІ ПЕЧІНКИ ЩУРІВ ПРИ ХРОНІЧНІЙ АЛКОГОЛЬНІЙ ІНТОКСИКАЦІЇ

Надмірне споживання алкоголю є глобальною проблемою охорони здоров'я у всьому світі, що призводить до понад 3 мільйонів смертей щороку. Метою роботи було вивчити біохімічні маркери метаболізму міжклітинного матриксу печінки щурів за умов моделювання хронічної алкогольної інтоксикації. Експерименти виконані на 30 білих статевозрілих щурах-самцях лінії Вістар, вагою 180-220 г. Тварини були розділені на 2 групи: I – контрольна; II група – група хронічної алкогольної інтоксикації. Виведення тварин з експерименту відбувалося на 10, 14, 21 та 28 добу. В гомогенаті печінки щурів визначали загальну концентрацію глікозаміногліканів та їх фракції, концентрацію вільного оксипроліну та сіалових кислот. Нами встановлено, що найбільшої деполімеризації зазнають протеоглікани позаклітинного матриксу печінки щурів на 21 день хронічної алкогольної інтоксикації. Найвища інтенсивність колагенолізу спостерігається на 14 добу хронічної алкогольної інтоксикації. Глікопротеїни печінки щурів зазнають найбільшого катаболізму на 28 добу хронічної алкогольної інтоксикації. Хронічна алкогольна інтоксикація на 28 день призводить до посилення розпаду глікопротеїнів та протеогліканів позаклітинного матриксу печінки, та посилює інтенсивність колагенолізу.

Ключові слова: алкоголь, печінка, глікозаміноглікани, сіалові кислоти, оксипролін, позаклітинний матрикс.

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Excessive alcohol consumption is a global public health problem worldwide with enormous social, economic and medical consequences, resulting in more than 3 million deaths each year [10]. The liver undergoes the earliest and greatest degree of tissue damage from excessive alcohol consumption, as it is the primary site of ethanol catabolism. Alcohol abuse is the world's third risk factor for disease and

disability. Alcohol is the cause of 60 types of diseases and injuries, which in turn can cause at least 200 others [11].

Chronic alcohol abuse leads to liver damage, which occurs in stages: fatty liver dystrophy occurs asymptomatically after acute or minor chronic alcohol consumption, refers to the reverse conditions. Steatohepatitis is a necrotic-inflammatory form of liver damage caused by significant exposure to alcohol. Liver fibrosis leads to excessive accumulation of complex proteins of the extracellular matrix (ECM), mainly collagens of various types. Cirrhosis, the terminal stage, is manifested by impaired ECM remodeling and severe liver failure [7]. Fatty liver dystrophy, alcoholic hepatitis and steatofibrosis can develop as independent diseases or by continuation of each other.

Liver ECM occupies about 3 % of liver volume. Under physiological conditions, the ECM of the liver is limited by portal tracts, walls of sinusoids, and central veins. The proteins most commonly found in the ECM of the liver are collagenous (most common types I, III, V (in the portal system and wall of the central vein) and IV (in the walls of sinusoidal capillaries)). The ECM of the liver includes glycoproteins such as laminin I and II, fibronectin, osteopontin, oligomeric cartilage protein, fibromodulin, tenascin, nidogen, and SPARC (secreted protein, acidic and rich in cysteine). The proteoglycans of the ECM of the liver include heparan sulfate, dermatan sulfate, chondroitin sulfate, perlecan, hyaluronic acid, biglycan, and decorin, the main function of which is structural, which consists in the formation of the framework of the liver parenchyma, as well as promoting cell proliferation, migration, and differentiation [2].

Liver fibrosis is a potentially reversible pathophysiological event that leads to excessive ECM deposition and is a dynamic and highly integrated pathological process that occurs during chronic liver injury [4]. The development of liver fibrosis leads to an increase by 3-5 times in ECM volume compared to a healthy liver. In the early stages of liver fibrosis, significant changes occur in the perisinusoidal space of Disse, where, due to the activation of stellate cells, fibrillogenesis of mostly collagen types I and III occurs. In addition, sinusoidal cells change their phenotype and endothelial cells lose their fenestrations, which disrupts the interaction between hepatocytes and the blood supply. In this context, ECM remodeling plays an important role in the pathogenesis of alcoholic liver injury at different stages of the development of alcoholic liver disease [3].

The purpose of the study was to study the biochemical markers of rat liver intercellular matrix metabolism under the conditions of chronic alcohol intoxication modeling.

Materials and methods. Experiments were performed on 30 white, sexually mature male Wistar rats, weighing 180-220 g. The animals were divided into 2 groups: I – control group (n=6); II group – the group of chronic alcohol intoxication, which included animals on which we modeled alcoholic hepatitis (n=24) by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal injection of 16.5 % ethanol solution in 5 % glucose solution, at the rate of 4 ml/kg of body weight [8]. The control group included animals that were subjected to similar manipulations throughout the study period, but were injected with a physiological solution. The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on days 10, 14, 21 and 28 by bloodletting under thiopental anesthesia. The object of research was blood serum and liver. During the experiments, the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) were followed 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 serum of rats, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutaminyltranspeptidase (γ-GTP) was determined using diagnostic kits, produced by NPP "Filisit-Diahnostyka". We also calculated the de Ritis coefficient (AST / ALT).

The total concentration of glycosaminoglycans (GAG), GAG fractions (heparin-heparan, keratan-dermatan and chondroitin), the concentration of free oxyproline and sialic acids were determined in the rat liver homogenate [8].

Statistical processing of the results of biochemical studies was carried out using a pairwise comparison using the 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 $p<0.05$.

Results of the study and their discussion. Biochemical analysis of the blood serum of rats in chronic alcohol intoxication group revealed an increase in AST activity on the 10th, 14th, and 21st days of the experiment, by 12.1, 1.78, and 1.19 times respectively, compared to the control group (Table 1), and

on the 28th day of the experiment, a 1.6-fold decrease in AST activity was detected compared to the control group.

Table 1

Biochemical markers of cytolytic syndrome in blood serum of rats under the conditions of chronic alcohol intoxication, M±m

Parameters	Groups				
	Control	10th day	14th day	21st day	28th day
AST activity, mmol/h per L	1.71±0.07	20.69±0.81*	3.05±0.14*^	2.04±0.06*^	1.07±0.08*^
ALT activity, mmol/h per L	1.22±0.06	0.59±0.05*	1.72±0.05*^	1.45±0.1^	0.94±0.16^
De Ritis coefficient	1.41±0.06	4.43±0.21*	1.79±0.11^	1.45±0.11	1.32±0.23
γ-GTP activity, μcat / L	0.67±0.04	0.41±0.04*	0.15±0.02*^	0.1±0.01*^	0.11±0.01*

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

ALT activity in the blood serum of rats from chronic alcohol intoxication group on the 10th and 28th day of the experiment was reduced by 2.07 and 1.3 times, respectively, compared to the control group. On the 14th and 21st days of the experiment, the activity of ALT in the blood serum of rats increased by 1.41 and 1.19 times compared to the control group.

The de Ritis coefficient was increased 3.14 times on the 10th day of the experiment compared to the control group.

The activity of γ-GTP in the blood serum of rats from chronic alcohol intoxication group decreased by 1.63 times on the 10th day, 4.47 times on the 14th day, 6.7 times on the 21st day, and 6.09 times on the 28th day compared to a control group of rats.

During biochemical studies of the liver of rats, it was established that the total concentration of GAG on the 10th, 21st, and 28th days of chronic alcohol intoxication was increased by 1.16, 1.36, and 2.32 times, respectively, and on the 14th day, it was decreased by 1.12 times compared to with control (Table 2). On the 14th day of the experiment, the total concentration of GAG in the liver of rats decreased by 1.3 times compared to the 10th day of the experiment. On the 21st day of chronic alcohol intoxication, the total concentration of GAG in the liver of rats increased by 1.53 times compared to the 14th day of the experiment. On the 28th day of the experiment, the total concentration of GAG in the liver of rats decreased by 1.03 times compared to the 21st day of the experiment.

Table 2

Biochemical parameters in the liver of rats under conditions of chronic alcohol intoxication, M±m

Biochemical parameters	Groups				
	Control, n=6	10th day, n=6	14th day, n=6	21st day, n=6	28th day, n=6
Total concentration of glycosaminoglycans, μmol/l	2.62±0.02	3.04±0.03*	2.34±0.05*^	3.57±0.02*^	3.45±0.03*^
Concentration of heparin-heparan fraction of GAG, μmol/l	1.81±0.02	0.9±0.06*	1.47±0.09*^	1.32±0.02*	1.7±0.06^
Concentration of keratan-dermatan fraction of GAG, μmol/l	0.27±0.004	1.21±0.707*	0.34±0.079	0.28±0.007	0.69±0.013*^
Concentration of the chondroitin fraction of GAG, μmol/l	0.59±0.009	1.42±0.03*	0.42±0.1^	1.61±0.05*^	0.77±0.017*^
Concentration of free oxyproline, μmol/g	1.28±0.02	2.82±0.32*	3.001±0.03*	2.63±0.04*^	2.94±0.07*^
Concentration of sialic acids, mg/g	1.26±0.04	4.4±0.07*	5.54±0.11*^	5.76±0.45*	6.48±0.03*

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

The concentration of the heparin-heparan fraction of GAG in the liver of rats on the 10th, 14th, and 21st days of chronic alcohol intoxication was reduced by 2.01, 1.23, and 1.37 times, respectively, compared to the control group. On the 14th day of the experiment, the concentration of the heparin-heparan fraction of GAG in the liver of rats increased by 1.63 times compared to the 10th day of the experiment. On the 28th day of chronic alcohol intoxication, the concentration of the heparin-heparan fraction of GAG in the liver of rats increased by 1.29 times compared to the 21st day of the experiment.

The concentration of the keratan-dermatan fraction of the GAG fraction in the liver of rats on the 10th and 28th day of chronic alcohol intoxication increased by 4.48 and 2.48 times, respectively, compared to the control group. On the 28th day of the experiment, the concentration of the keratan-dermatan fraction of GAG in the liver of rats increased by 2.46 times compared to the 21st day of the experiment.

The concentration of the chondroitin fraction of GAG in the liver of rats on the 10th, 21st, and 28th day of chronic alcohol intoxication increased by 2.41, 2.73, and 1.31 times, respectively, compared to the

control group. On the 14th day of the experiment, the concentration of the chondroitin fraction of GAG in the liver of rats decreased by 3.38 times compared to the 10th day of the experiment. On the 21st day of chronic alcohol intoxication, the concentration of the chondroitin fraction of GAG in the liver of rats increased by 3.83 times compared to the 14th day of the experiment. On the 28th day of the experiment, the concentration of the chondroitin fraction of GAG in the liver of rats decreased by 2.09 times compared to the 21st day of the experiment.

The concentration of free oxyproline in the liver of rats on the 10th, 14th, 21st, and 28th day of chronic alcohol intoxication increased by 2.2, 2.34, 2.05, and 2.3 times, respectively, compared to the control group. On the 21st day of the experiment, the concentration of free oxyproline in the liver of rats decreased by 1.14 times compared to the 14th day of the experiment. On the 28th day of simulation of chronic alcohol intoxication, the concentration of free oxyproline in the liver of rats increased by 2.3 times compared to the 21st day of the experiment.

The concentration of sialic acids in the liver of rats on the 10th, 14th, 21st, and 28th day of chronic alcohol intoxication increased by 3.49, 4.4, 4.57, and 5.14 times, respectively, compared to the control group. On the 14th day of the experiment, the concentration of sialic acids in the liver of rats increased by 1.26 times compared to the 10th day of the experiment.

Thus, we found that ECM proteoglycans underwent the greatest depolymerization on the 21st day of chronic alcohol intoxication due to an increase in the content of the chondroitin fraction of GAG, against the background of the absence of changes in the concentration of the keratan-dermatan fraction of GAG and a decrease in the concentration of the heparin-heparan fraction of GAG. The highest intensity of collagenolysis was observed on the 14th day of chronic alcohol intoxication, as evidenced by the highest content of free oxyproline in the liver. Glycoproteins underwent the greatest catabolism on the 28th day of chronic alcohol intoxication, as evidenced by an increase in neuraminic acid concentration in the liver of the studied animals.

Proteoglycans are non-collagenous ECM proteins, the carbohydrate part of which consists from GAG heteropolysaccharides, which play an important role in the structural architecture of tissues and cell proliferation. In healthy liver tissue, the main type GAG is heparan sulfate, located on the cell surface; however, chronic liver disease can lead to differential expression and structural changes of other proteoglycan subclasses [13]. Heparin and its sulfated form have powerful antioxidant and anti-inflammatory properties due to their ability to bind α1-microglobulin [1]. Therefore, a sharp decrease in the concentration of this fraction of GAG on days 10, 14 and 21 may be associated with the use of these compounds as additional antioxidants. In our previous studies, it was shown that during these experimental periods there was a deficit in the activity of antioxidant enzymes and an increase in the intensity of oxidative damage to protein and lipid polymers of the liver [9]. This also explains the tendency to increase the concentration of the heparin-heparan fraction of GAG on the 28th day of the experiment, since during this period there was an increase in the activity of enzymatic and non-enzymatic antioxidants [9].

Sialic acids or N-acetylneuraminic acids are a diverse group of 9-carbon carboxylated monosaccharides present at the outermost end of N-linked and O-linked oligosaccharide chains of glycoconjugates. Glycoconjugates are components of the outer surface of cells, and their carbohydrate structures change dramatically during cell development. They are characteristically expressed at different stages of differentiation and are recognized by specific antibodies. Aberrant expression of cell surface carbohydrates in humans is very often associated with malignant transformation [5]. Sialic acids affect the structure and function of glycoconjugates and act as ligands for lectins, antibodies and enzymes. They mediate cell-cell recognition, communication, aggregation, development, carbohydrate-protein interaction, controlling the life span of glycoconjugates in the body, mediating bacterial and viral infections, tumor growth and metastasis, playing a role in immunology, microbiome, cell signaling. It is believed that the total level of sialic acids is affected by endogenous or bacterial sialidase, but no experimental confirmation has been found [12].

Various liver diseases can change the concentration of sialylated glycoproteins in the liver. On the other hand, it is well known that changes in protein glycosylation play an important role in the pathogenesis and progression of liver diseases. Since the most common disorders of glycosylation depend on an increase in neurominidase, which cuts off sialic acid residues from glycoconjugates and/or a decrease in enzymes for the synthesis and binding of neuraminic acids to oligosaccharide chains, these processes can lead to an increase in free sialic acid and indicate a violation of the formation of higher levels of organization of glycoconjugates and, as a result, their dysfunction [6]. In the scientific literature, there are data on the effect of ethanol with chronic excessive consumption to lead to desialylation of glycoconjugates.

Conclusion

Chronic alcohol intoxication on the 28th day leads to increased breakdown of glycoproteins and proteoglycans of the extracellular matrix of the liver, and increases the intensity of collagenolysis.

Analysis of the dynamics of changes in the metabolism of the extracellular matrix of the liver showed that proteoglycans undergo the greatest breakdown on the 21st day of chronic alcohol intoxication due to an increase in the concentration of the chondroitin fraction of GAG. The highest intensity of collagenolysis is observed on the 14th day of chronic alcohol intoxication, and glycoproteins undergo the greatest catabolism on the 28th day of chronic alcohol intoxication.

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