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EXTRACELLULAR MATRIX OF RAT LIVER UNDER THE CONDITIONS OF COMBINING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME AND CHRONIC ALCOHOL INTOXICATION

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The extracellular matrix of the liver is a highly dynamic structure that undergoes constant controlled remodeling to maintain homeostasis and suffers from changes during the development of pathological processes. The aim of this study was to establish the effect of systemic inflammatory response syndrome on the background of chronic alcohol intoxication on the concentration of oxyproline, glycosaminoglycans and sialic acids in the liver of rats. The study was performed on 24 male Wistar rats. Animals were divided into 4 groups of 6 animals: I – control; II – systemic inflammatory response syndrome group; III – alcoholic hepatitis group and IV – animals with a combination of systemic inflammatory response syndrome and chronic alcohol intoxication. The concentrations of glycosaminoglycans, heparin-heparan, keratan-dermatan and chondroitin fractions of glycosaminoglycans, free oxyproline and sialic acids were studied in the liver tissue homogenate. The combination of systemic inflammatory response syndrome and chronic alcohol intoxication leads to increased intensity of collagenolysis, glycoprotein catabolism and reduced intensity of breakdown of proteoglycans of connective tissue of the liver. However, it changes the ratio of individual fractions of glycosaminoglycans in the direction of reducing the concentration of anti-inflammatory and increasing regenerative.

Keywords: liver, alcoholic hepatitis, oxyproline, glycosaminoglycans, sialic acids.

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ПОЗАКЛІТИННИЙ МАТРИКС ПЕЧІНКИ ЩУРІВ ЗА УМОВ ПОЄДНАННЯ СИНДРОМУ СИСТЕМНОЇ ЗАПАЛЬНОЇ ВІДПОВІДІ ТА ХРОНІЧНОЇ АЛКОГОЛЬНОЇ ІНТОКСИКАЦІЇ

Позаклітинний матрикс печінки – це високодинамічна структура, яка піддається постійному контрольованому ремоделюванню для підтримання гомеостазу і зазнає змін під час розвитку патологічних процесів. Метою даної роботи є встановлення впливу синдрому системної запальної відповіді на фоні хронічної алкогольної інтоксикації на концентрацію оксипроліну, глікозаміногліканів та сіалових кислот в печінці щурів. Дослідження проведене на 24 щурах-самцях лінії «Вістар». Тварини були поділені на 4 групи по 6 тварин: I – контрольна; II – тварини, яким моделювали синдром системної запальної відповіді; III – тварини, яким моделювали алкогольний гепатит та IV – тварини, яким моделювали поєднання синдрому системної запальної відповіді та хронічну алкогольну інтоксикацію. В гомогенаті тканин печінки досліджували загальну концентрації глікозаміногліканів, гепарин-гепаранової, кератан-дерматанової та хондроїтинової фракцій глікозаміногліканів, вільного оксипроліну та сіалових кислот. Поєднання синдрому системної запальної відповіді та хронічної алкогольної інтоксикації призводить до посилення інтенсивності колагенолізу, катаболізму глікопротеїнів та зниження інтенсивності розпаду протеогліканів сполучної тканини печінки, проте змінює співвідношення окремих фракцій глікозаміногліканів у бік зменшення концентрації протизапальних та збільшення регенераторних.

Ключові слова: печінка, алкогольний гепатит, оксипролін, глікозаміноглікани, сіалові кислоти.

The study is a fragment of the research project "Features of the development of pathological changes in the digestive system under different conditions and the development of methods for their correction", state registration No. 0120U100502.

In the presence or absence of infections, systemic inflammatory response syndrome (SIRS) is a major determinant of multiorgan failure and mortality in alcoholic hepatitis, and the mechanisms involved in the development of SIRS under these conditions should be investigated, according to Michelena J. et al. [8, 11].

The extracellular matrix is a highly dynamic structure that is present in all tissues and is constantly undergoing controlled remodeling, including quantitative and qualitative changes in macromolecules mediated by specific enzymes responsible for the degradation of biopolymers. The function of the extracellular matrix goes beyond providing physical support for the integrity and elasticity of tissues. It is a dynamic structure that is constantly being rebuilt to control the homeostasis of tissues and organs. It is well known that the terminal stage of alcoholic liver disease is fibrogenesis and cirrhosis, but what mechanisms directly lead to the development of liver fibrosis during combination of SIRS and long-term use of alcohol remain open.

Ethanol metabolism in the liver affects the intracellular signaling pathways and disrupts the transcriptional control of genes, leading to fat accumulation, fibrogenesis and activation of innate and adaptive immunity. Reactive oxygen species (ROS), acetaldehyde and aldehydes formed during lipid peroxidation induce collagen synthesis due to their ability to form protein products, which activate profibrogenic pathways that depend on transforming growth factor-β (TGF-β) in stellate liver cells [7]. Acetaldehyde alters the intestinal barrier and promotes the translocation of lipopolysaccharides (LPS) to the liver, destroying intercellular contacts in the mucous membrane of the human colon. Acetaldehyde and

LPS induce Kupffer cells to release ROS and proinflammatory cytokines and chemokines that promote neutrophil infiltration. In addition, alcohol consumption suppresses natural killer cells, which are cytotoxic to liver stellate cells and thus perform an important antifibrotic function in the liver [5].

Acetaldehyde is one of the main triggers of alcoholic fibrogenesis in the liver. Experimental studies have shown that acetaldehyde can stimulate the synthesis of fibril-forming collagen and structural glycoproteins of the intercellular matrix by stellar liver cells. In addition, acetaldehyde promotes the remodeling of the intercellular matrix by activating the matrix metalloproteinase-2 (MMP-2) and inhibiting the matrix metalloproteinase-1 (MMP-1), which leads to sclerosis of the intercellular matrix.

Glycosaminoglycans (GAG) play an important role in virtually all physiological processes in the body and are necessary to maintain homeostasis. Changes in the concentration and structure of GAG are observed in many pathological conditions and are used as biomarkers of disease progression [12]. Key events in the inflammatory process are regulated by GAG, especially those that cover the surface of endothelial cells and leukocytes.

The purpose of the study was to establish the effect of systemic inflammatory response syndrome on the background of chronic alcohol intoxication on the concentration of oxyproline, glycosaminoglycans and sialic acids in the liver of rats.

Materials and methods. The experiments were performed on 24 white adult male Wistar rats weighing 180–220 g. Animals were divided into 4 groups: I – control (n=6); Group II – animals (n=6), in which we simulated SIRS by intraperitoneal administration of 0.4 μg/kg of bacterial lipopolysaccharide S. typhi (pyrogenal) in the first week 3 times a week, then once a week throughout the experiment; Group III – animals (n=6), in which we simulated alcoholic hepatitis by forced intermittent alcoholism for 5 days, repeated two days later by intraperitoneal administration of 16.5 % ethanol solution in 5 % glucose solution, at a rate of 4 ml/kg body weight. Then they were converted to 10 % ethanol as the only source of drink [2]. Group IV consisted of animals (n=6), in which we simulated chronic alcohol intoxication as in group III and injected pyrogenal according to the scheme of group II.

The control group included animals that were subjected to similar manipulations throughout the study, but they were injected saline. Conditions for keeping animals in the vivarium were standard. Removal of animals from the experiment occurred on 63rd day by taking blood from the right ventricle of the heart under thiopental anesthesia. The object of research was blood serum and liver. The experiments followed the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and in accordance with the "General Principles of Animal Experiments" approved by the First National Congress of Bioethics, and the requirements of the "Procedure for scientific research, animal experiments" (2012).

The total concentration of glycosaminoglycans (GAG) by method of Sharaev PN (1987), concentrations of GAG fractions (heparin–heparan, keratan–dermatan and chondroitin) by method of Volpi N. (1996), the concentration of free oxyproline by method of Tetyanets SS (1985) and sialic acids [1] were determined in rat liver homogenate.

Statistical processing of biochemical research results was performed using pairwise comparison using the nonparametric Mann-Whitney method. All statistical calculations were performed in Microsoft Office Excel and its extension Real Statistics 2019. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. The influence of SIRS on the biochemical parameters of rat liver.

We found that under the conditions of administration of bacterial lipopolysaccharide, the concentration of GAG in the liver of rats statistically significantly increases by 1.03 times compared with the control group (table 1).

Under these conditions, the concentration of heparin-heparan fraction in rat liver decreased by 1.39 times, and the concentration of keratan-dermatan and chondroitin fractions increased by 1.3 times and 1.71 times, respectively, compared to the control group. Evaluating the metabolism of collagen fibers under the conditions of modeling the systemic inflammatory response, we found that the content of free oxyproline increased by 2.45 times compared to the control. The concentration of sialic acids in the liver of rats under SIRS conditions is significantly increased by 3.96 times compared to the control group. Thus, SIRS leads to increased collagenolysis and catabolism of glycoproteins of amorphous connective tissue of the liver. Against the background of increased breakdown of proteoglycans of connective tissue of the liver of rats, the ratio of individual fractions of GAG is shifted towards the predominance of chondroitin fraction.

The effect of prolonged alcohol intoxication on the biochemical parameters of the rat liver.

Prolonged administration of ethanol reduced the concentration of GAG in the liver by 1.27 times compared to the control group. The concentration of keratan–dermatan fraction of GAG in the liver of rats

increased by 3.11 times, and the concentration of heparin–heparan and chondroitin fractions of GAG decreased by 2.32 times and 1.2 times, respectively. The concentration of free oxyproline increased 2.25 times, and the concentration of sialic acids increased 6.09 times compared to the control group. Thus, prolonged alcohol intoxication of rats leads to increased collagenolysis and catabolism of glycoproteins of the liver's amorphous connective tissue. Against the background of increased degradation of proteoglycans of connective tissue of the rat liver, the ratio of individual fractions of GAG shifts the predominance of keratan–dermatan fraction.

Table 1

Biochemical changes in rat liver under conditions of combination of SIRS and alcohol intoxication (M±m)

Biochemical parameters	Groups			
	Control, n=6	SIRS, n=6	Alcohol intoxication, n=6	Alcohol intoxication and SIRS, n=6
Total Concentration of glycosaminoglycans, μmol/l	2.62±0.02	2.69±0.02*	2.07±0.02*^	1.9±0.02*^#
Concentration of heparin-heparan fraction, μmol/l	1.81±0.02	1.3±0.02*	0.78±0.01*^	0.26±0.006*^#
Concentration of keratan-dermatan fraction, μmol/l	0.27±0.004	0.35±0.007*	0.84±0.009*^	0.3±0.009*^#
Concentration of chondroitin fraction, μmol/l	0.59±0.009	1.01±0.02*	0.49±0.006*^	1.26±0.002*^#
Concentration of free oxyproline, μmol/g	1.28±0.02	3.14±0.004*	2.88±0.05*^	3.1±0.03*#
Concentration of sialic acids, mg/g	1.26±0.04	4.99±0.09*	7.67±0.02*^	9.63±0.21*^#

Notes: * – $p<0.05$ compared to the control group; ^ – $p<0.05$ compared to SIRS group; # – $p<0.05$ compared to alcohol intoxication group

The effect of SIRS on the background of prolonged alcohol intoxication on the biochemical parameters of the rat liver.

Under the combined effects of SIRS and prolonged alcohol intoxication, we found that the concentration of GAG in the liver of rats decreased by 1.38 times compared with the control group, by 1.42 times compared to the group of animals with SIRS and by 1.09 times compared to group of animals with prolonged alcohol intoxication. The concentration of heparin-heparan fraction GAG in the liver of rats decreased by 6.96 times under conditions of SIRS on the background of prolonged alcohol intoxication compared to the control group, by 5 times compared to the group of animals with SIRS and by 3 times compared to the group of rats with prolonged alcohol intoxication. The concentration of keratan–dermatan fraction GAG in the liver of rats increased by 1.11 times under conditions of SIRS on the background of prolonged alcohol intoxication compared to the control group and decreased by 1.17 times compared to the group of animals with SIRS and by 2.8 times compared to the group of rats with by prolonged alcohol intoxication. The concentration of chondroitin fraction GAG in the liver of rats increased by 2.14 times under conditions of SIRS on the background of prolonged alcohol intoxication compared to the control group, by 1.25 times compared to the group of animals with SIRS and by 2.57 times compared to the group of rats with prolonged alcohol intoxication.

Analyzing the metabolism of collagen fibers in the liver stroma of animals with the combined effects of SIRS and prolonged alcohol intoxication, we found that the concentration of free oxyproline in the liver of rats increased by 2.42 times compared to controls and by 1.08 times compared to rats of prolonged alcohol intoxication. The concentration of sialic acids in the liver of rats increased by 7.64 times under conditions of SIRS on the background of prolonged alcohol intoxication compared to the control group, by 1.93 times compared to the group of animals with SIRS and by 1.26 times compared to the group of rats with prolonged alcohol intoxication.

Heparan sulfate, which is secreted by mast cells together with histamine, provides an interaction between leukocytes and vascular endothelium. During the adhesion of leukocytes to endothelial cells, heparan sulfate on the surface of endothelial cells binds to L-selectins on leukocytes, which leads to parietal rolling (standing) of leukocytes. Due to the interaction of integrin on leukocytes with the molecule of intercellular adhesion-1 (ICAM-1) on the surface of the endothelium increases the adhesion of leukocytes to the endothelium, which reduces their mobility and morphological changes necessary for leukocyte migration across the endothelial barrier. Syndecan, a heparin sulfate, containing proteoglycan, plays a major role in the migration of leukocytes through the vascular endothelium. Thus, heparan sulfate plays a key role in regulating transendothelial migration of leukocytes into the site of inflammation. At the same time, desulfated heparin has an anti-inflammatory effect by inhibiting neutrophil elastase without any anticoagulant effects [12]. Thus, an increase in the heparin-heparan fraction of GAG in the group of animals with SIRS and isolated alcohol intoxication can be considered an adaptive response to liver cell damage. And their reduction in group with combined influence of SIRS and alcohol intoxication testifies to loss of compensatory possibilities of an organism.

Dermatan sulfate has an inhibitory effect on P-selectin in the experiment, which reduces the ability of phagocytes to migrate to the site of inflammation [12]. Also, in experimental work it was shown that keratan sulfate has a pronounced ability to block the adhesion of leukocytes, on the other hand, keratan sulfate stimulates the activation of phagocytes through toll-like receptors type 4 (TLR-4) and can bind TGF-β, stimulating the development of fibrosis [4].

In recent years, chondroitin 4-sulfate has become the focus of attention because of the important role it plays in wound healing, neuronal growth, axon regeneration, cell adhesion and division, and its modulating effect on growth factors. According to Campo GM et al. Chondroitin-4-sulfate reduces liver tissue damage not only by reducing ROS generation but also by inhibiting caspases and blocking the activation of MMP-2 and MMP-9, which are activated by translocation of nuclear factor kappa B (NF-κB) into the cell nucleus, further limiting damage liver [3]. Therefore, the highest concentration of this fraction of GAG in the liver of rats in the group of combined SIRS and alcohol intoxication can be explained by the greatest damage to non-collagenous structures of the connective tissue of the liver.

LPS-stimulated liver macrophages express MMP-9 through the NF-κB and activator protein 1 (AP-1) signaling pathways [6]. There are data from studies on increased IL-33/STAT-3-mediated expression of MMP-2 and MMP-9 [10].

Reactive oxygen species overproduction induced by excessive alcohol intake can increase the level of MMP-2 and MMP-9 in liver tissue. Increased expression of MMP-2 followed by a decrease in tissue inhibitors of matrix proteinases-1 (TIMP-1) may lead to increased fibrosis. Decreased expression of TIMP-1 may increase the release of MMP-2, facilitating the breakdown of extracellular proteins of the liver connective tissue matrix [9]. This may explain the increase in oxyproline concentration in all study groups. Interestingly, despite similar mechanisms of collagen damage in SIRS and isolated alcohol intoxication, the synergism of these factors in the impact on collagenolysis is not observed.

Conclusions

1. The combination of systemic inflammatory response syndrome and chronic alcohol intoxication leads to increased intensity of collagenolysis and breakdown of glycoproteins of amorphous connective tissue of rat liver.

2. The development of systemic inflammatory response syndrome on the background of chronic alcohol intoxication leads to a decrease in the intensity of the breakdown of proteoglycans of amorphous connective tissue of the liver, but changes the ratio of individual proteoglycan fractions towards the reduction of anti-inflammatory and increase of regenerative glycosaminoglycans.

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