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INFLUENCE OF TRANSCRIPTION FACTOR KB ON REMODELING OF EXTRACELLULAR MATRIX OF RAT LIVER UNDER CONDITIONS OF CHRONIC ALCOHOL INTOXICATION

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Liver fibrosis is a common tissue reaction associated with chronic liver damage resulting from prolonged parenchymal cell damage and inflammation. The fibrogenic response is characterized by the progressive accumulation of extracellular matrix components enriched in collagen fibrils and impaired extracellular matrix remodeling. The aim of this study was to establish the effect of transcriptional factor κB on the concentration of oxyproline, glycosaminoglycans and sialic acids in the liver of rats under conditions of chronic alcohol intoxication. The study was performed on 24 male Wistar rats. The animals were divided into 4 groups of 6 animals: I – control; II – animals with inhibition of κB; III – animals with chronic alcohol hepatitis and IV – animals with κB inhibition on the background of chronic alcohol intoxication. We studied the total concentration of glycosaminoglycans, concentration of heparin-heparan, keratan-dermatan and chondroitin fractions of glycosaminoglycans, free oxyproline and sialic acids in the liver tissue homogenate. Prolonged alcohol intoxication of rats leads to remodeling of the extracellular matrix as evidenced by increased collagenolysis and catabolism of glycoconjugates of amorphous connective tissue of the liver. The use of the inhibitor of the transcription factor κB in the background of prolonged alcohol intoxication of animals reduced the concentration of GAG by 1.43 times, lowered oxyproline concentration by 1.6 times and decreased the concentration of sialic acids by 1.14 times compared to chronic alcohol intoxication group. Transcriptional factor NF-κB under conditions of chronic alcohol intoxication affects the architectural remodeling of connective tissue of rat liver by increasing degradation of collagen fibers and destruction of glycoproteins and proteoglycans of the extracellular matrix.

Keywords: liver, alcoholic hepatitis, rats, NF-κB, extracellular matrix.

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ВПЛИВ ТРАНСКРИПЦІЙНОГО ФАКТОРУ κВ НА РЕМОДЕЛЮВАННЯ ЕКСТРАЦЕЛЮЛЯРНОГО МАТРИКСУ ПЕЧІНКИ ЩУРІВ ЗА УМОВ ХРОНІЧНОЇ АЛКОГОЛЬНОЇ ІНТОКСИКАЦІЇ

Фіброз печінки – це поширена тканинна реакція, пов'язана з хронічним ураженням печінки, що є результатом тривалого ураження паренхіматозних клітин та запалення. Фіброгенна відповідь характеризується прогресуючим накопиченням компонентів позаклітинного матриксу, збагачених колагеновими фібрилами, і порушенням ремоделювання екстрацелюлярного матриксу. Метою даної роботи є встановлення впливу транскрипційного фактору κВ на концентрацію оксипроліну, глікозаміногліканів та сіалових кислот в печінці щурів за умов хронічної алкогольної інтоксикації. Дослідження проведене на 24 щурах-самцях лінії «Вістар». Тварини були поділені на 4 групи по 6 тварин: I – контрольна; II – тварини, яким інгібували транскрипційний фактор κВ; III – тварини, яким моделювали хронічний алкогольний гепатит та IV – тварини, яким інгібували транскрипційний фактор κВ на фоні хронічної алкогольної інтоксикації. В гомогенаті тканин печінки досліджували загальну концентрації глікозаміногліканів, гепарин-гепаранової, кератан-дерматанової та хондроїтинової фракцій глікозаміногліканів, вільного оксипроліну та сіалових кислот. Тривала алкогольна інтоксикація організму щурів призводить до ремоделювання екстрацелюлярного матриксу про що свідчить посилення колагенолізу та катаболізму глікокон'югатів аморфної речовини сполучної тканини печінки. Застосування інгібітора транскрипційного фактору κВ на фоні тривалої алкогольної інтоксикації тварин знижує концентрацію ГАГ в 1,43 рази, в 1,6 рази знижує концентрацію оксипроліну та знижує концентрацію сіалових кислот в 1,14 рази порівняно з групою щурів, яким моделювали хронічну алкогольну інтоксикацію. Транскрипційний фактор NF-kB за умов хронічної алкогольної інтоксикації впливає на архітектурне ремоделювання сполучної тканин шляхом збільшення деградації колагенових волокон та руйнування глікопротеїнів і протеогліканів екстрацелюлярного матриксу печінки щурів.

Ключові слова: печінка, алкогольний гепатит, щури, NF-kB, екстрацелюлярний матрикс.

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Chronic liver damage, regardless of the underlying disease, leads to a gradual change in the physiological architecture of the liver and to excessive production of extracellular matrix, which eventually leads to cirrhosis [10]. Liver fibrosis is a common tissue reaction associated with chronic liver damage resulting from prolonged parenchymal cell damage and inflammation. The fibrogenic response is characterized by the progressive accumulation of extracellular matrix components enriched in collagen fibrils and impaired extracellular matrix metabolism. This process is due to the heterogeneous population of hepatic myofibroblasts, which are mainly derived from liver stellate cells and portal fibroblasts. Regression of fibrosis can be achieved by successful control of chronic liver damage due to cessation of fibrogenic response and restoration of fibrolytic pathways. Understanding the complex network of

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transcriptional cascades underlying liver fibrogenesis has identified a large number of antifibrotic targets, but no antifibrotic drug has yet undergone clinical trials [4].

Experimental studies have shown that intensification of lipid peroxidation in the liver precedes the initial stages of fibrosis and is associated with increased production of profibrogenic transforming growth factor- β1 (TGF-β1) by Kupffer cells. Ethanol-induced lipid peroxidation triggers the activation of nuclear factor kappa B (NF-κB), a promoter of the collagen 2 (I) gene in stellate liver cells by stimulating the cascade of kinases [11]. Thus, oxidative stress directly contributes to liver fibrosis by activating NF-κB.

Fibrosis is a dynamically active reaction that is regulated by many factors. Analysis of protein and carbohydrate components of fibrous tissue metabolism in the liver will clarify the role of each of the components of the extracellular matrix in the complex system of interaction of liver cells [5].

Glycosaminoglycans (GAG) as heteropolysaccharides of the extracellular matrix of the liver are actively involved in the formation of fibrous tissue in the liver as a result of liver damage by reactive oxygen species. Heparin can prevent the development of liver fibrosis by affecting the signaling pathway initiated by TGF-β [12].

The final stage of alcoholic liver disease, which develops as a result of chronic alcohol abuse, is the development of liver fibrosis, which turns into cirrhosis. Chronic alcohol abuse activates the NF-κB transcription factor and increases the production of TGF-β by Kupffer cells of the liver [13]. Thus, changes in the composition of the extracellular matrix of the liver may have pathogenetic significance in the development of alcoholic cirrhosis. In the current scientific literature, there is a limited amount of data on the role of activation of the transcription factor NF-κB in changes in the fractional composition of GAG under conditions of chronic alcohol abuse.

The purpose of the study was to establish the influence of the transcriptional factor κB on the concentration of oxyproline, glycosaminoglycans and sialic acids in the liver of rats under conditions of chronic alcohol intoxication.

Materials and methods. The experiments were performed on 24 white adult male Wistar rats weighing 180–220 g. The animals were divided into 4 groups: I – control (n=6); II group – animals (n=6), which received ammonium pyrrolidine dithiocarbamate (PDTC) at a dose of 76 mg/kg 3 times a week throughout the experiment (Control+PDTC group); III group – animals, on which we modelled alcoholic hepatitis (n=6) by forced intermittent alcoholization for 5 days, repeated two days later by intraperitoneal administration of 16.5 % ethanol solution in 5 % glucose solution, at the rate of 4 ml/kg body weight (Alcohol intoxication group). Then they were converted to 10 % ethanol as the only water source [9]. Group IV – animals (n=6), on which we modelled alcohol intoxication as in group III and injected PDTC as in group II (Alcohol intoxication+PDTC group).

The control group included animals that were subjected to similar manipulations throughout the study but injected 0.9 % sodium chloride solution. Conditions for keeping animals in the vivarium were standard. Removal of animals from the experiment occurred on day 63 by taking blood from the right ventricle of the heart under thiopental anesthesia. Serum and liver were studied. The experiments 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 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 the method of Tetyanets SS (1985) and sialic acids [1] were determined in rat liver homogenate.

Statistical processing of biochemical results was performed using pairwise comparison using the nonparametric Mann-Whitney U-test. 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. *Influence of κB transcription factor on biochemical parameters of rat liver.* We found that under the conditions of administration of the NF-κB inhibitor, the concentration of GAG in the liver of rats was reduced by 1.67 times compared with the control group. Under these conditions, the concentration of heparin-heparan fraction in the liver of rats decreased by 3.35 times, the concentration of keratan-dermatan decreased by 3 times and the chondroitin fraction lowered by 1.55 times under conditions of NF-κB inhibitor administration compared with the control group. Evaluating the metabolism of collagen proteins under the conditions of administration of the NF-κB inhibitor, we found that the content of free oxyproline increased by 1.34 times compared to the control. The concentration of sialic acids in the liver of rats under the conditions of administration of the NF-κB inhibitor

increased by 2.35 times compared with the control group. Thus, the introduction of NF-κB inhibitor leads to increased collagenolysis and catabolism of glycoproteins of amorphous connective tissue of the liver against the background of reduced proteoglycan breakdown but maintains the ratio of individual fractions of GAG with a predominance of heparin-heparan fraction (Table 1).

Table 1

Indicators of connective tissue remodelling in rat liver under conditions of inhibition of transcription factor κB and chronic alcohol intoxication (M±m)

Biochemical parameters	Groups			
	Control	Control + PDTC	Alcohol intoxication	Alcohol intoxication + PDTC
Total Concentration of glycosaminoglycans, μmol/l	2.62±0.02	1.57±0.07*	2.07±0.02*^	1.45±0.06*#
Concentration of heparin-heparan fraction, μmol/l	1.81±0.02	0.54±0.008*	0.78±0.01*^	0.61±0.036*#
Concentration of keratan-dermatan fraction, μmol/l	0.27±0.004	0.09±0.004*	0.84±0.009*^	0.32±0.007*^#
Concentration of chondroitin fraction, μmol/l	0.59±0.009	0.38±0.04*	0.49±0.006*	0.67±0.028*^#
Concentration of free oxyproline, μmol/g	1.28±0.02	1.72±0.05*	2.88±0.05*^	1.8±0.03*#
Concentration of sialic acids, mg/g	1.26±0.04	2.96±0.03*	7.67±0.02*^	6.74±0.08*^#

*– $p<0.05$compared to control group, ^– $p<0.05$compared to Control+PDTC group, #– $p<0.05$ compared to alcohol intoxication group

The effect of prolonged alcohol intoxication on the biochemical parameters of the liver of rats. Prolonged alcohol intoxication of rats leads to increased collagenolysis and catabolism of glycoproteins of amorphous connective tissue of the liver. Against the background of increased degradation of proteoglycans of the extracellular matrix of the liver of rats, the ratio of individual fractions of GAG is shifted towards the predominance of a keratan-dermatan fraction [7].

Influence of transcriptional factor κB on biochemical parameters of rat liver under conditions of prolonged alcohol intoxication. Under the combined action of the transcription factor κB inhibitor and prolonged alcohol intoxication, the concentration of GAG in the liver of rats decreased by 1.81 times compared with the control group, by 1.43 times compared with the alcohol intoxication group. The concentration of heparin-heparan fraction of GAG in the liver of rats decreased by 2.97 times during inhibition of the transcription factor κB activation in the background of prolonged alcohol intoxication compared with the control group and decreased by 1.28 times compared with the alcohol intoxication group. The concentration of keratan-dermatan fraction of GAG in the liver of rats increased by 1.19 times under the conditions of PDTC administration under the background of prolonged alcohol intoxication compared with the control group and by 3.56 times compared to the PDTC group, but decreased by 2.63 times compared to alcohol intoxication group. The concentration of chondroitin fraction of GAG in the liver of rats increased by 1.14 times under the action of the inhibitor of the transcription factor κB on the background of prolonged alcohol intoxication compared with the control group, by 1.76 times compared to the PDTC group and by 1.37 times compared to alcohol intoxication group.

Analyzing the metabolism of collagen proteins in the liver stroma of animals, with a combined effect of κB transcription factor inhibitor and long-term alcohol intoxication, we found that the concentration of free oxyproline increased by 1.41 times compared to the control group and decreased by 1.6 times compared with alcohol intoxication group. The concentration of sialic acids in the liver of rats increased by 5.35 times under the conditions of PDTC injection on the background of prolonged alcohol intoxication compared with the control group, by 2.28 times compared with the PDTC group and decreased by 1.14 times compared with alcohol intoxication group.

Based on the detoxifying function of the liver in hepatocytes there is a significant expression of cytochrome P-450 genes, which can lead to excessive formation of free oxygen radicals in the liver, even under physiological conditions. Therefore, when the NF-κB transcription factor is blocked, independent activation of other redox-sensitive transcription factors, such as activator protein-1 (AP-1), is possible. AP-1 has a transcriptional control zone in common with NF-κB. Thus, AP-1 can independently NF-κB and activate the transcription of the inducible isoform of NO synthase [2]. In the case of excessive activation of AP-1, the decrease in the concentration of heparin-heparan fraction GAG can be explained by the effect of AP-1 on the expression of matrix metalloproteinase-7, which cleaves syndecan-1 (a variety of heparan fraction GAG) from the hepatocyte membrane [3]. The decrease in the concentration of heparin-heparan fraction GAG in during chronic alcohol intoxication can be explained by ethanol-induced activation of the transcription factor NF-κB due to increased production of reactive oxygen species. The decrease in the concentration of heparin-heparan fraction GAG in the blockade of the transcription factor NF-κB in chronic alcohol intoxication is probably associated with NF-κB-independent activation of other redox-sensitive transcription factors such as AP-1.

Given the anti-inflammatory properties of the keratan-dermatan fraction of GAG increase in the concentration of this fraction under the conditions of chronic alcohol intoxication, may be an adaptive response to the development of alcoholic hepatitis [6]. There are limited data in the scientific literature on the relationship between the transcription factor NF-κB and the keratan-dermatan fraction of GAG. However, based on the data of our study, the activation of NF-κB is a necessary condition for adaptive growth of the concentration of keratan-dermatan fraction of GAG.

The increase in the concentration of free oxyproline in the PDTC group may be the result of the development of oxidative lesions of the extracellular matrix by reactive oxygen species, due to activation of the transcription factor AP-1 [2]. An increase in the concentration of free oxyproline during chronic alcohol intoxication may be associated with an increase in the content of collagen fibers in the liver due to the development of alcoholic cirrhosis and liver fibrosis [4]. The use of the NF-κB transcription factor activation inhibitor reduces the concentration of free oxyproline in the liver, which may indicate a decrease in collagen fibers and some effectiveness of PDTC as an antifibrotic agent in alcoholic liver disease.

Sialic acids are involved in the activation of the transcription factor Nrf-2, which controls a number of antioxidant genes [8]. Therefore, an increase in the concentration of sialic acids during the blockade of NF-κB transcription factors may indicate the development of oxidative lesions of the liver parenchyma, which requires further study. The increase in the concentration of sialic acids during chronic alcohol intoxication may be caused by the development of alcohol-induced oxidative stress, due to the accumulation of acetaldehyde, mitochondrial dysfunction and stress of the endoplasmic reticulum.

Conclusions

Transcriptional factor NF-κB under conditions of chronic alcohol intoxication plays a leading role in the architectural remodeling of the extracellular matrix of rat liver by increasing the catabolism of collagen proteins and glycoconjugates of amorphous connective tissue.

Under conditions of chronic alcohol intoxication, there is an NF-κB-dependent redistribution of liver glycosaminoglycan fractions, in the direction of decreasing chondroitin glycosaminoglycan fraction on the background of an increase of heparin-heparan and keratan-dermatan fractions.

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