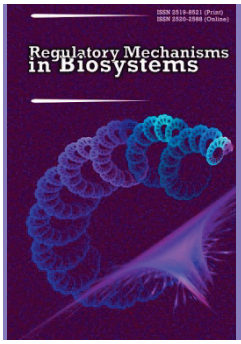

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Influence of doxorubicin on the extracellular matrix of the liver of rats under conditions of chronic alcoholic hepatitis

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The liver has a high regenerative potential that is dependent on many factors, in particular adenosine monophosphate kinase signaling, however, long-term alteration, such as daily alcohol consumption, turns regeneration into a chronic disease such as fibrosis, the end stage of which is cirrhosis. Hepatic extracellular matrix proteins are important triggers of enhanced stellate cell function during the progression of liver fibrosis. The experiments were performed on Wistar rats divided into four groups: control group; doxorubicin injection group (1.25 mg/kg); chronic alcohol hepatitis group; injection of doxorubicin during alcoholic hepatitis group, where 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. Cells with Mallory-Denk bodies were present in the liver of rats from the alcohol hepatitis group, which consisted of tangled balls of intermediate filaments and showed eosinophilia in the cytoplasm of degenerative hepatocytes. There were also necrotic changes in cells. Sinusoidal capillaries were locally dilated. In the central sections of the liver lobules of rats injected with doxorubicin against the background of chronic alcoholic hepatitis, the central veins were expanded, their endothelium was thinned. Sinusoidal capillaries were locally expanded, there were no blood cells in their lumens. The nuclei of the vast majority of hepatocytes were in a state of karyopyknosis, karyorrhexis, and karyolysis. Mallory-Denk bodies were present in the cytoplasm of cells. Administration of doxorubicin to animals with chronic alcoholic hepatitis leads to an increase in the total concentration of glycosaminoglycans, the concentration of chondroitin sulfates, a decrease in the heparin-heparan fraction of glycosaminoglycans and no changes in concentration of the keratan-dermatan fraction in the liver of rats compared to the control group. In the group of rats injected with doxorubicin against the background of chronic alcoholic hepatitis, the concentrations of total glycosaminoglycans, heparin-heparan fraction, and chondroitin fraction significantly increased, and the content of the keratan-dermatan fraction of glycosaminoglycans significantly decreased compared to animals with chronic alcoholic hepatitis. In rats injected with doxorubicin against the background of chronic alcoholic hepatitis, the content of free oxyproline decreased by 1.25 times, sialic acids – by 1.36 times, compared to the group of animals with chronic alcoholic hepatitis. Administration of ethanol or doxorubicin in combination with ethanol to rats causes morphological changes in the liver that are characteristic of chronic alcoholic hepatitis. Administration of doxorubicin to rats leads to degenerative morphological changes in the liver lobules. The administration of doxorubicin prevents alcohol-induced collagenolysis and the breakdown of glycoproteins, but increases the breakdown of proteoglycans due to an increase in the content of chondroitin and heparin-heparan fractions.

Keywords: adenosine monophosphate kinase; oxyproline; sialic acids; glycosaminoglycans; inflammation; ethanol intoxication.

Introduction

The extracellular matrix of the liver is a complex cross-linked network of macromolecular proteins that form a critically important microenvironment of hepatocytes to provide structural support and regulate organ homeostasis (Ortiz et al., 2021). The extracellular matrix not only provides structure to the liver and support for cells within the tissue, but also acts as a reservoir for growth factors and cytokines and as a signaling center through which cells can receive information from the environment and vice versa (Arteel & Naba, 2020). In addition to its mechanical and biochemical properties, extracellular matrix supports hydration and, interacting with cell surface receptors, regulates cell differentiation, adhesion, migration and proliferation. Thus, the extracellular matrix creates a complex microenvironment that is particularly dynamic in nature and undergoes continuous remodeling not only during development but also during regeneration and damage repair. Accordingly, the well-coordinated regulation of extracellular matrix remodeling is essential for the maintenance of homeostasis and to prevent the onset and progression of liver diseases (Arriazu et al., 2014). The liver has a high regenerative potential, however, when the damage is permanent and long-term, such as daily alcohol consumption, the regeneration turns into a chronic disease such as fibrosis (Cordero-Espinoza & Huch, 2018). Liver fibrosis is usually preceded by oxidative-nitrosative damage (Mykytenko et al., 2022), which leads to the development of alcoholic hepatitis (Sørensen et al., 2022), followed by the activation of the main type of fibrotic cells in the liver, hepatic stellate cells (Lin et al., 2023; Torres et al., 2023). It has become clear that extracellular matrix proteins are the triggers of increased hepatic stellate cells' function during the progression of liver fibrosis (Yan et al., 2021).

It is known from the literature that the serine/threonine protein kinase, adenosine monophosphate kinase (AMPK), plays an important role in the regulation of cellular stress, energy homeostasis and fibrosis. AMPK is activated by phosphorylation of Thr-172 on the catalytic subunit of AMPKα, but can also be regulated by oxidative stress (Au-Yeung et al., 2023). AMPK activation has protective effects against oxidative stress and inflammation in various cell types, tissues, and organs. Velagapudi et al. (2019) demonstrated that activation of the AMPK/Nrf2/HO-1 signaling pathway in BV-2 microglia leads to suppression of neuroinflammation. Du et al. (2019) found that modulation of the AMPK/Nrf2 pathway pre-

vents angiotensin II-induced cardiac fibrosis and dysfunction. Lee et al. (2023) observed that activation of the AMPK/Nrf2 signaling pathway protects cells from CCl_4-induced oxidative stress and inflammation. This protective potential is closely related to the activation of Nrf2 signaling. Su et al. (2023) demonstrated that AMPK/DRP1 phosphorylation in hepatic stellate cells induces mitochondrial fission and decreases the expression of α-smooth muscle actin and collagen.

Overall, AMPK activation protects against oxidative stress, nonalcoholic fatty liver disease, fibrosis, and metabolic syndrome, but chronic activation can also have serious adverse effects in the form of cardiac hypertrophy and cancer (Tian et al., 2023). On the other hand, blockade of AMPK cascade activation may have negative consequences for liver metabolism. Knockout of the AMPK cascade genes in the liver of mice significantly increases oxidative damage in the development of nonalcoholic steatohepatitis (Zhao et al., 2020). Blockade of AMPK activation by a specific inhibitor (compound C) also increases oxidative liver damage under conditions of excess alcohol intake (Nagappan et al., 2019). Doxorubicin, a drug widely used in the chemotherapy of various cancers, is a potent inhibitor of AMPK (Andugulapati et al., 2022). There are few studies that demonstrate the dependence of liver extracellular matrix changes under conditions of chronic alcoholic hepatitis on AMPK activity.

The purpose of this work is to determinate the influence of doxorubicin on the content of glycosaminoglycans, oxyproline and sialic acids in the liver of rats under conditions of chronic alcoholic hepatitis.

Materials and methods

The animal studies were performed in accordance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines 2.0 (Percie du Sert et al., 2020) and Guide for the Care and Use of Laboratory Animals (2011). Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this area. The animals were kept in a vivarium accredited in accordance with the "Standard rules of order, equipment and maintenance of experimental biological clinics (vivarium)". Devices used for research were subject to metrological control. All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (record No. 197 from 23.09.2021). The animals were kept in cages with 12-hours of daylight, at the air temperature of 20–23 °C with free access to food at the vivarium of Poltava State Medical University. Animals were removed from the experiment on the 63rd day by blood sampling from the right ventricle of the heart under thiopental anesthesia.

The experiments were performed on 24 white, sexually mature male Wistar rats, weighing 180–220 g. The animals were divided into 4 groups: I – control group (n = 6); II – group – animals (n = 6) which received doxorubicin hydrochloride (doxorubicin, S.C. Sindan-Pharma S.R.L.), as inhibitor of AMP-activated protein kinase, at a dose of 1.25 mg/kg intraperitoneally four times a week (Yarmohmmadi et al., 2017) for 63 days (doxorubicin injection group); III group (alcohol hepatitis group) – animals, on which we simulated chronic alcoholic hepatitis (n = 6) by forced intermittent alcoholization for 5 days by intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 mL/kg body weight once a day. Afterwards, there was a break for two days. Then the animals again received intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 mL / kg body weight a day for 5 days. Then they were converted to 10% ethanol as the only source of drink for 51 days, total experimental procedure lasted 63 days (Mykytenko et al., 2022). IV group – animals (n = 6), on which we simulated chronic alcohol hepatitis as in group III and administered doxorubicin hydrochloride according to the scheme of group II (injection of doxorubicin during alcoholic hepatitis group). 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).

In the serum of rats we determined the activity of γ-glutaminyltranspeptidase using a diagnostic kit, manufacturer NPP "Philisit-Diagnostics". To determine the total concentration of glycosaminoglycans, the carbazole method was used, which is based on determining the content of uronic acids after acid hydrolysis of glycosaminoglycans, based on the colour reaction of uronic acid with carbazole (Akimov et al., 2019). To define the concentration of different fractions (heparin-heparan, keratan-dermatan and chondroitin) of glycosaminoglycans in tissue homogenate, we used the method of sequential precipitation proposed by Volpi (1996). After sequential precipitation, we used the carbazole method to determine concentrations of different fractions. The concentration of free oxyproline was measured colorimetrically after the oxidation of oxyproline to pyrrole-2-carboxylic acid. Its concentration was estimated by the content of the coloured product formed in the reaction of the reaction product of pyrrole-2-carboxylic acid with paradimethylaminobenzaldehyde (Akimov et al., 2019). Sialic acids were measured as follows. 0.2 mL of 10% liver homogenate and 0.8 mL of distilled water were added to centrifuge tubes with a capacity of 10 mL, then 1 mL of a 10% solution of trichloroacetic acid was added. The test tubes were placed in a water bath for 5 minutes at the temperature of boiling water. Then the test tubes were placed in cold water with ice for 5 min. After that, they were centrifuged at 3000 rpm for 15 minutes. After centrifugation, 0.4 mL of the supernatant was taken and transferred to a new centrifuge tube with the addition of 5 mL of acetic-sulfuric acid reagent (95 mL of glacial acetic acid and 5 mL of concentrated sulfuric acid). After that, the samples were placed in a water bath for 30 min and cooled under running water to room temperature. Absorbance was measured on a Ulab 101 spectrophotometer at a wavelength of λ = 540 nm against a blank sample (containing only the acetic-sulphuric acid reagent) (Mykytenko et al., 2022). The fragments of the liver were removed and fixed with a 10% neutral formalin solution. The material was washed and prepared for paraffin embedding according to standard techniques (Bagrij et al., 2016). Sections of 5–7 μm thick were obtained Histo-Line microtome. Histological sections were stained with hematoxylin and eosin. Series of histological slide photomicrographs from objectives 4x and 10x were captured by a microscope MICROmed Fusion FS-7630 (Ningbo Zhanjing Optical Instruments Co., Ltd, China, 2019) attached to a MICROmed MDC-500 (Ningbo Zhanjing Optical Instruments Co., Ltd, China, 2019) digital 5.0 Mpx camera. Photo fixation was performed in Vividia Ablescope software.

Data was analyzed for normality of distribution using the Shapiro-Wilk test. In cases of normal distribution we used the ANOVA test to evaluate differences between groups followed by pairwise comparisons by the Student t-test. In cases of distribution different from normal we used nonparametric ANOVA by the Kruskal-Wallis method followed by pairwise comparisons by the Mann-Whitney U-test. To avoid the multiple comparisons problem we used Bonferroni correction. Differences between groups were considered statistically significant if $P < 0.05$. Data represented as mean (x) ± standard error (SE).

Results

Changes in activity of γ-glutaminyltranspeptidase in blood serum of rats. Analysis of the marker enzyme of alcoholic liver disease in blood serum, namely, γ-glutaminyltranspeptidase revealed that the activity of γ-glutaminyltranspeptidase in the doxorubicin injection group increased by 1.55 times, in the alcohol hepatitis group it decreased by 5.58 times, and in the injection of doxorubicin during alcoholic hepatitis group it increased by 1.97 times in comparison to the control group of rats. The introduction of doxorubicin against the background of alcohol intoxication increased the activity of γ-glutaminyltranspeptidase by 16.58 times in compared to the alcohol hepatitis group (Fig. 1).

Influence of doxorubicin on the morphological parameters of the liver of rats under the conditions of chronic alcoholic hepatitis modeling. Liver rats from control group had a lobular structure. Externally, each of the lobes was surrounded by a well-defined connective tissue that formed the extracellular matrix of the organ. Central veins were located in the center of the lobes. Hepatic beams formed by hepatocytes were visualized radially from them, with sinusoidal capillaries between them (Fig. 2a). On the periphery of the lobules in rats from the control group, triads from the artery, vein, and bile duct were localized. Blood filling of the vascular system of the lobules was moderate.

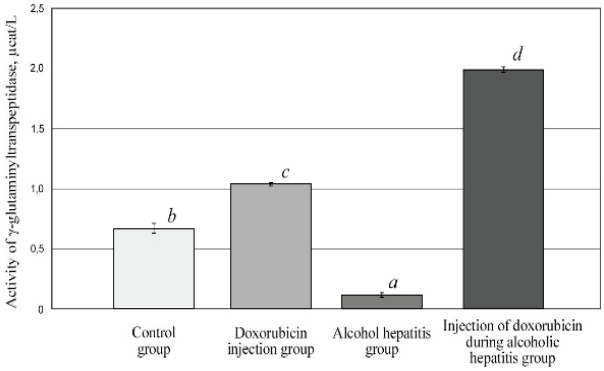

Fig. 1. Activity of γ-glutaminyltranspeptidase in blood serum of rats under the conditions of administration of doxorubicin and modeling of chronic alcoholic hepatitis (x ± SE, n = 6): different letters indicate values that differed one from another significantly within one line of the table according to the results of comparison using nonparametric ANOVA by Kruskal-Wallis method followed by pairwise comparisons by Mann-Whitney U-test with Bonferroni correction

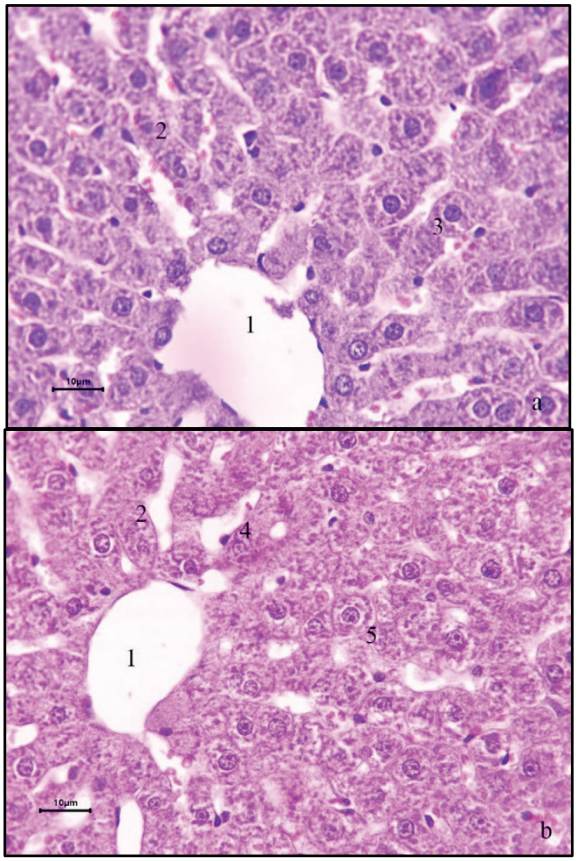

Fig. 2. The central part of the liver lobe of a rat: *a* – control group, *b* – doxorubicin injection group; *1* – central vein, *2* – liver beams, *3* – sinusoidal capillary, *4* – dilated sinusoidal capillaries, *5* – degenerative changes in hepatocyte; hematoxylin-eosin staining

There were degenerative changes in the liver of rats from the doxorubicin injection group, which were more pronounced in the central zones of the lobes. The endothelium of the central veins was thin, the nuclei of the endotheliocytes had a form of thin basophilic strips. There were signs of perivascular hyperhydration of an amorphous substance. Sinusoidal capillaries were dilated, their blood supply was uneven. Hepatocytes showed polymorphism, and moderately expressed phenomena of fatty dystrophy. There were no binucleated cells. Hepatocytes with pyknotically altered nuclei were present locally (Fig. 2b). In the liver of rats with chronic alcohol intoxication, there were foci of hepatocyte swelling and local necrotic changes. At the site of dead hepatocytes in the central parts of the lobules there were areas of proliferative cell activity (Fig. 3).

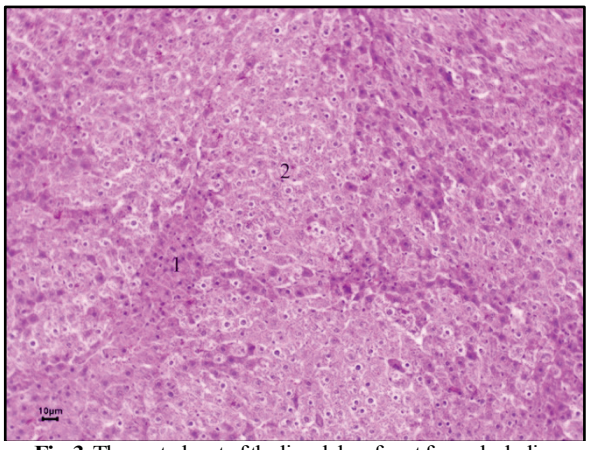

Fig. 3. The central part of the liver lobe of a rat from alcoholic hepatitis group: *1* – area of proliferation, *2* – area of cellular dystrophy; hematoxylin-eosin staining

Cells with Mallory-Denk bodies were present in the liver of rats from the alcohol hepatitis group, which consisted of tangled balls of intermediate filaments and showed eosinophilia in the cytoplasm of degenerative hepatocytes. There were also necrotic changes in cells (Fig. 4a). Sinusoidal capillaries were locally dilated.

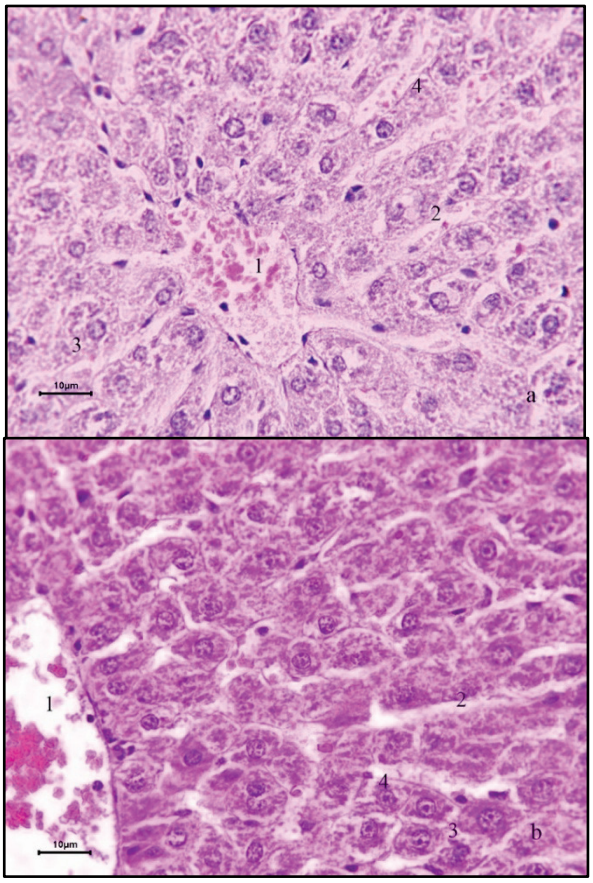

Fig. 4. The central part of the liver lobe of a rat: a - alcohol hepatitis group; b - injection of doxorubicin during alcoholic hepatitis group; 1 – central vein. 2 – hepatocyte necrosis. 3 – Mallory-Denk bodies. 4 – dilated sinusoidal capillary; hematoxylin-eosin staining

In the central sections of the liver lobules of rats injected with doxorubicin against the background of chronic alcoholic hepatitis, the central veins were expanded, their endothelium was thinned. Sinusoidal capillaries were locally expanded, there were no blood cells in their lumina. The nuclei of the vast majority of hepatocytes were in a state of karyopyknosis, karyorrhexis, and karyolysis. Mallory-Denk bodies were present in the cytoplasm of cells (Fig. 4b).

The effect of doxorubicin on the biochemical parameters of extracellular matrix of the liver of rats. We established that under the conditions of administration of doxorubicin, the total concentration of glycosaminoglycans increased by 1.26 times due to the heparin-heparan fraction of glycosaminoglycans in the liver of rats, which increased by 1.31 times and the chondroitin fraction of glycosaminoglycans, which increased by 1.15 times, but the concentration of the keratan-dermatan fraction decreased by 2.08 times compared to the control group (Table 1). Evaluating the metabolism of collagen proteins under the conditions of administration of an AMP-kinase inhibitor, we note an increase in the content of free oxyproline by 1.5 times compared to the control. The concentration of sialic acids in the liver of rats also increased under the conditions of administration of doxorubicin by 2.87 times compared to the control group. Thus, the introduction of doxorubicin led to increased collagenolysis and catabolism of glycoproteins of the extracellular matrix of the liver, and the following changes in the ratio of glycosaminoglycans fractions: an increase in the heparin-heparan and chondroitin fractions against the background of a decrease in the concentration of the keratan-dermatan fraction.

Table 1

Biochemical indicators of extracellular matrix of the liver of rats under conditions of chronic alcoholic hepatitis and administered doxorubicin hydrochloride (x ± SE, n = 6)

Biochemical parameters	Control group	Doxorubicin injection group	Alcohol hepatitis group	Injection of doxorubicin during alcoholic hepatitis group
Total concentration of glycosaminoglycans, μmol/L	2.621 ± 0.023^{b}	3.311 ± 0.008^{d}	2.074 ± 0.025^{a}	2.940 ± 0.008^{c}
Concentration of heparin-heparan fraction, μmol/L	1.814 ± 0.017^{c}	2.384 ± 0.014^{d}	0.780 ± 0.013^{a}	1.402 ± 0.016^{b}
Concentration of keratan-dermatan fraction, μmol/L	0.268 ± 0.005^{b}	0.130 ± 0.005^{a}	0.842 ± 0.009^{c}	0.284 ± 0.005^{b}
Concentration of chondroitin fraction, μmol/L	0.591 ± 0.009^{b}	0.678 ± 0.008^{c}	0.489 ± 0.006^{a}	1.182 ± 0.008^{d}
Concentration of free oxyproline, μmol/g	1.284 ± 0.015^{a}	1.918 ± 0.011^{b}	2.880 ± 0.053^{d}	2.301 ± 0.023^{c}
Concentration of sialic acids, mg/g	1.263 ± 0.037^{a}	3.619 ± 0.025^{b}	7.669 ± 0.016^{d}	5.644 ± 0.090^{c}

Note: different letters indicate values that differed one from another significantly within one line of the table according to the results of comparison using nonparametric ANOVA by Kruskal-Wallis method followed by pairwise comparisons by Mann-Whitney U-test with Bonferroni correction.

The effect of chronic alcoholic hepatitis on the biochemical indicators of extracellular matrix of the liver of rats. We have described the effects of chronic alcoholic hepatitis on biochemical markers of the extracellular matrix metabolism in the rat liver in detail in our previous publications (Mykytenko et al., 2022). Long-term alcohol intoxication leads to increased collagenolysis and catabolism of glycoproteins of the extracellular matrix of the liver. Against the background of an increase in the breakdown of extracellular matrix proteoglycans of the liver of rats, the ratio of individual fractions of glycosaminoglycans shifts towards the predominance of the keratan-dermatan fraction.

The effect of doxorubicin on the biochemical indicators of extracellular matrix of the liver of rats against the background of chronic alcoholic hepatitis. We found that the introduction of doxorubicin against the background of chronic alcoholic hepatitis led to an increase in the total concentration of glycosaminoglycans in the liver of rats by 1.12 times compared to the control group and by 1.42 times compared to the alcohol hepatitis group and a decrease by 1.13 times compared to doxorubicin injection group (Table 1). The concentration of the heparin-heparan fraction of glycosaminoglycans in the liver of rats decreased by 1.29 times in the injection of doxorubicin during alcoholic hepatitis group compared to the control group and by 1.7 times compared to the doxorubicin injection group and increased by 1.79 times compared to the alcohol hepatitis group. The concentration of the keratan-dermatan fraction of glycosaminoglycans in the liver of rats increased by 2.15 times compared to the doxorubicin injection group and decreased by 3 times compared to the alcohol hepatitis group. The concentration of the chondroitin fraction of glycosaminoglycans in the liver of rats increased 2-fold in the injection of doxorubicin during alcoholic hepatitis group compared to the control group, 1.74-fold compared to the doxorubicin injection group and 2.41-fold compared to the alcohol hepatitis group.

Analyzing the metabolism of extracellular matrix collagen proteins of the liver in the injection of doxorubicin during alcoholic hepatitis group, we found that the concentration of free oxyproline increased by 1.8 times compared to the control group and by 1.20 times compared to the doxorubicin injection group and decreased by 1.25 times compared to the alcohol hepatitis group. The concentration of sialic acids in the liver of rats from the injection of doxorubicin during alcoholic hepatitis group increased 4.48 times compared to the control group, 1.56 times compared to the doxorubicin injection group and decreased 1.36 times compared to the alcohol hepatitis group.

Discussion

An increase in the concentration of the heparin-heparan fraction of glycosaminoglycans in the liver when doxorubicin is administered can be considered as an adaptive response to doxorubicin-induced oxidative damage to hepatocytes (Pantea et al., 2023). An increased concentration of the heparin-heparan fraction of glycosaminoglycans can activate hepatocyte proliferation through the midkine-associated cascade, which acquires the ability to activate due to the reduction of AMPK expression by doxorubicin (Ou et al., 2020; Wang et al., 2023). Presumably, this is the same mechanism that ensures an increase in the concentration of the heparin-heparan fraction in the group of animals administered doxorubicin against the background of chronic alcohol intoxication in relation to the group of rats with chronic alcoholic hepatitis.

According to Shrikanth et al. (2021) inhibition of AMPK in kidney cells does not affect the content of the keratan-dermatan fraction of glycosaminoglycans. Our experimental results differ from the data obtained by Shrikanth et al. (2021) and indicate a decrease in the concentration of the keratan-dermatan fraction of glycosaminoglycans under the conditions of inhibition of the AMPK cascade by doxorubicin, which may be related to the different organs studied and the different pharmacological substances used for the inhibition of AMPK.

An increase in the concentration of the chondroitin fraction of glycosaminoglycans under the conditions of doxorubicin administration can be considered an adaptive response to the doxorubicin-induced decrease in the activity of the AMPK cascade, since the chondroitin-containing glycosaminoglycans decorin can contribute to the activation of the AMPK cascade through vascular endothelial growth factor receptor 2 (Neill et al., 2021). A decrease in the concentration of the chondroitin fraction of glycosaminoglycans in the alcohol hepatitis group may be a consequence of a decrease in decorin content and a sign of the inflammatory process in the liver (Li et al., 2023). An increase in the concentration of the chondroitin fraction of glycosaminoglycans in the liver in injection of doxorubicin during alcoholic hepatitis group may be a sign of the combination of toxic effects of these substances and threaten the development of liver fibrosis (Dudás et al., 2001; Sedeman et al., 2022).

An increase in the intensity of collagenolysis and desialylation of collagen and non-collagen proteins in the liver of rats administered doxorubicin may be associated with the development of oxidative damage to hepatocytes (Ahmed et al., 2022). A decrease in the intensity of collagenolysis in the liver of animals administered doxorubicin under conditions of chronic alcoholic hepatitis may be a consequence of an increase in the concentration of the chondroitin fraction of glycosaminoglycans in this group of rats. Decorin has an inhibitory effect on the activity of matrix metalloproteinase 14, which increases under the conditions of the development of alcoholic hepatitis (Itaba et al., 2019; Rivet et al., 2023). However, under these conditions, the risk of developing liver fibrosis increases due to Decorin-induced enhancement of the effect of transforming factor β (Du et al., 2023).

Under the conditions of alcoholic hepatitis, according to Gruszewska et al. (2014), the total concentration of sialic acids increases significantly,

which may be associated with the activation of neuraminidase in liver cells, as observed in steatosis according to Pilling et al. (2021). Increased concentration of sialic acids due to desialylation processes can lead to sialylation of various protein and lipid molecules, which is a process not controlled by enzymes. Changes in galactosylation, fucosylation, and sialylation are now well-established factors that govern the differential function of IgG, ranging from inhibitory/anti-inflammatory effects to complement activation and promotion of antibody-dependent cellular cytotoxicity (Cobb, 2020). Chung et al. (2014) published the results of experimental studies where increased galactosylation and total sialylation induce more persistent antibody-dependent cellular phagocytosis. However, there is inconsistency in the literature regarding the effect of IgG sialylation on antibody-dependent cellular phagocytosis activity. In the article by Lin et al. (2015) IgG, which has a biantennary N-glycan structure with two terminal alpha-2,6-linked sialic acids, was found to be an optimal structure that has high activity against cancer, influenza, and inflammatory diseases and is optimized for antibody-dependent cellular phagocytosis. However, in a scientific study by Quast et al. (2015) additional sialylation of α2,6-IgG reduced complement-mediated cytotoxicity, and in studies by Thomann et al. (2015) α2,6-sialylation of IgG1 had no significant effect on antibody-dependent cellular cytotoxicity. This threatens the involvement of immune mechanisms in the processes of liver damage under the conditions of alcohol abuse. According to the results of our research, under the conditions of inhibition of the AMPK cascade by doxorubicin, desialylation of non-collagenous proteins of the liver is reduced compared to the group of animals with alcoholic hepatitis, which can have a significant impact on the course of inflammation under conditions of chronic alcoholic hepatitis. Despite significant progress in the understanding of sialylation processes, for example, that α2,6-sialylated IgG/Fc can actively suppress inflammation, this topic remains incompletely researched and of great interest to researchers. One reason is that sialylation does not always suppress inflammation or disease. The effect is likely dependent on context, underlying disease mechanisms, and other metabolic factors and modulators that remain to be studied.

Conclusions

Administration of ethanol or doxorubicin in combination with ethanol to rats causes morphological changes in the liver that are characteristic of chronic alcoholic hepatitis. Administration of doxorubicin to rats leads to degenerative morphological changes in the liver lobules. The administration of doxorubicin prevents alcohol-induced collagenolysis and the breakdown of glycoproteins, but increases the breakdown of proteoglycans due to an increase in the content of chondroitin and heparin-heparan fractions. Thus, blockade of AMPK activation by doxorubicin during chronic alcoholic hepatitis, despite limiting the destruction of collagen fibers and decrease in intensity of desialylation processes, is a dangerous approach to treatment of alcoholic hepatitis because it leads to increase in glycosaminoglycans concentration, which can impair normal liver regeneration. Since blockade of AMPK cascade showed adverse effects during chronic alcohol hepatitis, it is a plausible and promising approach to artificially stimulate AMPK activation during chronic alcohol hepatitis for prevention or treatment or, at least, partial alleviation of biochemical and morphological changes caused by development of chronic alcohol hepatitis.

The authors declare that there is no conflict of interest.

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