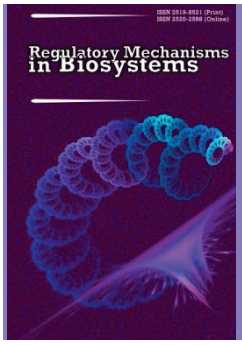

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Influence of nuclear factor κB and adenosine monophosphate-activated protein kinase on the vascular bed of the liver under the conditions of modeling chronic alcoholic hepatitis

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Chronic alcohol use activates the transcription factor κB (NF-κB) in liver sinusoidal endothelial cells (LSECs), macrophages and other cells of the liver parenchyma, which controls the regulation of the expression of pro-inflammatory cytokines that activate signaling pathways of immune regulation of liver inflammation and vascular tone. AMP-activated protein kinase (AMPK) is an important immunometabolic regulatory factor in macrophages and, as a metabolic sensor, AMPK in vascular cells may be involved in the regulation of blood flow. The aim of the work was to find out the effect of modulators of the transcription factor κB and AMP-activated protein kinase on the morphometric parameters of the vascular bed of the liver of rats under the conditions of modeling chronic alcoholic hepatitis. Simulation of chronic alcoholic hepatitis leads to a violation of the exchange of metabolites between the central and interlobular veins, which creates prerequisites for the development of hypoxic damage to hepatocytes, as evidenced by a decrease in the diameters of lobular arterioles and venules. Administration of ammonium pyrrolidinedithiocarbamate and bacterial lipopolysaccharide, which affect NF-κB signaling under the conditions of modeling chronic alcoholic hepatitis, helps to restore the exchange of metabolites between the central and interlobular veins, which is evidenced by an increase in the lumens of lobular arterioles and venules. The introduction of phenformin and doxorubicin, which affect AMPK under the conditions of modeling chronic alcoholic hepatitis, prevents the development of hypoxic damage to hepatocytes, as evidenced by an increase in the diameters of lobular arteries and veins, and leads to intensification of interlobular blood circulation.

Keywords: AMP-activated protein kinase; NF-κB; chronic alcoholic hepatitis; nitrous oxide; inflammation; ethanol intoxication.

Introduction

The liver is strategically positioned for its metabolic and immune function, as it receives 70–80% of its blood flow from the gastrointestinal tract via the hepatic portal vein and the remainder via the hepatic artery. The liver is exposed to countless microbial and food antigens in specialized capillaries of the liver's microcirculatory system called the hepatic sinuses (Chemych et al., 2023; Sumtsova et al., 2024). These capillaries are formed by liver sinusoidal endothelial cells (LSECs), which not only form a unique barrier between blood flow and the parenchyma of the organ, but also play an integral role in physiological and pathological processes in the liver (Wilkinson et al., 2020). Numerous studies have established that after taking high concentrations of ethanol, an excessive amount of acetaldehyde stimulates the expression of aldehyde dehydrogenase, contributes to the development of hypoxia and swelling of hepatocytes, leads to the destruction of hepatocytes and the structure of the sinuses of the liver, compression of blood vessels in the microcirculation system and, as a result, leads to impaired liver blood circulation. In addition, ethanol can directly cause vasoconstriction of the portal vein, which leads to portal hypertension, and the disruption of microcirculation in the hepatic lobe, due to hypoxia, will lead to increased liver damage. Thus, disruption of microcirculation is one of the leading mechanisms of alcoholic liver damage (Zhanget et al., 2016; Takei et al., 1999).

Capillarization of the liver sinuses is a pathology associated with chronic liver diseases and is characterized by thickening of the endothelium with the loss of fenestrae in LSECs (defenestration) and the formation of a basement membrane beneath them, ultimately resulting in the phenotypic transformation of LSECs into a systemic vascular type of endothelium, which is usually is localized in the capillaries of most human organs and tissues. These structural changes were named by Schaffner and Popper in 1963 as capillarization of the hepatic sinuses (Mak et al., 2022). According to Mak & Mei (2017), sinus capillarization occurs due to the deposition of collagen I, III, V, VI and XVIII types and non-collagen proteins. The expansion of the space of Disse from the normal width of 0.5 μm to several μm occurs due to the deposition of fibronectin and proteoglycans, which leads to perisinus fibrosis. It is worth noting that LSECs can defenestrate even before the appearance of the basement membrane. Capillarization of the sinuses of the liver leads to a violation of the lobular microcirculation, which in turn will reduce the bidirectional exchange of substances in the space of Disse between blood in the sinuses and parenchymal cells and lead to hepatocellular dysfunction (Martinez-Hernandez & Martinez, 1991).

The fenestrae of LSECs are dynamic structures. A number of studies indicate that substances such as ethanol, drugs, toxins, growth factors, hormones, viruses and nutrition modulate the dynamics of fenestrae formation and disappearance in LSECs and lead to capillarization of liver sinuses. Chronic liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease and chronic viral hepatitis are known to lead to sinus capillarization (Mak et al., 2022).

Pathogenetic mechanisms regulating the loss of fenestrae are not fully established, but several important molecules and pathways have been identified that regulate fenestration and play an important role in maintai-

ning liver microcirculation. Vascular endothelial growth factor (VEGF) production by hepatocytes and hepatic stellate cells (HSCs) supports the fenestrated phenotype of LSECs. VEGF is a key regulator of LSECs phenotype (Jin et al., 2023). As a result of experimental studies, it was established that VEGF acts through two signaling pathways: NO-dependent and NO-independent. In the NO-dependent pathway, nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) activates the NO-cGMP (cyclic guanosine monophosphate) signaling pathway, which ensures the formation of fenestrae in LSECs (Iwakiri & Trebicka, 2021; Wang & Peng, 2021).

Thus, as endothelial cells, LSECs also play an important role in regulating the microcirculation of the hepatic lobule. NO and endothelin-1 (ET-1), which are mainly produced by LSECs under physiological conditions, are two of the most potent vasoactive substances in the liver, causing multidirectional effects: vasodilation and vasoconstriction, respectively (Wilkinson et al., 2020). Under physiological conditions, in response to shear stress, which is friction between the vascular endothelium and blood flow, LSECs increase NO synthesis and inhibit ET-1 expression (Wang & Peng, 2021). Meanwhile, as mentioned earlier, NO-dependent signaling pathways ensure the physiological functioning of LSECs and maintain the resting state of HSCs and Kupffer cells (KC). (Wilkinson et al., 2020). These results indicate that LSECs may be involved in inflammation and fibrosis under pathological conditions through NO signaling. In addition, carbon monoxide and metabolites of the cyclooxygenase pathway, such as prostacyclin, have also been implicated in the regulation of hepatic sinus blood flow (Wan et al., 2022; Minciuna et al., 2024). Thus, the LSECs-related microcirculatory regulation process in the liver not only copes with circadian changes in hepatic blood flow, mainly caused by digestion, but also participates in the maintenance of liver homeostasis, including the prevention of inflammation and fibrosis under pathological conditions.

AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, is expressed in both endothelial and smooth muscle cells of liver vessels. Previous studies have established that the predominant isoform expressed in vascular endothelial cells is α1. Both α1 and α2 catalytic subunits are expressed in arterial smooth muscle cells, although their relative proportions differ between different arteries (Goirand et al., 2007). Vasodilation is a vital mechanism of systemic blood flow regulation that occurs during periods of increased energy demand. As a metabolic sensor, vascular AMPK may be involved in the metabolic regulation of blood flow. Hypoxia increases the AMP/ATP ratio in pulmonary artery smooth muscle cells and induces a twofold increase in AMPK activity (Evans & Hardie, 2020). Indeed, pharmacological activation of AMPK with AICAR results in relaxation of preconstricted mouse aorta (Gourand et al., 2007), and AICAR treatment of insulin resistance syndrome reduces blood pressure in rats (Viollet et al., 2009). Endothelial nitric oxide synthase (eNOS), an important modulator of angiogenesis and vascular tone, is a target of AMPK. It has been clearly established that AMPK can associate with and phosphorylate eNOS in cardiomyocytes and endothelial cells, thereby increasing eNOS activity and NO synthesis. AMPK activation by AICAR stimulates NO synthesis in human aortic endothelial cells (Goirand et al., 2007; Viollet et al., 2009).

AMPK activation may be an additional mechanism by which hypoxia or metabolic changes can induce vasorelaxation of large vessels, thereby increasing oxygen availability in peripheral tissues. Thus, AMPK appears to be a novel player in the complex signaling pathways that regulate vascular tone.

Chronic alcohol consumption activates the transcription factor κB (NF-κB) in LSECs, macrophages, and other liver parenchymal cells (Liu et al., 2021; Cui et al., 2023), which regulates the expression of proinflammatory cytokines that activate immune regulation of liver inflammation by the signaling pathways (Wang et al., 2021). LSECs identified as an important source of CXCL expression in the human liver regulated by TNFα/NF-κB signaling (Liu et al., 2021). AMPK is a key immunometabolic regulatory factor in macrophages. AMPK activation is required for efficient polarization of M1 macrophages to M2 macrophages. Antiinflammatory activity of AMPK in macrophages is associated with reduced degradation of IκB, increased activity of AKT and inactivation of glycogen synthase kinase 3 beta (GSK3-β). Inhibition of GSK3-β enables cAMP response element-binding protein (CREB) to compete for the nuclear coactivator CREB-binding protein (CBP) required for the function of NF-κB, thereby reducing the expression of proinflammatory genes (Cui et al., 2023). Thus, a logical question arises as to how AMPK and NF-κB change the resistant, exchangeable and capacitive link of the microcirculatory channel of the liver under the conditions of chronic alcoholic hepatitis? And can AMPK and NF-κB be used as targets for microcirculatory normalization in alcoholic liver disease in the future?

The aim of the work was to find out the effect of modulators of the transcription factor κB and AMP-activated protein kinase on the morphometric parameters of the vascular bed of the liver of rats under the conditions of modeling chronic alcoholic hepatitis.

Materials and methods

The animal studies were performed in accordance with revelant legislation and received approval from 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. The 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 60 white, sexually mature male Wistar rats, weighing 180–220 g. The animals were divided into 10 groups: I – control group (n = 6); II group (chronic alcoholic hepatitis group, ChrAlc) – animals, with simulated chronic alcoholic hepatitis (n = 6), which recieved intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution for 5 days 4 mL/kg a day). After two days break they received injection again. Afterwards they had 10% ethanol as the only source of drink for 51 days. The experiment lasted 63 days (Mykytenko et al., 2023); III group (PDTC group) – animals (n = 6), which received ammonium pyrrolidinedithiocarbamate (PDTC, Sigma-Aldrich, USA), as NF-κB inhibitor, intraperitoneally at a dose of 76 mg/kg three times a week throughout the experiment (Qin et al., 2014); IV group (LPS group) – animals (n = 6), which were administered NF-κB activator, namely pyrogenal (bacterial lipopolysaccharide (LPS) from *S. typhi*) intraperitoneally at the dose of 0.4 μg/kg by the following scheme: in the first week 3 times a week, thereafter once a week throughout the experiment (Mykytenko et al., 2022); V group (phenformin group) – animals (n = 6), which were administered AMPK activator, namely phenformin hydrochloride (phenformin, Sigma-Aldrich, USA) orally at a dose 10 mg/kg daily throughout the experiment (Mykytenko et al., 2023); VI group (doxorubicin group) – animals (n = 6), which were administered AMPK inhibitor, namely doxorubicin hydrochloride (doxorubicin, S.C. Sindan-Pharma S.R.L.) intraperitoneally at the dose of 1.25 mg/kg four times a week throughout the experiment (Yarmohmmadi et al., 2017); VII group (PDTC ChrAlc group) – animals (n = 6), on which we simulated chronic alcoholic hepatitis as in group II and administered PDTC according to the scheme of group III; VIII group (LPS ChrAlc group) – animals (n = 6), on which we simulated chronic alcoholic hepatitis as in group II and administered LPS according to the scheme of group IV; IX group (phenformin ChrAlc group) – animals (n = 6), on which we simulated chronic alcoholic hepatitis as in group II and administered phenformin according to the scheme of group V; X group (doxorubicin ChrAlc group) – animals (n = 6), on which we simulated chronic alcoholic hepatitis as in group II and administered doxorubicin according to the scheme of group VI.

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). We determined the metric indicators of the internal diameter of the vessels of the liver lobe - arteries, arterioles, venules and veins in the triad zone; lumen of the capillaries of the central and peripheral zones of the liver lobe, for which 10 determinations were made in each section in each group in one field of view.

Processing of the results of the morphometric study was carried out using the Kruskal-Wallis ANOVA method with subsequent use of pairwise comparisons according to the Mann-Whitney exact test and taking

into account the Bonferroni correction for multiple comparisons. The difference was considered statistically significant at $P < 0.05$.

Results

Changes in the vascular bed of rat liver under conditions of chronic alcoholic hepatitis modeling. On the 63rd day of simulation of chronic alcoholic hepatitis, we observed an increase in the average values of the lumen of the central veins of the hepatic lobe by 1.16 times compared to the control group of rats. Under these conditions, the lumen diameter of interlobular arteries decreased by 2.19 times, arterioles of the hepatic lobule decreased by 1.49 times, venules of the hepatic lobule decreased by 2.17 times, and interlobular veins decreased by 1.65 times compared to the control (Table 1).

Changes in the vascular bed of the liver of rats under the conditions of administration of ammonium pyrrolidinedithiocarbamate (PDTC) against the background of modeling chronic alcoholic hepatitis. Under the conditions of PDTC administration, the average diameter of the sinusoidal capillaries of the central zone of the hepatic lobule of rats increased by 1.43-fold, and the diameter of the sinusoidal capillaries of the peripheral zone of the hepatic lobule increased by 1.31-fold compared to the control (Table 1). The diameter of the lumen of the central veins of the hepatic lobe under the conditions of PDTC administration increased by 1.26 times compared to the control. The average lumen diameter of the interlobular arteries of rats under the conditions of PDTC administration decreased by 1.44 times, and the arteriole of the hepatic lobule increased by 1.41 times compared to the control. The average lumen diameter of the interlobular veins of rats was reduced by 1.66 times under the conditions of PDTC administration, and the venule of the hepatic lobule was reduced by 1.14 times compared to the control group of animals.

Under the conditions of PDTC administration against the background of chronic alcoholic hepatitis simulation, the diameter of the sinusoidal capillaries of the central zone of the liver lobule of rats increased by 1.25 times, and the diameter of the sinusoidal capillaries of the peripheral zone of the liver lobule increased by 1.28 times compared to the control. The diameter of the lumen of the central veins of the hepatic lobe under the conditions of PDTC administration against the background of simulation of chronic alcoholic hepatitis increased 1.76 times compared to the control. The average diameter of the lumen of the interlobular arteries of rats under the conditions of PDTC administration against the background of chronic alcoholic hepatitis simulation decreased by 1.16 times compared to the control. The lumen diameter of the interlobular veins of rats decreased by 1.16 times under the conditions of PDTC administration against the background of chronic alcoholic hepatitis simulation, and the venule of the hepatic lobule decreased by 1.71 times compared to the control group of rats.

Table 1

Changes in the vascular bed of the liver of rats under the conditions of administration of ammonium pyrrolidinedithiocarbamate against the background of chronic alcoholic hepatitis modeling ($x \pm SE$, $n = 6$)

Vessel lumen	Control	Ammonium pyrrolidinedithiocarbamate group	Chronic alcoholic hepatitis group	Ammonium pyrrolidinedithiocarbamate with chronic alcoholic hepatitis group
Lumen of sinusoidal capillaries of the central zone of the hepatic lobe, μm	6.80 ± 0.25^{a}	9.69 ± 0.31^{d}	7.31 ± 0.15^{b}	8.51 ± 0.26^{c}
Lumen of sinusoidal capillaries of the peripheral zone of the hepatic lobe, μm	5.86 ± 0.15^{a}	7.70 ± 0.17^{b}	5.75 ± 0.17^{a}	7.52 ± 0.21^{b}
Central vein, μm	57.4 ± 1.4^{a}	72.1 ± 1.6^{c}	66.5 ± 1.3^{b}	100.8 ± 3.9^{d}
Interlobular artery, μm	34.8 ± 1.6^{d}	24.2 ± 0.9^{b}	15.9 ± 0.8^{a}	29.9 ± 1.1^{c}
Lobular arteriola, μm	9.97 ± 0.49^{b}	14.05 ± 0.54^{c}	6.67 ± 0.41^{a}	9.57 ± 0.44^{b}
Lobular venula, μm	18.19 ± 0.61^{d}	15.93 ± 0.65^{c}	8.37 ± 0.20^{a}	10.63 ± 0.48^{b}
Interlobular vein, μm	42.35 ± 1.66^{d}	25.53 ± 1.22^{a}	25.66 ± 2.42^{b}	36.56 ± 1.48^{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.

Under conditions of administration of PDTC against the background of chronic alcoholic hepatitis simulation, the diameter of sinusoidal capillaries around the central vein of the hepatic lobe of rats increased by 1.16 times, and the diameter of sinusoidal capillaries around the hepatic triad increased by 1.31 times compared to the chronic alcoholic hepatitis group. The diameter of the lumen of the central veins of the hepatic lobe under the conditions of PDTC administration against the background of chronic alcoholic hepatitis simulation increased by 1.52 times compared to the chronic alcoholic hepatitis group. The average lumen diameter of the interlobular arteries of rats under the conditions of PDTC administration on the background of chronic alcoholic hepatitis simulation increased by 1.88 times, and the arteriole of the hepatic lobule increased by 1.43 times compared to the chronic alcoholic hepatitis group. The diameter of the lumen of the interlobular veins of rats increased by 1.42 times under the conditions of PDTC administration against the background of simulation of chronic alcoholic hepatitis, and the venules of the hepatic lobule increased by 1.27 times compared to the chronic alcoholic hepatitis group.

Changes in the vascular bed of the liver of rats under the conditions of administration of bacterial lipopolysaccharide (LPS) against the background of simulation of chronic alcoholic hepatitis. Under the conditions of LPS administration, the diameter of the sinusoidal capillaries of the central zone of the liver lobe of rats increased by 1.29 times, and the diameter of the sinusoidal capillaries of the peripheral zone increased by 1.20 times compared to the control (Table 2). The diameter of the lumen of the central veins of the hepatic lobe under the conditions of LPS administration increased by 1.13 times compared to the control. The average diameter of the lumen of the arterioles of the hepatic lobule of rats under the conditions of LPS administration increased by 1.72 times compared to the control. The lumen diameter of the interlobular veins of rats increased by 1.55 times under the conditions of LPS administration, and the venules of the hepatic lobule increased by 1.17 times compared to the control group of rats.

Under the conditions of administration of LPS against the background of simulation of chronic alcoholic hepatitis, the diameter of the sinusoidal capillaries of the central zone of the liver lobe of rats increased by 1.34 times, and the average diameter of the sinusoidal capillaries of the peripheral zone increased by 1.41 times compared to the control. The diameter of the lumen of the central veins of the hepatic lobe under the conditions of LPS administration against the background of chronic alcoholic hepatitis simulation increased by 1.15 times compared to the control. The diameter of the lumen of the interlobular arteries of rats under the conditions of LPS administration against the background of chronic alcoholic hepatitis modeled decreased by 1.21 times, and the arteriole of the hepatic lobule increased by 1.84 times compared to the control. The lumen diameter of the interlobular veins of rats increased by 1.55 times under the conditions of LPS administration against the background of chronic alcoholic hepatitis simulation, and the venules of the hepatic lobule increased by 1.35 times compared to the control group of rats.

Under the conditions of LPS administration against the background of chronic alcoholic hepatitis simulation, the diameter of sinusoidal capillaries in the central zone of the hepatic lobe of rats increased by 1.25 times, and the diameter of sinusoidal capillaries around the hepatic triad increased by 1.44 times compared to the group of chronic alcoholic hepatitis rats. The diameter of the lumen of the interlobular arteries of rats under the conditions of LPS administration against the background of chronic alcoholic hepatitis simulation increased by 1.82 times, and the arteriole of the hepatic lobule increased by 2.75 times compared to the chronic alcoholic hepatitis group. The lumen diameter of the interlobular veins of rats increased by 2.56 times under the conditions of LPS administration against the background of chronic alcoholic hepatitis simulation, and the venules

of the hepatic lobule increased by 2.93 times compared to the chronic alcoholic hepatitis group.

Changes in the vascular bed of the liver of rats under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis. Under the conditions of administration of phenformin hydrochloride, the diameter of the sinusoidal capillaries of the central zone of the liver lobe of rats increased by 1.15 times compared to the control (Table 3). The diameter of the lumen of the central veins of the hepatic lobe under the conditions of administration of phenformin hydrochloride increased by 1.16 times compared to the control. The diameter of the lumen of the interlobular arteries of rats under the conditions of administration of phenformin hydrochloride decreased by 1.28 times, and the arteriole of the hepatic lobe of rats increased by 1.38 times compared to the control. The lumen diameter of the interlobular veins of rats increased by 1.48 times under the conditions of administration of phenformin hydrochloride, and the venules of the hepatic lobule increased by 1.53 times compared to the control group of rats.

Under the conditions of administration of phenformin hydrochloride to rats against the background of simulation of chronic alcoholic hepatitis, the diameter of the sinusoidal capillaries of the central zone of the liver lobe of rats increased by 1.20 times compared to the control. The diameter of the lumen of the central veins of the hepatic lobe under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis increased by 1.15 times compared to the control. The average diameter of the lumen of the interlobular arteries of rats under the conditions of administration of phenformin hydrochloride against the background of chronic alcoholic hepatitis modeled decreased by 1.24 times, and the arteriole of the hepatic lobule increased by 1.38 times compared to the control. The diameter of the lumen of the interlobular veins of rats increased by 1.43 times under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis, and the venules of the hepatic lobule increased by 1.25 times compared to the control group of rats.

Under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis, the diameter of the sinusoidal capillaries of the central zone of the liver lobe of rats increased by 1.12 times, and the diameter of the sinusoidal capillaries of the peripheral zone increased by 1.08 times compared to the chronic alcoholic hepatitis group. The diameter of the lumen of the interlobular arteries of rats under the conditions of administration of phenformin hydrochloride against the background of chronic alcoholic hepatitis simulation increased by 1.77 times, and the arteriole of the hepatic lobule increased by 2.07 times compared to the chronic alcoholic hepatitis group. The average diameter of the lumen of the interlobular veins of rats increased by 2.36 times under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis, and the venules of the hepatic lobule increased by 2.73 times compared to the chronic alcoholic hepatitis group.

Table 2

Changes in the vascular bed of the liver of rats under the conditions of administration of bacterial lipopolysaccharide (LPS) against the background of simulation of chronic alcoholic hepatitis ($x \pm SE$, $n = 6$)

Vessel lumen	Control	Bacterial lipopolysaccharide group	Chronic alcoholic hepatitis group	Bacterial lipopolysaccharide with chronic alcoholic hepatitis group
Lumen of sinusoidal capillaries of the central zone of the hepatic lobe, μm	6.80 ± 0.25^{a}	8.80 ± 0.24^{b}	7.31 ± 0.15^{a}	9.13 ± 0.15^{b}
Lumen of sinusoidal capillaries of the peripheral zone of the hepatic lobe, μm	5.86 ± 0.15^{a}	7.05 ± 0.14^{b}	5.75 ± 0.17^{a}	8.26 ± 0.17^{b}
Central vein, μm	57.4 ± 1.4^{a}	64.8 ± 1.6^{b}	66.5 ± 1.3^{b}	66.3 ± 1.0^{b}
Interlobular artery, μm	34.8 ± 1.6^{c}	34.9 ± 1.1^{c}	15.9 ± 0.8^{a}	28.9 ± 1.4^{b}
Lobular arteriola, μm	9.97 ± 0.49^{b}	17.18 ± 0.57^{c}	6.67 ± 0.41^{a}	18.34 ± 0.66^{c}
Lobular venula, μm	18.19 ± 0.61^{b}	21.27 ± 0.59^{c}	8.37 ± 0.20^{a}	24.56 ± 0.67^{d}
Interlobular vein, μm	42.35 ± 1.66^{b}	65.85 ± 1.52^{c}	25.66 ± 2.42^{a}	65.72 ± 1.77^{c}

Note: see Table 1.

Table 3

Changes in the vascular bed of the liver of rats under the conditions of administration of phenformin hydrochloride against the background of simulation of chronic alcoholic hepatitis ($x \pm SE$, $n = 6$)

Vessel lumen	Control	Phenformin group	Chronic alcoholic hepatitis group	Phenformin with chronic alcoholic hepatitis group
Lumen of sinusoidal capillaries of the central zone of the hepatic lobe, μm	6.80 ± 0.25^{a}	7.82 ± 0.18^{b}	7.31 ± 0.15^{a}	8.17 ± 0.18^{b}
Lumen of sinusoidal capillaries of the peripheral zone of the hepatic lobe, μm	5.9 ± 0.2^{a}	6.4 ± 0.2^{c}	5.8 ± 0.2^{a}	6.2 ± 0.1^{b}
Central vein, μm	57.4 ± 1.4^{a}	66.5 ± 1.7^{b}	66.5 ± 1.3^{b}	65.9 ± 1.4^{b}
Interlobular artery, μm	34.81 ± 1.61^{c}	27.16 ± 0.80^{b}	15.87 ± 0.79^{a}	28.08 ± 0.79^{b}
Lobular arteriola, μm	9.97 ± 0.49^{b}	13.76 ± 0.34^{c}	6.67 ± 0.41^{a}	13.79 ± 0.28^{c}
Lobular venula, μm	18.19 ± 0.61^{b}	27.82 ± 0.79^{d}	8.37 ± 0.20^{a}	22.83 ± 0.46^{c}
Interlobular vein, μm	42.4 ± 1.7^{b}	62.8 ± 1.5^{c}	25.7 ± 2.4^{a}	60.4 ± 1.7^{c}

Note: see Table 1.

Table 4

Changes in the vascular bed of the liver of rats under the conditions of administration of doxorubicin hydrochloride against the background of simulation of chronic alcoholic hepatitis ($x \pm SE$, $n = 6$)

Vessel lumen	Control	Doxorubicin group	Chronic alcoholic hepatitis group	Doxorubicin with chronic alcoholic hepatitis group
Lumen of sinusoidal capillaries of the central zone of the hepatic lobe, μm	6.80 ± 0.25^{a}	7.01 ± 0.16^{a}	7.31 ± 0.15^{a}	7.14 ± 0.19^{a}
Lumen of sinusoidal capillaries of the peripheral zone of the hepatic lobe, μm	5.86 ± 0.15^{b}	6.33 ± 0.15^{b}	5.75 ± 0.17^{a}	7.14 ± 0.18^{c}
Central vein, μm	57.4 ± 1.4^{a}	60.4 ± 1.2^{a}	66.5 ± 1.3^{b}	76.5 ± 1.8^{c}
Interlobular artery, μm	34.81 ± 1.61^{d}	19.49 ± 0.44^{b}	15.87 ± 0.79^{a}	22.02 ± 0.72^{c}
Lobular arteriola, μm	9.97 ± 0.49^{b}	13.69 ± 0.19^{c}	6.67 ± 0.41^{a}	13.56 ± 0.23^{c}
Lobular venula, μm	18.19 ± 0.61^{b}	17.81 ± 0.53^{b}	8.37 ± 0.20^{a}	24.21 ± 0.64^{c}
Interlobular vein, μm	42.35 ± 1.66^{b}	52.41 ± 1.02^{c}	25.66 ± 2.42^{a}	67.97 ± 1.86^{d}

Note: see Table 1.

Changes in the vascular bed of the liver of rats under the conditions of administration of doxorubicin hydrochloride against the background of simulation of chronic alcoholic hepatitis. Under the conditions of administration of doxorubicin hydrochloride, the average diameter of the lumen of the interlobular arteries of rats decreased by 1.79 times, and the arteriole of the hepatic lobule of rats increased by 1.37 times compared to the control (Table 4). The diameter of the lumen of the interlobular veins of rats increased by 1.24 times under the conditions of administration of phenformin hydrochloride compared to the control group of rats.

Under the conditions of administration of doxorubicin hydrochloride to rats against the background of simulation of chronic alcoholic hepatitis, the diameter of the sinusoidal capillaries of the peripheral zone of the liver lobe increased by 1.22 times compared to the control. The average diameter of the lumen of the central veins of the hepatic lobe under the conditions of administration of doxorubicin hydrochloride against the background of simulation of chronic alcoholic hepatitis increased by 1.33 times compared to the control. The diameter of the lumen of the interlobular arteries of rats under the conditions of administration of doxorubicin hydrochloride against the background of chronic alcoholic hepatitis modeled decreased by 1.58 times, and the arteriole of the hepatic lobule increased by 1.36 times compared to the control. The average diameter of the lumen of the interlobular veins of the rats increased by 1.6 times under the conditions of administration of doxorubicin hydrochloride against the background of chronic alcoholic hepatitis simulation, and the venules of the hepatic lobule increased by 1.33 times compared to the control group of rats.

Under the conditions of administration of doxorubicin hydrochloride against the background of simulation of chronic alcoholic hepatitis, the diameter of the sinusoidal capillaries of the central zone of the liver lobe increased by 1.24 times compared to the chronic alcoholic hepatitis group. The lumen diameter of the central veins of the hepatic lobule under the conditions of administration of doxorubicin hydrochloride against the background of simulation of chronic alcoholic hepatitis increased by 1.15 times compared to the chronic alcoholic hepatitis group. The lumen diameter of the interlobular arteries of rats under the conditions of administration of doxorubicin hydrochloride against the background of chronic alcoholic hepatitis simulation increased by 1.39 times, and the arteriole of the hepatic lobule increased by 2.03 times compared to the chronic alcoholic hepatitis group. The lumen diameter of the interlobular veins of rats increased by 2.65 times under the conditions of administration of doxorubicin hydrochloride against the background of chronic alcoholic hepatitis simulation, and the venules of the hepatic lobule increased by 2.89 times compared to the chronic alcoholic hepatitis group.

Discussion

A decrease in the mean diameter of the central and interlobular veins in the chronic alcoholic hepatitis group may lead to impaired detoxification function of the liver, because the physiological movement of blood from the central vein of the hepatic lobule (portal vein basin) to the interlobular vein (inferior vena cava basin) through the lobular capillaries is reduced. Therefore, under the conditions of chronic alcoholic hepatitis, there is a violation of the exchange of metabolites between the central vein and the interlobular vein. According to scientific research, long-term alcohol consumption leads to increased expression of cyclooxygenase 2 (COX-2), which is accompanied by an increase in the level of prostaglandin E2 in liver tissue, which should create a vasodilating effect on the venous section of the vascular bed (Kline & Yamamoto, 2022). According to our research, the diameter of the venous vessels of the vascular bed of the liver decreases under the conditions of long-term alcohol consumption, which may be a consequence of the excessive effect of endothelin-1 on the vessel wall, which also increases with long-term exposure of alcohol to liver tissue (Kline & Yamamoto, 2022). Also in the scientific literature, there are data that under conditions of excessive intake of alcohol, an increase in COX-2 activity in the liver has a protective effect (Chen et al., 2021).

A decrease in the average diameter of interlobular arteries and lobular arterioles under the conditions of modeling chronic alcoholic hepatitis (ChrAlc) can lead to the development of hypoxia and dystrophy in the liver tissue due to a decrease in the supply of oxygen and substrates for oxidation. A decrease in the tone of the arteries of the vascular bed of the liver may be associated with the dissociation of the endothelial isoform of NO-synthase from its substrate under conditions of chronic excess alcohol intake (Yao & Abdel-Rahman, 2021). Our previous studies also confirm this possibility, since the activity of constitutive isoforms of NO-synthase in the liver of rats under conditions of chronic alcoholic hepatitis significantly increases against the background of increasing competition with the inducible isoform of NO-synthase for the reaction substrate (Mykytenko et al., 2022).

An increase in the average diameter of the interlobular veins under the conditions of LPS administration, which is known from the literature to activate the NF-κB transcription factor through TLR-4, may be associated with increased expression of COX-2 under the influence of LPS (Tsukayama et al., 2021). The increase in the diameter of the interlobular veins under the conditions of inhibition of the activation of the transcription factor NF-κB by the administration of PDTC may be related to the ability of PDTC to reduce the expression of the endothelin-1 genes (Wynants et al., 2013).

The increase in the average diameters of interlobular arteries and lobular arterioles in the PDTC and LPS groups, inhibition and activation of the transcription factor NF-κB, respectively, can be explained by a decrease in the competition between endothelial and inducible NO-synthase isoforms for the substrate. What is confirmed by the results of our previous studies: the introduction of bacterial LPS against the background of modeling chronic alcoholic hepatitis (LPS ChrAlc group) leads to increased activity of constitutive NO-synthase isoforms and decreases the activity of inducible NO-synthase (Mykytenko et al., 2022). Administration of PDTC, an inhibitor of the activation of the transcription factor NF-κB, under the conditions of chronic alcoholic hepatitis (PDTC ChrAlc group) led to an increase in the activity of constitutive NO-synthase isoforms and a decrease in the activity of inducible NO-synthase in the liver of rats (Mykytenko et al., 2022).

The changes observed in the vasculature of the liver of rats under the conditions of administration of phenformin against the background of modeling chronic alcoholic hepatitis (phenformin ChrAlc group) can be explained by the ability of phenformin to activate AMPK, which leads to an increase in the expression of endothelial NO-synthase genes, contributing to the reduction of hepatocyte hypoxia due to the expansion of the arterial (resistive) link of the microcirculatory channel, and reduces the expression of endothelin-1, which contributes to the expansion of the venous (capacitive) link of the microcirculatory channel of the liver (Jin et al., 2021).

The simultaneous increase in the volume of resistive and capacitive links of the microcirculatory channel of the liver of rats under the conditions of administration of doxorubicin against the background of modeling chronic alcoholic hepatitis (doxorubicin ChrAlc group) may be associated with the ability of doxorubicin to reduce the sensitivity of the endothelium to endothelin-1 (Bosman et al., 2021). Another mechanism of the expansion of the lumens of the central veins, interlobular veins and lobular venules can be the increased expression of COX-2, which is due to the toxicity of doxorubicin in relation to hepatocytes (Ekinci Akdemir et al., 2021).

Therefore, hepatic microcirculation is the basis of normal biosynthesis, metabolism and clearance of substances. Effective perfusion and proper shear stress within the microvessels support adequate fluid exchange and normal cellular phenotype and balance the liver microcirculation.

Conclusion

Simulation of chronic alcoholic hepatitis leads to a violation of the exchange of metabolites between the central and interlobular veins, which creates the prerequisites for the development of hypoxic damage to hepatocytes, as evidenced by a decrease in the diameters of the lobular arteries and veins.

Administration of ammonium pyrrolidinedithiocarbamate and bacterial lipopolysaccharide, which affect NF-κB signaling under the conditions of modeling chronic alcoholic hepatitis, promotes the restoration of metabolite exchange between central and interlobular veins, as evidenced by an increase in the lumens of lobular arterioles and venules. The introduction of phenformin and doxorubicin, which affect AMPK under the conditions of modeling chronic alcoholic hepatitis, prevents the development of hypoxic damage to hepatocytes, as evidenced by an increase in the diameters of lobular arterioles and venules, and leads to intensification of interlobular blood circulation.

Current research higlights the reaction of the miscrovascular bed of the liver to excessive alcohol consumption and reveals the role of NF-κB and AMPK signaling in its development. This information will provide grounds for further research into drugs targeting NF-κB and AMPK signaling as potential treatment of alcoholic hepatitis.

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