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CHANGES IN THE MICROCIRCULATORY BED OF THE LIVER DURING ALCOHOLIC HEPATITIS MODELING

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It is well known that chronic alcohol consumption leads to multiple organ damage and microcirculation disorders of organs and tissues. The aim of the work was to study the morphometric indicators of the microcirculatory bed of rat liver during alcoholic hepatitis modeling. The experiments were performed on 30 white, mature male Wistar rats, weighing 180–220 g. The animals were divided into 2 groups: I – control; group II– animals, on which we modeled alcoholic hepatitis according to Yu.M. Stepanov. (2017). The diameter of the sinusoidal capillaries around the central vein and the hepatic triad was increased at all time points compared to the control. The diameter of the lumen of the central vein of the hepatic lobe of rats increased on the 1st, 5th and 7th day of the experiment. The diameter of the lumen of the interlobular artery of rats was decreased during the studied periods, and the interlobular vein was increased. The diameter of the lumen of the arteriole increased on the 3rd day of the experiment, and the diameter of the lumen of the venule of the lobule of the rats decreased on the 1st day of the experiment. The microcirculatory channel of the liver of rats under the conditions of simulation of chronic alcoholic hepatitis in the first week demonstrates an increase in the exchange of metabolites between the central vein and the vein of the triad, which is accompanied by their expansion and threatens the development of venous hyperemia.

Key words: chronic alcohol hepatitis, liver, microcirculatory channel, rats.

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ЗМІНИ В МІКРОЦИРКУЛЯТОРНОМУ РУСЛІ ПЕЧІНКИ ЗА УМОВ МОДЕЛЮВАННЯ АЛКОГОЛЬНОГО ГЕПАТИТУ

Загальновідомо, що хронічне споживання алкоголю призводить до поліорганного ушкодження і порушення мікроциркуляції органів і тканин. Метою роботи було вивчити морфометричні показники мікроциркуляторного русла печінки щурів за умов моделювання алкогольного гепатиту. Експерименти виконані на 30 білих статевозрілих щурах-самцях лінії Wistar, вагою 180-220 г. Тварини були розділені на 2 групи: I – контрольна; II група – тварини, яким моделювали алкогольний гепатит за Степановим Ю.М. (2017). Діаметр синусоїдних капілярів навколо центральної вени і печінкової тріади збільшується на всіх досліджуваних строках порівняно з контролем. Діаметр просвіту центральної вени печінкової часточки щурів збільшується на 1, 5 та 7 добу експерименту порівняно з контролем. Діаметр просвіту міжчасточкової артерії щурів зменшується в досліджувані терміни, а міжчасточкової вени збільшується порівняно з контролем. Діаметр просвіту артеріоли печінкової часточки щурів збільшився на 3 добу експерименту, а діаметр просвіту венули печінкової часточки щурів зменшився на 1 добу експерименту порівняно з контролем. Мікроциркуляторне русло печінки щурів за умов моделювання хронічного алкогольного гепатиту в перший тиждень демонструє посилення обміну метаболітами між центральною веною та веною тріади, що супроводжується їх розширенням та загрожує розвитком венозної гіперемії.

Ключові слова: хронічний алкогольний гепатит, печінка, мікроциркуляторне русло, щури.

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It is well known that chronic alcohol consumption leads to multiple organ damage and microcirculation disorders of organs and tissues. Regarding the effects of ethanol on the liver there are several facts: small amounts of ethanol increase hepatic blood flow and prevent intestinal ischemia/reperfusion (I/R)-induced hepatic microvascular dysfunction and subsequent liver injury. While high amounts of ethanol alone cause hepatic microvascular dysfunction and exacerbate I/R-induced hepatic microvascular dysfunction and subsequent liver injury. Vasoactive molecules and oxidative stress are also involved in liver damage. Ethanol administration causes an increase in vasoactive molecules, such as endothelin and nitric oxide, and oxidative stress [3-5]. Hyperactivation of reticuloendothelial cells lining the sinusoids of the liver (Kupffer cells (macrophages) and sinusoidal endothelial cells) can narrow the lumen of the sinusoid, which impairs perfusion in the microcirculatory channel of the liver and contributes to the severity of the disease in alcoholic hepatitis [9]. In animals with endotoxemia, even a small amount of ethanol causes liver microvascular dysfunction. Chronic ethanol consumption exacerbates endotoxin-induced hepatic microvascular dysfunction. Thus, chronic alcohol consumption changes the microcirculation of the liver and, depending on the duration and dose, can have different consequences.

The purpose of the study was to assess the morphometric indicators of the microcirculatory bed of rat liver during alcoholic hepatitis modeling.

Materials and methods. The experiments were performed on 30 white mature male Wistar rats, weighing 180–220 g. The animals were divided into 2 groups: I – control (n=6); II group – animals, on

which we modeled alcoholic hepatitis (n=24) by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal injection of a 16.5 % ethanol solution in a 5 % glucose solution, at the rate of 4 ml/kg of body weight [1]. 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). The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on days 1, 3, 5, and 7 by bloodletting under thiopental anesthesia. The object of research was the liver. During the experiments, the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) were followed in accordance with the "General Principles of Animal Experiments" approved by the First National Congress on Bioethics, and the requirements of the "Procedure for Scientific Experiments" institutions of experiments, experiments on animals" (2012).

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's 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. The morphometric parameters of the inner diameter of the vessels of the microcirculatory bed of the liver lobe were determined, as well as capillary lumen around the central vein and hepatic triad.

Processing of the results of the morphometric study was carried out using one-factor analysis of variance according to the Khruskal-Wallis method with subsequent use of pairwise comparisons according to the Mann-Whitney exact test and taking into account the Bonferroni correction for multiple comparisons. All statistical calculations were performed in the Microsoft office Excel program and its extension Real Statistics 2019. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. According to the results of morphometric studies of the microcirculatory channel of the liver of rats, it was established that the diameter of the sinusoidal capillaries around the central vein increased by 1.24 times on the 1st day of the experiment, by 1.12 times on the 3rd day, by 1.25 times on the 5th day, 7 days – 1.26 times compared to the control (Fig. 1). On the 3rd day of the experiment, the diameter of the sinusoidal capillaries around the central vein of the liver lobe of rats decreased by 1.11 times compared to the diameter on the 1st day, on the 5th day it increased by 1.12 times compared to the diameter on the 3rd day of the experiment.

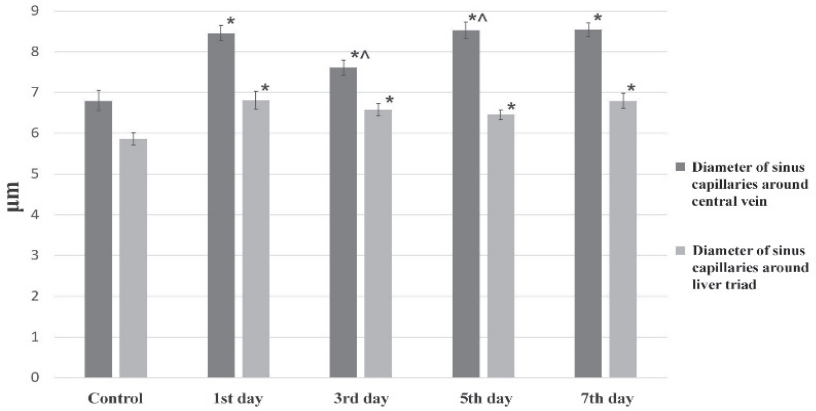

Fig. 1. Diameter of sinusoidal capillaries under conditions of alcoholic hepatitis modeling. * – $p<0.05$ compared with the control group of rats; ^ – $p<0.05$ compared with the previous term of the experiment.

The diameter of the sinusoidal capillaries around the hepatic triad of rats increased on the 1st day of the experiment - by 1.16 times, on the 3rd day – by 1.12 times, on the 5th day - by 1.1 times, on the 7th day - by 1.16 times compared to control (Fig. 1). The diameter of the lumen of the central vein of the hepatic lobule of rats increased by 1.11, 1.1, and 1.38 times on the 1st, 5th, and 7th day of the experiment, respectively, compared to the control (Fig. 2).

On the 7th day of the experiment, the diameter of the lumen of the central vein of the hepatic lobe of rats increased by 1.26 times compared to the diameter of the lumen on the 5th day of the experiment.

The diameter of the lumen of the interlobular vein of rats increased by 1.51, 1.24, 1.33 and 1.16 times on the 1st, 3rd, 5th and 7th day of the experiment, respectively, compared to the control. On the 3rd day of the experiment, the diameter of the lumen of the interlobular vein of rats decreased by 1.22 times compared to the diameter of the lumen on the 1st day of the experiment. On the 5th day, it increased by 1.07 times compared to the diameter of the lumen of the interlobular vein on the 3rd day of the experiment, and on the 7th day of the experiment, the diameter of the lumen of the interlobular vein of rats decreased by 1.15 times compared to the diameter of the lumen on the 5th day.

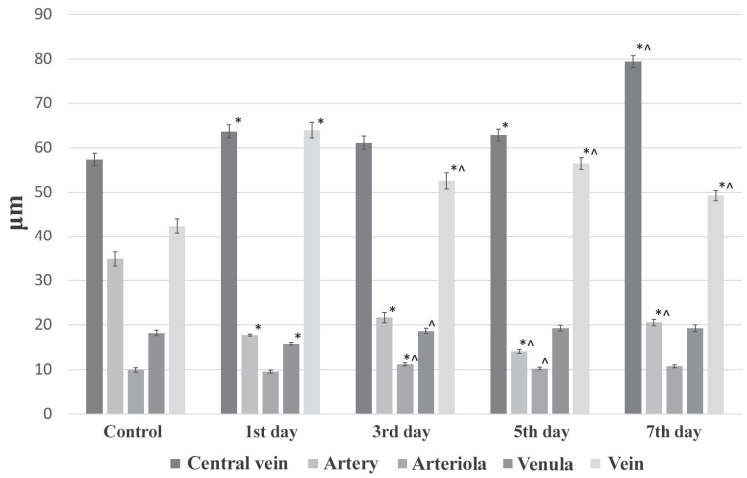

Fig. 2. The diameter of the vessels of the microcirculatory bed of the liver under the conditions of alcoholic hepatitis modeling– p <0.05 compared with the control group of rats; ^ – p <0.05 compared with the previous term of the experiment.

The diameter of the lumen of the interlobular artery of rats decreased by 1.97 times on the 1st day of the experiment, by 1.61 times on the 3rd day, by 2.47 times on the 5th day, and by 1.69 times on the 7th day compared to the control. On the 5th day of the experiment, the diameter of the lumen of the interlobular artery of rats decreased by 1.54 times compared to the diameter of the lumen of the artery on the 3rd day, and on the 7th day of the experiment, the diameter of the lumen increased by 1.46 times compared to the diameter of the lumen on the 5th day of the experiment.

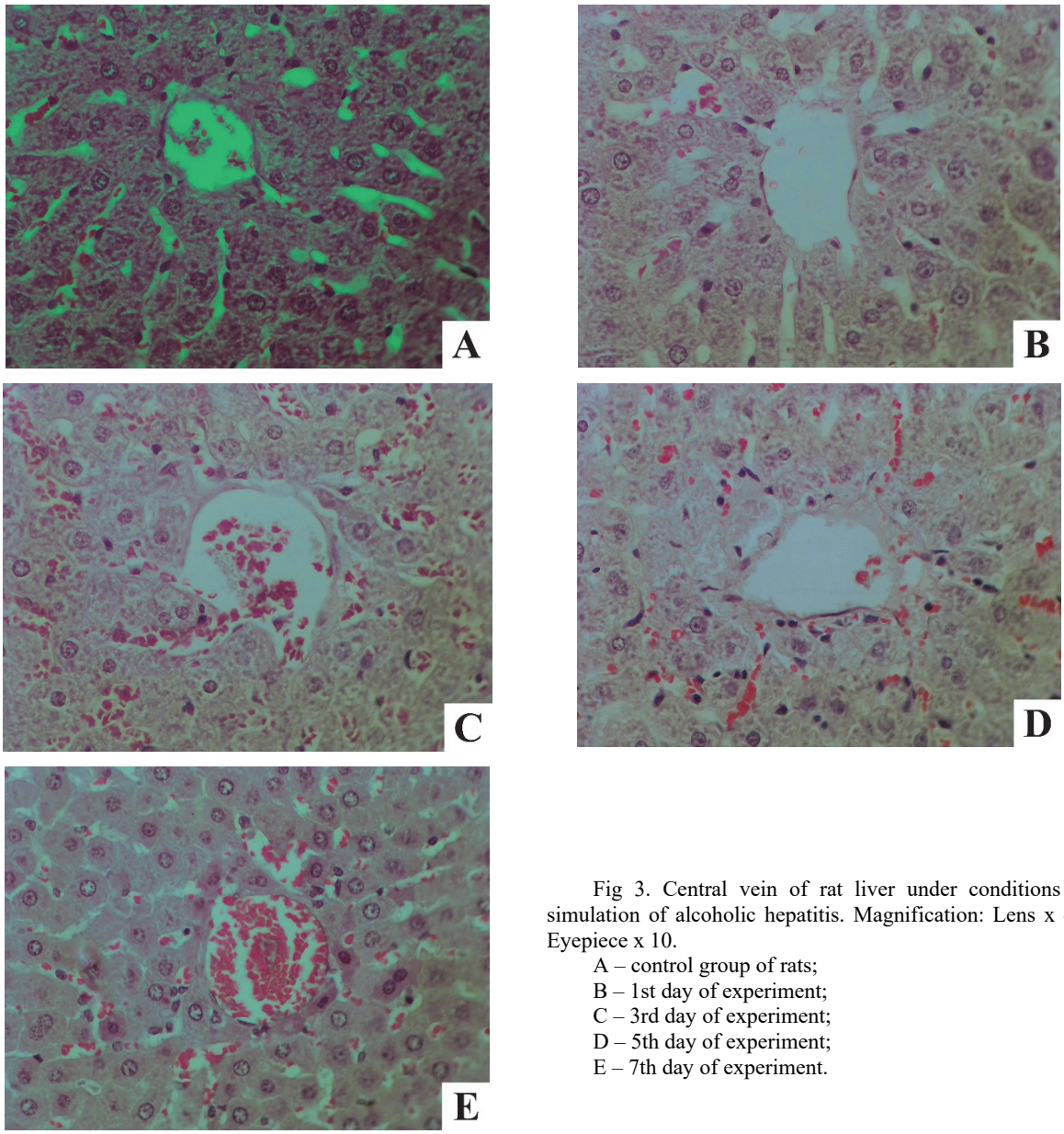

Fig 3. Central vein of rat liver under conditions of simulation of alcoholic hepatitis. Magnification: Lens x 40, Eyepiece x 10.
A – control group of rats;
B – 1st day of experiment;
C – 3rd day of experiment;
D – 5th day of experiment;
E – 7th day of experiment.

The diameter of the lumen of the arteriole of the hepatic lobule of rats increased by 1.12 times on the 3rd day of the experiment compared to the control. On the 3rd day of the experiment, the diameter of the lumen of the arteriole of the hepatic lobule of rats increased by 1.18 times compared to the diameter of the

lumen on the 1st day of the experiment, on the 5th day of the experiment, the diameter of the lumen of the arteriole decreased by 1.09 times compared to the diameter of the lumen on the 3rd day of the experiment.

The diameter of the lumen of the venule of the hepatic lobule of rats decreased by 1.15 times on the 1st day of the experiment compared to the control. On the 3rd day of the experiment, the diameter of the lumen of the venule of the liver lobe of rats increased by 1.18 times compared to the diameter of the lumen of the venule on the 1st day of the experiment.

The diameter of the central vein of the liver of rats expands from the first day of simulation of chronic alcoholic hepatitis and remains increased during most of the experimental periods studied (with the exception of 3rd day) (Fig. 3).

At the same time, the lumen of the interlobular artery of the triad decreases from the first day of the experiment, and the diameter of the interlobular vein of the triad is enlarged (Fig. 4).

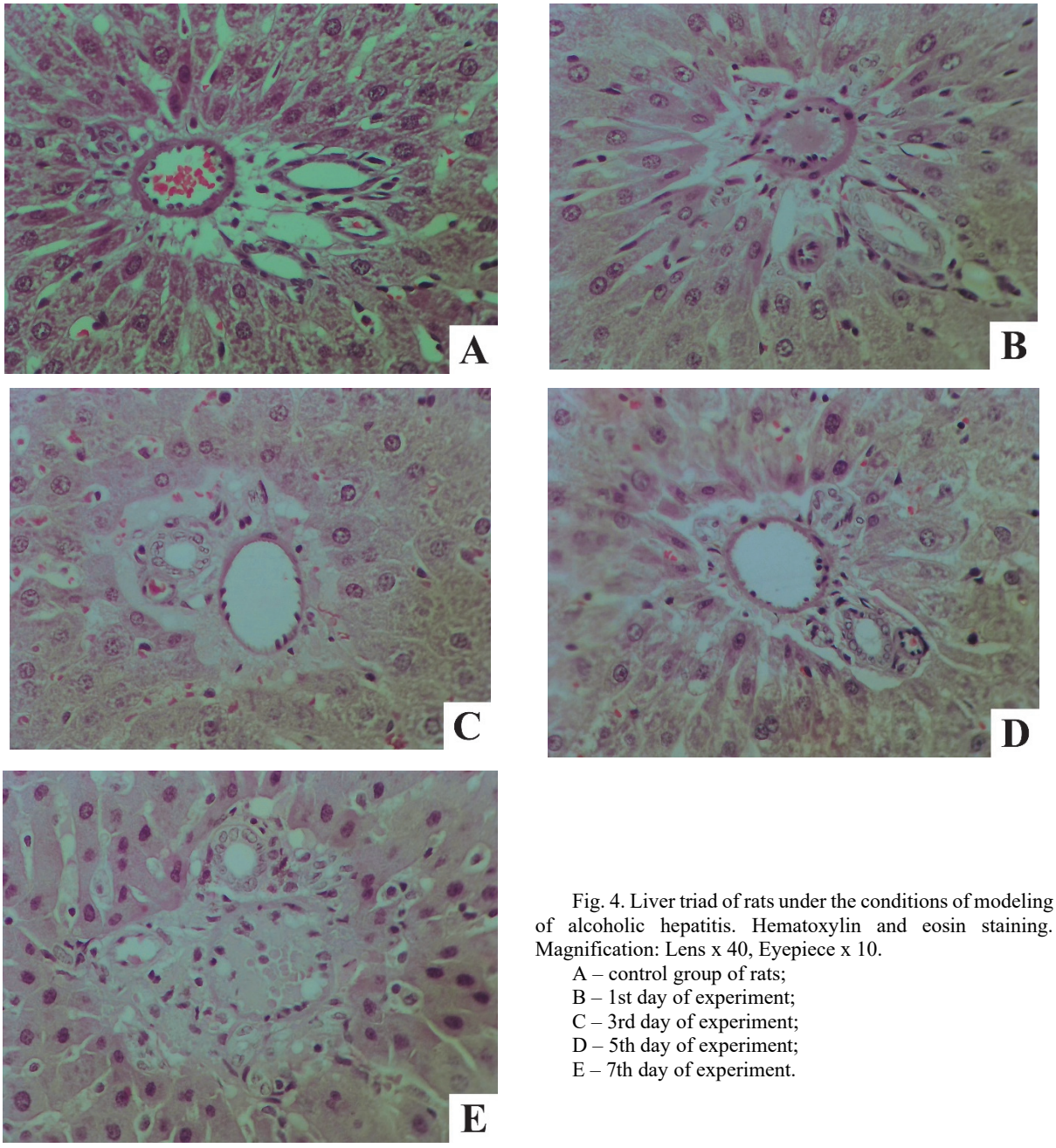

Fig. 4. Liver triad of rats under the conditions of modeling of alcoholic hepatitis. Hematoxylin and eosin staining. Magnification: Lens x 40, Eyepiece x 10.
A – control group of rats;
B – 1st day of experiment;
C – 3rd day of experiment;
D – 5th day of experiment;
E – 7th day of experiment.

Taking into account the physiological flow of blood plasma, which is directed from the central vein of the lobule to the triad vein, passing through the hepatic beams (which are formed by hepatocytes), it can be assumed that the simulation of chronic alcoholic hepatitis is accompanied by an increase in the flow of plasma in the liver in order to cleanse it faster of toxins. A decrease in the lumen of the triad interlobular artery, which provides substrates for energy generation and oxygen for hepatocytes, may threaten the development of hepatocyte hypoxia. The absence of statistically significant changes in the diameters of arterioles and venules of the triad, compared to the control, may indicate the absence of a direct harmful effect

of alcohol on hepatocytes at this stage of experimental modeling of chronic alcoholic hepatitis. An increase in the lumen of the capillaries located around the triad and the central vein may indicate the presence of molecules that have a vasodilating effect in the plasma passing through these capillaries.

To evaluate the potential molecular mechanisms that lead to the morphological and metric changes described above, it is necessary to take into account the features of the model of chronic alcoholic hepatitis chosen by us [9]. In the terms described by us, alcohol was administered to animals intraperitoneally, therefore, in addition to the direct effect of ethanol on liver tissue, it is worth considering possible changes in the metabolism of adipose tissue of the peritoneum. Ethanol has a direct effect on the metabolism of adipocytes, which was shown in in vitro experiments conducted by Patel D. et al. [7]. In the experiments of Patel D. et al. it was shown that ethanol leads to a decrease in the secretion of adiponectin by adipocytes into the blood and increases the secretion of leptin, resistin, IL-6 and TNF-α, and directly in adipocytes the expression of inducible NO-synthase, PPAR-γ and CYP2E1 genes increases [7]. There are also reports that ethanol and its metabolites formed by oxidative and non-oxidative pathways, regardless of the way they enter the body, can cause the death of adipocytes [8]. Alcohol-induced adipocyte death is also accompanied by the release of a significant amount of pro-inflammatory cytokines into the blood [4].

Leptin can widen the lumen of vessels by acting on endotheliocytes, which, in turn, increase under the influence of leptin the expression of the endothelial isoform of NO-synthase, thus increasing the production of such a powerful vasodilator as nitric oxide (NO) [11]. What explains the absence of a vasoconstrictor effect of alcohol on blood vessels, which is associated with an increase in acetylation of Hsp90 and a decrease in the activity of the endothelial isoform of NO-synthase [10]. At the same time, leptin can increase the activity of the sympathetic nervous system, which is accompanied by the development of "arterial stiffness" [2]. This explains the decrease in the diameter of the lumen of the arteries of the triad observed in our study during all the studied periods. The expansion of capillaries around the triad and the central vein indicates the predominance of the vasodilating action of leptin over the influence of the sympathetic nervous system on these vessels. Based on the above and the features of the model of chronic alcoholic hepatitis chosen by us, it is quite likely that the changes in the microcirculatory channel of the liver during the first week of the experiment are largely due to the effect of ethanol injections on peritoneal adipocytes.

Conclusion

The microcirculatory channel of the liver of rats under the conditions of simulation of chronic alcoholic hepatitis in the first week demonstrates an increase in the exchange of metabolites between the central vein and the vein of the triad, which is accompanied by their expansion and threatens the development of venous hyperemia.

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