3. Turovaia AYu, Kade AKh. Eksperymentalnoe modelyrovanye ostroho peryodontyta u krys Fundamentalnye yssledovanyia. 2010; 10: 46–50 [In Russian]
4. Al-Shammar NM, Shafshak SM, Ali MS. Effect of 0.8 % Hyaluronic Acid in Conventional Treatment of Moderate to Severe Chronic Periodontitis J. Contemp Dent Pract, 2018 May 1;19(5):527–534.
5. Casale M, Moffa A, Vella P. Hyaluronic acid: Perspectives in dentistry. A systematic review. International Journal of Immunopathology and Pharmacology. 2016; 29(4):572–582.
6. Debnath Tirthankar, Chakraborty Abhijit, Tamal Kanti Pal. A clinical study on the efficacy of hydroxyapatite - Bioactive glass composite granules in the management of periodontal bony defects. J Indian Soc Periodontol. 2014; 18(5):593–600.
7. Kaneko Aoi, Marukawa Eriko, Harada Hiroyuki. Hydroxyapatite nanoparticles as injectable bone Substitute Material in a Vertical Bone Augmentation Model. In vivo. 2020;34:1053–1061.
8. Leal AMC, Beserra Dos Santos MV, da Silva Filho EC, Menezes de Carvalho AL, Tabchoury CPM, Vale GC. Development of an Experimental Dentifrice with Hydroxyapatite Nanoparticles and High Fluoride Concentration to Manage Root Dentin Demineralization. Int J Nanomedicine. 2020 Oct 5; 15:7469–7479.
9. Mendes TD, Araújo VA, Filho JL, Monteiro LB, Guimarães MV. The use of Hyaluronic Acid as an adjuvant Therapeutic Approach to Non Surgical Periodontal Therapy for Periodontitis. Braz. J. of Develop. Curitiba. 2020; 6(5):29219–29234.
10. Salwowska NM, Bebenek KA, Żądło DA, Wcisło - Dziadecka DL. Physiochemical properties and application of hyaluronic acid: a systematic review. Journal of Cosmetic Dermatology. 2016; 15:520–526.
11. Subramaniam S, Fang YH, Sivasubramanian S, Lin FH, Lin CP. Hydroxyapatite-calcium sulfate-hyaluronic acid composite encapsulated with collagenase as bone substitute for alveolar bone regeneration. Biomaterials. 2016 Jan; 74: 99–108. doi: 10.1016/j.biomaterials.2015.09.044. PMID: 26454048.

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MORPHOLOGICAL AND FUNCTIONAL CHANGES OF THE HEPATIC VASCULAR BED UNDER THE CONDITIONS OF MODELING ALCOHOLIC HEPATITIS

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The experiments were performed on 30 white adult outbred male rats weighing 180–220 g. Animals were divided into 2 groups: I – □□tr□1 (□=6); □□ – animals with simulated alcoholic hepatitis (n=24). We studied the total NO synthase activity, peroxynitrite, nitrites and nitrosothiols concentrations in rat liver homogenate. We also studied morphometric parameters of hemomicrocirculatory tract of the rat liver. On the 7th day of modeling of alcoholic hepatitis interparticle arteries and veins and lobular arterioles narrow. The central vein dilates. The sinusoidal capillaries around the hepatic triad and central vein dilate. Alcoholic hepatitis increases the total activity of NO synthases, the concentration of peroxynitrites and nitrosothiols, but reduces the concentration of nitrites in the liver of rats on day 7 of the experiment. Nitric oxide and its metabolites play an important role in the regulation of the resistant part of the hemomicrocirculatory tract of the rat liver in the first three days of the experiment. Further redistribution of nitric oxide cycle metabolites leads to an increase in the concentration of peroxynitrite, which is a factor of secondary alteration.

Keywords: liver, alcoholic hepatitis, rats.

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МОРФО-ФУНКЦІОНАЛЬНІ ЗМІНИ СУДИННОГО РУСЛА ПЕЧІНКИ ЗА УМОВ МОДЕЛЮВАННЯ АЛКОГОЛЬНОГО ГЕПАТИТУ

Експерименти виконані на 30 білих статевозрілих безпородних щурах-самцях, вагою 180-220 г. Тварини були розділені на 2 групи: I – контрольна (n=6); II група – тварини, яким моделювали алкогольний гепатит (n=24).

В гомогенаті печінки щурів визначали активність загальної NO-синтази, концентрації пероксинітриту, нітритів та нітрозотіолів. Також визначали морфометричні параметри гемомікроциркуляторного русла печінки щурів. На 7 добу моделювання алкогольного гепатиту міжчасточкові артерії і вени, часточкові артеріоли звужуються. Центральна вена розширюється. Синусоїдні капіляри навколо печінкової тріади і центральної вени розширяються. Алкогольний гепатит збільшує загальну активність NO-синтаз, концентрацію пероксинітритів та нітрозотіолів, проте знижує концентрацію нітритів у печінці щурів на 7 добу експерименту. Оксид азоту і його метаболіти грають важливу роль в регуляції резистентної ланки гемомікроциркуляторного русла печінки щурів перші три доби експерименту, подальший перерозподіл метаболітів циклу оксиду азоту призводить до збільшення концентрації пероксинітриту, який виступає фактором вторинної альтерації.

Ключові слова: печінка, алкогольний гепатит, щури.

Depending on age, alcohol abuse is the fifth risk factor for premature death and disability worldwide. Alcohol is a leading risk factor for mortality and overall disease complications in the 15-59 age group [1]. According to WHO statistics, alcohol causes about 2.5 million deaths annually [2]. Alcohol consumption is a major etiological factor in the pathogenesis of chronic liver diseases such as fatty liver disease, alcoholic hepatitis, liver fibrosis or cirrhosis and hepatocellular carcinoma [3].

Current data suggest that the pathogenesis of alcoholic hepatitis is multifactorial and is the result of a complex interaction of ethanol metabolism, inflammation and immunological reactions [4]. Despite the large amount of data from experimental and clinical studies, the pathogenesis of alcoholic hepatitis has not been definitively elucidated. Therefore, the search for new links in the pathogenesis of alcoholic hepatitis is an urgent and important problem today.

Hemomicrocirculatory tract of the liver plays an important role in the development of alcoholic hepatitis. The reaction of arteries and arterioles to inflammatory mediators, permeability of sinusoidal capillaries, intercellular cooperation of immune cells in the lumen of blood vessels and endothelial damage cause the development of inflammation in the liver. NO synthases regulate the tone of the hemomicrocirculatory tract of the liver. Therefore, the study of the role of nitric oxide and its metabolites in the development of alcoholic hepatitis is a topical issue today.

To study the role of nitric oxide in the pathogenesis of inflammatory alcoholic liver disease we used an experimental model in rats. Considering that nitric oxide, synthesized by endothelial NO synthase, is beneficial for liver function because it regulates blood circulation and blood cell interaction. The main issue is the role of nitric oxide, which was synthesized by inducible NO synthase, because it can play both beneficial and harmful role in liver homeostasis during inflammation [5].

The purpose of the research was to study the morphometric and biochemical parameters of the hemomicrocirculatory tract of the liver of rats during modeling of alcoholic hepatitis.

Materials and methods. The experiments were performed on 30 white adult outbred male rats weighing 180-220 g. Animals were divided into 2 groups: I – control (n=6); II – animals with simulated alcoholic hepatitis (n=24). We simulated alcoholic hepatitis by the method of forced intermittent alcohol consumption 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 of body weight [2]. The control group included animals that were subjected to similar manipulations throughout the study, but were injected saline. Conditions for keeping animals in the vivarium were standard. Removal of animals from the experiment occurred on 1, 3, 5 and 7 days by bloodletting under thiopental anesthesia. Serum and liver were studied. During the experiments, the recommendations of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" were followed (Strasbourg, 1986). We performed experiment in accordance with the "General Principles of Animal Experiments" approved by the First National Congress of Bioethics, and the requirements of the "Procedure for conducting scientific experiments, experiments on animals" (2012).

In the serum of rats, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined using diagnostic kits, produced by NPP "Filisit-Diahnostyka". We also calculated the de Ritis coefficient (AST/ALT).

The activity of total NO synthase (NOS), peroxynitrite, nitrite (NO2-) and nitrosothiols concentrations were determined in the rat liver homogenate [6, 7].

Total NO-synthase activity was evaluated by the increase of nitrites after incubation of 10 % liver homogenate (0.2 ml) for 30 min in the incubation solution (2.5 ml of 0.1 M trisbuffer, 0.3 ml of 320 mM aqueous solution of L-arginine and 0.1 ml of 1 mM NADPH+H+ solution).

The first aliquot (A1=0.2 ml) was taken immediately after mixing the homogenate with the incubation solution (solution 1). To 0.2 ml of solution 1 was added 1.8 ml of distilled water, 0.2 ml of 1 % sulfanilic acid and after 10 min was added 0.2 ml of 0.1 % 1-naphthylamine. Then the concentration of nitrites was determined in solution 1.

The reaction was stopped by adding 0.02 ml of 0.02 % sodium azide and took a second aliquot (A2=0.2 ml) to assess the growth of nitrites (solution 2). To 0.2 ml of solution 2 was added 1.8 ml of distilled water, 0.2 ml of 1 % sulfanilic acid and after 10 min was added 0.2 ml of 0.1 % 1-naphthylamine. Then the concentration of nitrites was determined in solution 2.

Nitrite concentration was measured on a Ulab-101 spectrophotometer on wavelength 540 nm. The concentration was calculated as C=A*0.30104 (μmol/g). The total NOS activity was calculated by the formula: NOS=(A2-A1)*2057/N (μmol/min*g), where N is the protein concentration calculated by the biuret method g/L [6].

The concentration of nitrosothiols was determined by the difference between the concentration of nitrites before and after incubation with 2 % mercuric chloride solution spectrophotometrically at a wavelength of 540 nm [7].

The concentration of peroxynitrites was determined after adding to 0.1 ml 10 % liver homogenate 1 ml of 5 % potassium iodide, dissolved in 3.9 ml of 0.2 M phosphate buffer (pH=7.0). After mixing and centrifugation (1,000 rpm) for 10 min, the supernatant was examined spectrophotometrically at a

wavelength of 355 nm against control (1 ml of solution containing 1 ml of 5 % potassium iodide and 4 ml of 0.2 M phosphate buffer, pH 7.0). The concentration of peroxynitrites was calculated by the formula: C=A * 20; μmol/g [6].

For morphometric studies, the liver was fixed in 10 % neutral formalin, poured into paraffin blocks, from which semi-thin sections were prepared, which were stained with hematoxylin and eosin. Morphometric examination and microphotography were performed using a microscope Biorex-3 BM-500T with a digital microphoto nozzle DCM 900 with programs adapted for research data. The outer (Do) and inner (Di) diameters of the vascular wall of the central vein, interparticle arteries and veins, arterioles and venules of hepatic lobes and subparticle vein were determined. We also studied lumen of capillaries around the central vein and hepatic triad.

Statistical processing of the results of biochemical studies was performed using pairwise comparison using the nonparametric Mann-Whitney method. Processing of the results of the morphometric study was performed using one-way analysis of variance by the method of Kruskal-Wallis with the subsequent use of pairwise comparison according to the exact Mann-Whitney test and taking into account the correction for the multiplicity of comparisons according to Bonferroni. All statistical calculations were performed in Microsoft office Excel and its extension Real Statistics 2019. The difference was considered statistically significant if $p<0.05$. Data in tables represented as mean ± standard error of mean (M±m).

Results of the study and their discussion. Biochemical analysis of serum of rats from simulated alcoholic hepatitis group revealed a decrease in the activity of AST on day 7 of the experiment by 1.73 times compared to the control ($p<0.05$) and by 2.1 times compared to the activity of AST on the 5th day of the experiment ($p<0.05$) (table 1).

Table 1

Biochemical parameters of rat serum under the conditions of modeling of alcoholic hepatitis (M±m)

Parameters	Groups of animals				
	Control	1st day	3rd day	5th day	7th day
AST activity, mmol/h per L	1.71±0.07	1.69±0.07	2.04±0.06	2.08±0.12	0.99±0.06*^
ALT activity, mmol/h per L	1.22±0.06	1.25±0.07	2.37±0.1*^	2.68±0.12*	0.95±0.06^
De Ritis coefficient	1.41±0.06	1.37±0.06	0.87±0.03*^	0.78±0.05*	1.04±0.006*^

* – $p<0.05$ compared with the control group of rats; ^ – $p<0.05$ compared with the previous term of the experiment.

The activity of ALT in the serum of rats from simulated alcoholic hepatitis group on days 3 and 5 of the experiment increased by 1.94 and 2.2 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the activity of ALT in the serum of rats increased by 1.9 times compared to the activity of ALT on day 1 of the experiment ($p<0.05$). On the 7th day of the experiment, the activity of ALT in the serum of rats decreased by 2.82 times compared to the activity of ALT on the 5th day of the experiment ($p<0.05$).

The de Ritis coefficient on days 3, 5, and 7 of the experiment was reduced by 1.62, 1.81, and 1.36 times, respectively, compared to the control ($p<0.05$), indicating cytolysis of hepatocytes.

On the 3rd day of the experiment, the de Ritis coefficient decreased by 1.57 times compared to the indicator on the 1st day of the experiment ($p<0.05$). On the 7th day of the experiment, the de Ritis coefficient increased 1.33 times compared to the 5th day of the experiment ($p<0.05$).

In biochemical studies of rat liver, it was found that the total NO synthase activity on the 1st, 3rd and 7th day of the experiment was increased by 5.89, 4.05 and 4.74 times, respectively, compared to the control ($p<0.05$) (table 2). On the 5th day of the experiment, the total NO-synthase activity in the liver of rats decreased by 4.53 times compared to the total NO-synthase activity on the 3rd day of the experiment ($p<0.05$). On day 7 of the experiment, total NO-synthase activity in the liver of rats increased by 5.29 times compared to total NO-synthase activity on day 5 of the experiment ($p<0.05$).

The concentration of nitrites in the liver of rats on day 1 of the experiment was reduced by 1.57 times compared to the control ($p<0.05$), on the 3rd, 5th and 7th day of the experiment increased by 1.52, 2.42 and 1.81 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the concentration of nitrites in the liver of rats increased by 2.38 times compared to the concentration of nitrites on day 1 of the experiment ($p<0.05$). On the 5th day of the experiment, the concentration of nitrites in the liver of rats increased by 1.59 times compared to the concentration of nitrites on the 3rd day of the experiment ($p<0.05$). On the 7th day of the experiment, the concentration of nitrites in the liver of rats decreased by 1.34 times compared to the concentration of nitrites on the 5th day of the experiment ($p<0.05$) (table 2).

Table 2

Biochemical parameters in the liver of rats under the conditions of modeling of alcoholic hepatitis (M±m)

Parameters	Groups of animals				
	Control	1st day	3rd day	5th day	7th day
NOS, μmol/min * g	0.19±0.02	1.12±0.16*	0.77±0.07*	0.17±0.02^	0.9±0.05*^
NO_2^-, nmol/g	7.14±0.17	4.56±0.66*	10.84±0.43*^	17.28±0.76*^	12.92±0.19*^
Nitrosothiols, μmol/g	0.36±0.02	0.97±0.2*	0.87±0.1*	2.71±0.1*^	1.64±0.07*^
$ONOO^-$, μmol/g	0.45±0.01	0.31±0.03*	8.63±1.17*^	6.96±0.33*	11.87±1.07*^

* – p <0.05 compared to the control group of rats; ^ – p <0.05 compared to the previous term of the experiment.

The concentration of nitrosothiols in the liver of rats on 1, 3, 5 and 7 days of the experiment was increased by 2.69, 2.42, 7.53 and 4.56 times respectively compared to the control (p<0.05). On day 5 of the experiment, the concentration of nitrosothiols in the liver of rats increased by 3.11 times compared to the concentration of nitrosothiols on day 3 of the experiment (p<0.05). On the 7th day of the experiment, the concentration of nitrosothiols in the liver of rats decreased by 1.65 times compared to the concentration of nitrosothiols on the 5th day of the experiment (p<0.05).

The concentration of peroxynitrite in the liver of rats on day 1 of the experiment was reduced by 1.45 times compared to the control (p<0.05), on the 3rd, 5th and 7th day of the experiment increased by 19.18, 15.47 and by 26.38 times, respectively, compared to the control (p<0.05). On day 3 of the experiment, the concentration of peroxynitrite in the liver of rats increased by 27.84 times compared to the concentration of peroxynitrite on day 1 of the experiment (p<0.05). On the 7th day of the experiment, the concentration of peroxynitrite in the liver of rats increased by 1.71 times compared to the concentration of peroxynitrite on the 5th day of the experiment (p<0.05).

When conducting a morphometric study of the hemomicrocirculatory tract of the liver of rats we found out, that the diameter of the sinusoidal capillaries around the central vein on 1st day of the experiment increased by 1.22 times and on 3rd day 1.37 times compared to the control (p<0.05) (table 3). On day 5 of the experiment, the diameter of the sinusoidal capillaries around the central vein of the hepatic lobe of rats decreased by 1.2 times compared to the control (p<0.05) and by 1.65 times compared to the diameter on the 3rd day (p<0.05) (table 3).

Table 3

Morphometric parameters of the hemomicrocirculatory tract of rats under the conditions of modeling of alcoholic hepatitis (M±m)

Parameters		Groups of animals				
		Control	1st day	3rd day	5th day	7th day
Lsv, μm		3.97±0.15	4.88±0.15*	5.44±0.13*	3.3±0.15*^	6.41±0.2*^
Lst, μm		2.48±0.11	3.24±0.1*	0.89±0.02*^	1.75±0.07*^	3.26±0.12*^
Central vein, μm	Do	36.24±0.28	37.14±0.64	31.95±0.45*^	16.13±0.1*^	27.55±0.51*^
	Di	33.99±0.24	32.2±0.47	27.35±0.25*^	13.51±0.11*^	24.42±0.51*^
Interlobular artery, μm	Do	13.8±0.11	14.4±0.79	25.2±0.15*^	26.62±0.16*^	15.31±0.64^
	Di	6.84±0.07	5.49±0.34	14.46±0.05*^	16.08±0.14*^	8.67±0.6^
Lobular arteriola, μm	Do	11.05±0.17	7.1±0.45*	10.73±0.27^	12.41±0.19*^	8.57±0.35*^
	Di	9.66±0.11	5.39±0.41*	7.58±0.18*^	8.91±0.04*^	5.07±0.22*^
Lobular venula, μm	Do	16.58±0.51	7.25±0.45*	11.52±0.47*^	11.51±0.06*	11.52±0.1*
	Di	12.06±0.28	4.47±0.51*	9.44±0.5*^	9.29±0.05*	9.37±0.11*
Interlobular vein, μm	Do	52.42±2.37	20.42±0.15*	21.17±0.41*	46.82±1.27^	27.09±0.95*^
	Di	42.88±2.04	17.4±0.13*	17.6±0.42*	40.15±0.82^	22.7±0.79*^
Sublobular vein, μm	Do	74.59±0.92	68.15±0.33*	99.33±0.62*^	46.95±0.21*^	56.84±0.66*^
	Di	64.64±1.1	62.45±0.31	93.91±0.47*^	42.72±0.14*^	52.93±0.8*^

Note: Lsv – Lumen of sinusoidal capillaries around the central vein, Lst – Lumen of sinusoidal capillaries around the hepatic triad. * – p <0.05 compared with the control group of rats; ^ – p <0.05 compared with the previous term of the experiment.

On day 7 of the experiment, the diameter of the sinusoidal capillaries around the central vein of the hepatic lobe of rats increased by 1.61 times compared to the control (p<0.05) and 1.94 times compared with the diameter on the 5th day of the experiment (p<0.05).

The diameter of the sinusoidal capillaries around the hepatic triad of rats on day 1 of the experiment increases 1.31 times compared to the control ($p<0.05$). On day 3 of the experiment, the diameter of the sinusoidal capillaries around the hepatic triad of rats decreased by 2.79 times compared to the control ($p<0.05$) and by 3.64 times compared to the diameter on 1st day ($p<0.05$). On the 5th day of the experiment, the diameter of the sinusoidal capillaries around the hepatic triad of rats increased by 1.97 times compared to the diameter on the 3rd day ($p<0.05$) and by 1.42 times compared to the control ($p<0.05$). On day 7 of the experiment, the diameter of the sinusoidal capillaries around the hepatic triad of rats increased by 1.31 times compared to the control ($p<0.05$) and by 1.86 times compared to the diameter of sinusoidal capillaries on the 5th day ($p<0.05$).

The diameter of the lumen of the central vein of the hepatic lobe of rats decreased on 3rd, 5th and 7th day of the experiment by 1.24, 2.52 and 1.39 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the diameter of the lumen of the central vein of the hepatic lobe of rats decreased by 1.18 times compared to the diameter of the lumen on day 1 ($p<0.05$). On the 5th day of the experiment, the diameter of the lumen of the central vein of the hepatic lobe of rats decreased by 2.02 times compared to the diameter of the lumen on the 3rd day ($p<0.05$). On day 7 of the experiment, the diameter of the lumen of the central vein of the hepatic lobe of rats increased by 1.81 times compared to the diameter of the lumen on day 5 ($p<0.05$).

The diameter of the lumen of the interlobular artery of rats increased on 3rd and 5th days of the experiment by 2.11 and 2.35 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the diameter of the lumen of the interlobular artery of rats increased by 2.63 times compared to the diameter of the lumen on day 1 ($p<0.05$). On the 5th day of the experiment, the diameter of the lumen of the interlobular artery of rats increased by 1.11 times compared to the diameter of the lumen on the 3rd day ($p<0.05$). On the 7th day of the experiment, the diameter of the lumen of the interlobular artery of rats decreased by 1.85 times compared to the diameter of the lumen on the 5th day ($p<0.05$).

The diameter of the lumen of the arterioles of the hepatic lobe of rats decreased on 1st, 3rd, 5th and 7th days of the experiment by 1.79, 1.27, 1.08 and by 1.91 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the diameter of the lumen of the arterioles of the hepatic lobe of rats increased by 1.41 times compared to the diameter of the lumen on day 1 ($p<0.05$). On the 5th day of the experiment, the diameter of the lumen of the arterioles of the hepatic lobe of rats increased by 1.18 times compared to the diameter of the lumen on the 3rd day ($p<0.05$). On the 7th day of the experiment, the diameter of the lumen of the arterioles of the hepatic lobe of rats decreased by 1.76 times compared to the diameter of the lumen on the 5th day ($p<0.05$).

The diameter of the venula lumen of the hepatic lobe of rats decreased on 1st, 3rd, 5th and 7th days of the experiment by 2.7, 1.28, 1.3 and by 1.29 times, respectively, compared to the control ($p<0.05$). On day 3 of the experiment, the diameter of the venula lumen of the hepatic lobe of rats increased by 2.11 times compared to the diameter of the lumen on day 1 ($p<0.05$).

The diameter of the lumen of the interlobular vein of rats decreased on 1st, 3rd and 7th days of the experiment by 2.46, 2.44 and by 1.89 times, respectively, compared to the control ($p<0.05$). On the 5th day of the experiment, the diameter of the lumen of the interlobular vein of rats increased by 2.28 times compared to the diameter of the lumen on the 3rd day ($p<0.05$). On the 7th day of the experiment, the diameter of the lumen of the interlobular vein of rats decreased by 2.28 times compared to the diameter of the lumen on the 5th day ($p<0.05$).

The diameter of the lumen of the sublobular vein of the rat liver increased on day 3 of the experiment by 1.45 times compared to the control ($p<0.05$). On the 5th and 7th day of the experiment, the diameter of the lumen of the sublobular vein of rats decreased by 1.51 and 1.22 times, respectively, compared to the diameter of the lumen of the sublobular vein of the control group of animals ($p<0.05$). On the 3rd day of the experiment, the diameter of the lumen of the sublobular vein of the liver of rats increased by 1.5 times compared to the diameter of the lumen on the 1st day ($p<0.05$). On the 5th day of the experiment, the diameter of the lumen of the sublobular vein of the rat liver decreased by 2.2 times compared to the diameter of the lumen on the 3rd day ($p<0.05$). On the 7th day of the experiment, the diameter of the lumen of the sublobular vein of the rat liver increased by 1.24 times compared to the diameter of the lumen on the 5th day ($p<0.05$).

Hemomicrocirculatory tract of the liver plays an important role in the development of inflammation regardless of alteration factors. Any inflammatory process in the liver begins with a local increase in the permeability of the endothelium of sinusoidal capillaries under the action of mediators synthesized by stellate reticuloendotheliocytes. The reaction to alcohol intoxication from resistant, metabolic and

capacitive links of the hemomicrocirculatory tract may be the result of action of pro- and anti-inflammatory mediators. We set ourselves the task of elucidating the contribution of the activity of NO synthases and nitric oxide metabolites to the reaction of the hemomicrocirculatory tract of the rat liver in a model of alcoholic hepatitis.

On the first day of modeling of alcoholic hepatitis biochemical markers of the cytolytic process in the liver without changes. The reaction of the hemomicrocirculatory tract was vasoconstriction of lobular arterioles, venules and interlobular veins with simultaneous expansion of sinusoidal capillaries throughout the hepatic beam (Fig. 1B).

A sharp increase in total NO synthase activity leads to an increase in nitrosothiols a labile nitric oxide buffer in the vascular wall. The decrease in peroxynitrite and nitrites on the first day of the experiment indicates a cytoprotective effect of redistribution of metabolites of the nitrogen oxide cycle.

On the third day of the experiment, a sharp increase in ALT in the serum and a decrease of the de Ritis coefficient indicates a cytolytic syndrome in the liver.

The interlobular artery is dilated (fig. 1 C). Particular arterioles and venulas also expand compared to the previous period of the experiment, but do not reach the diameter of the vessels in the control group of animals. The interlobular vein is almost unchanged compared to the previous term of the experiment. The sublobular vein of the liver is dilated.

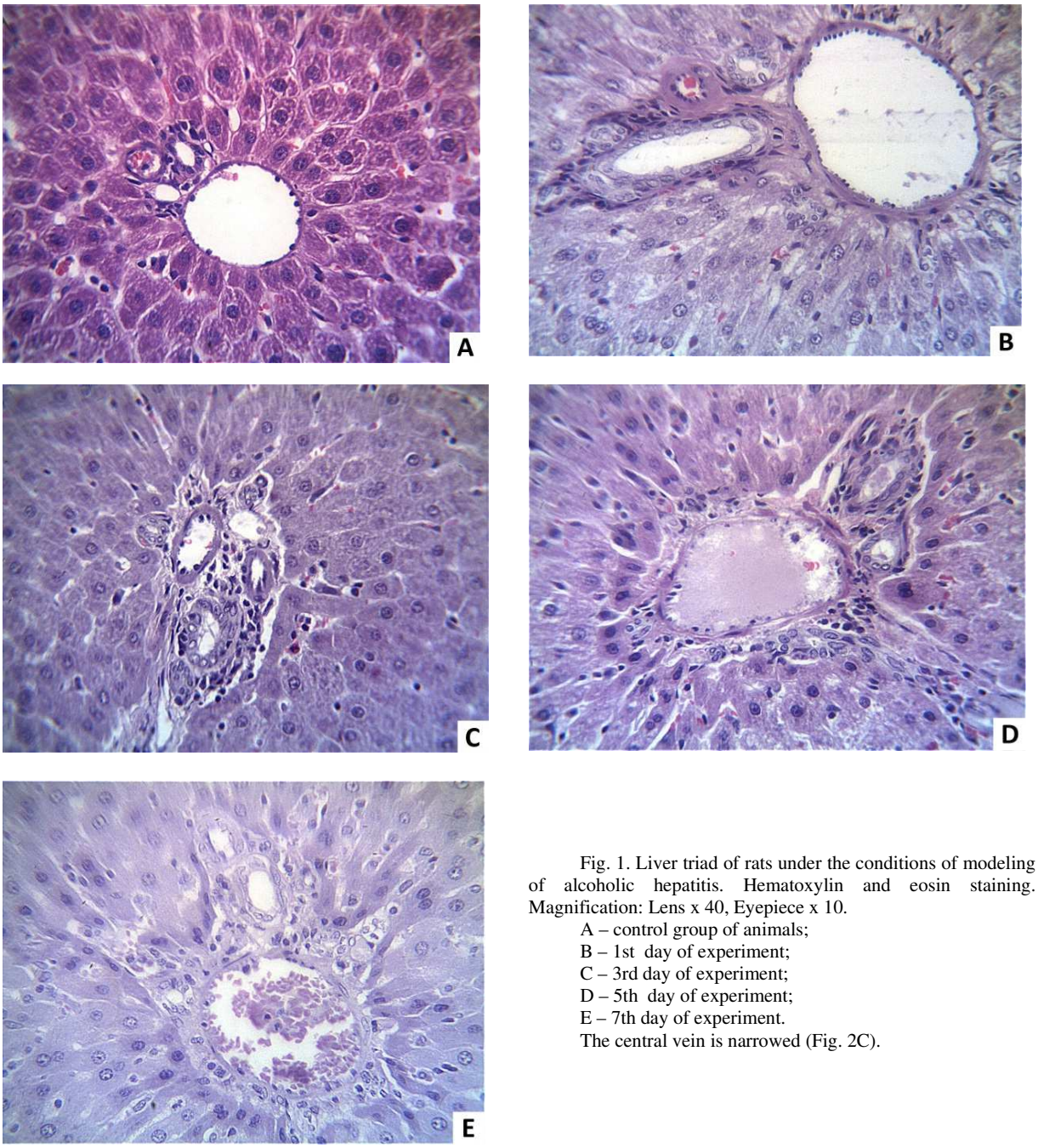

Fig. 1. Liver triad of rats under the conditions of modeling of alcoholic hepatitis. Hematoxylin and eosin staining. Magnification: Lens x 40, Eyepiece x 10.

A – control group of animals;
B – 1st day of experiment;
C – 3rd day of experiment;
D – 5th day of experiment;
E – 7th day of experiment.
The central vein is narrowed (Fig. 2C).

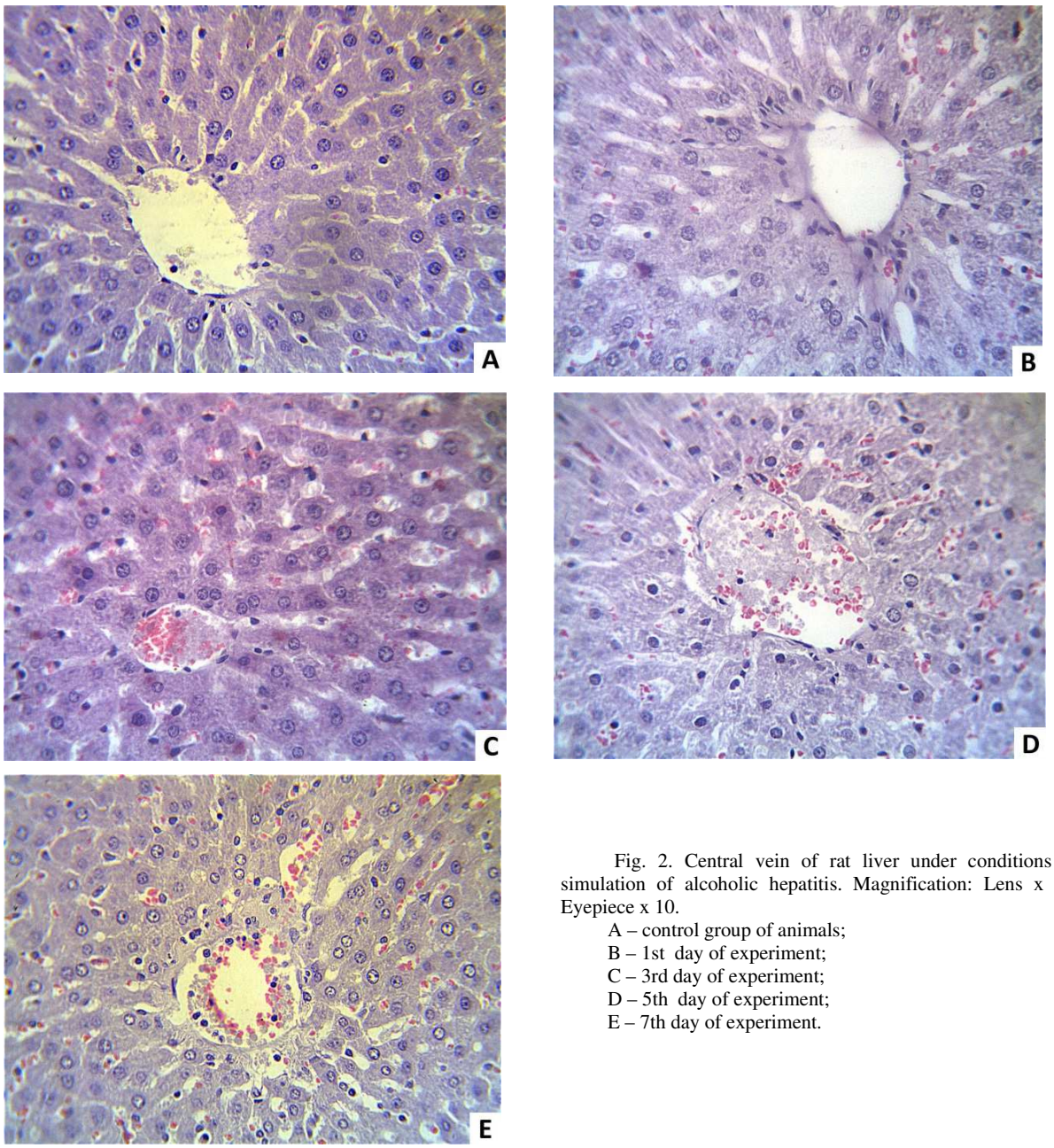

Fig. 2. Central vein of rat liver under conditions of simulation of alcoholic hepatitis. Magnification: Lens x 40, Eyepiece x 10.
A – control group of animals;
B – 1st day of experiment;
C – 3rd day of experiment;
D – 5th day of experiment;
E – 7th day of experiment.

Sinusoidal capillaries are narrowed around the hepatic triad and dilated around the central vein. Arterial hyperemia occurs in the liver, which is accompanied by an increase in peroxynitrite and nitrites, which indicates the possible development of nitrosative stress. The increase in the concentration of peroxynitrite on the third day of the experiment is associated with an increase in the formation of reactive oxygen species under the action of ethanol by hepatocytes due to cytochrome P-450 (CYP2E1) and Kupffer cells due to xanthine oxidase and NADPH oxidase [9] with simultaneously high total NO synthase activity, which produces an increased concentration of NO. Vasodilation of the resistant link of the hemomicrocirculatory tract is partly due to the increased concentration of nitric oxide produced by NO synthases.

On the fifth day of the experiment, we observed a high level of ALT in the blood serum and a reduced de Ritis coefficient. The interlobular artery and the lobular arteriole continue vasodilation (Fig. 1D). Lobular venula is almost unchanged from the previous term of the experiment. The interlobular vein expands in comparison with the previous term of experiment and almost reaches level of diameter in control group. Sublobular vein of the liver is narrowed. The central vein continues vasoconstriction (Fig. 2D). Sinusoidal capillaries expand around the hepatic triad compared to the previous term of experiment and narrow around the central vein. Venous hyperemia occurs in the liver accompanied by an increase in the concentration of peroxynitrites, nitrites and nitrosothiols against the background of a decrease in the total activity of NO synthases. This indicates a violation of the nitric oxide influence on the regulation of the

resistant part of the liver hemomicrocirculatory tract and its subsequent transformation into cytotoxic forms. Dilated arterial vessels on the background of reduced activity of NO synthases may be the result of increased expression of cyclooxygenase-2 under the influence of ethanol [10].

It is well known fact from the literature [8] that alcohol intoxication leads to NO-independent vasoconstriction, so increased activity of total NO-synthase may be a compensatory mechanism in response to vasoconstriction.

On the seventh day of the experiment, the level of ALT in the serum decreases, but the reduced de Ritis coefficient indicates a high activity of cytolysis. Interlobular arteries and veins, lobular arterioles narrow (fig. 1E). The lobular venula is practically without changes in diameter in comparison with the previous term of experiment. The sublobular vein of a liver expands in comparison with the previous term of experiment. The central vein dilates (fig. 2E). The sinusoidal capillaries around the hepatic triad and central vein dilate. Increased total activity of NO-synthase against the background of arterial vasoconstriction indicates the absence of a significant role of nitric oxide in maintaining the tone of the resistant link of the hemomicrocirculatory tract at this stage of progression of alcoholic hepatitis. Nitric oxide mostly metabolizes to peroxynitrite, which increases nitrosative stress in the liver.

Conclusion

The hemomicrocirculatory tract of the rat liver in the model of alcoholic hepatitis consistently demonstrates the development of arterial hyperemia, which turns into venous hyperemia. Nitric oxide and its metabolites play an important role in the regulation of the resistant part of the hemomicrocirculatory tract of the rat liver in the first three days of the experiment. On days 5 and 7 of the experiment, the role of nitric oxide in the regulation of vascular tone is significantly reduced. Further redistribution of nitric oxide cycle metabolites leads to an increase in the concentration of peroxynitrite, which is a factor of secondary alteration.

References

1. Akimov OYe, Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. Ukr. Biochem. J. 2016; 88 (6): 70–75. doi: https://doi.org/10.15407/ubj88.06.070

2. Ceni E, Mello T, Galli A. Pathogenesis of alcoholic liver disease: role of oxidative metabolism. World J Gastroenterol. 2014; 20(47): 17756–72. doi: 10.3748/wjg.v20.i47.17756

3. Chayanupatkul M, Liangpunsakul S. Alcoholic hepatitis: a comprehensive review of pathogenesis and treatment. World J Gastroenterol. 2014; 20(20): 6279–86. doi: 10.3748/wjg.v20.i20.6279.

4. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D. et al. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. Proc. Natl. Acad. Sci. USA. 1993; 90: 10957–10961.

5. Kudo R, Yuui K, Kasuda S, Hatake K. Effect of alcohol on vascular function. Nihon Arukoru Yakubutsu Igakkai Zasshi. 2015; 50(3): 123–34.

6. Lee DH, Kim DH, Hwang CJ, Song S, Han SB, Kim Y, et al. Interleukin-32γ attenuates ethanol-induced liver injury by the inhibition of cytochrome P450 2E1 expression and inflammatory responses. Clin Sci (Lond). 2015; 128(10): 695–706. doi: 10.1042/CS20140576.

7. Molina PE, Nelson S. Binge Drinking's Effects on the Body. Alcohol Res. 2018; 39(1): 99–109.

8. Stepanov YuM, Didenko VI, Dynnik OB, Konenko IS, Oshmianskaia NYu, Galinsky AA. Association of morphological changes in the liver parenchyma and its rigidity under the conditions of the experimental modeling of alcoholic and toxic hepatitis. Journal of the NAMSU. 2017; 23 (3-4): 196–204.

9. Xu L, Yu Y, Sang R, Li J, Ge B, Zhang X. Protective Effects of Taraxasterol against Ethanol-Induced Liver Injury by Regulating CYP2E1/Nrf2/HO-1 and NF-κB Signaling Pathways in Mice. Oxid Med Cell Longev. 2018; 2018: 8284107.

10. Zhang X, Jin L, Tian Z, Wang J, Yang Y, Liu J, Chen Y, Hu C, Chen T, Zhao Y, He Y. Nitric oxide inhibits autophagy and promotes apoptosis in hepatocellular carcinoma. Cancer Sci. 2019; 110(3): 1054–1063. doi: 10.1111/cas.13945.

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