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INFLUENCE OF NANOSIZED CERIUM DIOXIDE ON THE VASCULAR BED OF THE LIVER UNDER THE CONDITIONS OF CHRONIC ALCOHOLIC HEPATITIS MODELING

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Chronic alcohol consumption changes the microcirculation of the liver and, depending on the duration and dose of ethanol, can have various negative consequences. Nanosized cerium dioxide can be an effective means of pathogenetic correction of ethanol-induced changes in the liver. The aim of the work was to study the effect of nanosized cerium dioxide on the morphometric indicators of the vascular bed of rat liver under the conditions of chronic alcoholic hepatitis modeling. The experiments were performed on 24 white, mature male Wistar rats, weighing 180–220 g. Animals modeled chronic alcoholic hepatitis according to Stepanov Yu.M. (2017) and nanosized cerium dioxide was administered intragastrically at a dose of 1 mg/kg in a volume of 2.9 ml/kg. The morphometric parameters of the inner diameter of the vessels of the liver lobe were determined; capillary lumen around the central vein and hepatic triad. Simulation of chronic alcoholic hepatitis leads to expansion of the central vein and narrowing of interlobular arteries and veins and lobular arterioles and venules. The introduction of nanodispersed cerium dioxide against the background of modeling chronic alcoholic hepatitis expands alcohol-narrowed lumens of interlobular arteries and veins and lobular arterioles and venules, without affecting the diameter of the central vein.

Key words: nanosized cerium dioxide, vascular bed, chronic alcoholic hepatitis, liver, rats.

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ВПЛИВ НАНОДИСПЕРСНОГО ДІОКСИДУ ЦЕРІУ НА СУДИННЕ РУСЛО ПЕЧІНКИ ЗА УМОВ МОДЕЛЮВАННЯ ХРОНІЧНОГО АЛКОГОЛЬНОГО ГЕПАТИТУ

Хронічне вживання алкоголю змінює мікроциркуляцію печінки і, в залежності від тривалості і дози етанолу, може мати різні негативні наслідки. Нанодисперсний діоксид церію може бути ефективним засобом патогенетичної корекції етанол-індукованих змін в печінці. Метою роботи було вивчити вплив нанодисперсного діоксиду церію на морфометричні показники мікроциркуляторного русла печінки щурів за умов моделювання хронічного алкогольного гепатиту. Експерименти виконані на 24 білих статевозрілих щурах-самцях лінії Wistar, вагою 180-220 г. Тварини моделювали хронічний алкогольний гепатит за Степановим Ю.М. (2017) і внутрішньошлунково вводили нанокристалічний діоксид церію в дозі 1 мг/кг об'ємом 2,9 мл/кг. Визначали морфометричні параметри внутрішнього діаметра судинного русла печінкової часточки; просвіт капілярів навколо центральної вени та печінкової триади. Моделювання хронічного алкогольного гепатиту призводить до розширення центральної вени та звуження міжчасточкових артерій та вен і часточкових артеріол та венул. Введення нанодисперсного діоксиду церію на фоні моделювання хронічного алкогольного гепатиту розширює звужені алкоголем просвіти міжчасточкових артерій та вен і часточкових артеріол та венул, не впливаючи на діаметр центральної вени.

Ключові слова: нанодисперсний діоксид церію, судинне русло, хронічний алкогольний гепатит, печінка, щури.

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All functions of the liver, starting with the biosynthesis and catabolism of proteins, fats and carbohydrates and ending with the detoxification of xenobiotics, the formation of urea and the body's immune protection, are closely dependent on adequate microcirculation. Adequate nutrient perfusion, oxygen supply, and balanced vasomotor control with appropriate intercellular communication are required to maintain liver homeostasis [13]. Deterioration of liver homeostasis, as observed with chronic alcohol consumption, changes the microcirculation of the liver and, depending on the duration and dose of ethanol, can have different consequences [10]. Changes in the microcirculation of the liver under conditions of chronic ethanol intoxication are associated with the development of inflammation, oxidative stress and fibrosis of the liver beams.

Recent advances in nanotechnology offer promising agents that can effectively scavenge reactive oxygen species (ROS) and have anti-inflammatory effects. Among them is nanosized cerium dioxide (NCD), also known as nanocerium, which is used in the biomedical field to treat pathologies including oxidative stress and inflammation. NCD acts similarly to superoxide dismutase and catalase [3]. Recent studies have shown that the antioxidant capacity of NCD is 9 times higher than that of known antioxidants [4]. NCD prevents increased expression of interleukin-6 (IL-6) and tissue inhibitor of metalloproteinase-2 (TIMP-2) genes in cirrhotic rats. In addition, studies have shown that NCD is nontoxic at therapeutic doses, relatively stable, and accumulates in the liver upon administration [15]. In the scientific literature, there is a paucity of data regarding the possible effect of NCD on the microcirculatory channel of the liver of rats under conditions of chronic alcoholic hepatitis. But it is obvious that such an influence can be.

The purpose of the study was to assess the effect of nanosized cerium dioxide on the morphometric indicators of the vascular bed of rat liver under the conditions of chronic alcoholic hepatitis modeling.

Materials and methods. The experiments were performed on 24 white, mature male Wistar rats, weighing 180–220 g. The animals were divided into 4 groups of 6 animals each: I – control; II – group of rats that were intragastrically injected with nanosized cerium dioxide (NCD) [1] at a dose of 1 mg/kg in a volume of 2.9 ml/kg; III – group of animals on which chronic alcoholic hepatitis was modeled by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal administration of a 16.5% ethanol solution in a 5% glucose solution, at the rate of 4 ml/kg of body weight. After that, they were transferred to 10% ethanol as the only source of drinking [9]. IV group consisted from animals on which chronic alcoholic hepatitis was modeled as in group III and nanosized cerium dioxide was administered according to the scheme of group II.

The control group included animals that were subjected to similar manipulations throughout the study period, but were injected with a physiological solution. The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 63rd day by blood sampling from the right ventricle of the heart under thiopental anesthesia.

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 animal experiments 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.

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. Following morphometric parameters of the inner diameter of the vessels of the liver lobe were determined: capillary lumen around the central vein and hepatic triad.

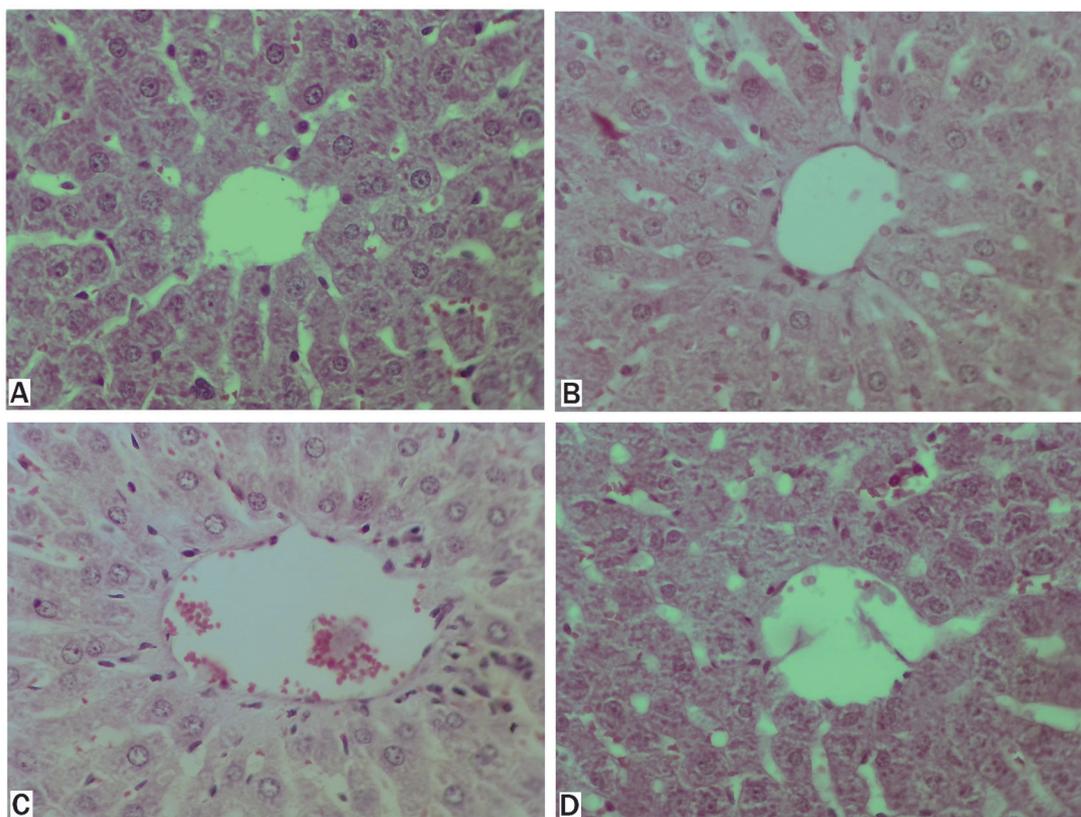


Fig. 1. Photomicrographs of the central vein of the liver of rats under the conditions of correction of chronic alcoholic hepatitis with nanosized cerium dioxide. Hematoxylin-eosin staining. Magnification: Lens x 40, Eyepiece x 10. A – control group; B – NCD group; C – chronic alcoholic hepatitis; D – correction of chronic alcoholic hepatitis with NCD.

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. Under the conditions of NCD administration, the diameter of sinusoidal capillaries around the central vein of the liver of rats increased by 1.09 times compared to the control (Fig. 1).

The diameter of the lumen of the interlobular artery of rats under the conditions of NCD administration decreased by 1.49 times, and the arterioles of the hepatic lobule increased by 1.5 times compared to the control (Fig. 2).

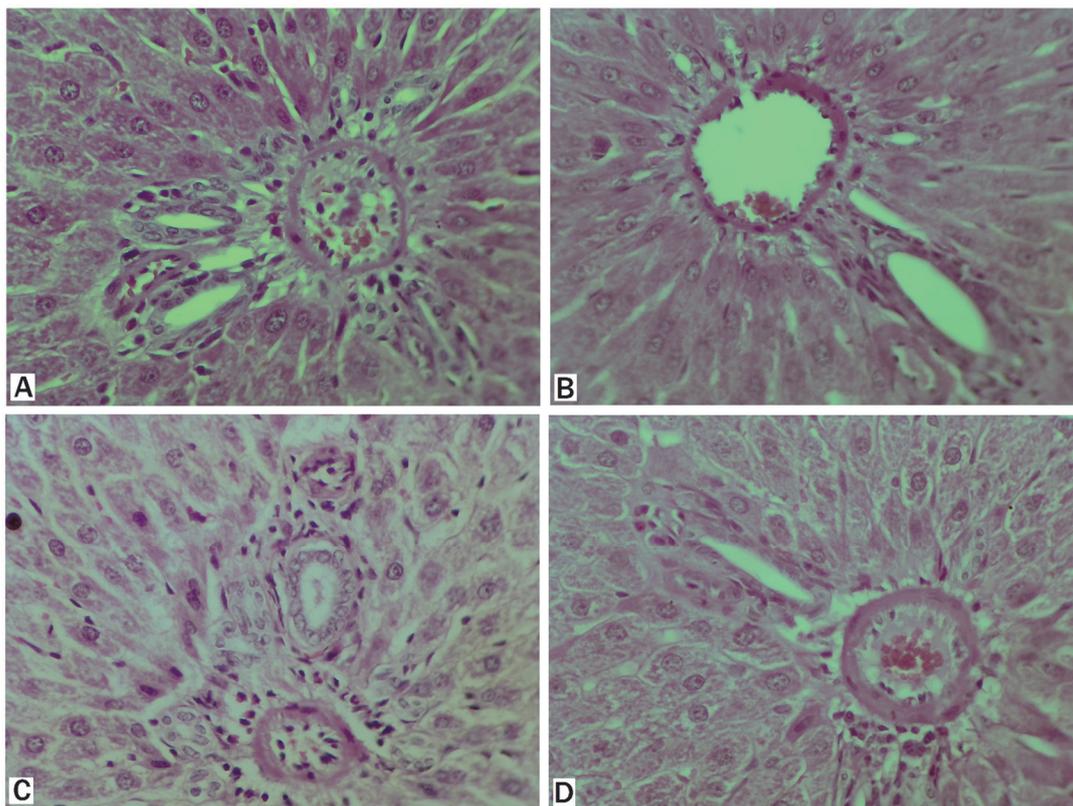


Fig. 2. Photomicrographs of the hepatic triad of rats under the conditions of correction of chronic alcoholic hepatitis with nanodispersed cerium dioxide. Hematoxylin-eosin staining. Magnification: Lens x 40, Eyepiece x 10. A – control group; B – NCD group; C – chronic alcoholic hepatitis; D – correction of chronic alcoholic hepatitis with NCD.

The diameter of the lumen of the interlobular vein of rats increased by 1.48 times under the conditions of NCD administration, and the venules of the hepatic lobule decreased by 1.11 times compared to the control group of animals (Table). Thus, the introduction of NCD reduced the flow of arterial blood to the hepatic lobe, which was evidenced by a decrease in the lumen of the interlobular arteries. An increase in the lumen of the interlobular veins probably indicated venous stasis. At the same time, the need of hepatocytes for metabolites most likely increased, as was evidenced by an increase in the lumens of the lobular arterioles and venules. Such changes in the microcirculatory channel of the liver under the conditions of NCD administration indicate a certain toxicity of the drug to liver cells, which may be due to the pro-oxidant effect of NCD under the conditions of its accumulation in hepatocytes [2]. The development of oxidative stress in hepatocytes due to NCD aggregation leads to the expansion of lobular vessels (arterioles and venules) and interlobular veins due to the activation of cyclooxygenase. The absence of changes in the diameter of the central vein can be explained by the fact that, under physiological conditions, the flow of metabolites is directed from the central vein to the interlobular vein, which also indicates the absence of violations of intralobular fluid flow under the conditions of NCD administration. The narrowing of the interlobular artery may be related to the ability of NCD to act as an “interceptor” of nitric oxide [5]. Thus, NCD has negligible toxicity that does not disrupt the functional state of the hepatic lobule.

Under the conditions of chronic alcoholic hepatitis modeling, the diameter of the lumen of the central vein of the hepatic lobe of rats increased by 1.16 times compared to the control and by 1.13 times

compared to the group of animals that were injected with NCD. The diameter of the lumen of the interlobular artery of rats under the conditions of chronic alcoholic hepatitis modeling decreased by 2.19 times compared to the control and by 1.47 times compared to the group of animals that were injected with NCD. The diameter of the lumen of the arteriole of the hepatic lobule of rats decreased by 1.49 times compared to the control and by 2.25 times compared to the group of animals that were injected with NCD. The diameter of the lumen of the venule of the hepatic lobule of rats decreased by 2.17 times compared to the control and by 1.96 times compared to the group of animals injected with NCD. The diameter of the lumen of the interlobular vein of rats under the conditions of chronic alcoholic hepatitis modeling decreased by 1.65 times compared to the control and by 2.44 times compared to the group of animals that were injected with NCD. Therefore, the introduction of alcohol leads to the expansion of the central lobar vein against the background of narrowing of the interlobular artery and vein, which creates conditions for the formation of blood stasis, which goes from the central vein to the interlobular vein. The narrowing of lobular arterioles and venules indicates a metabolic disorder in the hepatic lobule, namely a decrease in the exchange of metabolites between hepatocytes and the vascular bed. The described changes in the vascular bed of the liver may be a consequence of alcohol-induced damage to hepatocytes and the inflammatory process. Alcohol-induced chronic inflammation of the liver is accompanied by a decrease in the metabolic activity of hepatocytes, their apoptosis, and the development of fatty dystrophy and fibrosis [8] (Table 1).

Table 1

Changes in the vascular bed of the liver of rats under the conditions of administration of nanosized cerium dioxide (NCD) against the background of chronic alcoholic hepatitis modeling (M±m)

Vessel lumen	Groups of animals			
	Control	NCD	Alcoholic hepatitis	Alcoholic hepatitis + NCD
Lsv, μm	6.8±0.25	7.4±0.18*	7.31±0.15	7.23±0.16
Lst, μm	5.86±0.15	5.99±0.17	5.75±0.17	6.31±0.17^
Central vein, μm	57.39±1.35	58.89±1.39	66.46±1.27**	60.76±1.29^
Interlobular artery, μm	34.81±1.61	23.38±0.42*	15.87±0.79**	27.86±0.89**^
Lobular arteriola, μm	9.97±0.49	15.0±0.24*	6.67±0.41**	14.49±0.26**
Lobular venula, μm	18.2±0.61	16.44±0.51*	8.37±0.2**	19.04±0.45**^
Interlobular vein, μm	42.35±1.66	62.53±2.22*	25.66±2.42**	49.1±1.6**^

Note: Lsv – Lumen of sinusoidal capillaries around the central vein, Lst – Lumen of sinusoidal capillaries around the hepatic triad. * – p<0.05 compared to a control group of rats; ** – p<0.05 compared to NCD group; ^ – p<0.05 compared to the alcoholic hepatitis group.

Under the conditions of administration of NCD to rats with chronic alcoholic hepatitis, the diameter of sinusoidal capillaries around the hepatic triad of rats increased by 1.1 times, and the diameter of the central vein decreased by 1.09 times compared to the group of animals with chronic alcoholic hepatitis. Under the conditions of the introduction of NCD against the background of chronic alcoholic hepatitis modeling, the diameter of the lumen of the interlobular artery of rats decreased by 1.25 times compared to the control, but increased by 1.19 times compared to the group of animals injected with NCD and by 1.76 times compared to rats with chronic alcoholic hepatitis. The diameter of the lumen of the arteriole of the hepatic lobule of rats in this group increased by 1.45 times compared to the control and decreased by 1.04 times compared to the group of animals that were injected with NCD. The lumen diameter of the venule of the hepatic lobule of rats in the NCD correction group of chronic alcoholic hepatitis increased by 1.16 times compared to the group of animals injected with NCD and by 2.27 times compared to rats with chronic alcoholic hepatitis. The diameter of the lumen of the interlobular vein under the conditions of the introduction of NCD on the background of chronic alcoholic hepatitis modeling increased by 1.16 times compared to the control and by 1.91 times compared to rats with chronic alcoholic hepatitis, but decreased by 1.27 times compared to the group of animals, which were administered NCD. Thus, under the conditions of the introduction of NCD against the background of simulation of chronic alcoholic hepatitis in the vascular bed of the liver, the expansion of lobular arterioles and venules was observed, which indicates the improvement of microcirculation, and, therefore, the strengthening of the metabolic exchange between the hepatocyte and the vascular channel. Expansion under these conditions of interlobular veins and arteries on the background of a decrease relative to the chronic alcoholic hepatitis simulation group of the lumen of the central vein may be a sign of restoration under the influence of NCD of the microcirculation between the central and interlobular veins. Such positive changes in the vascular bed of the liver after the use of NCD for the correction of alcoholic hepatitis may be due to the strengthening of antioxidant protection in liver tissues after the introduction of NCD [11]. Thus, the use of NCD under the conditions of chronic alcoholic hepatitis can be considered as a means of pathogenetic therapy, which enhances antioxidant

protection in the liver and acts as a direct antioxidant that corrects alcohol-induced production of reactive oxygen species [14].

In addition to its antioxidant effects, NCD can affect vessels and alter their response to relaxant and contractile agonists. NCD increases the bioavailability of NO and increases vascular sensitivity to sodium nitroprusside. However, NCD reduces bradykinin-induced vasodilation and reduces angiotensin II-induced vascular smooth muscle contraction [6]. The decrease in the sensitivity of blood vessels to angiotensin II under the influence of NCD explains the expansion of interlobular arteries and lobular arterioles in the NCD administration group against the background of chronic alcoholic hepatitis modeling, since alcohol can increase the effect of angiotensin II on blood vessels [12]. Despite the high level of nitric oxide production during excess alcohol intake, its bioavailability for the vascular endothelium may decrease due to the formation of active forms of nitrogen (peroxynitrite) during the interaction of nitric oxide with the superoxide anion radical, the production of which also increases under conditions of excess alcohol intake [7]. Therefore, the increase in the bioavailability of nitric oxide under the influence of NCD may also be the reason for the expansion of interlobular vessels in the NCD administration group against the background of modeling chronic alcoholic hepatitis.

Conclusion

Simulation of chronic alcoholic hepatitis in rats leads to dilation of the central vein and narrowing of interlobular arteries and veins and lobular arterioles and venules.

The introduction of nanosized cerium dioxide against the background of chronic alcoholic hepatitis modeling expands alcohol-narrowed lumens of interlobular arteries and veins and lobular arterioles and venules, without affecting the diameter of the central vein, which improves microcirculation in the liver.

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