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MORPHOMETRIC CHARACTERISTICS OF RAT SALIVARY GLANDS HEMOMICROVASCULATURE CAPACITY COMPONENT UNDER NORMAL CONDITIONS AND IN ETHANOL CHRONIC INTOXICATION

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Data of the morphometric study on chronic ethanol intoxication are presented in the paper. It has been established that chronic ethanol intoxication affects the capacitive link of the hemomicrovasculature of the submandibular salivary glands. At the early stages of observation, venules dilation is determined, which is confirmed by a significant increase in the outer diameter and the lumen diameter with the vascular wall thickness reduction. From the twelfth day the tendency is observed towards the metric indices restoration. Normalization of the indices is not determined by the thirtieth day.

Key words: chronic ethanol intoxication, rats, salivary glands, venules.

The work is a fragment of the science research project "Experimental-morphological study on the effects of the cryopreserved placenta transplants and other exogenous factors on the morphofunctional state of certain internal organs", state registration number 0113U006185.

The problem of alcoholism is considered to be topical all over the world and ranks third after cardiovascular and oncological diseases by the mortality rate. According to the WHO statistics, there are about 140 million people in the world suffering from chronic alcoholism, but the fact should be assessed that more than half of them refuse to seek assistance of health professionals. In Ukraine, there is a high level of alcohol consumption by adolescents and student youth, which is directly a socio-demographic issue concerning the entire population. To fight against alcohol, medical and social diagnostic techniques are used in many countries to find the best solutions for treatment and recovery of alcohol dependent people [1].

Scientists are increasingly focusing on the study of salivary glands. The interest of researchers in studying the patterns of the salivary glands response to various stimuli has recently increased significantly, which is due to the diagnostic value of saliva as a highly informative object for the clinical assessment of the whole body's state. Large salivary glands undergo special changes in this case, being highly sensitive to the action of physiological, pathogenic factors [2].

In human and animal saliva, substances affecting hemomicrocirculatory hemostasis, blood clotting ability and fibrinolysis have been detected. Interaction of the hemostasis system with lipid peroxidation reactions and antioxidant system is well-known. It can also be traced at estimating the salivary glands functions [3].

The state of the hemomicrovasculature parts has significant impact on the organs functioning, particularly its capacity component, which provides a fully functional outflow of blood from tissues, which is a prerequisite for ensuring their normal life activity.

In the article of Yeroshenko G.A. and Senchakovich Yu.V. a change in the state of the hemomicrovasculature blood vessels at experimental hyposalivation of various genesis is observed - where a stable dilatation of the capacity component during the experiment [4].

Objectivization of histological data is possible using the morphometric method, which permits to establish the main tendencies of changes in structural components during the experiment [5].

The purpose of the work was to determine the dynamics of changes in the metric indices of the hemomicrovasculature of the rat submandibular glands in normal conditions and under chronic intoxication with ethanol.

Material and methods. The work was performed on 45 white outbred rats: 5 animals were in the control group, who received isotonic sodium chloride solution 4 times a day, and 40 rats were experimental ones, receiving 12 mg / kg of 40° ethanol intragastrically 4 times a day [6].

Animals were withdrawn from the experiment on the 5-th, 9-th, 12-th and 30-th days by overdose of thiopental anesthesia (25 mg / kg). Sections of the submandibular glands were encapsulated in epon-812 according to the generally accepted technique [7]. The semi-thin sections were stained with polychrome stain [8].

The mean values of the venules outer and lumen diameter were determined using a microscope with a digital Biorex 3 photomicrographic attachment with the software adapted for the present research. Statistical processing of morphometric data was performed using the Exel software [9]. The vascular wall thickness was calculated using the formula $Th_{vw} = D_{ex} - D_l / 2$.

Animal management and experiments were performed in accordance with the "General Ethical Rules for Animal Experiments" adopted by the 1st National Congress on Bioethics and the requirements

of the International Principles of the “European Convention for Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” [10].

Results of the study and their discussion. When carrying out a morphometric study, it was found that venules have a characteristic structure of the microvasculature vessels, with a smooth surface and a typical location near the cross-striated duct. Formed elements of blood were freely placed in the vessels lumen (fig. 1).

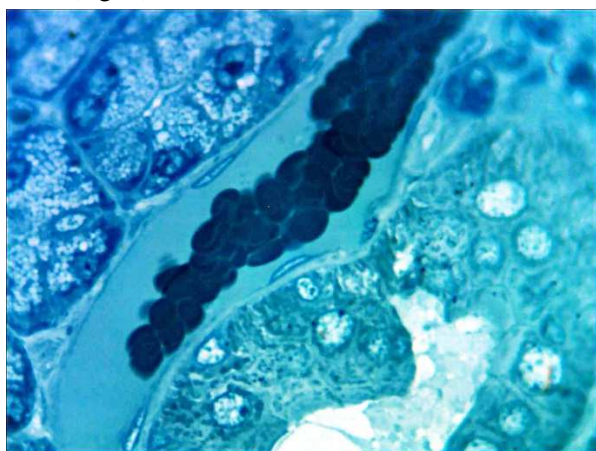


Fig. 1. Venule in a piece of the rat submandibular salivary gland in the control group of animals. Staining with methylene blue. Image magnification x1000.

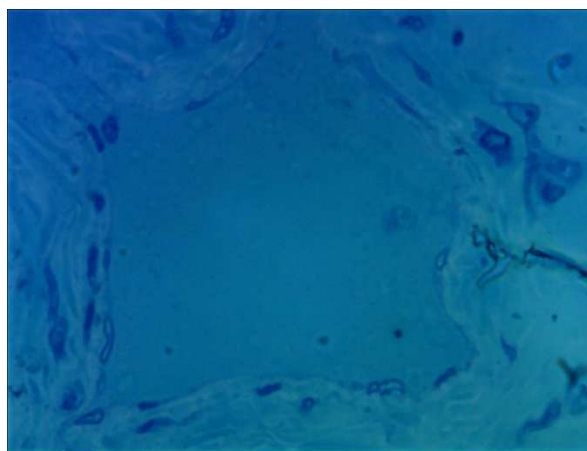


Fig. 2. Venule in the interstitial tissue of the rat submandibular salivary gland on the fifth day of the chronic ethanol intoxication experimental model. Staining with methylene blue. Image magnification x1000.

The average values of the outer diameter of the wall of the venule of the lobules of the submandibular gland were $16,31 \pm 0,08 \mu\text{m}$, and the internal diameter was $12,73 \pm 0,03 \mu\text{m}$. The thickness of the vascular wall of the venule was $1,74 \mu\text{m}$ (Table). The mean values of the venule wall's outer diameter of a submandibular gland piece were $16.31 \pm 0.08 \mu\text{m}$, and the lumen diameter equalled $12,73 \pm 0,03 \mu\text{m}$. The venule vascular wall's thickness was $1,74 \mu\text{m}$ (table).

Table

Mean values of the venule hemomicrovasculature diameters of rat submandibular gland pieces (μm)

Venules	D_{ex}	D_{l}	Th_{vw}
Control	16.31 ± 0.08	12.73 ± 0.03	1.74
5-th day	$19.90 \pm 0.05^*$	$16.91 \pm 0.04^*$	1.49
9-th day	$17.81 \pm 0.04^{*,**}$	$14.73 \pm 0.04^{*,**}$	1.54
12-th day	$15.46 \pm 0.12^{*,**}$	$11.74 \pm 0.12^{*,**}$	1.85
30-th day	$16.58 \pm 0.04^{*,**}$	$14.41 \pm 0.04^{*,**}$	1.09

Note: * - $P < 0.05$ compared to the control group; ** - $P < 0.05$ compared to the experimental group.

On the fifth day of the study, the outer diameter of the venule wall, making $19.90 \pm 0.05 \mu\text{m}$, which was larger by 22% than the indices in the control group animals ($p < 0,05$) and the diameter of the venule lumen was by 32,8% larger, too. Its mean values were $16.91 \pm 0.04 \mu\text{m}$, red blood cells were absent in the lumen (fig. 1). The vascular wall's thickness reduced by 14.4%, which made $1.49 \mu\text{m}$ (table).

On the ninth day of the experiment, the outer diameter of the venules reduced by 10.5% compared to the fifth day and was $17.81 \pm 0.04 \mu\text{m}$, but it was larger than the values in the control group by 9.2% ($p > 0.05$). The lumen diameter was $14.73 \pm 0.04 \mu\text{m}$, which was less than that of the fifth day by 12.9%, but it was larger by 15.7% compared with the control group ($p > 0.05$). The vascular wall's thickness had the following tendency to change: it reduced, compared to the fifth day of the experiment by 3.4%, and compared with the control group by 11.5%, and equalled $1.54 \mu\text{m}$ (tab.).

On the twelfth day, the venule outer diameter was $15.46 \pm 0.12 \mu\text{m}$, which is 13.2% less than that on the ninth day of the study, and less than that of the control group (5.2%) ($p < 0.05$). The mean values of the lumen diameter were $11.74 \pm 0.12 \mu\text{m}$, which is less than the previous study indices by 20.3%, which was also reliably less than the control group indices by 7.8% ($p < 0.05$). The vascular wall's thickness reliably increased by 20.1% compared to the ninth day and accordingly was greater by 6.3% compared to the control group ($p < 0.05$). Its mean values were $1.85 \mu\text{m}$ (table). The venule wall's swelling and the complete filling of the vessels with formed blood elements were observed (fig. 3).

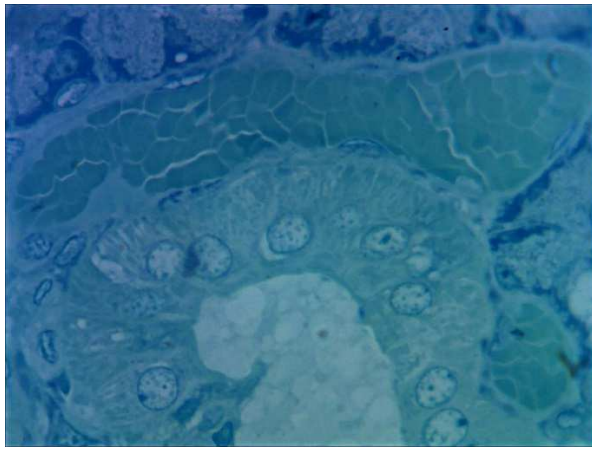


Fig. 3. Venule next to the cross-striated duct in a piece of the rat submandibular salivary gland on the twelfth day of the chronic ethanol intoxication experimental model. Staining with methylene blue. Image magnification x1000.

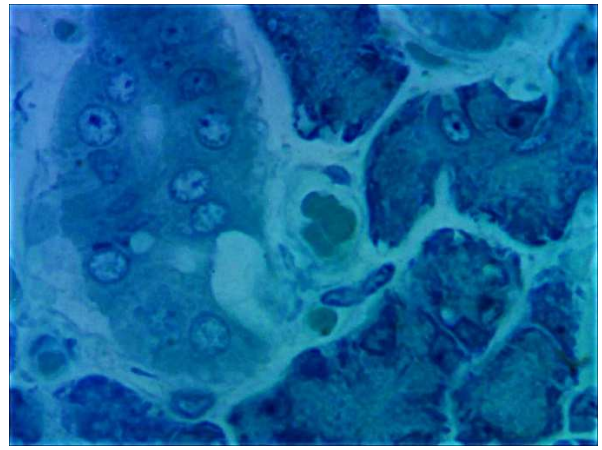


Fig. 4. Venule in a piece of the rat submandibular salivary gland on the thirtieth day of the chronic ethanol intoxication experimental model. Staining with methylene blue. Image magnification x1000.

On the thirtieth day of the experiment, the outer diameter of the submandibular gland's venule was $16.58 \pm 0.04 \mu\text{m}$, which exceeded that of the 12th day by 7.2%, and was also larger by 1.7% compared to the values in the control group of animals ($p < 0.05$). The venule's internal diameter also increased by 22.7% compared to the previous experiment and amounted to $14.41 \pm 0.04 \mu\text{m}$, which was also 13.2% larger than that of the control group animals, the blood cells completely filled the vessels lumen (fig. 4). The vascular wall's thickness reduced significantly by 41.1% compared to the twelfth day and was reliably less by 37.4% compared to the indices in the control group of rats ($p < 0.05$). Its mean values were $1.09 \mu\text{m}$ (table).

Conclusion

The performed morphometric study has established that chronic intoxication with ethanol affects the capacitive component of the submandibular salivary glands' hemomicrovasculature. At the early stages of observation, vasodilation is determined, which is confirmed by an increase in the outer and of the lumen diameters, with the vascular wall's thickness being reduced. Since the twelfth day, there is a tendency towards the restoration of metric parameters, but a vascular swelling is observed, as evidenced by an increase of the vascular wall's thickness, which gradually disappears until the end of the experiment. The indices normalization by the thirtieth day is not determined.

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Реферати

**МОРФОМЕТРИЧНА ХАРАКТЕРИСТИКА
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ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА
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ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ**
Єрошенко Г.А., Шевченко К.В., Якушко О.С.

**МОРФОМЕТРИЧЕСКАЯ ХАРАКТЕРИСТИКА
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СЛЮННЫХ ЖЕЛЕЗ КРЫС В НОРМЕ И ПРИ
ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ЭТАНОЛОМ**
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В роботі представлені дані морфометричного дослідження при хронічній інтоксикації етанолом. Встановлено, що хронічна інтоксикація етанолом впливає на емнісну ланку гемомікроциркуляторного русла часточок піднижньощелепної слинної залози. На ранніх термінах спостереження визначається розширення венул, що підтверджується достовірним збільшенням зовнішнього діаметру та діаметру просвіту із зменшенням товщини судинної стінки. З дванадцятої доби спостерігається тенденція до відновлення метричних показників. Нормалізація показників до тридцятої доби не визначається.

Ключові слова: хронічна інтоксикація етанолом, щури, слинні залози, венули.

Стаття надійшла 14.03.2018 р.

В работе представлены данные морфометрического исследования при хронической интоксикации этанолом. Установлено, что хроническая интоксикация этанолом влияет на емкостное звено гемомикроциркуляторного русла долек поднижнечелюстной слюнной железы. На ранних сроках наблюдения определяется расширение венул, что подтверждается достоверным увеличением наружного диаметра и диаметра просвета с уменьшением толщины сосудистой стенки. С двенадцатого дня наблюдается тенденция к восстановлению метрических показателей. Нормализация показателей до тридцатого дня не определяется.

Ключевые слова: хроническая интоксикация этанолом, крысы, слюнные железы, венулы.

Рецензент Проніна О.М.