препаратов плодов человека 160,0-450,0 мм теменно-копчиковой длины (4-9-й месяцы внутриутробного развития) с применением комплекса традиционных и новейших методов морфологического исследования (антропометрия, компьютерная томография, морфометрия, изготовление серий последовательных гистологических срезов, микроскопия, трехмерное компьютерное реконструирование, статистический анализ). Установлены закономерности индивидуальной анатомической изменчивости, полово-возрастные и конституционные особенности строения нижней челюсти в плодовом периоде онтогенеза человека. Определены критические периоды морфогенеза нижней челюсти в пренатальном периоде онтогенеза человека. Созданы реконструктивные и математические модели нижней челюсти для выяснения ее функциональной морфологии и ембриотопографии.

Ключевые слова: нижняя челюсть, плод, внутриутробное развитие, человек.

Стаття надійшла 7.05.18 р.

specimens of human fetuses 160,0-450,0 mm of their parietal-coccygeal length (4-9th months of the intrauterine development) were examined using a complex of traditional and up-to-date methods of morphological study (anthropometry, computed tomography, morphometry, making the series of sequential histologic sections, microscopy, three-dimensional computer reconstruction, statistical analysis). The regularities of individual anatomical variability, sex-age and constitutional peculiarities of the mandibular structure in the fetal period of human ontogenesis are determined. Critical periods of ontogenesis of mandibular morphogenesis at the pre-natal term of human ontogenesis are detected. Reconstructive and mathematical models of the mandible to determine its functional morphology and embryo topography are designed.

Key words: mandible, fetus, intrauterine development, human

Рецензент Єрошенко Г.А.

DOI 10.26724/2079-8334-2018-4-66-231-234
UDC 616.316.1:611.018

K.V. Shevchenko, V.I. Garets[1], L.Ya. Fedonyuk[2], K.S. Volkov[2], C.O. Nesteruk[2]
Ukrainian Medical Stomatological Academy, Poltava
[1]SE «Dnipropetrovsk Medical Academy of Health Ministry of Ukraine»
[2]SHEE "Ternopol state medical university named I.Ya. Horbachevskiyi", Ternopol

HISTOPHYSIOLOGY OF SUBMANDIBULAR SALIVARY GLANDS END PIECES IN RATS WITH CHRONIC ETHANOL INTOXICATION

E-mail: kvshevchenko2017@gmail.com

The paper presents the morphometric study of the submandibular salivary gland parenchyma in chronic intoxication with ethanol. It has been established, that chronic ethanol intoxication affects the morphofunctional state of the submandibular gland lobules' end pieces in rats, which is confirmed by the lumen diameter reduction and the epithelial cells height growth up to 15.44 ± 0.41 µm at the early stages of observation. Reduction in the external diameter and the lumen diameter with the reduced epithelial cells height were observed on the thirtieth day of the experiment. This is due to dystrophic changes in the cells of the of the submandibular glands end pieces in rats at the later stages of the experiment, and is confirmed by reduction in their size, the number of secretory granules and the increased optical density of cells.

Key words: chronic intoxication with ethanol, rats, submandibular salivary glands.

The study is a fragment of the research project "Experimental morphological study of of cryopreserved placenta transplants action and other exogenous factors on the morphofunctional status in a number of internal organs", state registration No. 0113U006185.

The alcohol consumption situation in Ukraine is currently quite threatening. Today, the level of alcohol consumption in Ukraine is one of the highest in the world and is about 12-13 liters of absolute alcohol per capita in a year (unofficial statistics reports 20 l). In Ukraine, over 40 thousand people die yearly due to alcohol [9, 6]. Chronic ethanol intoxication is manifested by a wide range of various negative factors effects on the body [3]. Specific receptors sensitive to ethanol do not exist. However, it interacts with many cell components, including extracellular and intracellular receptors located in the membranes of many organs, with secondary mediators of receptors and enzymatic cell systems, which are reflected in the clinical picture of intoxication [1].

All salivary glands of both humans and rats, according to the literature, are based on a single principle and are complex branched, alveolar-tubular glands consisting of end or secretory lobules, and of the outflow ducts system [14]. The function of salivary glands acini lies not only in the production of protein and mucosal secretion, but is also associated with osmotic transmural transfer of large fluid volumes into the end pieces lumens from the surrounding interstitium [5]. The secret of the salivary glands is saliva whose functions are diverse. First of all, it is established that quantitative and qualitative changes in saliva largely determine the teeth resistance to caries [2, 7].

In previous studies it has been established that chronic ethanol intoxication affects the resistive link of the hemomicrocirculatory bed of the rat's submandibular gland lobules, which is determined by vascular

spasm at the early stages of the observation, with an increase in the vascular wall's thickness [6]. The capacitive link of the submandibular salivary gland lobules at the early stages of observation responds by the venules expansion and is confirmed by a significant increase in the external diameter and the lumen diameter with a reduction of the vascular wall thickness [16].

Consequently, studying the patterns of the salivary glands reaction to various stimuli is of great importance, due to the diagnostic value of saliva as a highly informative object for the clinical status assessment of the whole body.

Application of the morphometric method permits an objective assessment of changes in the structural elements of organs after the action of various endogenous and exogenous factors [15].

The purpose of the present study was to establish structural changes of the submandibular glands' end pieces of rats in normal and in chronic ethanol intoxication.

Materials and methods. The work was performed on 45 white outbred rats. The total of 5 animals constituted the control group, being administered isotonic sodium chloride solution 4 times a day, and 40 experimental ones, which were injected intragastrically 4 times a day with 12 mg/kg of 40 ABV ethanol per day (in terms of pure alcohol) [11].

Animals were sacrificed on the 5th, 9th, 12th and 30th days by the thiopental anesthesia overdose (25 mg / kg). Fragments of the submandibular glands were embedded in epon-812 according to the generally accepted procedure [4]. The semi-thin sections were stained with methylene blue [12].

The mean values of the external diameter, lumen and height of the epithelial cells were determined using the Biorex 3 BM-500T microscope with a digital DCM-900 photomicrographic attachment with the research program adapted for the present study. Statistical processing of morphometric data was performed using the Exel software [13].

Animal management and experiments were carried out in compliance with the "General Ethical Rules for Animal Experiments" adopted by the 1st National Congress on Bioethics and with the requirements of the International Principles of the European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes [10].

Results of the study and their discussion. It was found by the morphometric study, that in the control group rats, the mean value of the external diameter in the submandibular glands end pieces was 36,86 ± 1,11 μm, the lumen diameter was 9,17 ± 0,33 μm, and the height of the epithelial cells was 14,74 ± 0, 65 microns (tab.).

Table

Morphometric indices of the submandibular glands end pieces (μm)

End pieces	External diameter	Lumen diameter	Epithelial cells height
Control group (n=5)	36,86±1.11	9,17±0,33	14,74±0,65
The 5th day (n=5)	37,00±1.06	7,92±0,42 *	14,38±0,56
The 9th day (n=5)	38,45±1.23	7,86±0,32 *	15,44±0,41 **
The 12th day (n=5)	34,07±2.23	7,83±0,12 *	13,84±0,81 *,**
The 30th day (n=5)	31,15±1.19 *,**	7,79±0,22 *	11,58±0,38 *,**

Note. * - P <0,05 in compared to the control group; ** - P <0,05 compared to the previous observation period.

In the histological study the end parts of the submandibular gland in the control group rats have a tubular shape and are separated by thin layers of interstitial connective tissue. In the end pieces, two types of cells are defined: seromucous and myoepithelial (fig. 1), it should be noted that the structural feature of these glands is the absence of clearly expressed mucous and serous secretory cells in the end pieces, as all epitheliocytes synthesize mainly a mixed secret.

On the fifth day of ethanol intoxication, the mean external diameter of the end pieces of the submandibular gland does not significantly change and amounts to 37,00 ± 1,06 μm. The lumen diameter is significantly reduced by 13,63% (p <0,05), which makes 7,92 ą 0,42 μm. The height of the epithelial cells does not change, its mean values are 14,38 ± 0,56 μm (table) and do not differ from the values in the control group.

On the ninth day of the experiment, the values of the end pieces external diameter tend to increase up to 38,45 ± 1,23 μm. The lumen diameter on the ninth day is 7,86 ± 0,02 μm, which is 14,29% lower than that in the control group rats (Fig. 2). The height of the epithelial cells compared to that in the control group remains unchanged, and increases by 7,37% (p <0,05) compared to the previous period of the experiment, its mean values making 15,44 ± 0,41 μm (table).

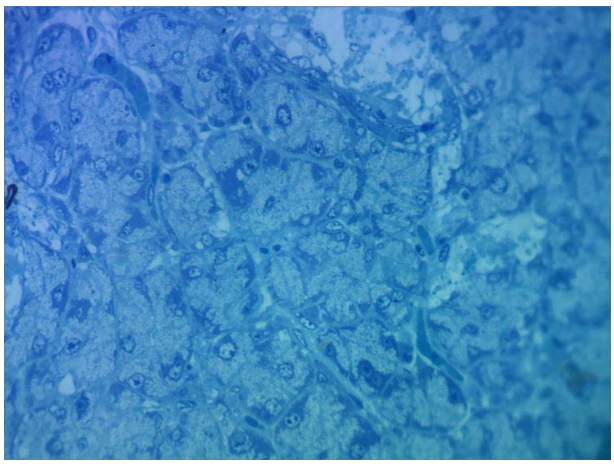

Fig. 1. Serocytes in the end pieces of the submandibular gland parenchyma in the control group rats. Staining with methylene blue. Magn. x400.

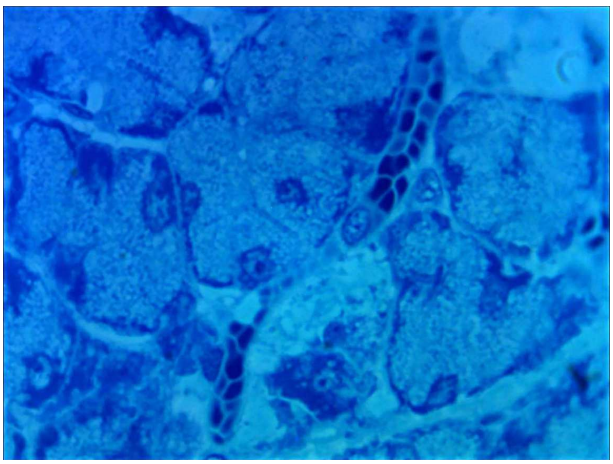

Fig. 2. The end pieces of the rats submandibular gland on the ninth day of the chronic ethanol intoxication experimental model. Staining with methylene blue. Magn. x1000.

On the twelfth day of study, the external diameter mean values of the submandibular salivary glands end pieces are 34,07 ± 2,23 μm, which is by 11,39%, reliably less than the value of the previous experiment and by 7,57% less than that in the control group rats. The lumen diameter is 7,83 ± 0,12 μm, which is by 14,61% reliably less than that in the control group and does not differ from the results of the previous day of the study. The epitheliocytes height amounts to 13,84 ± 0,81 μm and is by 10,36%% reliably less than on the ninth day of the experiment ($p < 0,05$), and its results are less by 6,11% than the values in the control group of animals (table). Seromucous cells are densely located; clearly visible light and dark areas are observed in the cytoplasm; the acini lumen is very small or almost undetectable; increased are interstitial layers of connective tissue in which the mastocytes degranulation and the organelles induration in the cytoplasmic matrix are observed (fig. 3).

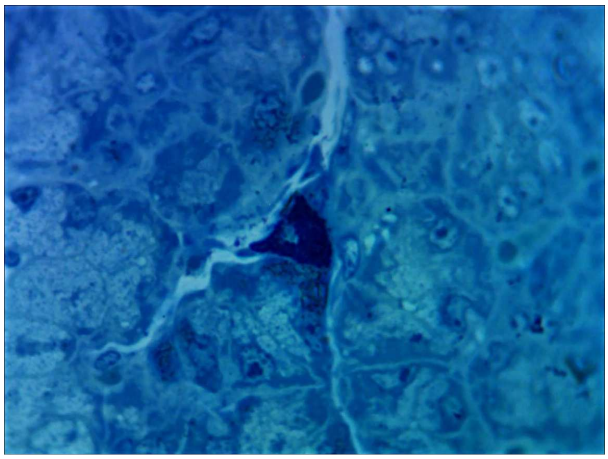

Fig. 3. Parenchyma of the rat submandibular gland on the twelfth day of the of chronic ethanol intoxication experimental model. Staining with methylene blue. Magn. x1000.

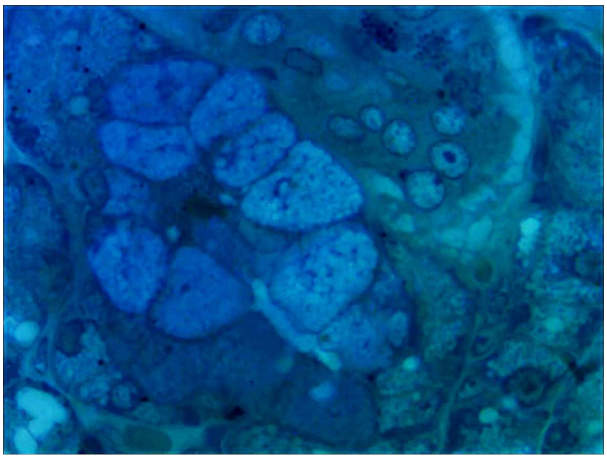

Fig. 4. Sliming of the submandibular gland acinus cells in rats on the 30th day of the chronic ethanol intoxication experimental model. Staining with methylene blue. Magn. x1000.

On the thirtieth day of ethanol intoxication there occurs a significant reduction in the external diameter of the submandibular salivary glands end pieces, their lumen diameter and the decrease in the epithelial cells height. The external diameter is 31,15 ± 1,19 μm, which is by 22,07% less than that on the 12th day of the experiment, being by 15,49% less than the similar value in the control group ($p < 0,05$). The lumen diameter is reduced by 0,52% compared to the twelfth day of the experiment and amounts to 7,79 ± 0,22 μm, which is also by 15,05% less than in the control group. The epithelial cells height on the thirtieth day is 11,58 ± 0,38 μm, being by 31,24% reliably lower than the similar value of the previous study period, and by 21,44% lower than that in the control group rats ($p < 0,05$) (table). The serocytes are smaller in size, on the basal parts of the cells there are interspaces with clefts, strongly developed interstitial connective tissue and well-visible parts of the acini, which have undergone complete sliming, got flattened and adjacent to the basement membrane by nuclei, turbid and foamy cytoplasm. Between them and the normal serocytes, a group of cells is noted with a dark cytoplasm, almost ivisible dark nuclei and a fuzzy boundary between the cells, which are clearly a transitional stage to complete sliming (fig. 4).

Conclusion

Chronic ethanol intoxication causes structural changes in the parenchyma of the submandibular salivary gland, which are manifested at the early stages of observation by the increased secretory activity of the end pieces cells. Inhibition of secretion is determined by the twelfth day of the experiment, which is morphometrically confirmed by the reliable decrease of the epithelial cells height by 6,11%, as compared to the control group. On the thirtieth day of observation, dystrophic changes in glandular cells and rearrangement of the secretory apparatus for the carbohydrates synthesis were established.

References

1. Afanasyev VV, Rubitel LT, Afanasyev LV. Ostraya intoksikatsiya etilovym alkogolem. S-Peterburg. 2002; 10-21. [in Russian]
2. Barchuk RR, Popadynets OH, Pastukh MB, Hryshchuk MI, Dubyna NM. Morfofunktsionalni osoblyvosti velykykh slynnykh zaloz v umovakh normy ta patolohiyi. Visnyk problem biolohiyi i medytsyny. 2016; 2: 1(128):318-22. [in Ukrainian]
3. Belov AA. Toksicheskoye deystviye alkogolya na krov. Eritrotsity i alkogol. Vestnik AGIUV. 2013; 4: 75-78. [in Russian]
4. Karupu VYa. Electronna microskopiya. Kyiv:Vyscha shkola; 1984. 207 p. [in Ukrainian]
5. Kostilenko YuP. Bazisnaya funktsiya slyunnykh zhelez. Poltava. 1999; 55 s. [in Russian]
6. Pishchenko H., Minchenko S. Viktymolohichni aspekty nehatyvnykh sotsialnykh yavyshch, povyazanykh iz zlochynnistyu. Pravo Ukrayiny. 2006; 5: 108. [in Ukrainian]
7. Timofeyev AA. Sekretornaya funktsiya slyunnykh zhelez u bolnykh s ostrymi odontogennymi vospalitelnymi zabolevaniyami chelyustey Sovremennaya stomatologiya. 2011; 4: 70-4. [in Ukrainian]
8. Shevchenko KV, Silkina YuV, Volkov KS. Morfometrychna kharakterystyka rezystyvnoyi lanky hemomikrotsyrkulyatornoho rusla slynnykh zaloz shchuriv pry khronichniy intoksykatsiyi etanolom., 2018; Morfolohiya. 12 (1), 51-54. [in Ukrainian]
9. Yahodynskyi VV. Profilaktyka nehatyvnykh yavyshch sered uchnivskoyi ta studentskoyi molodi. Kyiv: vydavnytstvo yevropeyskoho universytetu; 2003. 168 s. [in Ukrainian]
10. European convention for the protection of vertebrate animals used for experimental an others scientific purposes/ Strasbourg: Council of Europ, 1986. No 123.52p.
11. Ivanochko VM. Morphological state of structural components of the filtration and reabsorption barrier of the kidneys under normal conditions and at chronic alcoholization with drinks of various quality and strength]: extended abstract of Cand. Sci. Dissertation: speciality 14.03.01 "normal anatomy". Ternopil, 2003. 19 p.
12. Kazakova KS, Starchenko II, Yeroshenko GA. Method of semi-thin sections staining. Technical Innovation Certificate No.1880 issued by the Ukrainian Medical Stomatological Academy; 1999. September 15.
13. Lapach SN, Chubenko AV, Babich PN. Statistical methods in medical and biological research using Exel. Kyiv: Morion; 2000. 320 p.
14. Wong RJ, Randolph GW. Morphophysiology of the salivaryglands. Otolaryngology. Basel: Karger; 2006. 634 – 643.
15. Yeroshenko GA, Kostylenko YuP, Skrypnikov MS. Correlation relationship between the morphometric indices of large salivary glands of rats under normal conditions and after stimulation of the peripheral nervous system. World of Medicine and Biology. 2009; 3(1): 64-69.
16. Yerochenko GA, Shevchenko KV, Yakushko OS. Morphometric characteristic of rat salivary glands hemomicrovasculature capacity component under normal conditions and in ethanol chronic intoxication. World of medicine and biology. 2018; 3 (65), 149-152.

Реферати

ГІСТОФІЗІОЛОГІЯ КІНЦЕВИХ ВІДДІЛІВ ЧАСТОЧОК ПІДНИЖНЬОЩЕЛЕПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПРИ ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

Шевченко К.В., Гарець В.І., Федонюк Л.Я., Волков К.С., Нестерук С.О.

В роботі представлені дані морфометричного дослідження паренхіми піднижньощелепної слинної залози при хронічній інтоксикації етанолом. Встановлено, що хронічна інтоксикація етанолом впливає на морфофункціональний стан кінцевих відділів часточок піднижньощелепної залози щурів, що підтверджується зменшенням діаметру просвіту та збільшенням висоти епітеліоцитів до 15,44±0,41мкм на ранніх стадіях спостереження. Зменшенням діаметру зовнішнього та діаметру просвіту із зменшенням висоти епітеліоцитів на тридцяту добу експерименту. Що обумовлене дистрофічними змінами в клітинах кінцевих відділів піднижньощелепних залоз щурів на пізніх стадіях експерименту, та підтверджується зменшенням їх розмірів, кількості секреторних гранул та підвищенням оптичної щільності клітин.

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

Стаття надійшла 20.03.18 р.

ГИСТОФИЗИОЛОГИЯ КОНЕЧНЫХ ОТДЕЛОВ ДОЛЕК ПОДНИЖЕЧЕЛЮСТНЫХ СЛЮННЫХ ЖЕЛЕЗ КРЫС ПРИ ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ЭТАНОЛОМ

Шевченко К.В., Гарець В.И., Федонюк Л.Я., Волков К.С., Нестерук С.О.

В работе представлены данные морфометрического исследования паренхимы поднижнечелюстной слюнной железы при хронической интоксикации этанолом. Установлено, что хроническая интоксикация этанолом влияет на морфофункциональное состояние конечных отделов долек поднижнечелюстной слюнной железы крыс, что подтверждается уменьшением диаметра просвета и увеличением высоты эпителиоцитов до 15,44±0,41мкм на ранних стадиях наблюдения. Уменьшением наружного диаметра и диаметра просвета с уменьшением высоты епителиоцитов на тридцатые сутки эксперимента. Что объясняется дистрофическими изменениями в клетках конечных отделов поднижнечелюстных слюнных желез крыс на поздних стадиях эксперимента и подтверждается уменьшением их размеров, количества секреторных гранул с увеличением оптической плотности клеток.

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

Рецензент Єрошенко Г.А.