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ORIGINAL ARTICLES / PRACE ORYGINALNE

ORIGINAL ARTICLE
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REMODELING OF THE DUCT SYSTEM OF THE RAT SUBMANDIBULAR SALIVARY GLANDS IN CHRONIC ETHANOL INTOXICATION

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ABSTRACT

The aim: To determine the dynamics of changes in metric parameters of the duct system of rat submandibular glands in normal conditions and chronic ethanol intoxication.

Materials and methods: 50 albino outbred rats were involved in the experiment. 10 animals were assigned in the control group, 40 animals – in the experimental group. Animals were sacrificed on 5, 9, 12 and 30 days by overdose of thiopental anesthesia. Pieces of the submandibular glands were embedded into epon-812 according to the conventional technique.

Results: On day 5 of the experiment the lumen diameter of intercalated duct reduced by 9,15 % ($p < 0,05$). The lumen diameter of the striated ducts was by 5,29 % significantly greater than the values in controls ($p < 0,05$). The lumen diameter of the granular ducts reduced by 2,45 % ($p < 0,05$). On day 30 of the experiment the height of the epithelial cells of the intercalated ducts was by 8,47 % significantly less ($p < 0,05$), the height of the epithelial cells of the striated ducts was by 12,27 % less ($p < 0,05$) and the height of the epithelial cells of the granular ducts was by 11,96 % less ($p < 0,05$) than the values in controls.

Conclusions: No recovery of parameters occurs by day 30 of the experiment, indicating the depletion of the secretory epithelium of the duct system, due to dystrophic changes caused by vascular disorder in the microvasculature.

KEY WORDS: salivary gland, duct, rats, ethanol

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INTRODUCTION

Major salivary glands are located outside the oral cavity, and enter it via the excretory ducts [1]. The system of the excretory ducts of the salivary gland lobes consists of intercalated, striated and collecting excretory ducts [1, 2]. Intercalated ducts located between the acini and striated ducts. They are lined by the low-cuboidal or flattened epithelial cells with a poorly expressed organelle apparatus. Epithelial cells with clear cytoplasm are characterized by the presence of dense granules with mucoid secretion on the apical part of the cell [3]. According to the foreign publications, these granules are more often found in the cells of the ducts, adjacent to the acini. The outer layer of cells in the intercalated ducts is formed by the fusiform myoepithelial cells. Noteworthy, intercalated ducts contain cambial elements of the acini and the system of excretory dusts [4]. These elements are differentiated into the glandular cells or duct cells, enabling regeneration of above mentioned parts of the glands [5].

Striated ducts are presented in the form of wide tubules, lined by oxyphilic highly prismatic cells with orbicular centric nuclei. Apical part of the cell protrudes into the wide lumen and lined by short microvilli. It accumulates the secretory granules, which mainly contain kallikrein [6]. G.A. Yeroshenko reports that it is precisely these cells that are involved in the development of a number of substances and growth factors that are secreted by human salivary glands. Importantly, in rodents, and above all, in rats, these and other biologically active substances are produced more actively than in humans [7]. In the lobules of the rat submandibular glands, secretory granules in the epithelial cells of the granular ducts contain kallikrein and ensure local mechanisms for regulating blood flow to the vascular system of the salivary glands [8]. Salivary glands produce the saliva, which plays a significant role in maintaining the homeostasis of the oral cavity [9, 10]. Recently, the interest of researchers in the study of the patterns of the salivary glands response to various stimuli has increased significantly, which is due to the diagnostic value of saliva as a highly informative object for the clinical assessment of the state of the overall health [11].

The World Health Organization reports about 2.5 million alcohol-related deaths worldwide, accounting for 4% of all deaths, and alcohol is a causative factor for general illness and injuries. Currently, the alcohol situation in Ukraine is quite devastating. [12, 13]. Chronic ethanol intoxication is manifested by a wide range of effects of various negative factors on the body. Currently, alcohol remains one of the most common toxic factors in everyday life. [14].

Objectification of the findings of the study is achieved by the morphometric method, which enables detection

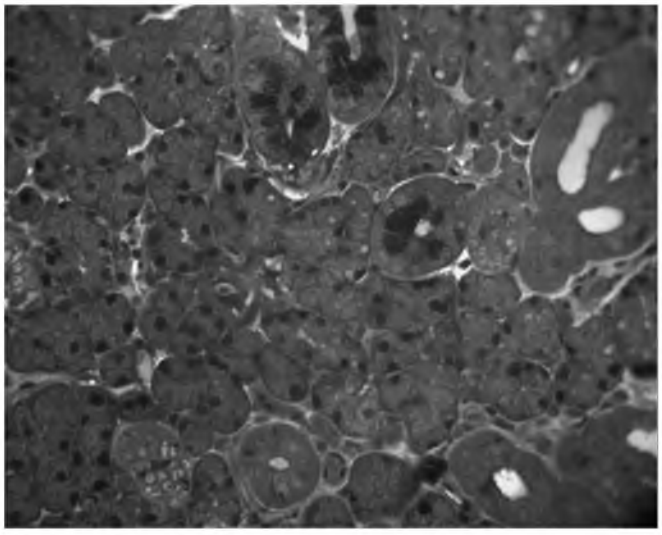

Fig. 1. Intercalated duct of the parenchyma of the lobule of the rat submandibular gland on day 9 of the experiment. Semi-thin section. Methylene blue stain. 400×magnification.

of changes in the structural elements of organs after the effect of various endogenous and exogenous factors [15].

THE AIM

The paper was aimed at the determination of the dynamics of changes of metric parameters of the duct system of the rat submandibular glands in normal condition and chronic ethanol intoxication.

MATERIALS AND METHODS

50 albino outbred rats were involved in the experiment. 10 animals were assigned in the control group and were administered with isotonic saline solution QID, delivered directly into the stomach. 40 animals were assigned in the experimental group, who were administered with 12 mg/kg ethanol 40° QID, delivered directly into the stomach [16]. Animals were withdrawn from the experiment on day 5, 9, 12 and 30 by thiopental anesthesia overdose (25 mg / kg). Pieces of the submandibular glands were embedded into epon-812 according to the conventional technique [17]. Semi-thin sections were stained with methylene blue. The mean values of the outer diameter, the diameter of the lumen of the ducts and the height of the epithelial cells were determined using a Biorex-3 BM-500T microscope with a digital microphotohead DCM with software, adapted to these studies. Statistical processing of morphometric data was performed using the Exel program [18]. Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics and the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [19].

RESULTS AND DISCUSSION

Morphometric study of the intercalated ducts of the submandibular glands of rats of control group has established that their outer diameter was 18,68±1,07 μm and the lumen diameter was 3,28±0,02 μm. The height of the epithelial cells was 7,08±0,07 μm (Table I).

On day 5 of the experiment the outer diameter of the intercalated ducts reduced by 9,04 % compared to the controls ($p< 0,05$). The lumen diameter reduced by 9,15 %. The height of the epithelial cells was 7,06±0,04 μm, showing no significant difference from the parameters in the controls ($p< 0,05$) (Table I).

On day 9 of the experiment it was established that the value of the outer diameter of the intercalated ducts of the rat submandibular glands no significant difference from the findings of the previous time period of the experiment ($p< 0,05$); however, it was by 11,64 % less than the values in the controls. The lumen diameter of the intercalated ducts reduced by 1,34 % compared with the day 5 of the experiment, though was significantly less by 10,37 % compared with the values in controls ($p< 0,05$). The height of the epithelial cells reduced by 4,96 % compared with the day 5 of the experiment that was significantly less by 5,22 % compared with the values in controls ($p< 0,05$). Its mean values were 6,71±0,06 μm (Table I).

The wall of the intercalated duct was formed by the cuboidal cells with basophilic cytoplasm and eccentric nucleus; the lumen of the duct was narrowed (Fig. 1).

On day 12 the outer diameter of the intercalated ducts was by 12,74 % significantly less than the values in controls ($p< 0,05$). The lumen diameter enlarged by 3,74 % compared with the values of day 9; however, it was by 7,01 % less than the value in controls ($p< 0,05$). The height of the epithelial cells reduced by 1,19% compared with the values of day 9 of the experiment and was by 6,63% significantly less than the values in control rats ($p< 0,05$) (Table I).

Table I. Morphometric parameters of the intercalated ducts of the submandibular glands (μm)

Intercalated ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	18,68±1,07	3,28±0,02	7,08±0,07
Day 5	16,99±1,05 *	2,98±0,02 *	7,06±0,04
Day 9	16,58±1,03 *	2,94±0,01 *,**	6,71±0,06 *,**
Day 12	16,30±1,03 *	3,05±0,01 *,**	6,63±0,04 *,**
Day 30	16,18±1,04 *	3,06±0,01 *	6,48±0,05 *,**

Note: * - $p < 0,05$ compared to the controls; ** - $p <0,05$ compared with the previous time period of observation.

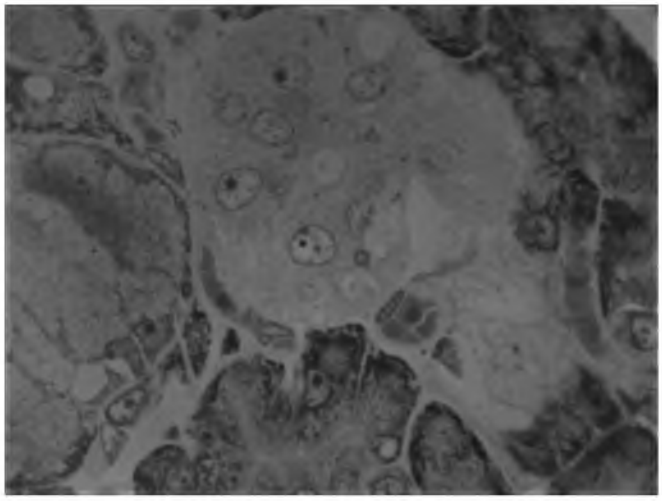

Fig. 2. Striated duct of the lobules of the rat submandibular gland on day 12 of the experiment. Methylene blue stain. 1000×magnification.

On day 30 of the experiment the outer diameter of the intercalated ducts of the submandibular glands differed insignificantly from the value of the previous time period of the experiment and was by 13,38 % significantly less than the values in control animals (p< 0,05). Similarly, the lumen diameter differed insignificantly from the value on day 12 of the experiment, though was by 6,71 % significantly less than the findings in controls (p< 0,05). The height of the epithelial cells was by 2,26 % less than the values in the previous time period of the experiment and by 8,47% significantly less than the values in controls (p< 0,05) (Table I).

The morphometry of the striated ducts of the submandibular glands of rats of control group has established that their outer diameter was 34,80±2,05 μm and the lumen diameter was 5,10±0,03 μm. The height of the epithelial cells was 14,43±1,07 μm (Table II).

On day 5 of the observation the outer diameter of the striated ducts enlarged insignificantly. The mean value of the lumen diameter was by 5,29 % significantly greater than the values in controls. The height of the epithelial cells was almost similar to the values in control rats (p< 0,05) (Table II).

On day 9 of the experiment the values of the outer diameter of the striated ducts of the submandibular glands did not differ from the values of the previous time period of the experiment and its values in controls (p< 0,05). The lumen diameter changed insignificantly compared with the values of the previous time period of the experiment; however, it was by 6,67 % significantly greater than the values in controls (p< 0,05). The height of the epithelial cells was by 10,26 % significantly greater than the values in control animals (p< 0,05) (Table II).

On day 12 of the experiment the outer diameter of the striated ducts of the submandibular glands reduced by 11,54 % compared with the previous findings of the experiment and differed insignificantly from the values in control rats (p< 0,05). The lumen diameter enlarged by 2,02 % compared with day 9 and was by 8,82 % significantly greater than its value in controls (p <0,05). The height of the epithelial cells of the striated ducts of the submandibular glands was by 12,88 % significantly less than the findings of day 9 of the experiment with no significant difference from the values in controls (p <0,05). (Table II).

The wall of the striated duct was formed by the clear epithelial cells. The nuclei were centric. Vacuoles were not numerous. The cytoplasm was homogenous. The basal striation was poorly identified (Fig. 2).

On day 30 of the experiment the outer diameter of the striated ducts was with no significant difference from the values of the previous time period of the observation and values in controls (p <0,05). The lumen diameter enlarged by 2,16 % compared with day 12 of the experiment and was by 11,18 % significantly greater than the values in controls (p<0,05). The height of the epithelial cells of the striated ducts of the submandibular glands was by 8,66 % significantly less than the values of the previous time period and by 12,27 % less than the values in control animals (p <0,05) (Table II).

The wall of the striated ducts was formed by the highly prismatic cells. The lumen of the ducts of rat submandibular glands was filled with optically clear secret. In the basal segments of the epithelial cells of the striated ducts vacuole-like extensions were visualized that become larger in size during the experiment (Fig. 3).

Morphometric study has established that the mean values of the outer diameter of the granular ducts of rats of control group were 38,38±0,05 μm, the lumen diameter was 8,56±0,06 μm, and the height of the epithelial cells was 15,47±0,43 μm (Table III).

On day 5 of the experiment the outer diameter of the granular ducts of the submandibular glands differed insignificantly from the values in control rats, whereas the lumen diameter reduced by 2,45 %. The height of the epithelial cells was by 12,61 % greater than the values in control group of animals (p <0,05). On day 9 of the experiment

Table II. Morphometric parameters of the striated ducts of the submandibular glands (μm)

Striated ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	34,80±2,05	5,10±0,03	14,43±1,07
Day 5	37,12±2,11	5,37±0,04 *	15,76±1,06
Day 9	37,61±1,94	5,44±0,05 *	15,91±1,09 *
Day 12	33,27±1,81 **	5,55±0,05 *,**	13,86±1,09 **
Day 30	33,48±1,72	5,67±0,03 *,**	12,66±1,09 *,**

Note: * - p < 0,05 compared to the controls; ** - p <0,05 compared with the previous time period of observation.

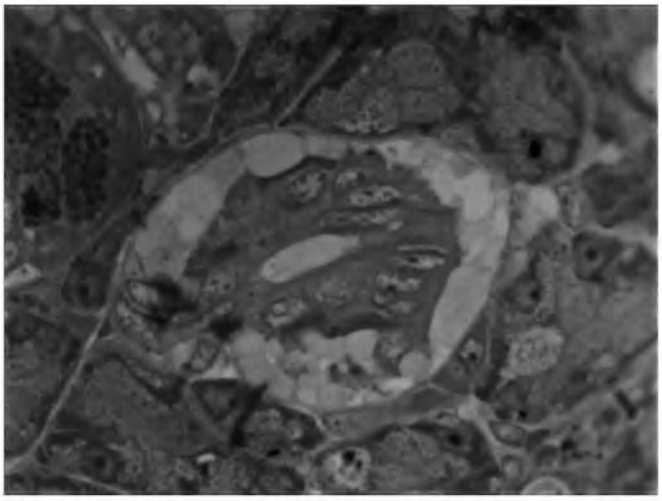

Fig. 3. Vacuoles in the basal part of the epithelial cells of the striated duct on day 30 of the experiment. Semi-thin section. Methylene blue stain. 1000×magnification.

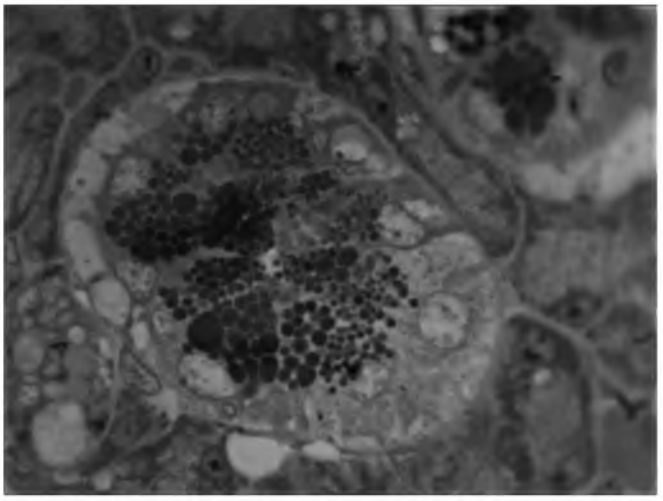

Fig. 4. Granules in the epithelial cells of the granular ducts in the lobules of the rat submandibular glands on day 12 of the experiment. Semi-thin section. Methylene blue stain. 1000×magnification.

Table III. Morphometric parameters of the granular ducts of the submandibular glands (μm)

Granular ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	38,38±0,05	8,56±0,06	15,47±0,43
Day 5	39,31±0,10	8,35±0,08 *	17,42±0,36 *
Day 9	41,19±0,17 *,**	9,07±0,09 *,**	17,91±0,54 *
Day 12	45,23±0,17 *,**	9,83±0,08 *,**	18,52±0,46 *,**
Day 30	33,58±0,05 *,**	9,36±0,07 *,**	13,62±0,35 *,**

Note: * - $p < 0,05$ compared to the controls; ** - $p < 0,05$ compared with the previous time period of observation.

the mean value of the outer diameter of the granular ducts of the rat submandibular glands was by 4,78 % greater than the values on day 5 and by 7,32 % greater than the values in controls ($p < 0,05$). The lumen diameter was by 8,62 % greater than the values of the previous time period of the experiment and by 5,96 % greater than the findings in the control group of animals. The height of the epithelial cells was differed insignificantly from the values of the previous time period of the experiment; however it was by 15,03 % significantly greater compared with findings in controls ($p < 0,05$). On day 12 of the experiment the outer diameter was by 9,81 % significantly greater than the values on day 9 and by 17,85 % greater than the values in controls ($p < 0,05$). The mean value of the lumen diameter was by 8,38% greater than the values of the previous time period of the experiment and by 14,84 % greater than the values in control group of animals ($p < 0,05$). The height of the epithelial cells was by 3,41 % greater than the value on day 9 of the experiment and by 19,72 % significantly greater than the findings in control rats ($p < 0,05$) (Table III).

The wall was formed by the single layer of the secretory columnar epithelial cells of variable size and amount that contained optically dense basophilic granules in the cytoplasm. In the basal segments of the epithelial cells of the granular ducts the vacuole-like extensions were visualized. The eccentric nuclei were located in the basal segment of the epithelial cells (Fig. 4).

On day 30 of the experiment the mean value of the outer diameter of the granular ducts of the rat submandibular glands was by 25,76 % significantly less than the values on day 12 and by 12,51 % less than the value in controls ($p < 0,05$). The lumen diameter also reduced by 4,78 % compared to day 12 and enlarged by 9,35 % compared with controls ($p < 0,05$). The height of the epithelial cells of the granular ducts of the submandibular glands was by 26,46 % less than the value in the previous time period of the experiment and by 11,96 % less than the findings in controls ($p < 0,05$) (Table III).

The previous experiments showed multidirectional response of the duct system on the state of salivary glands under the influence of various exogenous factors. In that case, on day 14 of the experiment under the effect of methacrylate the mean values of the outer diameter and lumen diameter of the intralobular ducts decreased, tending to reduce in size to the end of the experiment, which was the result of hyperhydration of the amorphous substance due to microcirculation disorder [20], in contrast to the effect of ethanol, when similar changes occurred in the intercalated ducts, which also responded by persistent narrowing with reduce in the height of the epithelial cells due to microcirculation disorder in the exchange section, which is confirmed by the reduced outer diameter by 19.5%, lumen diameter by 12.5% and thinning of the vascular wall by 30.4%, [21], which resulted in hyposalivation, since intercalated ducts regulate the quantitative aspect of salivation.

Noteworthy, the mean values of the outer and inner diameters of the striated and granular ducts increased with the subsequent regeneration, due to the compensatory reaction to the narrowing of the intercalated ones to increase the amount of saliva through the juxtacellular transport of liquid from the surrounding interstitium on the side of the striates ducts [9] and the provision of system of local mechanisms for regulating blood flow to the vascular system due to granular ducts with kallikrein granules, leading to depletion of the epithelial cells of the duct system of the submandibular salivary glands at the end of the experiment. It was confirmed experimentally by a decrease in the mean values of the height of the epithelial cells compared to the values in control group of rats, though tending to recovery since ethanol is a metabolite in contrast to methacrylate, which acts as a toxic substance, and the location of the submandibular glands outside the oral cavity, affected indirectly through the vessels of the microvasculature.

CONCLUSIONS

To conclude with, the duct system responds to the effect of chronic ethanol intoxication, which at the initial stage of the experiment is confirmed by the narrowing of the outer and inner diameters with reduced height of the epithelial cells of the intercalated ducts, enlargement of the outer and inner diameter with increased height of the epithelial cells of the striated and granular ducts with a tendency to regeneration in the second half of the experiment. However, no recovery of the parameters by day 30 of the experiment was registered, which obviously indicates the depletion of the secretory epithelium of the duct system, due to dystrophic changes caused by vascular disorder in the microvasculature, confirmed by changes in the diameters of the walls of the ducts with a decrease in the height of epithelial cells.

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