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MORPHOMETRIC CHARACTERISTICS OF STRUCTURAL CHANGES IN THE MAJOR SALIVARY GLANDS OF RATS DURING THE CHRONIC ALCOHOL POISONING

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ABSTRACT

The aim of the study was to establish the characteristics of restructuring of salivary glands of rats with chronic alcohol intoxication and their quantitative morphometric characteristics.The experiments were performed on rats, which were forcibly intragastricaly administered 25% solution of ethanol in a dose of 1.5% of body weight. Morphological studies were performed in 5, 9, 12, 16, 24, 28 days from the beginning of the experiment. Morphometric study included determination of square of nuclei and cytoplasm of serocytes; nuclear-cytoplasmic ratio; number of cells in an area of acini.During the study we found that chronic alcohol intoxication caused a restructuring of acini cells and stromal of the studied organs. The restructuring in the early stages of alcohol intoxication (from 5 to 9 days of observation) manifested a strong progressive hyperplasia of acini with the extension of the striped ducts and simultaneous narrowing of the intercalated duct size, due to the pressure of swollen cells of acini, that was especially characteristic for mandibular salivary gland. The cells of acini of both types of glands were increased in volume by increasing of hydrated cytoplasm.Between 12 to 16 days of the experiment we observed a certain stabilization of identified processes. However. The further observations with increasing duration of chronic alcohol poisoning revealed that secretory units of salivary glands, on the contrary, gradually exposed to the atrophic changes. This simultaneously increased the layer of connective tissue, a large number of fatty inclusion were observed in the glandulocytes. Atrophic changes were also observed in epithelial cells that line the ducts of the salivary glands.It should be noted that these processes in glands of both locations proceeded the same way, but the intensity of their development was different. More intense changes manifested in the parotid glands, which may be due to the morphological and functional of the differences discovered glands.The estimated histological features of the restructuring of the salivary glands and their differences depending on the type and location of gland had their objective quantitative morphometric confirmation.

Key Words:- Hyperemia, Edema, Atrophy, Sclerosis.

INTRODUCTION

Today, dentists increasingly face in their daily practice of oral pathology, caused by alcohol abuse (Riedel *et al.*, 2005 and Nuzhnyy *et al.*, 2003). A number of scientific studies confirmed that oral homeostasis is determined primarily by the functional activity of salivary glands and composition of oral fluid and the state of mucosa (Shepitko *et al.*, 2011). Specifically was found that in prolonged use of alcoholic beverages as a result of destruction of the salivary glands is reduced saliva production and developing obesity of parotid glands and, consequently, they increase in size (Barclay *et al.*, 2008).

Therefore, the study of structural changes in tissues of salivary glands may be important for the diagnosis and treatment of chronic alcohol poisoning.The

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purpose of the study. To estimate the features of structural adjustment of salivary glands of rats with chronic alcohol intoxication and give them a quantitative morphometric characteristics.

MATERIALS AND METHODS

Experiments were performed on 36 rats, which were forcibly administered intragastricaly the 25% solution of ethanol in a dose of 1.5% of body weight (at the rate of 4 g of 96% ethanol per 1 kg of body weight) (Alekseenko *et al*., 2013). The control group consisted of 12 intact animals. Morphological studies were performed in 5, 9, 12, 16, 24, 28 days from the beginning of the experiment. The histological sections of pieces of tissue of mandibular and parotid salivary glands were fixed with 10% neutral formalin, stained with hematoxylin and eosin, resorcinol-fuchsin by Veygert, as well as Van Hizon.

Morphometric study included the determination of square of nuclei and cytoplasm of serocytes; the number of cells in an area of acini. Sectional area of the cytoplasm of serocyte determined by the formula: Sc - Sn where Sc - sectional area of the cell, Sn - sectional area of nuclei. Defined as the nuclear-cytoplasmic ratio (ncr) in serocytes of acini. (Semenova *et al*., 2012 and Gubina-Vakulik *et al*., 2013).

The statistical analysis of the results was performed by variational statistics using the program «Microsoft Excel». We determined the average value (M), standard deviation (δ) and the average error (m). Statistical analysis of the results of research was carried by "Microsoft® Office Exel 2003". Typing and adjustments made using text editor "Microsoft® Office Word 2003". These software products are licensed.

All the experimental studies were conducted in compliance with the "Rules of work using experimental animals," as evidenced by the committee on bioethics SHEI "Ternopil State Medical University named after I. Horbachevskyi of Ministry of Health of Ukraine "(protocol number 24 of 27 August 2014).

RESULTS

When modeling the chronic alcohol poisoning in rats in the early stages of the experiment were recorded a tangible violation of organ hemodynamics in the bloodstream of major salivary glands. These disorders are manifested, first of all, by the expressed venous congestion. The arterial route responded by the increased tone with the narrowing of the lumen and the decreasing of capacity of small arteries with the simultaneous compensatory expansion and increase in capacity of larger caliber arteries. Later there was a progressive increase of uplink vasoconstriction with the corresponding decrease of capacity of intraorganic arteries at all levels of branching.

The revealed changes in the bloodstream were accompanied by an appropriate restructuring of acini cells and stromal of the studied organs. Such a restructuring in the early stages of alcohol intoxication (from 5 to 9 days of observation) manifested a strong progressive hyperplasia of acini with the extension of striped ducts while narrowing of size of intercalated ducts due to pressure of swollen acini cells that was especially characteristic for mandibular salivary glands (Fig. 1). The cells of acini of both types of glands were increased in volume by increasing their volume of hydrated cytoplasm.

Further observations with the increasing of duration of chronic alcohol poisoning revealed that secretory units of salivary glands, on the contrary, gradually exposed to the atrophic changes. This simultaneously increased the layer of connective tissue, a large number of fatty inclusion was detected in glandulocytes. Atrophic changes were observed also in the epithelial cells that line the ducts of the salivary glands (Fig. 2).

When conducting the quantitative analysis of morphometric parameters of acini of the parotid and mandibular glands of rats during the chronic alcohol poisoning are three periods of change.

The first period, early - up to 9 days of experimental observation during which, mainly by increasing the volume of the cytoplasm because of its hydration, the acini cell area increased and the acini themselves with simultaneous tendency to reduce the area of nuclei from which the nuclear cytoplasmic ratio also declined. The number of cells in the acinus did not increase, thus the hyperplasia of acini did not occur.

Second period - from 9 to 12 a day - adaptive, as it was characterized by the stabilization of processes, and even a partial restoration of studied parameters.

Third period - from 16 days to complete the observation on the 28 day of chronic alcohol intoxication can be estimated as the development of decompensation. This period was characterized by the progression of atrophic processes. This was manifested as a decrease in nuclear square, and a progressive decrease in the square of serocytes and acini formed by their clusters. And if on the 9-day, basic indicators significantly exceeded target figures, on the 28 day of observation they were significantly below the initial data (Table. 1 and 2).

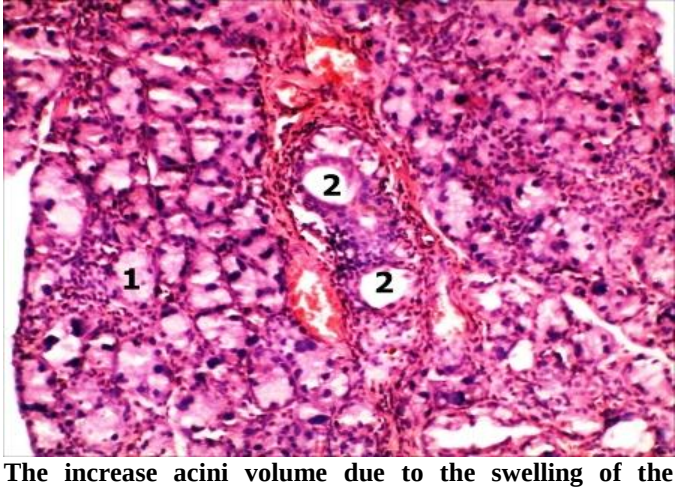
Table 1. Morphometric indices of acini cells of the parotid salivary glands of rats with chronic alcohol intoxication (M ± m)

The duration of the observation	Parameter					
	Suare of nucleus (mcm²)	Cell square (mcm²)	Cell square without nucleus (mcm²)	ncr %	number of serocytes on the cut of acinus	cross-sectional area of acinus (mcm²)
Control	9,48±0,54	59,44±0,82	49,97±1,01	19,06±1,34	5,17±0,31	220,06±5,05
5 days	8,62±0,68	65,82±0,74*	57,19±0,43*	15,09±1,21	5,00±0,58	242,61±6,94
9 days	8,27±0,60	67,92±0,84*	59,65±0,28*	13,85±0,96*	5,17±0,60	246,27±6,57*
12 days	9,13±0,62	63,42±1,18	54,29±0,71*	16,79±1,03	5,33±0,67	238,83±5,03
16 days	8,77±0,52	59,39±1,33	50,63±0,84	17,46±0,63	4,83±0,60	228,93±5,49
24 days	7,77±0,58	55,41±0,88*	49,99±1,23	15,45±0,83	4,50±0,62	208,80±4,52
28 days	7,58±0,42*	52,09±0,96*	44,51±0,60*	16,99±0,76	4,33±0,49	200,40±5,25
Note: * - p < 0,05 comparing to control						

Table 2. Morphometric indicators of acini cells of mandibular salivary glands of rats with chronic alcohol intoxication (M ± m)

The duration of the observation	Parameter					
	Suare of nucleus (mcm²)	Cell square (mcm²)	Cell square without nucleus (mcm²)	ncr %	number of serocytes on the cut of acinus	cross-sectional area of acinus (mcm²)
Control	19,18±1,04	98,96±1,04	79,78±0,68	24,07±1,40	12,67±0,67	348,65±5,04
5 days	18,15±0,87	106,18±1,41*	88,03±0,57*	20,59±0,87	13,00±0,68	380,28±6,68*
9 days	17,41±0,90	109,80±1,78*	92,39±1,09*	18,83±0,86*	12,50±0,67	388,38±6,59*
12 days	18,15±0,87	101,38±2,74	83,24±1,95	21,75±0,65	12,33±0,76	368,86±6,94
16 days	18,45±1,18	96,97±1,75	78,52±0,65	23,45±1,35	12,00±0,73	350,98±7,59
24 days	17,05±1,57	91,69±1,80*	74,64±0,57*	22,83±2,06	11,67±0,67	334,49±5,66
28 days	17,21±1,19	89,70±1,00*	72,50±0,44*	23,76±1,73	10,83±0,60	329,13±6,12
Note: * - p < 0,05 comparing to control						

Figure 1. The histological section of mandibular salivary gland of rat after 5 days of chronic alcohol intoxication. Stained with hematoxylin and eosin. x 140.



The increase acini volume due to the swelling of the cytoplasm of cells that form them – 1, extension of the duct lumen of the striped ducts - 2.

Figure 2. The histological cut of the parotid salivary gland of rat after 28 days of chronic alcohol intoxication. Stained with hematoxylin and eosin. x 120.

Connective tissue sheath surrounding the tubular gland system – 1, full-blooded vein - 2, extended striped duct - 3, extended push duct - 4.

It should be noted that these processes in both glands proceeded the same way, but the intensity of their development was different. More intense this changes manifested in the parotid glands, which may be due to the morphological and functional differences of the discovered glands. Thus, if on the 9 day of observation in the serocytes of the parotid salivary gland the cut area of cells has grown on 14%, the area of the cytoplasm - 19% and acini area - 12% with the reduction in cut area of nucleus by 13% and reducing the nuclear-cytoplasmic ratio on 27% , in the mandibular gland the growth of the first group of parameters constituted 11%, 16% and 11% respectively, while lowering the second group of parameters reached 9% and 12% respectively.

Concerning the third observation period, the atrophic processes also proceeded more rapidly in cells and acini of the parotid salivary gland. Thus, if on the 28 day of observation in the secretory cells of the parotid salivary gland the cut area compared with the control now has decreased on 9%, the area of the cytoplasm - 11% acini area - 9%, while the further reducing of nuclear cut area on 20% and decrease of nuclear-cytoplasmic ratio on 11% (less than the intensity of the decline in this indicator compared to 9 days is caused by the severe atrophic processes of cytoplasm with a significant decrease in its volume), in the mandibular salivary gland the intensity of reduction in morphometric parameters of the first group was only 10%, 9% and 6% respectively, while the lowering of the second group of indicators does not exceed 10% and 2% respectively.

DISCUSSION

In the course of our investigation it was found that chronic alcohol poisoning in major salivary glands of rats take place the same type of structural changes in the dynamics of which can be divided into three periods:

- Early or the period of acute lesions - up to 9 days of experimental observation during which, mainly by increasing the volume of the cytoplasm because of its hydration, increased the cell area of acini and acini themselves with the simultaneous tendency to reduce the area of nuclear and nuclear-cytoplasmic ratios;- the period of adaptation and relative compensation - from 9 to 12 days, which was characterized by stabilization of processes, and even a partial restoration of studied parameters;

- the development of decompensation - from 16 days till the end of the observation - a period that was characterized by the progression of atrophic changes. Such periods is consistent with modern concepts that have developed in recent decades (Meerson, 1978).

As for the uniformity of nature of development of the structural changes, it is due to the uniformity of the major pathogenetic mechanisms of their development: the impact of endogenous intoxication and the hemodynamic factor that occur under the influence of alcohol on the body (Razvodovskiy, 2004).

However, the different intensity of these processes in the salivary glands of various locations (most in parotid and less in mandibular) may be due to morphological and functional differences of the studied glands (Maier *et al.*, 1988).

CONCLUSIONS

1. During the chronic alcohol poisoning in major salivary glands of rats occur the same type of structural changes in the course of which are the following stages:
- alternative changes in the form of hydrotopic dystrophy of glandulocytes in the early period (up to 9 days of observation);
- period of relative adaptation and compensation (from 12 to 16 days observation);
- period of development of decompensation signs with the atrophy of parenchymal structures and stromal fibrosis progression (from 24 to 28 days observation).
2. More intensive were detected the histologically confirmed morphometric and structural changes occurring in the parotid salivary gland compared with mandibular.

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