ORIGINAL ARTICLE

CONTENTS

Quantitative morphological analysis of age structural changes in prostate of experimental animals with ethanol poisoning

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ABSTRACT

Aim: To find out the age remodeling of the structural components of the prostate gland at alcohol poisoning using quantitative morphological analysis.

Materials and Methods: The structure of the prostate gland of 4 white male rats groups were morphologically investigated. The 1 group included 30 control intact animals aged 8 months, the 2-nd group – 30 rats aged 24 months, the 3-rd group – 30 8-month-old animals with ethanol intoxication, and the 4-th group included 30 24-month-old rats with the specified simulated pathology. Ethanol intoxication was modeled by intragastric administration of 30% ethyl alcohol solution at a dose of 20 ml/kg once daily for 28 days. Rats were euthanized by bloodletting under general thiopental anesthesia 28 days after the beginning of the experiment. The area of glands, the height of glandular epithelial cells, the area of their nuclei and cytoplasm, the nuclear-cytoplasmic ratio in these cells and the stromal-parenchymal ratio in the organ were studied using light microscopy and were determined morphometrically. Morphometric parameters were processed statistically.

Results: It was established that with age in the intact prostate of laboratory sexually mature white male rats, the area of glands, the height of glandular epitheliocytes, the area of their nuclei and cytoplasm, with the stability of nuclear-cytoplasmic ratios in the epithelial cells of the glands, significantly decreases, and the stromal-parenchymal ratio in the organ under study increases. Long-term ethanol poisoning leads to pronounced structural changes in the prostate, which is characterized by pronounced atrophy of the glandular epithelium, a decrease in the area of the glands, a decrease in the height of epithelial cells, a violation of nuclear-cytoplasmic relations in them, an increase in stromal-parenchymal ratio, and a prominent growth of the muscle-elastic stroma. The revealed structural changes of the studied components of the prostate dominated in 24-month-old experimental animals.

Conclusions: Morphological analysis of the prostate gland established that morphometric and morphological changed significantly according to the age and were depend on the ethanol poisoning.

KEY WORDS: prostate gland, ethanol poisinisng, age, morphology, morphometry

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INTRODUCTION

Alcoholism is a widespread pathology that hs possibility to spread among people in the worlds, it often leads to disability and mortality of the population and is an important medical and social problem. Prolonged ethanol intoxication damages almost all organs and systems, the degree of functional and structural disorders of which is different and depends on the duration and severity of intoxication [1-4].

Ethanol and its metabolites have a powerful membranotropic effect, are complicated by increased lipoperoxidation, decreased antioxidant protection, deterioration of microcirculatory bed circulation, disruption of metabolic processes in the body, structural and functional changes in all organs and systems [5, 6].

It should be noted that until today, researchers are interested in the structural components of the prostate gland, its changes in pathological conditions [7-10]. In modern medical and biological scientific literature, there is not enough data on age-related changes in the structures of the prostate gland in conditions of long-term ethanol poisoning [11-13]. The study of the above will help to expand modern ideas about the structural and functional phenomena that occur in the prostate gland under the conditions of long-term alcohol consumption.

Because the prostate gland is an accessory gland of the male reproductive system that is found only in mammals [12, 14], it seems logical to use experimental animal model to investigate the mechanisms of ethanol poisonign.

Morphometry is widely used method to study the architectonics of the blood vessels and intraorgan vascular microcirculatory bed and the structure of intact organs and in various pathological conditions [14]. At the same time, it is known that the age-related

features of remodeling of the structural components of the prostate gland during long-term ethanol poisoning have not been studied in detail. When the structures of the prostate gland are damaged, male fertility, urination, hormonal homeostasis, and immune protection of the urinary system is deteriorate and decrease [15].

AIM

To find out the age remodeling of the structural components of the prostate gland at alcohol poisoning using quantitative morphological analysis.

MATERIALS AND METHODS

The work was performed on 120 laboratory sexually mature male rats, which were divided into 4 groups: 1 group included 30 control intact animals aged 8 months, the 2-nd group – 30 rats aged 24 months, the 3-rd group – 30 8-month-old animals with ethanol intoxication, and the 4-th group included 30 24-month-old rats with the specified simulated pathology. Experimental animals were the same in weight: 8-month-old rats (166-170 g), 24-month-old rats (295-300 g).

Ethanol intoxication was modeled by intragastric administration of 30% ethyl alcohol solution at a dose of 20 ml/kg once daily for 28 days. According to laboratory practice, intragastric administration of medicinal agents to experimental animals does not require anesthesia.

Rats were euthanized by bloodletting under general thiopental anesthesia 28 days after the beginning of the experiment using institution-approved methods. The dose of ethanol was calculated for each animal separately according to its weight.

Cut pieces were fixed in Bouin's solution, passed through ethyl alcohols of increasing concentration and placed in paraffin blocks. After deparaffinization, microtome sections with a thickness of 5-6 μm were stained with hematoxylin and eosin according to Van Gieson, Mallory, Masson, and toluidine blue [16] and were studied using light microscopy and morphometrical method.

Quantitative morphological analysis of prostatic gland was used to determine the area of glands, the height of glandular epithelial cells, the area of their nuclei and cytoplasm, the nuclear-cytoplasmic ratio in these cells, and the stromal-parenchymal ratio in the organ. Morphometric parameters were processed statistically.

50 measurements were performed on each histological specimen. Morphometry was performed with the help of a light microscope «Olimpus BX-2» with a digital video camera and a package of application programs «Video Test 5.0» and «Video size 5.0».

Statistical processing of digital data was carried out using Excel (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA) software. The analysis of the research results was carried out using parametric statistical methods, the choice of which was based on the correctness of the distribution of values. The processing of the results was carried out in the systematic statistical research Department of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine. For all indices, the average arithmetic mean of the sample (M) and the error of the average arithmetic mean (m) were calculated. The reliability of the difference in values between independent quantitative values was determined in the case of a normal distribution according to the Student's t-test, and it was considered statistical at a value of $p < 0.05$ [17, 18].

Laboratory animals were kept on the standard ration of the vivarium of I. Horbachevsky Ternopil National Medical University, all manipulations were carried out in compliance with the rules of the «European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes» (Strasbourg, 2005), according to the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty» dated February 21, 2006, and due to the requirements of the Commission on Bioethics of I. Horbachevsky Ternopil National Medical University.

RESULTS

The rats' prostate gand has a distinct morphological structure in spite of similar embryological origin, tissue and cellular composition, and molecular characteristics to human prostate gland [19-21].

As a result of morphological investigations, it was found that in group of the control animals the prostate gland was represented as lobulated organ and consisted of an acini and ducts with three types of epithelial cells: basal cells, columnar luminal secretory cells, and neuroendocrine cells [19]. The last had neural and epithelial characteristics, and were found in very small number between basal cells, were present in ducts and acini [20, 21]. The stromal component was not very well developed, it contains few smooth muscle cells [22].

Lobes of the prostate gland were surrounded by a thin connective tissue capsule, which was lined by simple squamous epithelium (mesothelium); capsule separated lobes from each other by fibrous connective tissue. The acini, which are the components of the prostatic lobes, were surrounded by a delicate and thin fibromuscular tunica, and were embedded in a loose connective tissue with large number of collagen fibers and few stromal cells [22, 23].

Table 1. Morphometric parameters of the structural components of the prostate gland (M±m)

Indicators	groups			
	1 (control)	2 experimental	3 experimental	4 experimental
Glands area, μm²	137452,3±724,2	115349,6±975,2***	33574,9±204,3***	22242,7±183,6***
Height of glandular epithelial cells,	16,9±0,3	14,8±0,3**	9,43±0,09***	6,51±0,05***
Nuclei area of epithelial cells, μm²	36,7±0,4	28,1±0,3**	23,8±0,3***	14,9±0,2***
Cytoplasm area of epithelial cells, μm²	262,4±2,1	220,3±1,8***	82,1±0,5***	45,2±0,4***
Nuclear-cytoplasmic ratio	0,14±0,01	0,13±0,01	0,29±0,02***	0,33±0,02***
Stromal-parenchymal ratio	0,14±0,01	0,17±0,01*	0,22±0,02**	0,28±0,02***

*-p<0,05; **-p<0,01; ***-p<0,001 comparatively to the 1-st group.

The morphometric parameters of the studied structures of the prostate gland are presented in Table 1.

A comprehensive analysis of the obtained quantitative morphological indicators established that they changed significantly according to the age and were depend on the ethanol poisoning. It was found that age led to remodeling of the studied structures of the prostate gland, which was confirmed by their morphometric indicators. Thus, the glands area of the organ in 24-month-old male laboratory white rats (the second experimental group) with a pronounced statistically significant difference (p<0.001) decreased by 16.1%, the height of glandular epithelial cells – by 10.1%, the area of nuclei and the area of the cytoplasm of the studied cells – by 23.4% and 16.0%, respectively. Established changes in the morphometric parameters of glandular epithelial cells indicated their age-related atrophy.

At the same time, the nuclear-cytoplasmic ratio in glandular epithelial cells did not change significantly, which indicated the stability of cellular structural homeostasis. With age, the stromal-parenchymal ratio in the prostate gland increased by 21.4% (p<0.05), which indicated an increase in the number of stromal structures in the examined organ.

With long-term ethanol poisoning, pronounced remodeling of the studied structures of the prostate gland was observed, which was confirmed by a change in their quantitative morphological indicators. Thus, the area of prostate glands in 8-month-old animals under the influence of ethanol decreased statistically significantly (p<0.001) by 4.1 times, in 24-month-old animals – by 5.2 times (p<0.001). The height of glandular epithelial cells changed almost similarly. Thus, in 8-month-old laboratory white male rats of the control group, the specified morphometric parameter was equal to (16.9±0.3) μm, and in ethanol poisoning – (9.43±0.09) μm. A statistically significant difference (p<0.001) was found between the given quantitative morphological indicators.

At the same time, the last morphometric parameter was smaller than the previous one by 44.2%, in 24-month-old experimental animals this decrease was 56.0% (p<0.001). The area of the nuclei of epithelial cells and the area of their cytoplasm changed almost similarly. Thus, the area of the nuclei of glandular epithelial cells in 8-month-old experimental animals with ethanol poisoning was statistically significantly (p<0.001) reduced by 35.1%, in 24-month-old animals by 46.9% (p<0.001), the area of cytoplasm of the studied cells was changed by 68.7% and 79.5% (p<0.001), respectively.

Uneven changes in the morphometric parameters of the nuclei and cytoplasm of glandular epithelial cells led to violations of the ratios between the indicated components of the nucleus and cytoplasm in the studied cells. Thus, in 8-month-old white rats, this morphometric parameter under the influence of ethanol increased with a high degree of statistically significant difference (p<0.001) from (0.14±0.01) to (o.29±0.02), i.e. in 2.07 times, in 24-month-old animals – 2.5 times (p<0.001). The established changes in the given morphometric parameters indicated a violation of structural cellular homeostasis.

It was also established that the stromal-parenchymal relations in the prostate gland were also changed in the simulated conditions of the experiment. Thus, in the 3rd experimental group (8-month-old animals with long-term ethanol consumption), the indicated morphometric parameter increased by 57.1% with a pronounced statistically significant difference (p<0.001), in 24-month-old animals – by 64.7% (p<0.001).

DISCUSSION

Light microscopy of the prostate gland during long-term ethanol poisoning showed an expansion, pronounced fullness of mainly venous vessels, and pronounced perivascular and stromal edema. In the venous vessels of the microcirculatory bed, the expansion of intraendothelial gaps, desquamation of the endothelium, swelling and fragmentation of the basement membrane of hemocapillaries, plasmarrhagia of the walls and paravasal tissues were observed. In the lumens of the glands there was a different unequal amount of

secretion, which was characterized by various tinctorial properties. Coagulation of contents was noted in some glands. Growth of the muscle-elastic stroma, reduction of folds and atrophic changes of the glandular epithelium, pronounced reduction of the glandular component of the prostate gland, dystrophic, necrobiotic changes of endothelial cells, epithelial cells, stromal structures, and the appearance of foci of cellular infiltration were noted. The revealed morphological changes prevailed in the prostate gland of 24-month-old laboratory sexually mature white male rats [10].

The conducted research and obtained results showed that with age in the intact prostate gland there is atrophy of glandular structures and a decrease in their number, the number of stromal structures also increases, which was objectively confirmed by an increase in the stromal-parenchymal ratio. Long-term ethanol poisoning leads to more pronounced atrophic processes, a decrease in the area of glands, the height of glandular epithelial cells, the area of their nuclei and cytoplasm, and disruption of cellular structural homeostasis in them. At the same time, the nuclear-cytoplasmic ratio increased markedly. The identified structural changes in the components of the prostate dominated in 24-month-old rats [15].

CONCLUSIONS

The conducted studies and obtained results show that with age in the intact prostate gland of laboratory sexually mature white male rats, the area of the glands, the height of the glandular epitheliocytes, the area of their nuclei and cytoplasm significantly decreases with the stability of the nuclear-cytoplasmic ratio in the epithelial cells of the glands, and the stromal-parenchymal ratio increases in the studied body.

Long-term ethanol poisoning leads to pronounced structural changes in the prostate gland, which is characterized by pronounced atrophy of the glandular epithelium, a decrease in the glands area, a decrease in the height of epithelial cells, a violation of their nuclear-cytoplasmic ratio, an increase in stromal-parenchymal ratio, and a prominent growth of the muscle-elastic stroma. The revealed structural changes of the studied components of the prostate dominated in 24-month-old experimental animals.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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