ORIGINAL ARTICLE

QUANTITATIVE MORPHOLOGICAL ASPECTS OF THE STUDY OF THE FEATURES OF THE REMODELING OF THE HEMOMICROCIRCULATORY CHANNEL OF THE TESTICLES UNDER THE ACTION OF ALUMINUM CHLORIDE ON THE BODY

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ABSTRACT

The aim: To conduct a morphometric analysis of the features of the remodeling of vessels of the hemomicrocirculatory channel of the testicles under the action of aluminum chloride on the body.

Materials and methods: The testicles of 52 white male Wistar rats were morphologically studied, which were divided into two groups: intact and animals injected with aluminum chloride at a dose of 100 mg/kg.

Results: The long-term effect of aluminum chloride on the body led to pronounced changes in the quantitative morphological indicators of the vessels of the hemomicrocirculatory channel of the testes compared to the control ones. The diameter of arterioles of the left testicle decreased by 18.4%, precapillary arterioles by 19.1%, hemocapillaries by 10.1%. The diameter of the postcapillary venules of the left testicle increased by 26.3%, the venules by 26.4%, and the density of microvessels decreased by 30.0%, which indicated the deterioration of its blood supply. The degree of remodeling of microvessels in the right testis was less pronounced. The diameter of arterioles of the right testicle decreased by 17.1%, precapillary arterioles by 18.4 %, and hemocapillaris by 9.1%. The diameter of the postcapillary venules of the right testicle increased by 25.5%, the venules by 27.0%, and the density of microvessels decreased by 25.8%.

Conclusions: Long-term action (during the month) of aluminum chloride on the body leads to pronounced remodeling of the vessels of the hemomicrocirculatory channel of the testicles, which is characterized by a pronounced narrowing of its supply (arterioles, precapillary arterioles), exchange (hemocapillaries) links.

KEY WORDS: morphometry, testicles, hemomicrocirculatory channel, aluminum chloride

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INTRODUCTION

Today, there is an increase in the artificial load on the environment, because of which the amount of chemicals and their metabolites, which can negatively affect the organs and systems of the body and worsen the course of various pathologies, is increasing. It is known that aluminum and its compounds have a toxic effect on living organisms due to their accumulation in organs and tissues, which is accompanied by a violation of normal metabolic processes and the development of pathology. Inorganic metal salts, which include aluminum chloride, are particularly harmful to living organisms [1].

This substance is widely used by man in various spheres of his activity: oil, as a catalyst, cosmetics industry for the production of antiperspirants and deodorants, and medicine. The reproductive system in men is very sensitive to the influence of exogenous factors and can be complicated by azoospermia (absence of spermatozoa in the ejaculate) [2, 3]. In recent decades, indicators of men's reproductive and sexual health have been decreasing in many countries of the world, but in Ukraine, they have a rapid and extremely negative trend [3]. Modern researchers emphasize the underestimation of the negative impact of environmental factors on the generative function in men.

It is also known that functional and structural changes in the vessels of the hemomicrocirculatory channel are the first early signs of the effects of negative factors of endogenous and exogenous origin on the body [4], which clearly occur under the conditions of increased formation of reactive oxygen species with a concomitant deficiency of antioxidant protection [5-8]. Against the background of age-related pathobiochemical

changes, asthenodepressive syndrome, memory and sleep disorders develop, and efficiency is reduced. Age-related androgen deficiency is manifested not only by sexual dysfunction, but also associated with age-related diseases (obesity, diabetes, coronary heart disease, osteoporosis, etc.), which exacerbate dysfunction of the male reproductive system [9-11].

Thus, in the process of spermatogenesis an important place belongs to the interaction of many factors, first – hormonal, as well as nervous, immune, genetic, which are based on subtle, still little studied molecular mechanisms of regulation. The vessels of the hemomicrocirculatory channel, where transcapillary exchange takes place, play an important role in the trophic supply of cells and tissues and in the pathomorphogenesis of their injuries. It should be noted that the features of the remodeling of the hemomicrocirculatory channel of the testes during the action of aluminum chloride on the body have not been sufficiently studied.

THE AIM

To carry out a morphometric analysis of the features of the remodeling of vessels of the hemomicrocirculatory channel of the testicles under the action of aluminum chloride on the body.

MATERIALS AND METHODS

The testes of 52 sexually mature white male Wistar rats, which were divided into two groups, were studied using a complex of morphological methods. The 1st group (intact) included 26 animals that were in normal vivarium conditions, the 2nd – 26 rats that were injected intraperitoneally with aluminum chloride at a dose of 100 mg/kg [12]. The experimental animals were euthanized one month after the start of the experiment.

The hemomicrocirculatory channel of the testes was studied by filling their vessels with a carcass-gelatin mixture, which was injected through the abdominal aorta. 3-4 hours after filling the bloodstream of the testicles with the indicated mixture, they were collected and fixed in a 10% neutral formalin solution for 2 weeks. Sections with a thickness of 30-40 μm were made on a freezing microtome, which were dehydrated in ethyl alcohol, clarified in methyl ether of salicylic acid and placed in polystyrene. Examination of micropreparations was performed on a Nicon Eclipse CI-E microscope. Microscopy of microscopic images was performed using a Sigeta M3CMOS 14000 camcorder and Toup View software on a personal computer. [13]. The diameters of arterioles (DA), precapillary arterioles (PA), hemocapillaries, postcapillary venules (PV), venules (V) and microvessel density (MD) per 1 mm^2 of the tissues of the left (LT) and right testicles (RT) were determined morphometrically [4]. Quantitative indicators were processed statistically. The processing of the obtained results was carried out in the department of systematic statistical research of I. Horbachevsky Ternopil National Medical University the STATISTIKA software package. The difference between the compared values was determined by the Student and Mann-Whitney test. Conducting experiments and euthanasia of experimental animals was carried out in compliance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" as well as the Law of Ukraine "On the Protection of Animals from Cruelty" (dated February 21, 2006).

RESULTS

A comprehensive analysis of the data (Table I) established that long-term exposure to aluminum chloride led to pronounced changes in the quantitative morphological indicators of blood vessels of the hemomicrocirculatory channel compared to control ones. Thus, the diameter of the arterioles of the left testicle in simulated experimental conditions with a pronounced statistically significant difference ($p<0.001$) decreased from (18.20±0.30) μm to (14.85±0.24) μm, by 18 4%.

The diameter of the precapillary arterioles of the specified testicle also changed similarly. Thus, the diameter of the precapillary arterioles of the intact left testicle was equal to (10.82±0.12) μm, and in the 2nd group of observations - (8.75±0.12) μm. The given morphometric parameters were statistically significantly ($p<0.001$) different from each other. At the same time, the last quantitative morphological indicator was lower than the similar control by 19.1%.

The lumen of the hemocapillaries of the left testicle in the studied conditions of the experiment also decreased by 10.1% with a high degree of statistically significant difference ($p<0.001$).

Quantitative morphological analysis established that the venous vessels of the hemomicrocirculatory channel of the left testicle expanded when aluminum chloride was applied to the body of experimental animals. Thus, in the simulated experimental conditions, the diameter of the post-capillary venules of the left testicle of the animals increased statistically significantly ($p<0.001$) by 26.3%, and the venules increased by 26.4% ($p<0.001$). At the same time, the density of microvessels per unit tissue area of the examined organ significantly

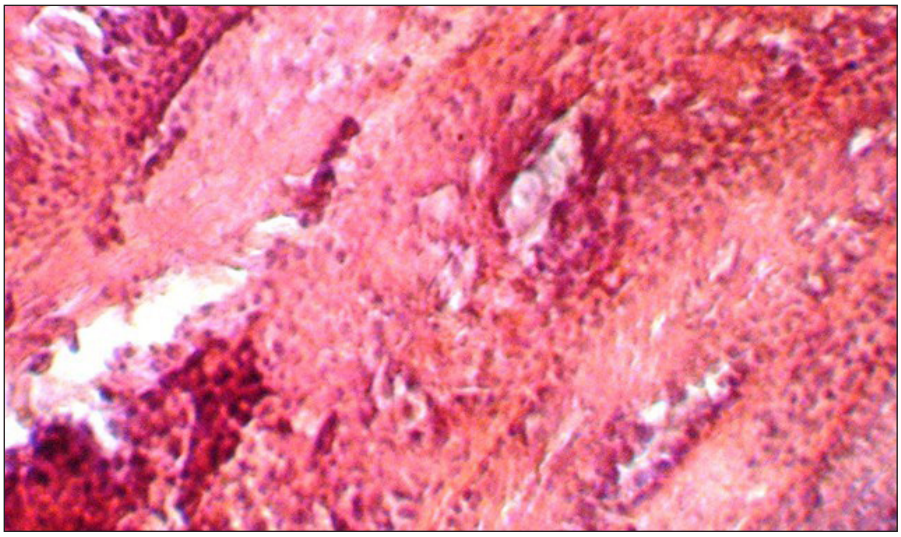

Fig. 1. Destructive processes in the vessel wall desquamation of endotheliocytes, narrowing of the lumen of the vessels of a laboratory sexually mature white male rat under the action of aluminum chloride on the body.

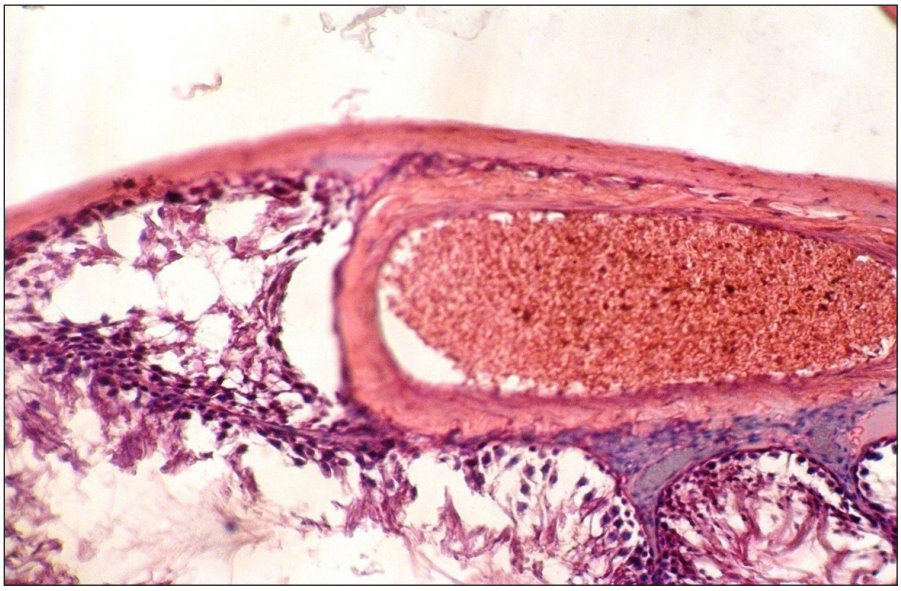

Fig. 2. Full-blooded vein, destructive processes in its wall, desquamation of spermatogenic epithelium in tortuous seminiferous tubules

decreased by almost 30.0% ($p<0.001$), which indicated the deterioration of its blood supply [2, 4].

The structural rearrangement of the vessels of the hemomicrocirculatory channel of the right testicle during the action of aluminum chloride on the body of experimental animals turned out to be similar to the one described above. It is worth noting that the analysis of the investigated morphometric parameters established that the degree of remodeling of the microvessels of the hemomicrocirculatory channel in the left and right testicles turned out to be different. Thus, the diameter of the arterioles of the right testicle in the simulated conditions of the experiment with a pronounced statistically significant difference ($p<0.001$) decreased by 17.1% compared to the similar control indicator, the diameter of the precapillary arterioles – by 18.4% ($p<0.001$), and hemocapillaries - by 9.1% ($p<0.001$).

Venous vessels of the hemomicrocirculatory channel (capillary venules and venules) expanded under the influence of aluminum chloride. Thus, the diameter of the postcapillary venules of the right testicle increased by 25.5%, and the diameter of the venules increased by 27.0% ($p<0.001$) with a high degree of statistically significant difference ($p<0.001$). The density of microvessels under these experimental conditions decreased from (3836.8±30.3) to (2846.9±22.1), so it is by 25.8% ($p<0.001$).

DISCUSSION

Man-made loads with aluminum compounds are accompanied by hemodynamic disorders, which are manifested to one degree or another by pronounced testicular ischemia. [1, 6, 7]. The cells of the spermatogenic epithelium show the greatest sensitivity to hypoxia. According to most authors [1, 3, 6, 7] violations of spermatogenesis are directly dependent on circulatory disorders and are irreversible.

Table I. Morphometric characteristics of the hemomicrocirculatory channel of the test animals (M±m)

Indicator	Group of animals	
	1st group (intact)	2nd group
Left testicle		
diameters of arterioles, μm	18.20±0.30	14.85±0.24***
precapillary arterioles, μm	10.82±0.12	8.75±0.12***
hemocapillaries, μm	6.12±0.09	5.50±0.09***
postcapillary venules, μm	12.58±0.15	15.90±0,15***
venules, μm	26.57±0.30	33.60±0.30***
microvessel density	3843.3±28.2	2690.5±21.3***
Right testicle		
diameters of arterioles, μm	18.22±0.30	15.10±0.27***
precapillary arterioles, μm	10.85±0.12	8.85±0.09***
hemocapillaries, μm	6.12±0.09	5.56±0.06***
postcapillary venules, μm	12.54±0.15	15.75±0.12***
venules, μm	26.53±0.30	33.70±0.27***
microvessel density	3836.8±30.3	2846.9±23.1***

***-$p<0.001$

The predominance of expansion of venous microvessels of the hemomicrocirculatory channel, venous full blood was complicated by hypoxia, in which oxygen transport to cells is disrupted, energy synthesis decreases intracellular ATP decreases, functional-metabolic and trophic disturbances occur, which significantly affects the vital activity of testicular structures. The latter is confirmed by the presence of dystrophy, necrobiosis of cells and tissues of the examined organ, infiltrative and sclerotic processes [1, 6].

The leading in of aluminum chloride into the body of sexually mature male laboratory rats leads to complex general biological processes that arise and develop in the organs and systems of the body during its adaptation to a new level of life [6, 9]. It should be noted that detailed and objective knowledge of compensatory-adaptive processes in cotyledons needs its own solution.

The obtained morphometric indicators of the studied vessels indicate that the influence of aluminum chloride leads to a pronounced structural rearrangement of the vessels of the hemomicrocirculatory channel of the testes. At the same time, it should also be noted that the microvessels of the supply and exchange links are narrowed, and those of the outlet part of the hemomicrocirculatory channel are significantly expanded. Pronounced expansion of post-capillary venules and venules led to venous congestion and stasis, which cause edema, plasmarrhagia in the vascular walls and perivasal stroma, which supported and intensified the state of hypoxia and deterioration of cell and tissue trophism. The latter contributed to the increase in edema and were complicated by dystrophic, necrobiotic changes in cells and tissues. Damage to endotheliocytes led to a decrease in the production of nitric oxide, an increase in the synthesis of endothelin-1, which increased vasospasm. The specified hemomicrocirculatory changes were accompanied by deterioration of blood rheology, increased permeability of the walls of microvessels, plasmarrhagia not only of their walls, but also of the paravasal stroma with blood proteins. At the same time, perivasal swelling, saturation of the surrounding stroma with proteins and blood-forming elements, hypoxia, defibrillation, disorganization and dissociation of fibrous structures, significant deterioration of the diffusion of nutrients and oxygen, which was complicated by dystrophic and necrobiotic changes of stromal structures, vascular endotheliocytes and spermatogenic epithelium, fibroplastic activity, polymerization and saturation of collagen fibrils with glycosaminoglycans [4, 6, 9].

During histological examination of micro-preparations, an increase in the lumens of mainly venous vessels of the testicles, especially post-capillary venules and venules, which are dilated, filled with blood, with foci of stasis, thrombosis, diapedesis, paravasal hemorrhages, was revealed. Hemocapillaries are mostly narrowed, spasmodic. The wall of arterioles is thickened, their lumen is narrowed, some endotheliocytes are dystrophically and necrobiotically altered and desquamated. In individual arterioles, the following were observed: destruction of membranes, structural changes of myocytes, phenomena of myoelastofibrosis, which are presented in Fig 1.

In the lumen of the venules, formed elements of the blood, mainly erythrocytes, were found, the shape of which was elongated oval. Edema was observed in the cytoplasm of endotheliocytes. Cytolemma of endotheliocytes of uneven thickness and contours. This structure formed protrusions of various shapes and sizes, as well as microvilli in the lumen of microvessels. The cytolemma of endotheliocytes also formed recesses in their cytoplasm.

Pronounced venous congestion was complicated by hypoxia, which led to dystrophic and necrobiotic changes in endotheliocytes, vascular myocytes, spermatogenic epitheliocytes, stromal structures, infiltration and sclerosis (Fig 2).

The contours of the walls of venous vessels are unclear, thickened. Swollen and sclerosed cells alternate, their contours are tortuous, twisted, the lumens are deformed. Endotheliocytes of venous vessels are increased in size with the phenomena of edema, dystrophy, and necrobiosis. There were cells with desquamation of endotheliocytes, pyknosis and lysis of their nuclei. Structural changes dominated in the left testicle, which is associated with the peculiarities of the venous outflow from the indicated organ [3, 4].

CONCLUSIONS

Long-term action (during the month) of aluminum chloride on the body leads to pronounced remodeling of the blood vessels of the hemomicrocirculatory channel of the testes, which is characterized by a pronounced narrowing of its supply (arterioles, precapillary arterioles), exchange (hemocapillaries) links and expansion of postcapillary venules and venules, venous congestion, hypoxia, dystrophic, necrobiotic changes in cells and tissues, infiltrative and sclerotic processes that dominate the left testicle.

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