ORIGINAL ARTICLE

ULTRAMORPHOMETRIC CHARACTERISTICS OF ACINI AND MICROVASCULATURE OF THE PANCREAS IN THE PRESENCE OF MODERATE DEHYDRATION

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ABSTRACT

The aim: The objective of our study was to evaluate the features of ultramorphometric characteristics of exocrine parenchyma and microvasculature of the pancreas in the presence of moderate dehydration by means of an experiment in laboratory rats.

Materials and methods: The experiment involved 20 mature white male rats divided into 2 groups: control and experimental (10 rats each). In the experimental group, moderate dehydration was simulated, i.e. the animals were deprived of water for 7 days, while the control rats were provided with a normal water supply during the study. Pancreatic parenchyma samples were fixed in phosphate-buffered glutaraldehyde solution and post-fixed in osmium tetroxide solution, dehydrated and embedded in a mixture of epoxy resins. Ultrastructural analysis was performed using JEOL JEM-1230 transmission electron microscope (Japan).

Results: Pancreatic electron microscopy in the presence of moderate dehydration demonstrated statistically significant changes in exocrinocytes area and exocrinocyte nucleus area which increased by 8.02% ($p = 0.028$) and 40.28% ($p < 0.001$), respectively. Among the vessels of microcirculation, the largest changes occurred in the capillaries: their lumen narrowed by 22.34% ($p = 0.002$) as compared with the control group. The cytoplasm of endothelial cells contained a large number of vacuoles and micropinocytotic vesicles.

Conclusions: Among the organelles of exocrinocytes, mitochondria appeared the most vulnerable to the effects of dehydration. They demonstrated polymorphic changes: a part of the mitochondria was hypotrophic and had partially reduced cristae, and another part was hypertrophic.

KEY WORDS: pancreas, dehydration, ultrastructure, morphometry

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INTRODUCTION

Nowadays, water is considered not only a natural resource but also a factor having social significance, since the availability of sufficient amount of proper quality water is one of the main milestones of safe living conditions and development of a country [1]. Most countries of the world, including Ukraine, entered the XXI century with a set of environmental, global, and regional problems [2]. The most threatening among them are the depletion and deterioration of water resources, i.e. sources of drinking water. Today, about 1.1 billion people on Earth still do not have permanent, secure access to water supply [3]. Insufficient water intake causes a pathological condition that is accompanied by metabolic disorders and has significant consequences for population health and working capacity.

In recent decades, there has been a high growth rate of the digestive system and endocrine system diseases, which optimize the fluid volume in the body [4]. The main reasons for the development of these diseases are not only genetic factors but rather the lifestyle and external adverse factors, such as intoxication, radiation, gravity load and hypoxic stress, salts of heavy metals, injuries, etc. [5], to which the pancreas is very sensitive.

The issue of morphofunctional changes in the pancreas under the influence of various exogenous factors is important for both clinicians and morphologists. Despite the significant amount of work devoted to this problem, many issues still remain debatable, in particular, the changes in the pancreatic morphology under the influence of various types of dehydration. Taking into account the abovementioned facts, we can assume that experimental and clinical studies on the different types and degrees of dehydration will help clinicians predict the course of pancreatic diseases and provide adequate treatment.

THE AIM

The objective to study the features of ultramorphometric characteristics of acini and microvasculature of the pancreas in the presence of moderate dehydration by means of an experiment in mature laboratory rats.

MATERIALS AND METHODS

To achieve this objective, the experiment involved 20 mature white male rats kept in a stationary vivarium. The experimental study was performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986); the Declaration of Helsinki of

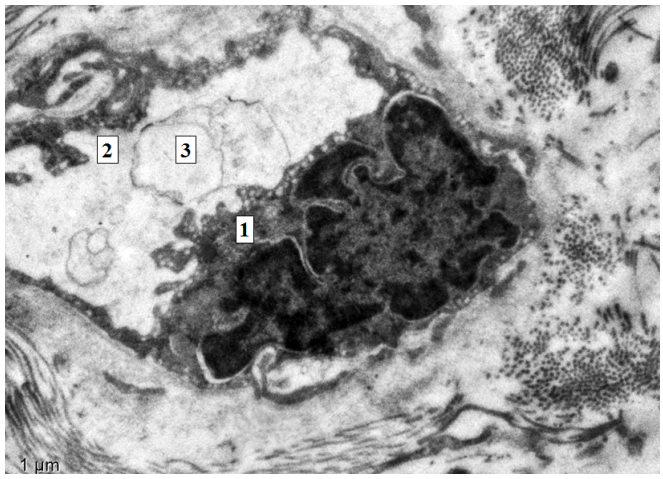

Fig. 1. Rat pancreatic capillary. Dehydration, Day 7. Endothelial cell cytoplasm (1), endothelial cell cytoplasmic processes (2), membrane structures (3). Contrast enhancement with uranyl acetate and lead citrate according to Reynolds method. x8000

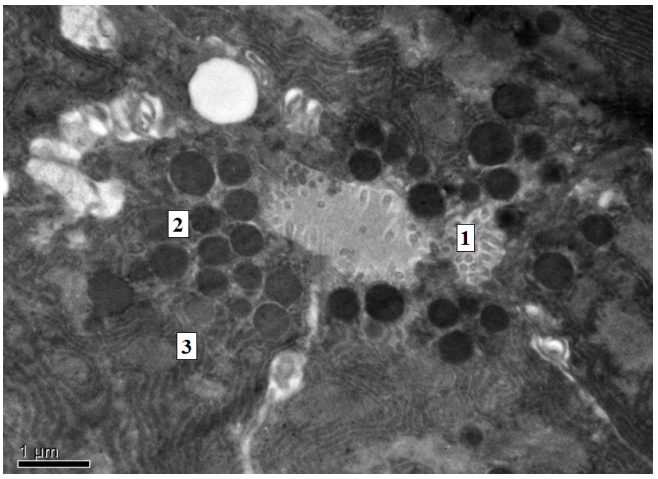

Fig. 2. Rat pancreatic acinus. Dehydration, Day 7. Acinar lumen (1), secretory granules (2), hypotrophic mitochondria (3). Contrast enhancement with uranyl acetate and lead citrate according to Reynolds method. x8000

Table I. The results of ultramorphometric studies of the pancreas in rats

Parameter	Control group (n = 10)	Dehydration (n = 10)
PA, μm²	983.4 ± 67.1	1058.8 ± 63.1
EA, μm²	149.5 ± 3.1	161.5 ± 4.5
ECA, μm²	138.7 ± 4.7	146.5 ± 4.4
ENA, μm²	10.7 ± 0.4	15.01 ± 0.4
N/C ratio, %	8.2 ± 0.9	10.2 ± 0.4
Da, μm	16.34 ± 0.49	14.49 ± 0.48
Dc, μm	7.92 ± 0.28	6.15 ± 0.30
Dv, μm	19.41 ± 0.59	15.89 ± 0.40

Note: The resulting values were presented as M ± m.

the General Assembly of the World Medical Association (1964–2000); the General Ethical Principles for Animal Experiments approved by the First National Congress on Bioethics (Kyiv, 2001); and the Law of Ukraine "On Protection of Animals From Cruelty" (2006).

Animals were divided into two groups: the control group (10 rats) and the experimental group (10 rats). In the experimental group, moderate dehydration was simulated according to the model of A. D. Sobolieva [6], i.e. the animals were deprived of water for 7 days. The control rats were provided with a normal water supply during the study. Upon reaching the required degree of dehydration, the animals were sacrificed using thiopental anesthesia overdose.

For ultrastructural examination, pancreatic samples were fixed in 4% phosphate-buffered glutaraldehyde solution and post-fixed in 1% OsO_4 solution, dehydrated, and embedded in a mixture of epoxy resins (epon and araldite). Ultrathin sections (40–60 nm) were performed on UMTP–6M ultramicrotome using glass knives. Contrast enhancement of ultramicrosections on copper grids was performed for 15 minutes with a 2% solution of uranyl acetate, followed by a lead citrate solution for 30 minutes according to Reynolds method. Ultrastructural analysis was performed using JEOL JEM-1230 transmission electron microscope (Japan). All measurements of ultrastructural components were performed using SEO Image Lab 2.0 electronic program. During the study, the following parameters were evaluated: pancreatic acini area, exocrinocytes area, exocrinocyte cytoplasm area, exocrinocyte nucleus area, the nuclear-cytoplasmic ratio of exocrinocytes, the inner diameter of arterioles, capillaries, and venules.

The results of ultramorphometric studies were analyzed by means of statistical methods using IBM SPSS Statistic 21 statistical program. The digital data were tested for normality of the distribution using the Kolmogorov-Smirnov test. Mean values were presented as M ± m. The Mann-Whitney test was used to compare the parameters in the experimental and control groups. Values of $p < 0.05$ (≤ 5%) were considered statistically significant.

RESULTS

The microscopical study in the control rats revealed that the pancreatic parenchyma was represented mainly by exocrine tissue – conical pancreatic acini, with the apical parts directed towards the secretory ducts, where the intercalated ducts of the pancreas originate from. The average size of the pancreatic acini was 983.4 ± 67.1 μm. In the acinar parenchyma, the islets of Langerhans of different shapes and sizes were seen. In the center of the pancreatic islets, there were β-cells that were localized close to each other and had oval or round shape and a light cytoplasm, while on the periphery of the islet, smaller and fewer α- and D- cells were observed. The average size of the islets of Langerhans was 13934.1 ± 688.3 μm. Endocrine cells were clearly separated from exocrine cells by connective tissue consisting of reticular, collagen, and elastic fibers.

On Day 7 of the experiment (upon reaching the average degree of dehydration), the cytoplasmic processes and cy-

toplasm of pancreatic capillary endothelial cells contained a large number of vacuoles and micropinocytotic vesicles. The luminal surface of endothelial cells had a significant number of cytoplasmic processes and reniform nodules. Endothelial cell nuclei had invaginations of the nuclear envelope and marginally placed chromatin (Fig. 1). Capillary lumens obtained irregular shape and significantly narrowed by 22.34% ($p = 0.0001$) vs. control group and contained thin membrane structures. Collagen fibers in the pericapillary zone generally maintained an orderly arrangement. The diameter of arterioles decreased by 11.32% ($p = 0.0001$), and the diameter of venules – by 18.12% ($p = 0.0001$) (Table I).

The lumens of the acini were narrowed and contained fine-grained secretory material. The acini area increased by 7.66% ($p > 0.05$) vs. the control group. The microvilli of the apical part of acinocytes were well visualized, but somewhat deformed. On the periphery of the apical cytoplasm, there were accumulations of electron-dense secretory granules of regular round shape; closer to the central part of the cell, large and irregularly shaped vacuoles were visualized. A part of mitochondria was hypotrophic and had partially reduced cristae; still, another part of mitochondria appeared to be hypertrophic (Fig. 2).

Exocrinocytes area and exocrinocyte cytoplasm area increased by 8.02% ($p = 0.028$) and 5.54% ($p = 0.326$). Exocrinocyte nucleus area and the nuclear-cytoplasmic ratio of exocrinocytes increased by 40.28% ($p < 0.001$) and 29.11% ($p = 0.96$) which is presented in Table I.

DISCUSSION

Today, there are a large number of scientific studies devoted to the study of the pathology of the pancreas, however, in the modern literature there are no systematized data on the direct anatomical and physiological features of the structure of this organ under the influence of various types of dehydration on the pancreatic parenchyma. The sources contain a lot of data on the influence of various factors on the structure of the pancreas, in particular, the group of authors P.N. Zamyatin, V.N. Likhman et al. [9] studied ultrastructural changes in pancreatic cells after modeling blunt trauma, the researchers noted changes in the structure of the pancreas, both dystrophic and destructive, revealed mitochondrial dysfunction in the form of focal lysis of membranes and granular endoplasmic reticulum, found that some of the mitochondria were hypotrophic and had partially reduced cristae, however, there were also those that were, on the contrary, hypertrophied.

A group of authors [10] studying the effect of opioids, such us nalbuphine, on the pancreatic parenchyma noted pronounced structural changes in the microvasculature in the form of micro- and macroangiopathy, followed by profound destructive changes that led to disorganization of the pancreatic tissue, which is consonant with our studies - a decrease in the area of arterioles, venules and capillaries, the gaps of which have acquired an irregular shape.

Back in 2008, a group of researchers in their work studied the effect of heavy metal salts on the pancreatic parenchyma [12]. The authors noted pronounced morphological changes in the pancreas, the intensity of which was associated with the duration of the experiment, first of all, they found mitochondrial edema with a decrease in the number of cristae, vacuolization of cisternae of the granular endoplasmic reticulum, clarification of the nuclear matrix, and chromatin condensation, however, further studies in this direction were not carried out. We have revealed a similar relationship between changes in the structure of the pancreas depending on the duration of dehydration, with the progression of the process, the cytoplasmic processes of the endothelial cells of the pancreatic capillaries and their cytoplasm contained a large number of vacuoles and micropinocytic vesicles, the nuclei of endothelial cells were with invagination of the nuclear membrane and marginally located chromatin.

In 2017, data were published on the effect of changes in water and electrolyte balance on the morphogenesis of the organ of the gastrointestinal tract - tongue [12]. The authors noted destructive processes in the microvessels, which were full-blooded, the walls were edematous, and their lumens were enlarged, which is consonant with the results of our studies.

CONCLUSIONS

Analysis of the exocrine parenchyma in the presence of moderate dehydration demonstrated statistically significant changes in exocrinocytes area and exocrinocyte nucleus area which increased by 8.02% ($p = 0.028$) and 40.28% ($p < 0.001$), respectively.

Analysis of ultramorphometric characteristics of the pancreas showed a significant decrease in the diameter of arterioles, capillaries, and venules among the vessels of microcirculation, but the largest changes occurred in the capillaries: their lumen narrowed by 22.34% ($p = 0.002$) as compared with the control group. The cytoplasm of endothelial cells contained a large number of vacuoles and micropinocytotic vesicles.

Among the organelles of exocrinocytes, mitochondria appeared the most vulnerable to the effects of dehydration. They demonstrated polymorphic changes: a part of mitochondria was hypotrophic and had partially reduced cristae, and another part was hypertrophic.

Prospects for future research. It is planned to study the features of ultramorphometric parameters of the pancreas in the presence of cellular dehydration of various degrees.

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The Authors declare no conflict of interest.

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