Section 5. Physiology

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MORPHOLOGICAL CHARACTERISATION OF THE PANCREAS IN GERONTOGENESIS UNDER EXPERIMENTAL HYPERGLYCAEMIA

Abstract. The article assessed the development of diabetes mellitus at a reduced dose of alloxan by morphological examination of the pancreas. Middle-aged and elder rats were used in the experiment. The development of hyperglycaemia and its further effects on the pancreas were confirmed during the experiment.

Keywords: experimental hyperglycaemia, pancreas, diabetes mellitus, morphology, β-cells, islets of Langerhans.

Introduction

Diabetes mellitus (DM) is a metabolic disease resulting from impaired insulin secretion by the pancreas, insulin response or a combination of these factors. In terms of prevalence, DM can be considered a pandemic of the 21st century [2]. Major health problems develop as a result of the resulting hyperglycaemia [3]. There are no curative treatments for these conditions, and a deeper understanding of the mechanisms of disease development is required [4].

Experimental research models are widely used to study the pathogenesis of the disease. The alloxan-induced diabetic animal model is widely considered to be the classical model. Alloxan causes destruction of the β-cells of the pancreatic islets of the pancreas [5]. But despite the similarity of subdiabetogenic to lethal doses of the drug, the wide range of recommended doses for modeling, the absence of morphological evidence of DM development in conditions of administration of certain doses, the wide range of hyperglycaemic values, the emergence of specific insular apparatus functioning during gerontogenesis, our work is relevant. We chose a dose of 120 mg/kg animal weight.

Materials and methods

The experiment was carried out on 60 white Wistar rats kept in standard vivarium conditions. Animals were represented by two age groups – middle aged rats (6–7 months) weighing 140–160 g and elder rats (18–22 months) weighing 260–340 g(according to classification of I. P. Zapadnyuk, 1983). Each age group was divided into intact (control) and experimental (with experimental hyperglycemia) groups. Hyperglycemia was modeled by intraperitoneal injection of alloxan monohydrate solution (120 mg/kg, Sigma, Germany).

The development of hyperglycaemia was monitored by blood glucose, which was determined (glucose oxidase method) using a Bionime portable glucometer. On the third day, animals were selected that had persistent hyperglycaemia with peripheral blood glucose higher than 28 mmol/l.

The animals were euthanized by inhalation anesthesia with ethyl ether for tissue sampling [6; 7]. After decapitation, the pancreas was extracted, and microslides of the pancreas were made according to standard histological techniques, followed by hematoxylin and eosin staining. Fragments of pancreas were preserved in 10% neutral formalin. The material was dehydrated in a Leica TP1020 automatic station and embedded in paraffin. Paraffin sections (5 μm) were placed on slides coated with poly-L-lysine film (Sigma). Morphometric analysis was carried out using a system of computer analysis of microscopic images, consisting of a Nikon Eclipse E400 microscope, a Nikon DXM1200 digital camera, a personal computer and Video-Test-Morphology4.0 software (Avtandilov grid).

The results were statistically processed using Student's t-test and determination of criterion of normality of values distribution of the studied series [1]. We adhered to General Ethical Principles of Animal Experiments (Kyiv, 2001) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg, 1986).

Results and discussion

To assess the peculiarities of the structure and functional activity of endocrine and exocrine parts of the pancreas in experimental animals we used standard criteria, including measurement of islets of Langerhans, number of islets per unit conventional area (per 0.1 mm^2), diameter of α- and β-cells, number of endocrine cells in pancreatic islets, area of parenchyma and organ stroma [8].

In the course of the study we found that in the middle-aged animals of the intact group there was a gradual decrease in the relative volume density of the stroma and, conversely, an increase in the relative volume density of the parenchyma (Fig. 1).

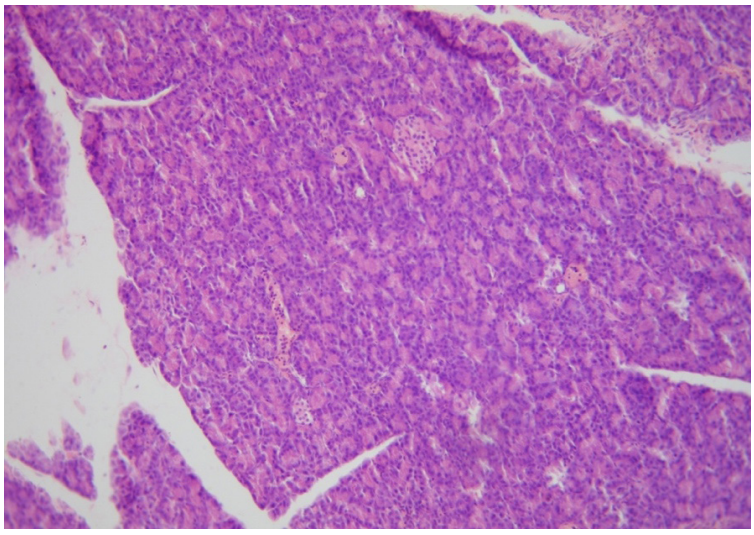

Figure 1. The pancreas of the middle-aged intact rat

3 groups of stromal changes were observed in animals with experimental hyperglycaemia: the occurrence of inflammatory infiltrate, fibrosis and vascular reaction. In most cases there was productive inflammation, which was either isolated in some lobules or diffusely distributed in the stroma of the organ. Foci of exudative inflammation were rare and detected around the excretory ducts. Fibrosis in most cases was combined with productive inflammation and venous vascular congestion. In some areas the parenchyma was separated by broad interlayers of connective tissue. Connective tissue development was more prominent in the immediate vicinity of the ducts (Figure 2). The excretory ducts were dilated and contained homogeneous oxyphilic contents.

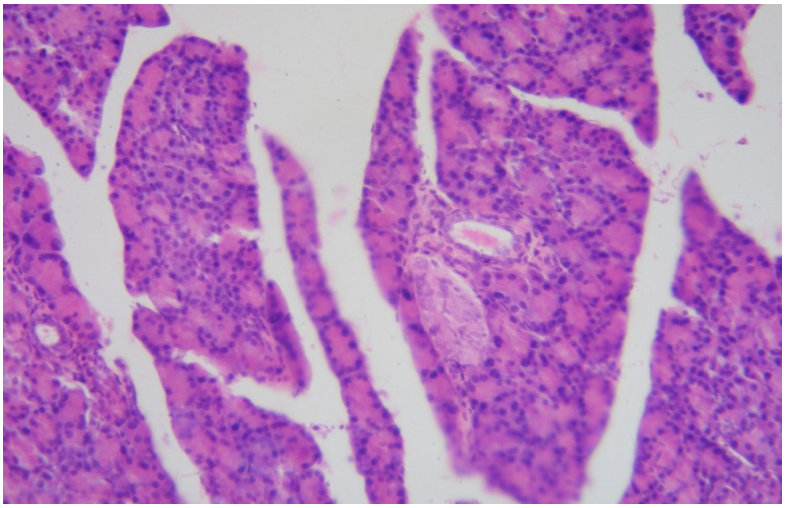

Figure 2. Pancreas of the middle-aged rat with experimental hyperglycaemia

Pancreatic exocrine tissue consists of 4 types of cells: acinar cells; centroacinarductal cells; mucin-secreting ductal cells; and connective interstitial cells [9].

The acini in the middle-aged intact animals consisted of a pyramidal, single-layered epithelium lining the basal membrane with curves directed towards the centre. Basal part of cell cytoplasm contained basophilic granules and stained more intensively in comparison with apical part of cell cytoplasm (by hematoxylin-eosin staining). Pancreatic secretory activity correlated with the size of acinocytes and their nuclei. Along the course of the ducts, cells with round nuclei, well visualized chromatin and 1 or 2 nuclei appeared. Mitotic markers were rarely observed (up to 1%). The small-calibre ducts were lined by flattened cube-shaped epithelium, the outlet terminal ducts by tall cylindrical epithelium and surrounded by dense fibrous connective tissue. Islets of Langerhans were few and irregularly rounded and accounted for 3% of the total structure of the pancreas. Diameter of islets ranged from 50 to 200 μm, most of them were 55–65 μm and 145–170 μm in diameter. Histologically, they were predominantly syncytium-like bands of heterogeneous prismatic cells. The boundaries of the islets were clear, and β-cells were predominantly located in the centre of the islets. Despite the rarity of mitoses, apoptosis markers (0.3%-0.5%) were found in islets of Langerhans.

In the exocrine part, histological examination in hyperglycemic middle-aged rats showed both focal and diffuse infiltration of the acini with lymphocytes. There was wrinkling of the affected acini, in isolated cases there was their enlargement with flattening of the epithelium. There were also areas of acinar dislocation into fatty tissue. Ducts with dilatation underwent the greatest changes. On sagittal slices the ducts had a tortuous course and were filled with condensed homogeneous masses. There was a decrease in accumulation of zymogen granules in acini of peripheral marginal sections and a high degree of their accumulation in

perinsular areas of lobules. In most animals there were foci of marked fatty dystrophy up to "fattening" of cells in exocrinocytes. This was accompanied by compensatory hypertrophy and hyperplasia of adjacent cells.

The islet part in this group decreased to 2%-2.5%, islet boundaries were blurred with moderately pronounced infiltration by lymphocytes at the periphery (initiation of insulinitis). Diameter of islets ranged from 50 to 150 μm with the predominance of islets with a diameter of 80–90 μm of irregular shape. We have noted heterogeneity of islets cell structure: in some cells there was active degranulation and hydrophic cytoplasmic dystrophy and prominent pycnosis of nuclei, in other part against the background of a low mitotic index (less than 1%) increased apoptosis up to 2.5%-3%.

In elder intact rats, the organ retained a lobular structure and was represented by tubular-alveolar structures. The outside of the pancreas was covered by a connective-tissue capsule dominated by fibrosis and venous vasculopathy (in contrast to young animals). From the capsule inside the organ there were thickened connective tissue interlayers that divided the pancreas into lobules, but the parenchymatous component decreased due to fibrosis and redistribution of adipose tissue. In the experimental group, organ fibrosis developed on the background of productive inflammation. There was noted a replacement of the parenchymatous comonte of the organ by the adipose tissue, in which there was an inflammatory infiltrate of lymphocytes, plasma cells, histiocytes and neutrophils. The pancreatic capsule was significantly thickened. On the capsule side, connective tissue bands with fatty tissue compounds were growing into the pancreas and squeezing the organ parenchyma (Fig. 3).

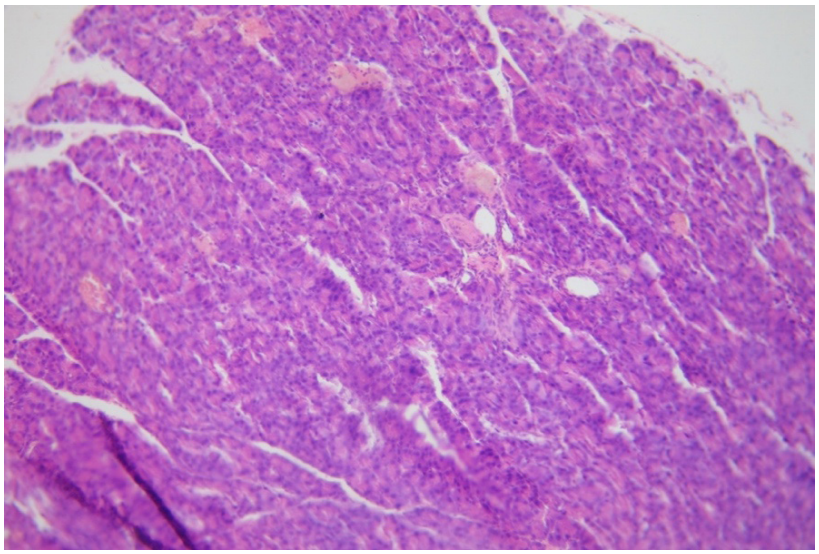

Figure 3. Elder intact rat pancreas

In animals without DM, the acinar structure and pyramidal shape of exocrinocytes was preserved, but in some acini the microvilli in the apical part of the cells were desquamated and lost. In some acini the heterogeneity of staining of basal and apical parts of cytoplasm was lost (basophilic and acidophilic component was leveled). Single exocrinocytes showed fine vacuolization of cytoplasm and pycnotic changes of nuclei. Small exit ducts were lined by cubic epithelium, terminal sections by cylindrical, but in some ducts there was flattening of epithelium and relative dilatation of duct with fibrosis of surrounding stroma and venous fullness. Mitotic patterns were absent. In old rats with DM, the gland retained a lobular structure, but the lobules varied in size, predominantly decreasing due to connective and adipose tissue overgrowth. In most cases the gland had acinar structure with single row pyramidal cells, however dystrophic changes prevailed in cells – diffuse hydrophic cytoplasmic dystrophy, pycnosis of nuclei and chromatin fragmentation in cells

with loss of apical microvessels, apical and then total desquamation of exocrinocytes into acinus lumen. In some animals, foci of fine fatty dystrophy were detected. On the background of dystrophic-degenerative changes of cells, there were also areas of compensatory adenomatosis of exocrinocytes. Among the dilated exit ducts with flattened epithelium there were ducts with epithelium hyperplasia. Fibrosis with residual lymphocytes and plasma cells developed around the vessels and the exit ducts. Fibrosis also developed and the ducts were tortuous, dilated and filled with homogeneous oxyphilic substance (Fig. 4).

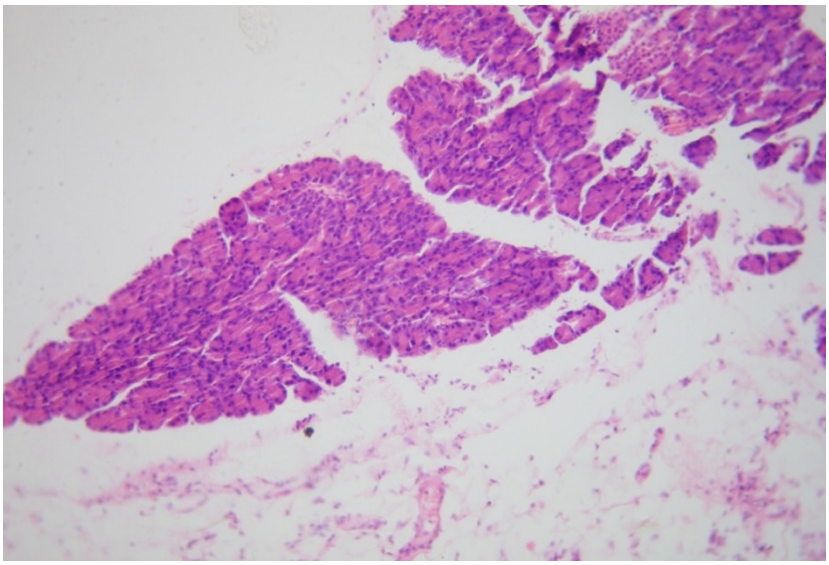

Figure 4. Pancreas of the elder rat with DM

Islets of Langerhans in the elder animals of the intact group were few in number, their shape was more rounded compared to younger animals and amounted to 1%-1.5%. The diameter of islets also ranged from 50 to 200 μm, but islets of different diameters were evenly distributed and no significant patterns of islet size were detected. The boundaries of islets were clear, and β-cells were located both in the centre of the islet and were diffusely scattered. Apoptosis markers were not observed, and individual cells with signs of cytoplasmic hydropic dystrophy and nuclear pycnosis were found in islet cells. In hyperglycaemic rats, the islet part was reduced to 1%-1.5% without signs of inflammatory infiltration. Islets of Langerhans had an irregular shape and blurred borders. The diameter of the islets decreased and ranged from 50 to 100 μm. Mitotic and apoptotic markers were absent. Degranulation, vacuolization of cytoplasm and nucleus, chromatin condensation and cell shrinkage developed in most islets.

Conclusion. The data obtained during morphological study of pancreas in rats of different age groups correlate with similar parameters of glycaemic levels and confirm the development of DM. The effect of the same dose of alloxan on rats of different ages was found to be slightly different. The damage to the exo- and endocrine parts of the BP of older rats was less pronounced, which may be related to age-related features of glucose metabolism, namely an increase in basal blood glucose and insulin levels.

References:

1. Lakin G. V. Biometriya.– M.: Vyisshaya shkola; 1990.– 352 p.
2. Semenko V. Serdyuk V. Savytskyi I. Development of experimental alloxan model of diabetes mellitus. International Journal of Endocrinology (Ukraine), [S. l.],– Vol. 13.– No. 4. 2017.– P. 276–280. DOI: 10.22141/2224–0721.13.4.2017.106657.

3. Sharofova M. U., Sagdieva Sh. S., and Yusufi S. D. "Diabetes mellitus: current state of the art (part 1)" Vestnik Avitsennyi,– Vol. 21.– No. 3. 2019.– P. 502–512.
4. Garbuzov V. V., Morozov V. A., Maslov A. V., Hafizov R. F. Diabetes mellitus: risk factors of development and complications. modern tendencies. Alleya nauki,– 2(1). 2020.– P. 171–177.
5. Gritsyuk M. I. Comparative characteristics of experimental models of diabetes mellitus / M. I. Gritsyuk, T. M. Boychuk, O. I. Petrishen // SvIt meditsini ta bIolog IYi. 2014.– No. 2(44).– P. 199–203.– Rezhim dostupu: URL: http://nbuv.gov.ua/UJRN/S_med_2014_2_57.
6. International guidelines for biomedical research using animals// Hronika VOZ. 1985.– T. 39.– No. 3.– P. 3–9.
7. European convention for the protection of vertebrate animals used for experimental and other scientific purposes.– Council of Europe, Strasbourg, 1986.– 53 p.
8. Yanko R. V., Chaka E. G., Levashov M. I. Age differences in the morphofunctional state of the pancreas in rats after the administration of magnesium chloride. Rossiyskiy fiziologicheskiy zhurnal im. I. M. Sechenova. 105, 4.– fev. 2019.– P. 501–509. DOI: https://doi.org/10.1134/S0869813919040
9. Balabina N. M. B20 Chronic pancreatitis: diagnosis, treatment and prevention in outpatient: a study guide / N. M. Balabina; GBOU VPO IGMU Minzdrava Rossii, Kafedra poliklinicheskoy terapii i obschey vrachebnoy praktiki.– Irkutsk: IGMU, 2016.– 91 p.