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RESEARCH ARTICLE

Morphological Analysis of Changes in The Liver and Pancreas in Metabolic Syndrome in An Experimental Animal Model

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Abstract: Metabolic syndrome (MS) is a complex of interrelated disorders including obesity, insulin resistance, dyslipidemia, and hyperglycemia, which lead to structural and functional changes in the internal organs. Of particular importance in the pathogenesis of MS are lesions of the liver (non-alcoholic fatty liver disease, NAFLD) and pancreas (non-alcoholic fatty pancreas disease, NAFLD). This article presents an analysis of morphological changes in the hepatobiliary system and pancreatic tissue in experimental rodent models of MS. The dynamics of steatosis, inflammatory infiltration, fibrosis, and cell apoptosis depending on the duration of dietary exposure are considered. Particular attention is paid to the correlation between morphometric parameters (pancreatic islet diameter, adipocyte area, degree of fatty infiltration) and metabolic disorders (insulin resistance, hyperglycemia, dyslipidemia). The role of intestinal endotoxemia and oxidative stress in the development of organ damage is discussed.

Key words: Metabolic syndrome, liver morphology, pancreas morphology, steatosis, non-alcoholic fatty liver disease, non-alcoholic fatty pancreas disease, experimental models, islets of Langerhans, apoptosis.

INTRODUCTION

Metabolic syndrome is one of the most pressing issues in modern medicine due to its high prevalence (20–40% of the adult population in developed countries) and its role as a key risk factor for the development of cardiovascular diseases, type 2 diabetes mellitus, and non-alcoholic fatty liver disease [1, 4]. According to current concepts, the underlying causes of metabolic syndrome are visceral obesity, insulin resistance, low-grade systemic inflammation, and oxidative stress [9, 10].

The liver and pancreas are the target organs most early and severely affected by MS. The liver develops a spectrum of changes, collectively known as NAFLD, ranging from simple steatosis to steatohepatitis, fibrosis, and cirrhosis [1, 3, 8]. NAFLD develops in the pancreas, characterized by ectopic fat accumulation in the acinar tissue and islet apparatus, leading to impaired insulin secretion and worsening IR [4, 6].

Animal models play a key role in studying the pathogenesis of MS and morphological changes in internal organs. The most adequate and reproducible models are diet-induced models in rats and mice using high-fat and high-carbohydrate diets [1, 4, 9]. Long-term feeding of such diets allows us to track the dynamics of structural changes in the liver and pancreas throughout the development of MS—from initial metabolic disturbances to terminal organ damage.

The aim of the study was to conduct a comprehensive morphological study of liver and pancreas changes in experimental metabolic syndrome in rodents and to identify correlations between structural changes and metabolic disturbances.

METHODS

For morphological analysis, the obtained tissues were fixed in 10% buffered formalin for 24 hours. Routine tissue processing was performed on a carousel processor STP 120, ThermoFisher, Germany, after which the samples were embedded in paraffin. Sections 3-4 μm thick were obtained on a rotary microtome HM 325 (TFS, USA). For staining the sections with

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hematoxylin and eosin, serial sections were dewaxed and dehydrated. Then they were kept for 2-5 minutes in a solution of Ehrlich's hematoxylin. The sections were washed in distilled water, followed by histological examination on an upright microscope Soptop. CX 40 (China). Staining was considered satisfactory if the nuclei had an intense red-violet color, nucleoli and chromatin clumps were visible within the nuclei, and the cytoplasm was not stained. Sections stained with hematoxylin and washed with water were transferred to distilled water for 3-5 minutes. To stain the cell cytoplasm, sections were placed in an eosin solution for 0.5-2 minutes. Staining was considered successful if the section had a uniform yellowish-pink color, against which the blue-stained nuclei were clearly visible. After staining in an eosin solution, sections were washed in distilled water, dehydrated with alcohol, cleared in xylene, and mounted in a preservative medium.

Images were captured using a Soptop OD2000Y detachable camera (China). Cells were imaged using ImageJ software version 1.51n (National Institutes of Health, USA) on the Java 1.8.0_345 platform (64-bit version). Cell density per mm2 was calculated, taking into account that the microscope eyepiece area at 20x resolution was 0.95 mm2.

RESULTS

Group No. 1, Experimental group, which was administered melatonin 10 mg/kg orally for 3 months, stained using the hematoxylin and eosin method.

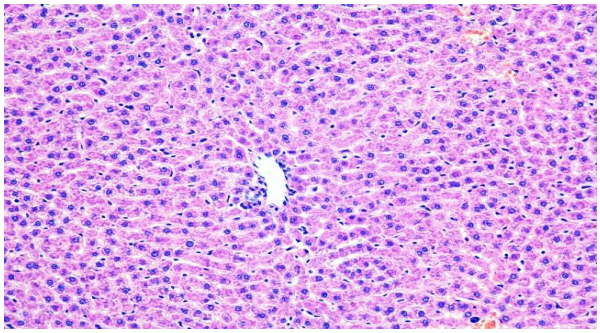

Fig. 1. Research using the method hematoxylin-eosin x20.

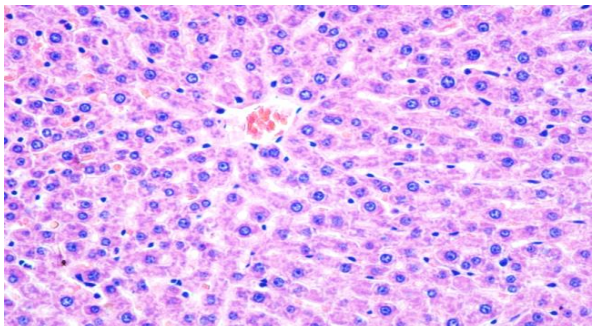

Fig. 2. Research using the method hematoxylin-eosin x40.

At low magnification, rat liver tissue reveals hepatoid glandular structures extending from the central vein to the portal veins as trabeculae, representing a lobe of the liver parenchyma. At high resolution, endothelial cells are visible between the trabeculae, forming sinusoids (black arrows) within which individual Kupffer cells "levitate." Between the endothelial cells and hepatocytes is the Disse space, where hepatic stellate cells are adjacent. The following changes can be clearly identified:

1. Absence of zonation: Almost all cells of the glandular acini are elongated, oval, or irregular in shape. The nuclei are large and spherical and occupy the center of the cell.

Heterochromatin is present as scattered clusters in the nucleoplasm and as a distinct band under the nuclear membrane. Each nucleus contains two or more clearly visible nucleoli. The cytoplasm of hepatocytes is filled with acidophilic granules.

2. Hepatocyte isometry: despite the poorly visualized boundaries of the hepatocyte membranes, at low magnification they form smooth strands and show the absence of any deviations in cell size.

Decreased number of inflammatory cells: inflammatory cells in the form of segmented leukocytes (black circle) and lymphocytes can occasionally be found in the portal veins.

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Group No. 2, Experimental group, which was treated with fats, Coca-Cola drink orally for 3 months, stained using the hematoxylin and eosin method.

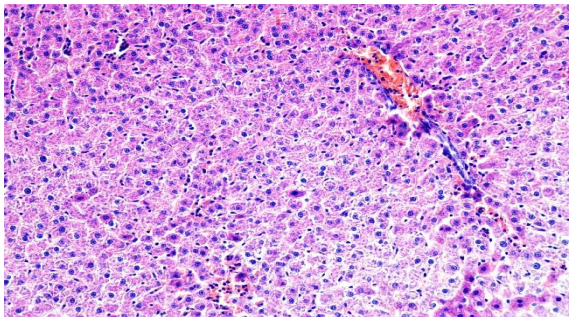

Fig. 3. Research using the method hematoxylin-eosin x20.

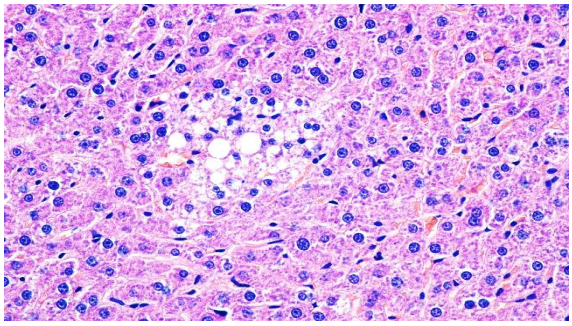

Fig. 4. Research using the method Hematoxylin- eosin x40.

At low magnification, the specimen is characterized by a fragmented liver parenchyma structure with isolated fatty foci, moderate portal reaction, and congestion. Moderate inflammatory cell infiltration, locally involving Kupffer cells, is noted in the periportal areas.

1. Single foci of steatosis: vacuolization of hepatocytes with degenerative changes, including mild granulation in the cytoplasm, also replacing vacuoles (black circle).

2. Small foci of inflammatory conglomerates: a moderate accumulation of inflammatory and Kupffer cells (black arrow) can be seen on the preparation; this tendency is observed especially in the periportal zones.

Portal reaction and congestion: manifest as bile duct proliferation in the area of Mallet, whereas one or two ducts should normally be present. Inflammatory cells in a state of "diapedesis" are also detected in the central veins.

The data from the group results show the absence of NAFLD or NASH, since when assessing the level of steatosis according to Kleiner et al ., 2005, showed a favorable outcome of the experimental groups Table 1, based on this, there was no need to evaluate the micropreparations according to Bedossa et al ., 2016 and Isak .

Histological feature	Description	Score	Group 1	Group 2
Steatosis (%)	≤ 5	0		
	5–33	1		
	33–66	2		
	≥ 66	3		
Lobular inflammation	Absent	0		
	≤ 2 foci	1		
	2–4 foci	2		
	≥ 4 foci	3		

Table 1. Assessment according to Kleiner et al. al ., 2005. Definitions and scoring of the NASH scoring system.

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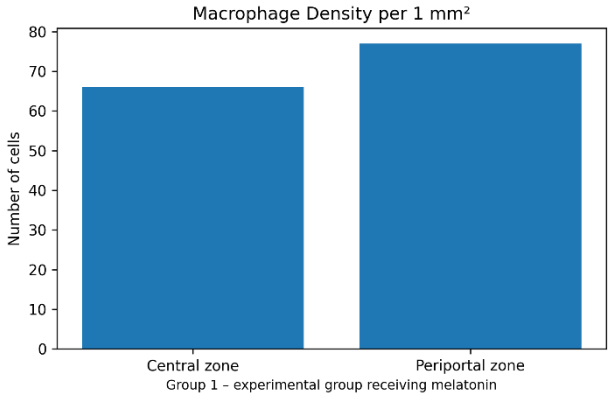

Nevertheless, a preliminary count of Kupffer cells in the periportal and central zones of the liver lobes was performed. In Group 1, the periportal zone contained 66.32 cells per mm², while in the central zone, 76.8 (Fig. 5), and in Group 2, 121 and 82, respectively. The differences in the figures between the two groups indicate the influence of melatonin in the anti-sleep process.

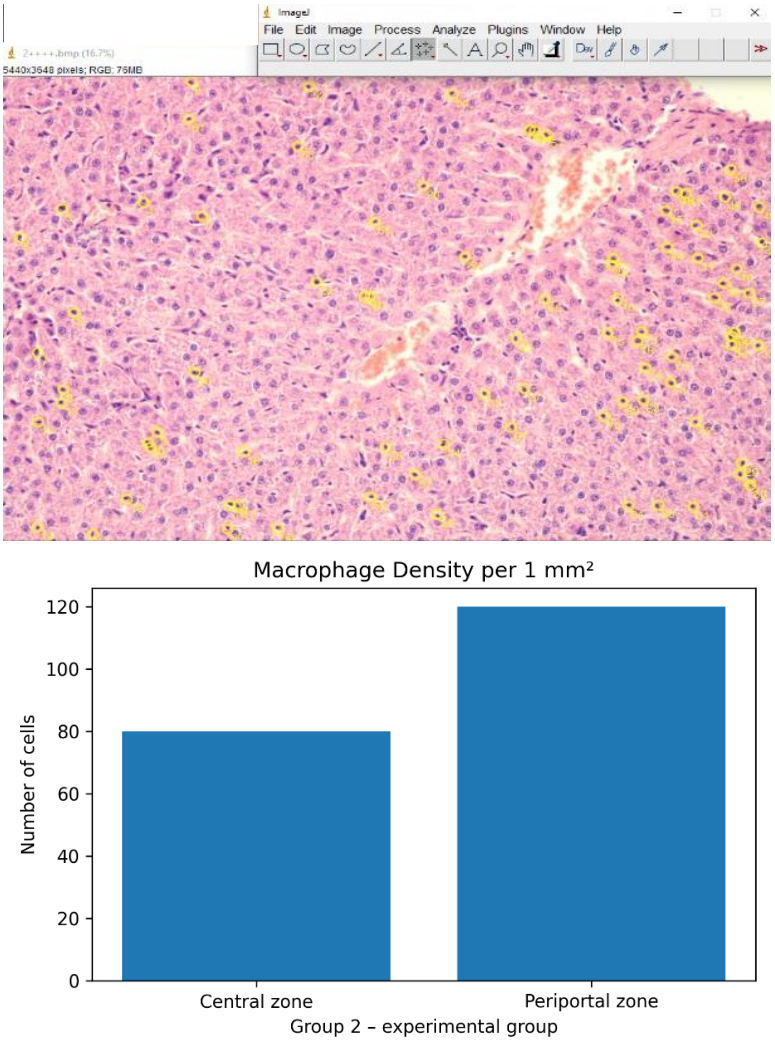

Fig. 5. Annotation of Kupffer cells, at 20x resolution Image J

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CONCLUSION AND DISCUSSION

Restoration of architecture due to isometries of units of hepatoid acini was clearly manifested in the liver of the first group Fig. 1; similar results were shown by the experiment in diabetic rats Alsharif et al., 2016, who received melatonin intraperitoneally at 10 mg/kg body weight for 4 weeks, but were distinguished only by moderate congestion, monocytic infiltration and mild periportal fibrosis. However, in the work of Agil et al., 2016, in experimental Zucker Diabetic Fatty rats treated with melatonin for 6 weeks, according to the assessment of Kleiner et al., 2005, 15% steatosis ($p < 0.001$) in the liver parenchyma remained. Also in this study, liver cells were isolated and mitochondria were measured under an electron microscope; thus, the area and number of mitochondria increased: number (284.19±17.46 pcs.), area (0.10±0.012 μm2) ($p < 0.001$), this speaks of the reparative properties of melatonin, since it neutralizes free radicals in hepatocytes, which ultimately reduces the release of pro-inflammatory cytokines (IL -6, TNF- a , C-reactive protein) [9] , and, consequently, the elimination of the involvement of immune cells in this process [10, 11] .

In the second group, most hepatocytes were vacuolated, and in isolated places foci with slight steatosis were visualized, and in the experiment of Auberval et al ., 2014, after 2 months of treatment with a high-fat diet, the liver tissue of Wistar rats had pronounced grade 3 steatosis according to Kleiner et al., 2005 [2] . It is worth noting that the number of resident macrophages was relatively higher than in the first group, since melatonin polarizes active macrophages into proregulatory ones. By the way, ROS in hepatocytes is a trigger for oxidative stress and the excretion of proinflammatory cytokines, which, in fact, activate immune cells (macrophages and T-lymphocytes) and Ito cells Fig. 2, and the starting point leading to chronic inflammation of the liver fibrogenesis.

In conclusion, melatonin at a dose of 10 mg/kg as a potential hepatoprotector has a beneficial effect on the functional parameters and histological signs of the liver in rats.

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