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ABSTRACT

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THE EFFECT OF ALLOXAN-INDUCED HYPERGLYCEMIA ON THE RENAL CORTEX

About 422 million people in the world suffer from diabetes mellitus. Among diseases, diabetes ranks third, and among endocrine disorders, it ranks first. Some issues concerning the pathogenesis of this disease are unclear. The main reason for diabetes damage is high blood glucose levels. Hyperglycaemia has a toxic effect on the vessels of the kidneys. The present study aims to investigate the impact of alloxan-induced diabetes mellitus on the remodeling of the renal cortex.

Materials and methods. We divided twenty-four mature white male rats into the control and experimental groups. We administered alloxan to experimental animals intraperitoneally at a single dose of 40 mg/kg. Blood glucose levels were measured 2, 12, and 24 hours after injection of alloxan and then weekly. The average glucose level remained 11.0 ± 2.0 mmol/l. Animals were sacrificed on days 14, 21, and 45. We stained histological preparations of kidneys with hematoxylin and eosin. The selected dose of alloxan and the method of its administration caused persistent hyperglycemia in rats and did not lead to their death.

Results. On the 14th day, the diseased kidney had a thickening of the glomerular capillary walls. Nephrons had a spherical shape with a slightly uneven surface. On the 21st day of the observation, it was more difficult to distinguish the cortex from the medulla. On the 45th day, the distal tubules lost their usual shape, became thinner, and were difficult to distinguish from other tubules by histological preparation. The cortex became spongy due to cystic dilation of the tubules.

Conclusions. All components of the renal cortex underwent daily changes. At the early stages of the experiment, it looked much denser compared to the cortical layer of the kidneys of animals in the control group. The number of subcapsular nephrons visually increased, and the renal capsule thickened. In the later period of the experiment, dilatation and blood fullness of glomerular capillary with their leukocyte infiltration were observed. In addition, there was a cystic expansion of the tubules, due to which the cortical layer of the kidney looked like a sponge.

Keywords: hyperglycemia, alloxan, kidney, cortex, medulla.

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ВПЛИВ АЛЛОКСАНОВОЇ ГІПЕРГЛІКЕМІЇ НА КІРКОВУ РЕЧОВИНУ НИРКИ

Близько 422 мільйонів людей у світі хворіють на цукровий діабет. Серед захворювань цукровий діабет посідає третє місце, а серед ендокринних захворювань – перше. Деякі питання щодо патогенезу цього захворювання залишаються неясними. Основною причиною ураження діабетом є високий рівень глюкози в крові. Гіперглікемія чинить токсичну дію на судини нирок. Метою цього дослідження є вивчення впливу цукрового діабету, індукованого алоксаном, на ремоделювання кори нирок.

Матеріали та методи. 24 білі 7-місячні щури-самці були розділені на дві групи: контрольну (6 тварин) і дослідну (18 тварин). Піддослідним тваринам аллоксан вводили один раз внутрішньочеревно в дозі 40 мг/кг. Рівень глюкози у щурів вимірювали через 2, 12 і 24 години після ін'єкції аллоксану, а потім щотижня. Середній рівень глюкози залишився 11,0 ммоль/л ± 2,0 ммоль/л. Тварини були виведені з експерименту на 14, 21 та 45 добу. Гістологічні препарати нирок фарбували гематоксиліном та еозином. Підібрана доза аллоксану та спосіб його введення викликає у щурів стійку гіперглікемію і не призводить до їх загибелі.

Результати. На 14-ту добу в хворій нирці спостерігається потовщення стінок капілярів клубочків. У кірковому шарі знаходяться численні клубочки нефронів. Вони мають сферичну форму з невеликою нерівністю поверхні. На 21-й день спостереження відрізнити кору від мозкової речовини важче. Однак кортикальний шар має більш нерівну поверхню, ніж у попередньому дослідженні. На 45-ту добу спостереження в нирках дистальні канальці втрачають свою звичну форму, стоншуються і їх важко відрізнити від інших канальців на гістологічному препараті. Кора стала губчастою внаслідок кістозного розширення канальців. Відбувається поступове посилення кровотоку, що є причиною функціональних і структурних порушень у нирках.

Висновки. Усі компоненти кіркового шару нирок щодня зазнають змін. На ранніх етапах експерименту він виглядає значно щільнішим порівняно з таким у тварин контрольної групи. Візуально збільшується кількість субкапсулярних нефронів, потовщується ниркова капсула. У більш пізній період досліду спостерігається розширення і кровонаповнення клубочкових капілярів з їх лейкоцитарною інфільтрацією. Крім того, спостерігається кістозне розширення канальців, за рахунок чого кортикальний шар нирки виглядає як губка.

Ключові слова: гіперглікемія, аллоксан, нирка, кіркова речовина, мозкова речовина.

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INTRODUCTION / ВСТУП

Diabetes mellitus is a chronic disease associated with hyperglycemia when bodies cannot cope with blood sugar (glucose) levels. Hyperglycemia is the most common feature in the diagnosis of diabetes [1]. The number of people that have this disease increases every day.

About 422 million people worldwide have diabetes (World Health Organization, 2020). An estimated 700 million adults worldwide will have diabetes by 2045. (International Diabetes Federation [IDF], 2020). Among diseases, diabetes ranks third place, and among endocrine diseases – first. Diabetes is ahead of tuberculosis in terms of the rate of the disease [2].

Scientists from all over the world are researching diabetes. Despite the rapid development of science, some questions about the pathogenesis of the disease are unclear [3]. Many scientists link the appearance of diabetes with urbanization, unhealthy habits, biochemical changes, high-sugar drinks, and a stressful lifestyle. However, some people are genetically prone to type 2 diabetes caused by pancreatic beta-cells dysfunction [4]. The main reason for diabetes damage is high blood glucose levels. It has a toxic effect on the vessels of the kidneys. Primarily it induces non-enzymatic glycosylation of proteins, oxidative stress, active growth factors, and cytokines that cause kidney damage at the cellular level. Diabetes mellitus causes functional, structural, and clinical abnormalities [7].

Alloxan is a structural analog of glucose. Most often, alloxan diabetes is caused by double subcutaneous administration of an aqueous solution of alloxan hydrate to animals (mice, rats, rabbits, and dogs) that were previously fasted during the day [5]. Alloxan-diabetic rats have increased kidney weight [6, 8]. This disease increases blood glucose, creatinine, and cholesterol and provokes high oxidative stress in the kidneys. As a result of the nephron's increasing filtration load, there is an excretion of proteins and glucose in the urine [9].

The present study aims to investigate the effect of alloxan diabetes mellitus on the kidney functions of diabetic rats.

Materials and Methods. We divided twenty-four mature white male rats into control and experimental groups. We administered alloxan to experimental animals one time intraperitoneally at a dose of 40 mg/kg. Blood glucose levels were measured 2, 12, and 24 hours after injection of alloxan and then weekly. The average glucose level remained at 11.0 ± 2.0 mmol/l. Animals were sacrificed on days 14, 21, and 45. Histological preparations of the kidneys were stained with hematoxylin and eosin. The selected dose of alloxan and the method of its administration cause persistent hyperglycemia in rats and do not lead to their death.

Results. In the kidneys of the control group of rats, the thin fibrous capsule directly adjacents to the cortex. The capsule, cortex, and medulla are microscopically distinguished. The glomeruli have a spherical shape. The capsule of nephrons has a precise contour and a rounded shape (Fig. 1).

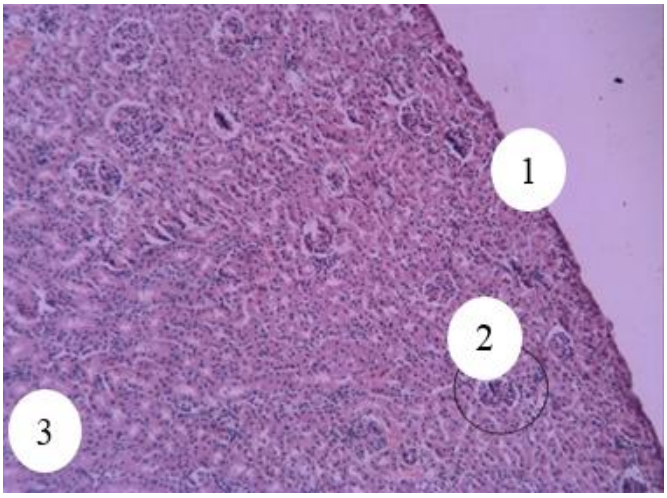

Figure 1 – The kidney of control rats x 40 (1 − kidney capsule, 2 – glomeruli, 3 – medulla)

On the 14th day of the observation in the experimental rat's capsule, the cortex and medulla are microscopically distinguished. There is a thickening of the cortical layer of the kidney of experimental animals. The diseased kidney has a thickening of the walls of the capillaries of the glomeruli. In the cortical layer, there are numerous glomeruli of nephrons. They are spherical with a little uneven surface. The glomerular capsule contains a rounded shape (Fig. 2).

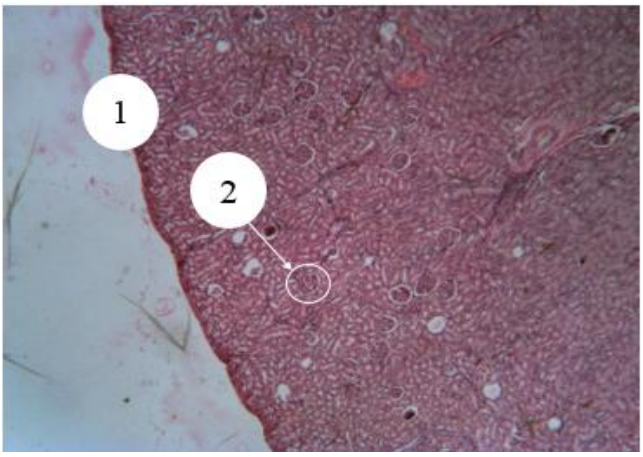

Figure 2 – The kidney of experimental rats on day 14 of alloxan-induced hyperglycemia x 40 (1− kidney capsule, 2 – glomeruli)

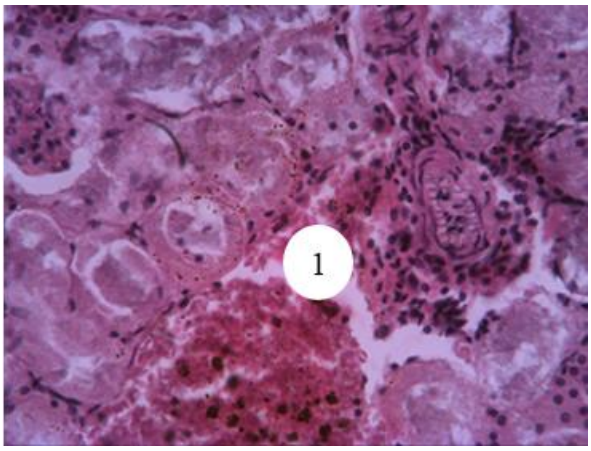

Figure 3 – The kidney of experimental rats on the day of 21 of alloxan-induced hyperglycemia x 400 (1 − Bowman's capsule)

On the 21st day of the observation, it is more difficult to distinguish the cortex from the medulla. However, the cortical layer has a more uneven surface than in the previous study. The glomeruli lose their rounded shape. The cavity between the outer and inner layers of the glomerular capsule (Bowman's capsule) slightly increases (Fig. 3). Renal capsule compaction is also observed. However, in the early development of experimental diabetes mellitus, disorders of the tubular nephron are not responsible for changes in renal function.

On the 45th day of the observation in the kidneys, the distal tubules lose their usual shape,

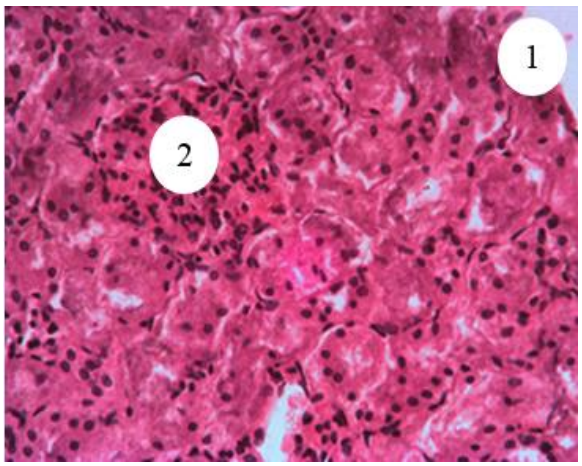

Figure 4 – The kidney of experimental rats on the 45-th day of alloxan-induced hyperglycemia x 400 (1 − spongy cortex, 2 – glomeruli in the renal cortex infiltrated with leukocytes)

become thinner, and are difficult to distinguish from other tubules by histological preparation. The cortex has become spongy due to cystic tubular dilatation. Glomerular capillaries are dilated and full of blood. This is explained by hyperfunction of the kidney under hyperglycemia, which is manifested by an increase in blood flow due to hyperfiltration (Fig. 4). Also, there is a thinning of the renal artery. The mesangial expansion and inflammation of interstitial cells in diabetic animals explains diffusely located lymphocytes and macrophages in the interstitial connective tissue of the kidney.

CONCLUSIONS / ВИСНОВКИ

All components of the renal cortical layer undergo daily changes under hyperglycemia. Already at the early stages of the experiment, the cortex looks much denser compared to the cortical layer of the kidneys of animals in the control group. The number of subcapsular nephrons visually increases, and the renal capsule thickens. In the later period of development of experimental diabetes mellitus, disorders of the glomeruli and tubular system are responsible for changes in renal function. Thus, dilatation and blood fullness of glomerular capillaries with their leukocyte infiltration is observed. In addition, there is a cystic expansion of the tubules, due to which the cortical layer of the kidney looks like a sponge.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

Such an experimental model can help conduct comprehensive research aimed at studying the mechanisms of diabetes mellitus, the effects of hyperglycemia on organs and tissues, and finding ways to correct complications of diabetes mellitus.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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None.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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