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UDC 612.392.6: 612.751.1: 616-092.9: 616.71-003.96
DOI – https://doi.org/10.14300/mnnc.2021.16041
ISSN – 2073-8137

PATHOBIOCHEMICAL CHANGES IN THE HISTOLOGICAL STRUCTURE OF KIDNEYS WITH EXPERIMENTAL HYPERVITAMINOSIS D

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ПАТОБИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ ГИСТОЛОГИЧЕСКОЙ СТРУКТУРЫ ПОЧЕК ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ГИПЕРВИТАМИНОЗЕ D

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This study focused on the nature and degree of pathobiochemical changes in kidney tissues from the post-lactation period of female rats and their offspring at different ages after exposure to hypervitaminosis D. The pathobiochemical cascade from hypervitaminosis D involved excessive calcium accumulation and was characterized by self-damage to cells and the development of hypoxia during gestation and lactation and in the post-lactation period in rats and their offspring. Hypervitaminosis D produced vascular disorders (plethora of vessels of the kidney, stasis and interstitial edema) leading to increased epithelial cell size and narrowing of the lumen of tubules. These disorders were combined with hyaline-droplet protein dystrophy of the convoluted tubules and fatty degeneration of the epithelium of collecting ducts. There were also multiple foci of hypereosinophilia and areas of calcification located in the vessel walls, glomeruli and tubules of kidneys.

Keywords: hypervitaminosis D, calcium, hypoxia, kidneys, experiment, epithelial cells

В условиях эксперимента на лабораторных животных изучены характер и степень патобиохимических изменений в тканях почек у беременных и их потомства, в различные возрастные периоды, при интоксикации витамином D. Патобиохимический каскад избыточного накопления кальция, характеризующийся повреждением клеточных мембран и развитием гипоксии, у беременных лабораторных животных и их потомков проявлялся сосудистыми нарушения (полнокровие сосудов почки, стаз и интерстициальный отек), приводящими к увеличению эпителиальных клеток в размерах и сужению просвета канальцев. Эти нарушения сочетались с гиалиново-капельной белковой дистрофией извитых канальцев почек и жировой дистрофией эпителия собирательных трубочек. Установлены множественные очаги гиперэозинофилии и участки обызвествления, расположенные в стенке сосудов, в клубочках и канальцах почек.

Ключевые слова: гипервитаминоз D, кальций, гипоксия, почки, эксперимент, эпителиальные клетки

For citation: Bondar T. P., Svetlitskiy K. S., Svetlitskaya Yu. S., Botasheva V. S. PATHOBIOCHEMICAL CHANGES IN THE HISTOLOGICAL STRUCTURE OF KIDNEYS WITH EXPERIMENTAL HYPERVITAMINOSIS D. *Medical News of North Caucasus.* 2021;16(2):180-183. DOI – https://doi.org/10.14300/mnnc.2021.16041

Для цитирования: Бондарь Т. П., Светлицкий К. С., Светлицкая Ю. С., Боташева В. С. ПАТОБИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ ГИСТОЛОГИЧЕСКОЙ СТРУКТУРЫ ПОЧЕК ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ГИПЕРВИТАМИНОЗЕ D. *Медицинский вестник Северного Кавказа*. 2021;16(2):180-183. DOI – https://doi.org/10.14300/mnnc.2021.16041

ATP – adenosine triphosphate

25(OH)D – 25-hydroxyvitamin D (calcidiol)

Recent experimental work on deciphering the metabolism of vitamin D and large-scale epidemiological studies have confirmed the essential role of active metabolites of vitamin D in maintaining health [1–3]. Views on the role of vitamin D have undergone significant evolution. It is known that an excess of vitamin D disrupts the function of many organs and systems, including kidney function, disrupting the glomerular and canaliculi apparatus (especially the proximal tubule) function, with a decrease in renal concentration function and glomerular filtration. Particular studies have targeted organs and tissues with receptors for vitamin D, and the genomic and extragenomic cellular effects of calcitriol, especially in children, starting with intrauterine development [4–6].

The purpose of this research was to determine the conditions of hypervitaminosis D in laboratory animals, and the nature and degree of pathobiochemical changes in kidney tissue in pregnant rats and their offspring at different ages during vitamin D toxicity.

Material and Methods. White Wistar rats were used in this study. Twelve post-lactation period female rats with an average weight of 200.1±8.9 g and approximately the same age, and 42 offspring were examined. Hypovitaminosis D was established in 12 female rats. After pregnancy was confirmed and hypovitaminosis D was established, all animals received a therapeutic dose (5000 IU/day) of cholecalciferol during pregnancy and lactation. Each animal examined throughout pregnancy and lactation was kept isolated and in stationary vivarium conditions with free access to water and food, under natural temperature and light conditions, the same time of day and season. Rat offspring received vitamin D at a therapeutic dosage after transition to self-feeding (in the second age period). As a control for this model, the content of total 25(OH) D was evaluated by quantitative analysis, as well as the levels of biochemical markers of vitamin D metabolism and densitometry data. All animals exhibited biochemical changes that characterized hypervitaminosis D. The results and a detailed description of the experimental model were published earlier [7, 8].

To study the nature and degree of changes in the histological structure of the kidneys, standard methods of preparing, fixing and staining (hematoxylin and eosin) histological tissue sections were used. Histological examination of organs in the post-lactation period of female rats and their offspring was carried out at 30, 60 and 90 days after birth.

Statistical processing of the data obtained during the study was carried out using a standard software package for applied statistical analysis, STATISTICA 10 (StatSoft, USA). When statistically significant differences between the groups were found, post hoc comparisons were made using the Mann–Whitney test.

Results and Discussion. Our research into pathobiochemical changes in rats with experimental hypervitaminosis D has revealed the nature and severity of histological changes to the structure of kidney cells.

At the initial stages of the pathobiochemical cascade of hypervitaminosis D, the process of intracellular calcium accumulation begins, leading to an overload of mitochondria and an increase in catabolic processes, as well as the activation of anaerobic glycolysis, accumulation of lactic acid and activation of lipid peroxidation. The disappearance of ATP leads to a rapid swelling of epithelial cells, increasing their size and resulting in narrowing of lumen in the tubules. The epithelium of individual renal tubules contained cytoplasmic hyaline-like protein masses intensely stained with eosin (Fig. 1a). Dead mitochondria form masses consisting of lipids. Small and large drops of lipids were observed in the cytoplasm of epithelial cells of the collecting tubules (Fig. 1b), and fat drops occupied the entire cytoplasm of some epithelial cells. In the later cells, the nucleus was pyknotic and pushed to the periphery or completely destroyed.

Mild to moderate interstitial edema and vascular disorders, such as plethora and stasis (Fig. 1c), were early manifestations of hypoxia. Additionally, agranular cytoplasm was observed in epithelial of the renal tubules (Fig. 1d). In the kidneys of post-lactation female rats with hypervitaminosis D, multiple small foci of calcification were found and were most often localized in the glomeruli, tubules and vascular wall (Fig. 1e).

Thus, hypoxic-ischemic damage developed in the kidneys of rats exhibiting hypervitaminosis D during pregnancy and the lactation period, indicated by granularity of the cytoplasm and signs of hyaline-droplet protein dystrophy in the epithelium of convoluted tubules. Degeneration with small and large fatty droplets was observed in the epithelium of collecting tubules on the entire surface of the kidney, and small foci of calcification were located in blood vessel walls in the glomeruli and tubules.

At 30 days after birth, offspring from dams with vitamin D toxicity exhibited normal kidney histology, although minor vascular disorders were revealed, as well as areas with hypereosinophilia in the epithelium of convoluted tubules (Fig. 2).

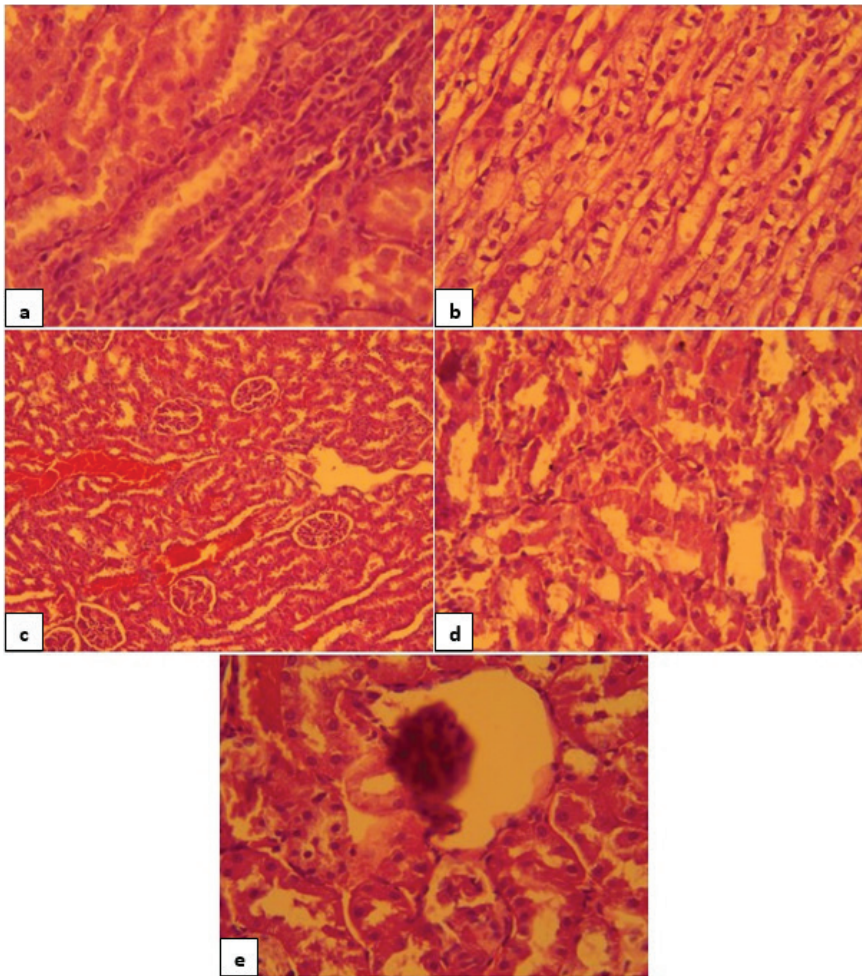

Fig. 1. Influence of hypervitaminosis D on the histological structure of kidney cells in rats in the post-lactation period: a) hyaline-drop protein dystrophy of the epithelium of convoluted tubules; b) fatty degeneration of the epithelium of collecting tubules; c) blood vessels of kidney stasis; d) granularity of the epithelial cytoplasm in renal tubules; e) small foci of calcification. Hematoxylin and eosin staining, ×400

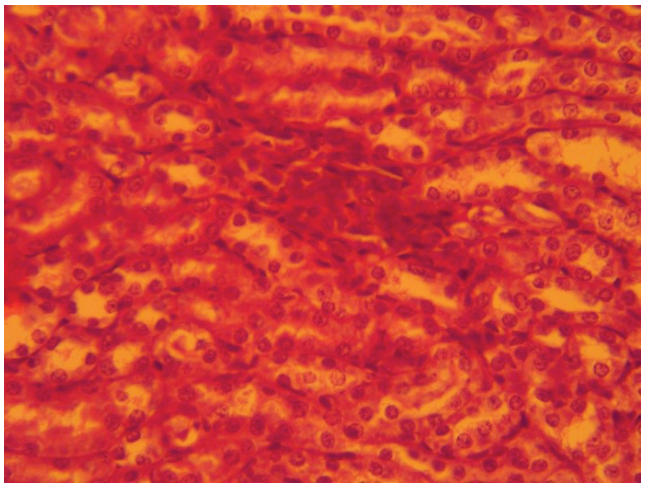

Fig. 2. Foci of hypereosinophilia in the epithelium of renal tubules in rat offspring 30 days of age. Hematoxylin and eosin staining, ×400

At 60 days after birth, vascular disorders persisted in the kidneys of offspring, in addition to plethora and stasis, and large aggregates of erythrocytes. The epithelial cytoplasm of convoluted tubules became homogeneous, but granularity remained in places. Proteinaceous and dense hyaline casts were visible in the lumen of tubules, as well as basophilic conglomerates consisting of calcareous masses (Fig. 3a). At 90 days after birth, widespread vascular disorders were found in the kidneys. The epithelium of renal tubules was homogeneous and large foci of hypereosinophilia, together with areas of calcification, were found in blood vessel walls (Fig. 3b).

Thus, experimental rat offspring at 30 days of age exhibited insignificant pathological changes in the kidneys, although there were vascular disorders and foci of cytoplasmic hypereosinophilia. In offspring at 60 days of age, these changes intensified and calcium conglomerates were found in the lumen of tubules. In 90-day-old experimental rats, foci of calcification appeared in the blood vessel walls.

There have been no studies examining the pathobiochemical changes in kidneys as a result of prolonged use of various dosages of vitamin D by pregnant women and their offspring. There is disagreement on the use of adequate doses of vitamin D, not only at different ages, but also during intrauterine development. In particular, the lack of work on the safe use of vitamin D has been noted by some international authors [9].

However, in an earlier study, nephrosclerosis and microcalcifications were observed in a 54-year-old man who took a daily dose of 8000–12000 IU vitamin D by mistake every day for 2.5 years [10]. These findings were consistent with the pathobiochemical changes obtained in our study.

In the current work, it is impossible to exclude the direct effect of hypercalcemia, which presents as significant damage to kidney tissues, such as deep dystrophic changes against the background of pronounced vascular plethora, and hypereosinophilia. The current data support the results of previous studies carried out by V. I. Strukov [11], showing that the kidneys of white rats displayed the phenomena of necronephrosis (death of the epithelium of convoluted tubules with signs of karyolysis), deposition of calcium salts, and electron microscopic examination showing a decrease in the amount mitochondria and the appearance of calcium salts in mitochondria.

Conclusions. By establishing hypervitaminosis D in pregnant and lactating female rats and examining their offspring at progressive age periods, this study revealed temporal pathobiochemical changes in the histological structure of the kidneys. The pathobiochemical cascade of excessive calcium accumulation inside cells, a decrease in energy metabolism, an increase in the permeability of cell membranes along with cellular acidosis and activation of lipid peroxidation, all characterize a biochemical adaptation to hypoxia. The kidneys of post-lactation rats with hypervitaminosis D were characterized by moderate vascular disorders, hyaline droplet degeneration in the epithelium of convoluted tubules and fatty degeneration of epithelium of collecting ducts. In addition, foci of cal-

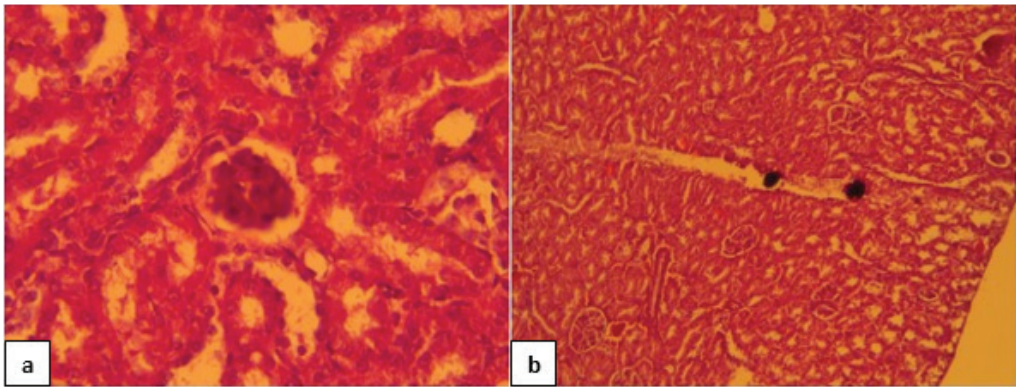

Fig. 3. Influence of hypervitaminosis D on the histological structure of kidneys from 60- and 90-day-old experimental rats: a, calcareous basophilic conglomerates in the lumen of convoluted tubules; b, foci of calcification in blood vessel walls. Hematoxylin and eosin staining, ×400

cification were found mainly located in the walls of blood vessels in glomeruli and tubules. At 30 and 60 days of age, offspring from these pregnant rats exhibited pathobiochemical changes characterized by minor structural and circulatory disorders, the development of edema and the appearance of foci of hypereosinophilia. At 90 days of age, the offspring displayed widespread vascular disorders with the epithelium of renal tubules acquiring a homogeneous structure and large foci of hypereosinophilia. Furthermore, calcareous conglomerates had formed in the lumen of tubules from offspring at 60 days of age, and by 90 days of age, calcification foci were also detected in blood vessel walls.

Experimental animal procedures. The study was conducted in accordance with the International Recommendations of the World Health Organization, the International Recommendations for Biomedical Research Using Animals (1985) as well as the national standard of the Russian Federation GOST R-53434-2009 «Principles of Reasonable Laboratory Practice» GLP rules. The experiments were carried out in accordance with the principles of humanity set out in the directives of the European Community (86/609 /EEC) and the Helsinki Declaration. The study protocol was approved by the Local Ethical Committee.

Disclosures: The authors declare no conflict of interest.

Acknowledgment. We thank Charles Allan, PhD, from Edanz (www.edanz.com/ac) for editing a draft of this manuscript.

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