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MORPHOLOGICAL CHANGES IN THE RAT KIDNEYS INFLUENCED BY SURFACTANT

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МОРФОЛОГІЧНІ ЗМІНИ В НИРКАХ ЩУРІВ ПІД ВПЛИВОМ СУРФАКТАНТУ

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МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ПОЧКАХ КРЫС ПОД ВЛИЯНИЕМ СУРФАКТАНТА

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Summary/Резюме

The present study is focused on elucidate the effect of Laprol-604 on morphological structure of rat kidneys. The model of intoxication was produced by oral administration of water solution Laprol-604 to rats of experimental I group.

The pathomorphological changes in the kidneys I group have been found. Atrophy of glomerular cells, Bowmens' capsular spaces have been extended; tubular epithelial cells have been degenerated. In addition, protein in the renal tubular lumen and interstitial edema have been detected. These morphological changes in the kidneys I group indicate significant load renal tissue.

Keywords*: kidney, Laprol-604, surfactant, polyols*

The present study is a fragment of the scientific research work of the KhMAPE "Pathochemical mechanisms of radiotoxins impact on the body and the principles of their early diagnosis and correction" (state registration number № 0117U000589).

В настоящем исследовании изучали влияние Лапрола-604 на почки взрослых крыс. Эксперимент проводили на половозрелых крысах-самцах линии Вистар с массой тела 190 ± 23 г. Животных рандомизировано разделили на две группы, по 10 особей в каждой. Модель интоксикации получили ежедневным внутрижелудочным введением водного раствора Лапрола-604 в дозе 1/10 LD50 крысам I группы. Лапрол-604 вводили в течение 30 дней эксперимента. Эвтаназию крыс выполнили под тиопенталовым наркозом в дозе 20 мг/кг. Почки крыс взвешивали и обрабатывали для морфологического исследования. Рассчитывали почечный индекс. Почечный индекс в I группе был заметно выше, чем в контрольной группе, p=0.0001. Введение Лапрола-604 вызвало патоморфологические изменения в почках крыс I группы. Так, были обнаружены: атрофия клубочковых клеток, расширение пространства капсулы Боумена; дегенерация эпителиальных клеток проксимальных извитых канальцев, наличие белковых структур

в просвете почечных канальцев и интерстициальный отек. Эти морфологические изменения в почках I группы свидетельствуют о значительной нагрузке на почечную ткань.

Ключевые слова: *почка, Лапрол-604, поверхностно-активное вещество, полиолы.*

У даному дослідженні вивчали вплив Лапролу-604 на нирки дорослих щурів. Експеримент проведено на статевозрілих щурах-самцях з масою тіла 190 ± 23 г. Тварин рандомізовано поділили на дві групи, по 10 щурів у кожній. Модель інтоксикації отримали щоденним пероральним введенням водного розчину Лапролу-604 у дозах 1/10 LD50 щурам I групи. Лапрол-604 вводили впродовж 30 днів експерименту. Сечу щурів збирали протягом доби за допомогою метаболічних кліток. Евтаназію здійснювали під тіопенталовим наркозом у дозі 20мг/кг. Нирки щурів зважували і обробляли для морфологічного дослідження. Розраховували нирковий індекс. Нирковий індекс у I групі був статистично значуще, ніж у контрольній групі, p = 0.0001. Введення Лапролу-604 викликало патоморфологічні зміни в нирках щурів I групи. Так, були виявлені: атрофія клубочкових клітин, розширення простору капсули Боумена; дегенерація епітеліальних клітин проксимальних звивистих канальців, наявність білкових структур в просвіті ниркових канальців і інтерстиціальний набряк. Ці морфологічні зміни в нирках I групи свідчать про значне навантаження на ниркову тканину.

Ключові слова: *нирка, Лапрол-604, поверхнево-активна речовина, поліоли.*

Introduction

Nonionic surfactants are widely used in different fields of chemical industry such as pharmaceutical, food, and agricultural industries. Nonionic surfactants are employed to improve drugs‘ solubility and enhance its bioavailability. The food industry uses nonionic surfactants to change the stability of various emulsions and to decrease the retrogradation of amylopectin. Nowadays, humans are influenced by surfactants by numerous pathways [1, 2]. Research data confirmed that nonionic surfactants are able to spread between waters, soils, atmosphere, and living organisms from different geographic regions. The negative effect of nonionic surfactants on animals has been studied [3, 4, 5]. Their polytrophic influence on organism are closely related to the membrane pathology [6, 7]. These effects have been attributed to the surfactant’s ability to increase plasma membrane fluidity. Nonionic surfactant is Laprol-604 has attracted our attention. This is a complex organic mixture of polyoxypropylene polyols. Laprol-604 is being produced industrially for several decades. Laprol-604 has valuable commercial properties that include water and oil solubility, enough stability and, thus, it has been extensive utilized. This polyol is a component of epoxide resin, enamels, varnishes, plastic, fiber, glues, emulsifiers [8]. But we are far from understanding their behavior of those pollutants, their impact on living organisms. Although there are studies show morphological changes and cell apoptosis of hepatocytes and kidney cells under influenced by surfactants, but the histopathological changes in kidney induced by Laprol-604 are not investigated. The aim of this study has been given to elucidate the influence of Laprol-604 on morphological structure of rat kidneys.

Contingents

The study has been performed on

twenty male Wistar rats weighing 190±23g (10-week-old). All the procedures were performed according to "General ethical principles of experiments on animals"(Ukrainian, 2001) that agreed with European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg, 18.03.1986 [9].

Object

Laprol-604 was provided from Science and Production Joint Stock Company "Sintez PAV". Laprol-604 was reported to be 96% pure by the supplier. For all study, it was diluted in deionized water and prepared fresh daily.

Methods

Male rats were randomly assigned to two groups of 10 rats each: control group and I group. The model of intoxication was produced by oral administration of water solution Laprol-604 to rats of I group. Laprol-604 has been administered to male rats of I group once daily by gavage at doses of 1/10 LD_{50} (median lethal dose) – 12.5g/kg for 30 days. The control group consisted of 10 intact *male rats were kept on a standard diet,* without Laprol-604 administration. On the thirty first experimental day rats' body weight were measured. Male rats were euthanized by intraperitoneal administration of thiopental (20 mg/kg) and subsequently decapitated, kidneys were quickly removed, washed thoroughly with normal saline to remove the residual blood. Then weighed and processed for morphological studies. Renal index was calculated by dividing total left and right kidney weight to the terminal body weight for each animal.

Kidneys were fixed in 10% neutral buffered formalin. The tissue samples were dehydrated and embedded in paraffin wax and manually sectioned with a microtome to obtain at 5-μm thickness sections. The sections were washed by xylene to remove paraffin, and then washed by alcohol in descending order 100, 95, 80, 70, 50% and deionized water, and stained with hematoxylin and eosin. Organizational structure of kidney tissue was visualized by a light microscope. The statistical data processing was carried out by using the STATISTICA 7,0. The median and interquartile range (IQR) were calculated. Mann-Whitney U test was used to compare the numerical values of two groups. All p values less than 0.05 were considered significant.

Results and discussion

Body weight and renal index were compared between control group and I group. The initial body weight of control group and I group was 202 [196; 205] g, 203.5 [197; 210] g, p=0.472, respectively. No significant differences of initial body weight were recorded. The weight difference between I and control groups was significantly after 30 days of experiment. The rats of I group had body weight 243.5 [234; 248] g that significantly lighter than those of the control group 265 [259; 272] g, p=0.0001. The renal index in I group was noticeable greater than that in control group, p=0.0001 (Figure 1).

No laboratory and histological signs of pathological disturbances were detected in the animals of the control group (Figure 2). A renal cortex with Malpighian corpuscles, convoluted parts of proximal and distal tubules, collecting ducts, as well as renal medulla with Henle's loops and collecting ducts were clearly detected. The Malpighian corpuscles were moderately dense with narrow Bowman's spaces. Glomerular cells are represented as densely arranged round shape creations. Glomerular capillary endothelial cells have oblong and dusky nuclei. Podocytes and mesangial cells have round and light stained nuclei. Tubules with dense row of nuclei are located to the nearest with glomerular vascular pole.

Capillary basal lamina is thin. The

proximal and distal convoluted tubules have a relatively regular distinct lumen. These tubules are lined by typical thick cubic epithelium. The cubic epithelium has round nuclei with dense, darkly stained, clumped chromatin. The median and IQR of the renal glomerulus diameter were 122 [121; 125] мм.

Approximately, two-thirds of the total number glomeruli of I group treated by Laprol-604 have been found either crumbly or compacted (Figure 3).

Decreasing of cellularity of glomeruli was marked that led to increase the Bowman capsule spaces and elevating the Malpighian corpuscles. The nuclei of glomerular endothelial cells are hyperchromic, the capillary lumen is not visible. The apical brush border of the proximal tubule epithelium is significantly destroyed, and their cytoplasm is clumping. Hyperchromic nuclei have karyopiknosis and karyolysis. The median diameter of the glomerulus is 116.5 [115; 118] μm that is significantly less than control data (p=0.0001).The proximal convoluted tubules showed degeneration of epithelial cells and marked swollen of their tubular lumina

The massive degeneration of epithelial cells lining the tubules with marked distortion and missing of their tubular lumina was detected. The squamous epithelium were found in the lumen of many tubules.

The pathological lesions of the kidney I group have been found. Atrophy of glomerular

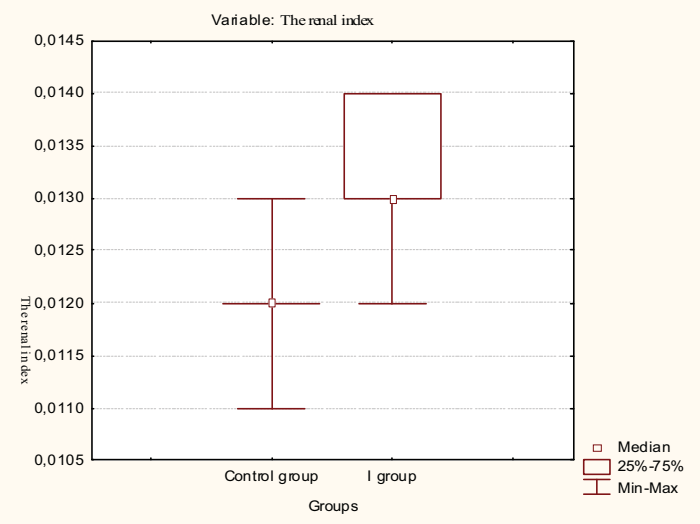

Fig. 1. The renal index of Wistar rats. Renal index was calculated by dividing total left and right kidney weight to the body weight of euthanized rats.

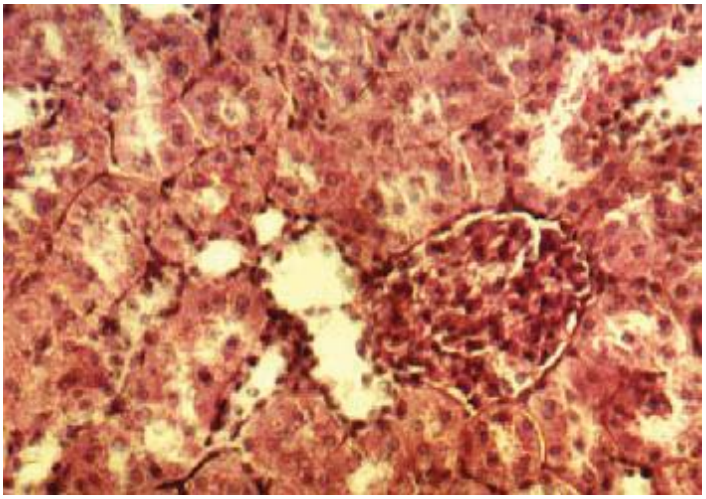

Fig. 2. Histological picture of rat's kidney control group paraffin section stained by hematoxylin and eosin (H & E stain X 200).
The normal structure of cortex and medulla of rats' kidney in the control group

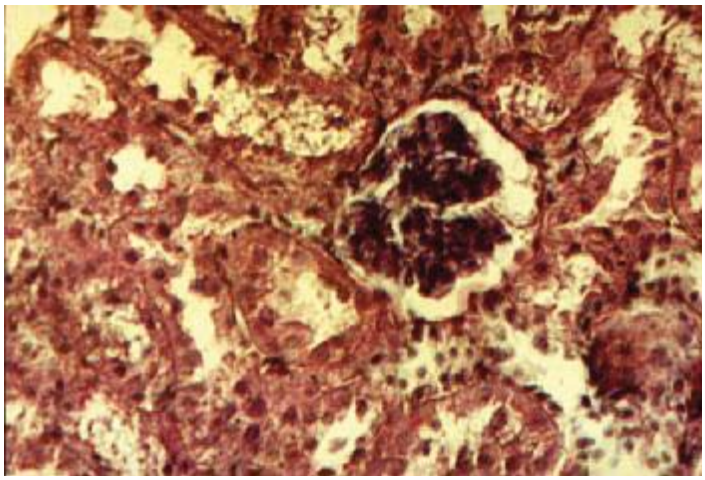

Fig. 3. Histological picture of rat's kidney I group paraffin section stained by hematoxylin and eosin (H & E stain X 200). Renal glomerular atrophy, distension of the renal glomerular capsular space and interstitial edema were found in kidneys of I group (H & E stain X 200).

cells, Bowmens' capsular spaces have been extended; tubular epithelial cells have been degenerated. In addition, protein in the renal tubular lumen and interstitial edema have been detected. These morphological changes in the kidneys I group indicate significant load renal tissue.

The present morphological manifestations of toxic damage kidney rats exposed to Laprol-604 confirm the biochemical disturbances have been determined in the blood serum and urine of these animals [10].

The pathomorphological changes have been found here therefore requires the study of nonionic surfactants could be furthered.

References

1. Petrovic M., Fernбndez-Alba A.R., Borrull F. et al. Occurrence and distribution of nonionic surfactants, their degradation products, and linear alkylbenzene sulfonates in coastal waters and sediments in Spain / M. Petrovic, A.R. Fernбndez-Alba, F Borrull , R.M. Marce, M.E. Gonzбlez, D. Barcely // Environmental Toxicology and Chemistry. – 2002. – №21. – P. 37-46.
2. Olkowska E., Ruman M., Kowalska A. and Polkowska Z. Determination of surfactants in environmental samples. Part III. Nonionic compounds/ E. Olkowska, M. Ruman, A. Kowalska and Z. Polkowska // Ecological Chemistry and Engineering. – 2013. – Vol. 20. – №3. – P. 449–461.
3. Yuan C.L, Xu Z.Z., Fan M.X. et al. Study on characteristics and harm of surfactants/ C.L Yuan, Z.Z. Xu, Fan M.X., Liu H.Y., Y.H. Xie, T. Zhu // Journal of Chemical and Pharmaceutical Research. – 2014. – Vol. 7№6. –P. 2233-2237.
4. Babienko V.V., Sakharova I.V., Danilchenko L.I. Hygienic evaluation of the influence of the nitrogen-containing surfactants under conditions of acute experiment// Journal of the Grodno State Medical University. – 2015.– №4. –P. 85-87. (in Russian) / Бабиенко В.В., Сахарова И.В., Данильченко Л.И. Гигиеническая оценка влияния азотсодержащих поверхностно-активных веществ в условиях острого эксперимента // Журнал Гродненского государственного медицинского университета. – 2015.– №4. – С. 85-87.
5. Popova T.M. The effects of Laprol-604 exposure on pregnant rats and their posterity / T.M. Popova // Clinical & experimental pathology. – 2017. – Vol. 16, №2 (60), part 2. – P. 61-64.
6. Cavanagh R. J., Smith P. A. and Stolnik S. Exposure to a nonionic surfactant induces a response akin to heat-shock apoptosis in intestinal epithelial cells: implications for excipients safety / R. J. Cavanagh, P. A. Smith and S. Stolnik // Mol. Pharmaceutics – 2019 – №16. – P. 618"631.
7. Popova T.M. The typical pathochemical reactions of the damaging impact of detergents as sources of radiotoxins / Popova T.M., Zharova NV, Kolesnik I.L. et al / / Problems of environment and medicine . – 2015. –Vol. 19 (5/6). – P. 11-16.
8. Zhukov V. Detergenty – modulyatory radiomimeticheskikh effektov / V. Zhukov. – Kharkov: Tornado, 2000. – 180 p. (in Russian) / Жуков В. Детергенты – модуляторы радиомиметических эффектов / В. Жуков. – Харьков: Торнадо, 2000. – 180 с.
9. Council of Europe [France]. Eroupean convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg,18.III.1986, http// conventions.coe.int/treaty/en/Treaties/Word/123.doc
10. Popova T.M. Impact of surfactant on renal function of adult Wistar rats / T.M. Popova // Actual problems of transport medicine. – 2018. – №1 (51) . –P. 91-98.

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