

УДК 611:61-018.7:[535.361+535.51]

T. M. Boychuk
O. I. Petryshen
H. M. Chernikova
*S. B. Yermolenko**

Bukovinian State Medical University,
Chernivtsi
* Y.Fedkovych Chernivtsi National
University

MORPHOLOGICAL, MORPHOMETRIC AND LASER POLARIMETRY CHARACTERISTICS OF EPITHELIAL TISSUE OF KIDNEYS THAT HAVE BEEN STRUCTURALLY REORGANIZED

Key words: *aluminum chloride, lead chloride, kidney, immobilizing stress, morphology, laser polarimetry.*

Abstract. *The article deals with the results of study of structural organization of epithelial tissue of the kidneys that were exposed to the combined influence of stress factor and metal salts. It has been determined, that of all the methods, used in the research, morphological changes are characterized by the method of laser polarimetry in full measure.*

Introduction

One of the organs which provide the stability of the internal medium of the organism is kidney. The excretion of the final products of metabolism (glomerular filtration, reabsorption, active secretion) is provided by specialized elements of kidney – nephrons [7,9]. Large amount of nephrons, typical tissue division in kidney, heterogeneous structure, organization of microcirculatory stream, wide ways of venous and lymphatic drainage, presence of specialized endocrine apparatus, that take part in regulation of hemodynamics, intro- and extrarenal nervous ligaments – all these factors determine the complicated construction of kidney, as a life important organ, that keeps permanency of internal medium of the organism.

The excretion of different xenobiotics pass through the kidney, that leads to morphological and functional disorders [4,6,8]. These substances include different chemical compounds of industrial processes. Among pollutants of technogenic origin one of the first places is occupied by compounds of different metals, and among them important places are taken by aluminum and lead salts [2,3].

As is generally known, substances that form aluminum and lead are concerned to be high molecular poisons, that are characterized by the slow excretion from the organism and have polytrophic effect, that in its turn leads not only to toxic damage of the organs, but also to the exacerbation of chronic attendant diseases.

Another harmful factor, the effect of which leads to morphological and functional disorders is stress. It has been proved, that stress initiates the development of adaptive reactions, and functional disorders. In response to this, adaptive and compensatory systems of the organism start functioning on the higher and more

intensive level to stabilize main homeostatic parameters. During long term and strong effect of stress factors the stress reaction can become pathogenic base for the development of various illnesses.

There are no published facts about the influence of aluminum and lead salts on an immobilizing background.

Research object

To research the influence of aluminum and lead salts combined with the effects of immobilizing stress on kidney morphology. As well as our work is directed to the research of the potentialities of the technique of laser polarimetry and spectropolarimetry of the epithelial tissue of kidney [1,5].

Material and methods

50 mature male albino rats, with the body weight 0,18-0,2 kg, kept in vivarium conditions under the constant temperature and air moisture, with free access to water and food, were studied by the complex of morphological and morphometrical methods. Animals were divided into 2 groups. I group – control (n=25), II group – research (n=25) in which animals during 14 days were injected intraventricularly by 1% starch suspension of aluminum chloride in a dose of 200mg/kg and lead chloride 50mg/kg. On the 14th day of the experiment group II of animals were effected by one hour immobilizing stress. Next step of the experiment was euthanasia of animals under light ether with further kidney removal. Kidneys were fixed in 10% solution of neutral formalin, dehydrated in ethyl alcohol of growing concentration and covered with paraffin. Microtome cuts, 6-8 mkm in thick were colored by

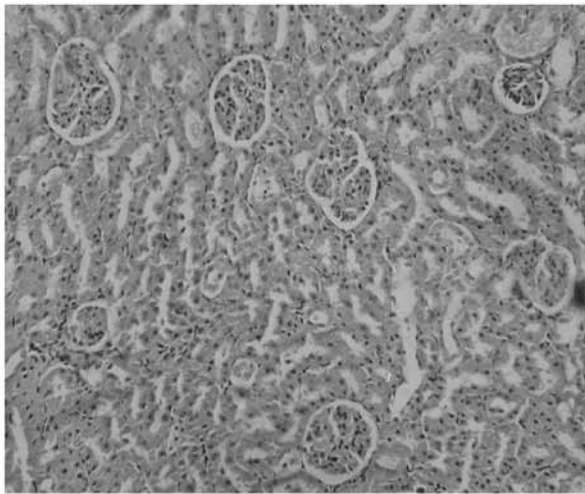


Figure 1. Structural organization of epithelial tissues of the kidneys control group objects 10, eyepiece 20

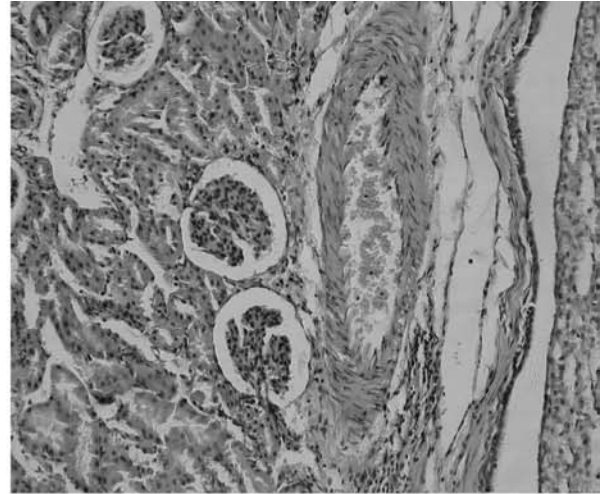


Figure 2. Structural organization of epithelial tissue of the kidney research group objects 10, eyepiece 20

0-0

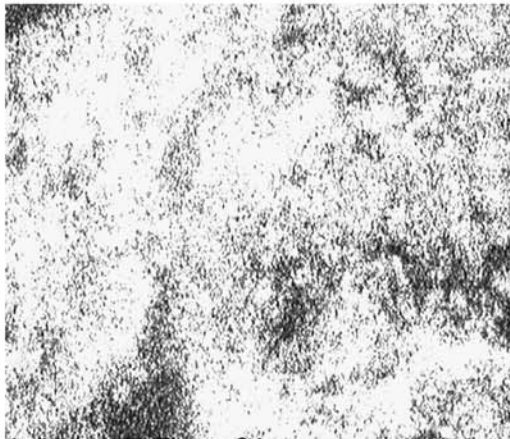


Figure 3

0-90

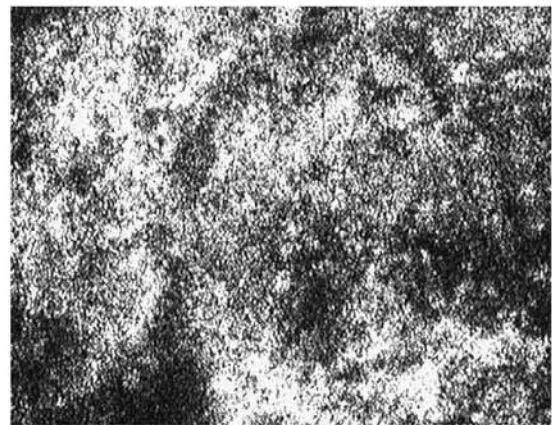


Figure 4

Polarization images of the epithelial tissue kidney I group

0-0



Figure 5

0-90

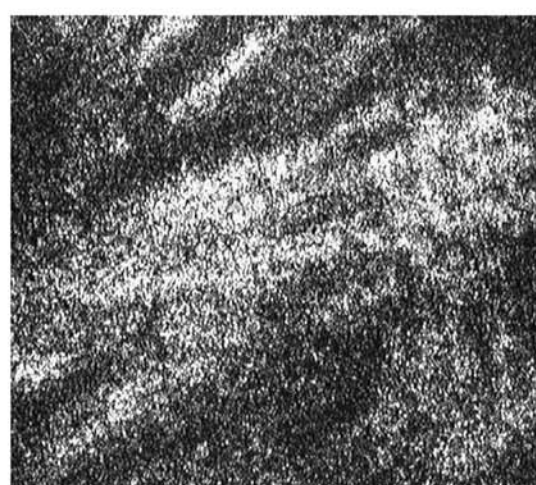


Figure 6

Polarization images of the epithelial tissue kidney II group

hematoksylin-eozyn. Morphometric methods of research were carried out by means of ocular micrometer and with the use of histometric methods of research by Palkovits and Zolnai.

For estimation of diagnostic possibilities of statistical and fractal analysis of the kidney epithelial tissue the histologic specimens in health sections of normal (I group) and group under study (II group) – were investigated.

The indices were processed statistically, the difference between the comparable indices was determined by the Student-criteria.

Discussion of the results of the research

Analyzing morphometric kidney indices of experimental animals, the thickness increase of the cortical (240±4,21 against 160±2,5 mkm in control group) and cerebral substance (128±1,2 against 96±1,6 mkm in control group) was stated. Experimental animals had the increase of nephron body sizes (117±10,25 x 104± 11,8 mkm against 81,25±5,15 x 81,25±4,75 mkm in control group) due to the volume growth of choroid glomus (91±2,5 Ч 104±4,5 mkm against 65±0,6 x 65±0,93 mkm in control group) and filtrating fissure (22,75±1,23 against 6,5±0,3 mkm in control group). Changes are also seen in the nephron tubules, the diameter becomes 2,5 times bigger in proximal part, Henle’s loop and moderate growth of the distal part.

Experimental animals also had morphological changes in the cells, that are the part of the renal tubules structure. There are significant hydropic changes and signs of ballonic dystrophy in epitheliocytes of proximal and distal parts of nephron (figure 2). Cell cytoplasm contains small and few large vacuoles, and perinuclear vacuoles in many epitheliocytes, that increases cell sizes (16,2 5±0,66 against 6,5±0,59 mkm in control group). Cell nuclei are hyperchromic, nuclei-cytoplasm Hertvig’s index is moved to the cytoplasm side. Local morphological changes, which are accompanied by dystrophic disorders of the cell structure are marked in part of epitheliocytes of proximal and distal tubes.

Moderate cases of stasis and sludge, vascular plethora, acute broadening of lymphatic vessels, stromal and perivascular oedemata, small centers of diapedic bleedings are found in blood micro-circulatory stream of kidney.

Histological sections of optically thick (reduction factor, geometrical thickness – 400) layers of epithelial tissue were used as the objects of investigation.

Polarization images of the healthy and morphologically modified kidney tissues obtained for coaxial (0-0) and crossed (0-90) polarizer and analyzer are presented in figure.

The mechanisms of interaction between laser radiation and this BT layer is described by the following matrix

$$\{F\} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & f_{22} & f_{23} & f_{24} \\ 0 & f_{32} & f_{33} & f_{34} \\ 0 & f_{42} & f_{43} & f_{44} \end{pmatrix} \quad (1)$$

where

$$\begin{aligned} f_{22} &= \cos^2 2\rho + \sin^2 2\rho \cdot \cos 2\delta; f_{23} = f_{32} = \cos 2\rho \sin 2\rho (1 - \cos 2\delta); \\ f_{24} &= -f_{42} = -\sin 2\rho \sin 2\delta; f_{33} = \sin^2 2\rho + \cos^2 2\rho \cos 2\delta; \\ f_{34} &= -f_{43} = \cos 2\rho \sin 2\delta; f_{44} = \cos 2\delta. \end{aligned} \quad (2)$$

Here:

ρ – the fibril orientation, which determines the direction of optical axis;

δ – the value of phase shift, which is added between the orthogonal components of the amplitude of the laser wave with of the λ length.

Polarization parameters (azimuth and ellipticity) in every point of the object field are determined according to the following algorithms:

$$\alpha = 0,5 \arctg \left(\frac{f_{32} S_2^0 + f_{33} S_3^0 + f_{34} S_4^0}{f_{22} S_2^0 + f_{23} S_3^0 + f_{24} S_4^0} \right); \quad (3)$$

$$\beta = 0,5 \arcsin \left(\frac{f_{42} S_2^0 + f_{43} S_3^0 + f_{44} S_4^0}{f_{22} S_2^0 + f_{23} S_3^0 + f_{24} S_4^0} \right); \quad (4)$$

where

$$S_{i=2,3,4}^0 = \begin{cases} \cos 2\alpha_0 \cos 2\beta_0; \\ \sin 2\alpha_0 \cos 2\beta_0; \\ \sin 2\beta_0. \end{cases} \quad (5)$$

Here:

$S_{i=2,3,4}^0$ – the Stokes vector parameters of the beam illuminating the BT;

α_0, β_0 – its azimuth and polarization ellipticity.

The intensity of every point of such image is determined by the expression

$$I = I_0 [\cos^2(\alpha \pm \Theta) + \tg^2 \beta \sin^2(\alpha \pm \Theta)] \quad (6)$$

where

Θ – orientation angle of the orientation of the passing axis of polarizer-analyzer, through which the BT is researched.

Statistical approach in the analysis of polarization images of biological tissues of the first,

$$M = \frac{1}{N} \sum_{i=1}^N |z_i|;$$

second,

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N z_i^2};$$

third and

$$A = \frac{1}{\sigma_S^3} \frac{1}{N} \sum_{i=1}^N z_i^3;$$

fourth orders

$$E = \frac{1}{\sigma_S^2} \frac{1}{N} \sum_{i=1}^N z_i^4,$$

were used as the analytical instrument for estimating the ensemble of random values of z .

Here: $N = m \times n$ – total number of pixels of CCD-camera.

Conclusions

1. Combined action of aluminum, lead salts and immobilizing stress leads to morphofunctional and dystrophical changes in renal tissue with the effects of hydropic and ballonic dystrophy in epitheliocytes of nephron tubules, which are accompanied by the effects of stasis and sludge with acute blood filling and broadening of lymphatic vessels, stromal and perivascular oedemata, small centers of diapedic bleedings.

2. The statistic moments of the 3rd and 4th orders appeared to be the most sensitive to pathological changes of orientation-phase structure of architectonics of the epithelial tissue kidney. Their value (asymmetry of distribution) changes within the limits of two orders.

Perspectives of the further research

Further studying of the influence of combined action of aluminum, lead salts and immobilizing stress on the kidney morphology will give the opportunity to reveal the dynamics of the development of compensatory-adaptive and reparative mechanisms as well as to develop methods of their correction.

References. 1. Ангельский О. В. Поляризационно-фазовая визуализация и обработка когерентных изображений фрактальных структур биотканей / О.В. Ангельский, А.Г. Ушенко, А.Д. Архелюк и др. // Журнал прикладной спектроскопии. – 2000. – Т.67. № 5. – с. 664-667; 2. Назаренко Г.И. Клиническая оценка результатов лабораторных исследований: 2-е издание, стереотипное / Г.И. Назаренко, А.А. Кишкун – М: Медицина, 2006 – 544 с; 3. Ушенко О.Г. Лазерная поляризационная технология биологических тканей: статистический та фрактальный підходи / [О.Г. Ушенко, В.П. Пішак, О.В. Ангельський, Ю.О. Ушенко]. – Чернівці, Колір-Друк, 2007. С. 341; 4. Ushenko A.G. *Polarization correlometry and wavelet analysis of dynamics of changes in orientation-phase structure of tissue architectonics* // Proc. SPIE. – 2002. – V.4900. – P.1323- 1326; 5. Ushenko A.G. *Laser polarimetry of pathological changes in biotissue* / A.G. Ushenko, O.V. Angelsky, D.N. Burkovets et al. // Proc. SPIE. – 2002. – V.4900. – P.1045-1049; 6. Angelsky O.V. *2-d tomography of biotissue images in pre-clinic diagnostics of their pre-cancer states* / O.V. Angelsky, Yu. Ya. Tomka, A.G. Ushenko et al. // Proc. SPIE. – 2005. – Vol. 5972. – P. 158-162. 7. Arthyr J. Vander, M.D. *Renal physiology* // McGraw-Hill, Inc. 2000. - P.15-68.

8. Cartana J., Romeu A., Arola LI. *Effects of copper, cadmium and nickel on liver and kidney glutathione redox cycle of rats* // Compar. Biochem. and Physiol. – 1992. – 101.-№ 2. – P.209-213. 9. Goethals F., Bocquet J.M., Allaey V., Roberfroid M. *The use of rat renal slices as an in vitro model to evaluate nephrotoxicity* // J. Pharm. Belg. – 1993. – 48.-№ 2. – P.130.

МОРФОЛОГІЧНА, МОРФОМЕТРИЧНА ТА ЛАЗЕРНОПОЛЯРИМЕТРИЧНА ХАРАКТЕРИСТИКА ЕПІТЕЛІАЛЬНОЇ ТКАНИНИ НИРОК, ЩО ЗАЗНАЛИ СТРУКТУРНОЇ РЕОРГАНІЗАЦІЇ

*Т.М.Бойчук, О.І.Петришен, Г.М.Чернікова,
С.Б. Єрмоленко*

Резюме. У роботі наведені результати вивчення структурної організації епітеліальної тканини нірок, що піддавалися комбінованому впливу стрес-фактора та солей металів. Визначено, що із використаних методів дослідження в більш повній мірі, морфологічні зміни характеризує саме метод лазерної поляриметрії.

Ключові слова. алюмінію хлорид, свинцю хлорид, нірка, іммобілізаційний стрес, морфологія, лазерна поляриметрія.

МОРФОЛОГИЧЕСКАЯ, МОРФОМЕТРИЧЕСКАЯ И ЛАЗЕРНОПОЛЯРИМЕТРИЧЕСКАЯ ХАРАКТЕРИСТИКА ЭПИТЕЛИАЛЬНОЙ ТКАНИ ПОЧЕК, КОТОРАЯ ПОДВЕРГЛАСЬ СТРУКТУРНОЙ РЕОРГАНИЗАЦИИ

*Т.Н.Бойчук, А.И.Петришен, Г.Н.Черникова,
С.Б. Ермоленко*

Резюме. В работе представлены результаты изучения структурной организации эпителиальной ткани почек, которые были подданы комбинированному влиянию стресс-фактора и солей металлов. Установлено, что из используемых методов исследования в более полной мере, морфологические изменения характеризует именно метод лазерной поляриметрии.

Ключевые слова. Алюминия хлорид, свинца хлорид, почка, иммобилизационный стресс, морфология, лазерная поляриметрия.

**Буковинський державний медичний
університет, г.Черновці
Черновицький національний університет
ім. Ю.Федьковича**

Clin. and experim. pathol. – 2013. – Vol.12, №3 (45). – P.28-31.

Надійшла до редакції 03.09.2013

Рецензент – проф. В.М.Шаплавський

*© Т.М.Boychuk, O.I.Petryshen, H.M.Chernikova, S.B.Yermolenko,
2013*