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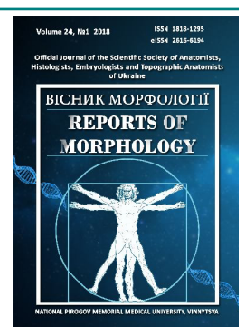
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Morphofunctional changes in kidneys of rats with gentamicin-induced acute kidney injury and use of melatonin

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Aminoglycosides are effective antibiotics, but their accumulation in kidney cortex causes nephrotoxic effects in 20-30% of patients, which significantly limits their use. For this reason, search for the new therapies aimed at prevention of gentamicin-induced acute kidney injury (AKI) is highly relevant. Thus, the objective of our research was to study the functional and histopathological changes in kidneys of rats with gentamicin-induced AKI, and estimate the renoprotective potential of pineal hormone melatonin, which possesses antioxidant, anti-inflammatory and immunomodulatory effects. The study was conducted on 24 non-linear male rats. Gentamicin-induced AKI was modeled by daily administration of 4% gentamicin sulphate (80 mg/kg) for 6 days. Melatonin (Sigma Aldrich, USA) was injected daily at a dose of 5 mg/kg. Functional state of kidneys was assessed by diuresis, creatinine clearance, urine protein excretion, fractional excretion of sodium, and plasma potassium level. Documentation of the pathological processes was performed by the computer morphometry of objects in histological preparations. Statistical analysis of the data was performed using SPSS 17.0 software. Administration of gentamicin resulted in a significant impairment of renal function of experimental animals. A decrease in creatinine clearance by 3.1 times along with a reduction of diuresis by 1.9 times, and an increase in plasma creatinine concentration by 2.6 times was observed. There also was an increase in urine protein level by 5.2 times, an elevation of fractional sodium excretion and a reduction of plasma potassium level. Use of melatonin caused a significant improvement of renal function comparing to model pathology group. Functional disturbances were accompanied with the significant histopathological changes in kidney tissue: necrosis of the 27.0±5.2% epithelial cells of proximal tubules with the signs of hydropic vacuolization (7.0±2.1%) or reversible hydropic swelling (76.0±1.5%) in the rest of cells; swelling or deformation of some glomeruli. In the medulla tubular lumen were dilated and partially filled with hyaline casts, tubular cells had signs of dystrophy. Use of melatonin contributed to the restraint of the histopathological changes, confirmed by the decrease of the prevalence and severity of tubular necrosis (1.2%), dystrophy (64.0±2.3%), and injury of glomeruli. Obtained results verify the significant nephroprotective effect of pineal hormone melatonin, providing a background for the further in-depth study of its renal effects as well as its prospects as a nephroprotector.

Keywords: *gentamicin-induced acute kidney injury, histopathological changes, melatonin, nephroprotection.*

Introduction

Aminoglycoside antibiotic gentamicin is widely used for the treatment and prophylaxis of Gram negative bacterial infections, though development of nephrotoxicity in 20-30% of patients significantly limits its use [2, 17, 20, 23].

Toxic influence of gentamicin on kidneys results from its accumulation in kidney cortex, where its concentration exceeds by more than 100-fold its serum level. Due to its proximal tubular reabsorption gentamicin is accumulated

in lysosomes of cells, inhibiting phospholipase and sphingomyelinase and leading to lysosomal phospholipidosis, accumulation of myeloid particles and cellular necrosis [2, 4]. It is also stated the interaction between gentamicin and prostaglandins, leading to decrease in glomerular filtration rate (GFR) [6]. It is verified that local oxidative stress in tubular cells plays a central role in pathogenesis of aminoglycosides toxicity [1, 4, 6,

7]. Gentamicin increases production of reactive oxygen species in mitochondria, which in turns inhibit respiratory chain activity and ATP production, stimulate release of cytochrome C and other proapoptotic factors, and disturb cellular functions by direct damage to proteins, lipids and nucleic acids, induce contraction of mesangial cells, cause dysfunction of endoplasmic reticulum, take part in development of inflammation. It results in disturbances of transmembrane transport of sodium ions, resulting in swelling and necrosis of cells [4, 17, 21]. Histological examination of experimental animals kidneys and biopsy of human kidneys reveals the swelling of proximal tubular cells, loss of brush border and tubular irregularity, as well as changes in intracellular organelles after one week of gentamicin use at therapeutic doses [3, 21].

Numerous experimental data verify an ability of antioxidants to limit a degree of kidney injury by aminoglycoside antibiotics due to minimization of direct toxic influence of gentamicin, prevention of vasoconstriction and contraction of mesangial cells, as well as anti-inflammatory effect [1, 2, 4, 7-12, 18, 20]. In this regard we focused our attention on melatonin as a possible remedy of pathogenetic correction of gentamicin-induced acute kidney injury (AKI) due to its antioxidant, anti-inflammatory and immunomodulatory effects [2, 6, 15, 25], which is confirmed by some studies [14, 15, 24]. Thus, use of pineal hormone may be considered as a potential therapeutic method of toxic AKI prevention.

Therefore, the objective of our research was to study the influence of melatonin on the histology and function of rats with gentamicin-induced AKI with estimation of its renoprotective potential.

Materials and methods

The study was conducted on 24 non-linear male rats weighting 130-180 g, maintained in the vivarium conditions with constant temperature and humidity, free access to water and food. Animals were randomly divided into 3 groups (n=8): I group - intact control, II group - gentamicin-induced nephropathy, modeled by daily intramuscular administration of 4% gentamicin sulphate (Galychpharm JSC, Ukraine) at a dose of 80 mg/kg for 6 days [22]. Melatonin (Sigma Aldrich, USA) was injected intraperitoneally at a dose of 5 mg/kg 40 min after every gentamicin injection [15]. Animals were sacrificed 24 h after last injection, while blood, urine samples and kidneys were collected for biochemical and histopathological assessments. All interventions were conducted in accordance with the criteria outlined in the European Union Directive 2010/63/EU "On the protection of animals used for scientific purposes" (2010).

Functional state of kidneys was assessed by diuresis, creatinine clearance, urine protein excretion, fractional excretion of sodium, and plasma potassium level [22]. Plasma and urine creatinine levels were determined using the Jaffe reaction; sodium and potassium levels - using electronic flame photometry method; urine protein content - using the sulfosalicylic acid precipitation test.

The kidneys of rats were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μ m and then stained with hematoxylin and eosin. The preparations were evaluated using light microscopy and photographed (Olympus C740UZ photo camera, Japan, LUMAM-R8 microscope, LOMO, Russian Federation). Documentation of the pathological processes was performed by the computer morphometry of objects in histological preparations using computer software "VideoTest - Razmer 5.0" (LLC "VideoTest", Russian Federation).

Statistical analysis of the data was performed using SPSS 17.0 software. All data are represented as a mean \pm standard error of the mean ($M \pm \sigma$). Estimation of the differences between the samples was conducted using parametric Student's t-test and nonparametric Mann-Whitney U test. The values $p < 0.05$ were considered statistically significant.

Results

Administration of gentamicin consecutively for 6 days resulted in a significant impairment of renal morphofunctional state of experimental animals (Tab. 1). A decrease in creatinine clearance by 3.1 times along with a reduction of diuresis by 1.9 times, and an increase in plasma creatinine concentration by 2.6 times was observed. There also was an increase in urine protein level by 5.2 times, an elevation of fractional sodium excretion and a reduction of plasma potassium level. Use of melatonin caused a significant improvement of renal function comparing to model pathology group.

Histopathological examination of rats with gentamicin nephropathy in comparison with intact control group (Fig. 1) revealed a significant impairment of kidney tissue structure, caused by toxic influence of gentamicin (Fig. 2). In the absence of cells without pathological changes, there is a necrosis $27.0 \pm 5.2\%$ of cortical tubular epithelial cells with deformation, swelling and atrophy of some glomeruli (Fig. 2A). In the renal cortex $7.0 \pm 2.1\%$ of epithelial cells exhibit signs of hydropic vacuolization, the remaining epitheliocytes ($76.0 \pm 1.5\%$) are in a state of reversible hydropic swelling. In the renal medulla (Fig. 2B) and papilla (Fig. 2C) tubular

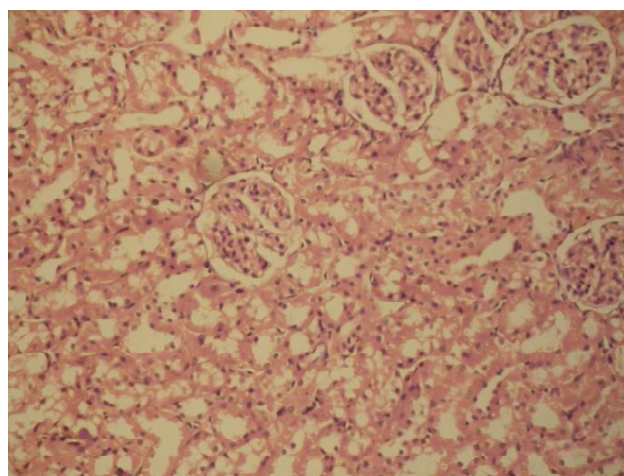


Fig. 1. Photomicrograph of kidney cortex section of intact rat (control). Staining with Haematoxylin and Eosin. (H&E). x100.

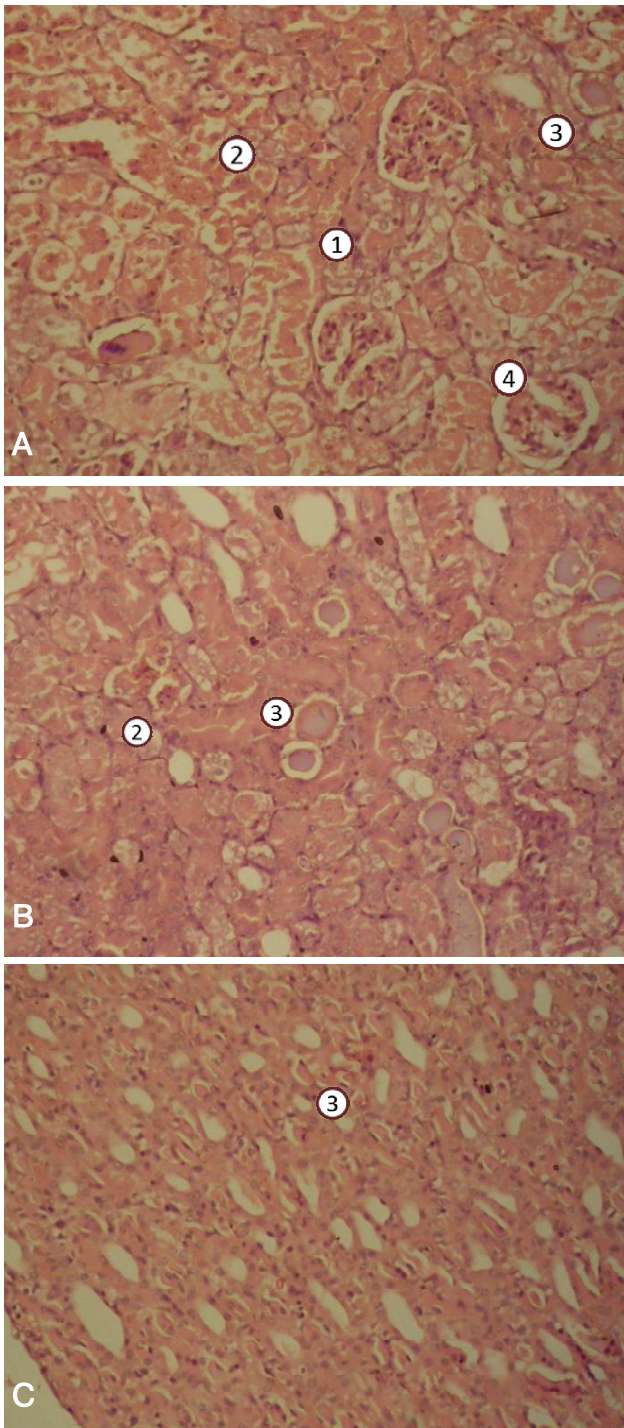


Fig. 2. Photomicrograph of kidney section (A - cortex, B - medulla, C - papilla) of rat with gentamicin nephropathy, 7th day. Epithelial necrosis (1), epithelial degeneration (2), hyaline casts (3), dilation of Bowman's space (4). H&E. x100.

cells degeneration, lumen dilation and deposition of hyaline casts are observed.

Melatonin co-administration ameliorated histopathological changes in kidneys. In the renal cortex areas of tubular epithelial necrosis are localized to 1.2%, reversible hydropic

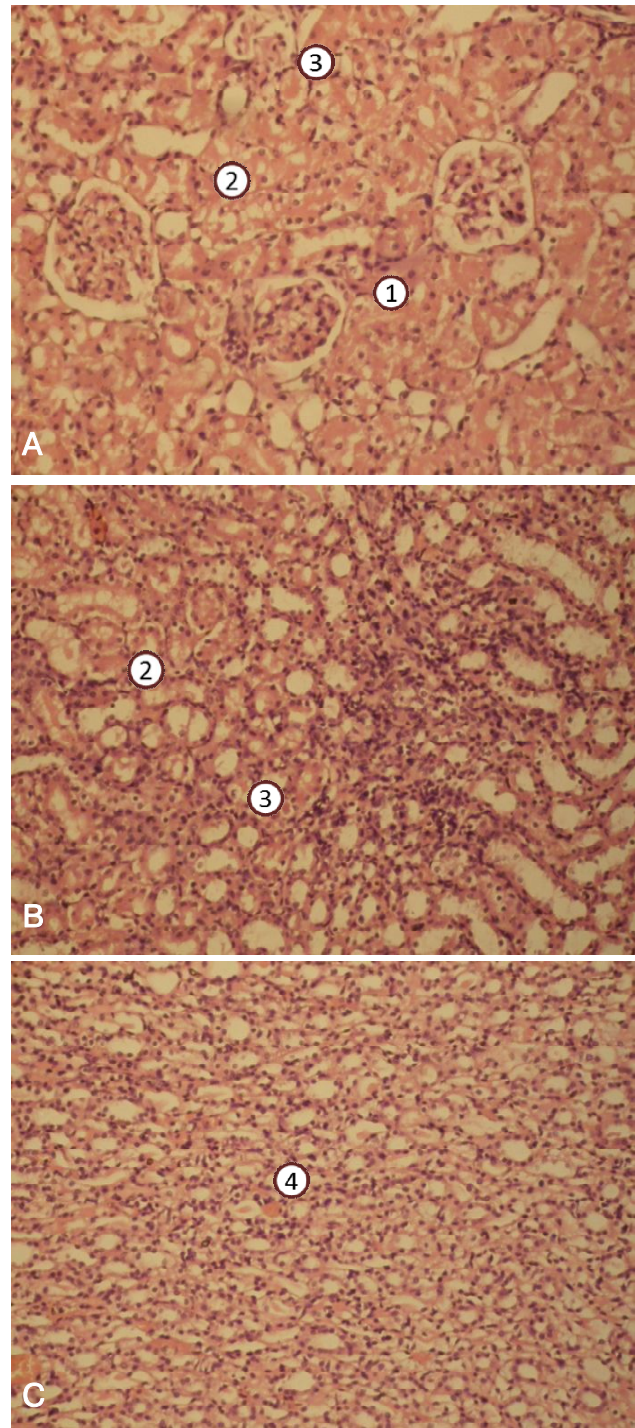


Fig. 3. Photomicrograph of kidney section (A - cortex, B - medulla, C - papilla) of rat with gentamicin nephropathy and use of melatonin, 5 mg/kg. Epithelial necrosis (1), epithelial degeneration (2), hyaline casts (3), hemorrhage (4). H&E. x100.

swelling is extended to $64.0 \pm 2.3\%$ of proximal tubular epitheliocytes, with $4.0 \pm 1.6\%$ of cells in a state of hydropic vacuolization, about 30.0% of the cells - without any signs of damage. Glomeruli of a normal structure and size, some with dilation of Bowman's space (Fig. 3A). Hyaline casts are

present in small amounts in the renal cortex, medulla and papilla, there are also isolated hemorrhages (Fig. 3B, 3C).

Discussion

Gentamicin-induced nephrotoxicity at the beginning of the development of the pathological process is characterized by the development of oliguric form of renal failure, accompanied by retention azotemia, proteinuria, increased loss of sodium and potassium ions with urine, resulting in hypokalemia (Table 1), which reflects the significant damage and death of proximal tubular cells along with injury of glomeruli, and corresponds to the results of similar experimental studies of other authors [1, 4, 8-12, 17, 18]. According to the obtained results of the morphological examination, histopathological changes, namely the necrosis and degeneration of the proximal tubular epithelial cells in the form of vacuolization and hydropic dystrophy, and glomerular damage (Figure 2) were revealed for gentamicin nephropathy [3, 8-12, 14]. It is known that the most important mechanism of gentamicin nephrotoxicity is the hyperproduction of reactive oxygen species, causing damage to proteins, DNA and peroxidation of lipids, with an alteration of the integrity of cellular membranes and development of morphofunctional disorders [1, 17]. This fact conditions numerous research on the effectiveness of prevention of the gentamicin nephropathy, using known and new antioxidants [1, 2, 4, 8-12, 20].

Literature data point to the nephroprotective activity of melatonin in AKI due to kidney ischemia-reperfusion [13], burn disease [5], some toxic models [14, 15, 19]. Probably, the protective effect of melatonin is due to its potent antioxidant and anti-inflammatory properties, as well as the ability to suppress apoptosis [6, 16].

According to the results of our study, the use of melatonin at a dose of 5 mg/kg in the prophylactic treatment regimen (administration 40 min after each injection of gentamicin) does not completely prevent kidney damage by gentamicin, but leads to an improvement in renal function, which is confirmed by a significant increase in diuresis, creatinine clearance, concentration of potassium ions in plasma, along with a decrease in proteinuria and fractional excretion of sodium ions compared to non-treated animals (Table 1). At that, the maintenance of glomerular filtration may be explained by the direct antioxidant activity of the drug, since reactive oxygen species and nitrogen oxides directly lead to a decrease in GFR [16]. The established renal effects of melatonin in toxic kidney damage correspond to the criteria of nephroprotective action under the conditions of AKI development [2, 8-12].

The protective effect of melatonin has been realized in

References

- [1] Adil, M., Kandhare, A., Dalvi, G., Ghosh, P., Venkata, S., Raygude, K., & Bodhankar, S. (2016). Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. *Renal Failure*, 38(6), 996-1006. <http://dx.doi.org/10.3109/0886022x.2016.1165120>
- [2] Ahmad, Q.Z., Jahan, N., Ahmad, G., & Tajuddin (2014). An appraisal of nephroprotection and the scope of natural products in combating renal disorders. *J. Nephrol. Ther.* 4, 170. doi:10.4172/2161-0959.1000170
- [3] Alarifi, S., Al-Doaiss, A., Alkahtani, S., Al-Farraj, S., Al-Eissa, M., & Al-Dahmash, B. (2012). Blood chemical changes

Table 1. Influence of melatonin on the state of kidney excretory function in conditions of gentamicin-induced acute kidney injury ($M \pm \sigma$, $n=7$).

Index	Intact control	AKI	AKI+ Melatonin
Diuresis, ml/2 hx100 g	4.649±0.193	2.464±0.187##	3.635±0.101**
Plasma creatinine, $\mu\text{mol/l}$	59.67±3.92	155.1±5.00##	106.4±5.60**
Creatinine clearance, ml/min	54.79±7.85	17.54±1.94##	30.29±2.44**
Urine protein, g/l	0.018±0.002	0.093±0.007##	0.058±0.005**
Fractional sodium excretion, %	0.673±0.069	3.426±0.716##	1.781±0.198**
Plasma potassium, $\mu\text{mol/l}$	5.393±0.266	4.357±0.261##	5.036±0.127*

Note: ## - statistical significance comparing to: intact control; ($p < 0.01$); AKI group * - ($p < 0.05$), ** - ($p < 0.01$).

the limitation the severity and prevalence of histopathological changes in the kidneys, indicating the cytoprotective activity of this hormone in relation to nephrons, the ability of the drug to ameliorate the toxic effects of gentamicin and prevent the development of renal failure.

The obtained results substantiate the further in-depth study of the renal effects of melatonin on various experimental models of acute kidney injury, including those considering the chronobiological peculiarities of the hormone action, and the prospects of its use as a nephroprotector in acute kidney injury of different genesis.

Conclusions

1. A 6-day consecutive administration of gentamicin at a dose of 5 mg/kg leads to an alteration of the morphofunctional state of rats kidneys, which is manifested by the development of oliguric form of renal failure.

2. Structural organization of the renal tissue in gentamicin-induced acute kidney injury is characterized by the occurrence of histopathological changes in both the glomerular and tubular apparatus of the nephron: necrosis of 27% of the epithelial proximal tubular cells and degenerative changes of various degrees of the remaining cells are observed.

3. According to research results, the protective effect of melatonin at a dose of 5 mg/kg is verified, which is manifested by restriction of nephrons damage and normalization of the structural organization of kidney tissue, as well as the preservation of renal function under the conditions of gentamicin-induced injury development.

- and renal histological alterations induced by gentamicin in rats. *Saudi Journal of Biological Sciences*, 19(1), 103-110. <http://dx.doi.org/10.1016/j.sjbs.2011.11.002>
- [4] Ali, B.H., Al Za'abi, M., Blunden, G., & Nemmar, A. (2011). Experimental gentamicin nephrotoxicity and agents that modify it: a mini-review of recent research. *BCPT*, 109, 225-232. doi:10.1111/j.1742-7843.2011.00728.x
- [5] Bai, X.-Z., He, T., Gao, J.-X., Liu, Y., Liu, J.-Q., Han, S.-C., ... Hu, D.-H. (2016). Melatonin prevents acute kidney injury in severely burned rats via the activation of SIRT1. *Scientific Reports*, 6, 32199. <http://doi.org/10.1038/srep32199>
- [6] Bharti, V. K., Srivastava, R. S., Subramanian, P., Warren Spence, D., Pandi-Perumal, S. R., Brown, G. M. (2011). Cerebral epiphyseal proteins and melatonin modulate the hepatic and renal antioxidant defense of rats. *Intern. J of Nephrology*. ArticleID 142896. doi:10.4061/2011/142896.
- [7] Casanova, A., Vicente-Vicente, L., Hernandez-Sanchez, M., Pescador, M., Prieto, M., Martinez-Salgado, C. ... Lopez-Hernandez, F.J. (2017). Key role of oxidative stress in animal models of aminoglycoside nephrotoxicity revealed by a systematic analysis of the antioxidant-to-nephroprotective correlation. *Toxicology*, 385, 10-17. <http://dx.doi.org/10.1016/j.tox.2017.04.015>
- [8] Diwan, P., Kakalij, R., Alla, C., Kshirsagar, R., Kumar, B., & Mutha, S. (2014). Ameliorative effect of *Elaeocarpus ganitrus* on gentamicin-induced nephrotoxicity in rats. *Indian Journal Of Pharmacology*, 46(3), 298. <http://dx.doi.org/10.4103/0253-7613.132163>
- [9] Ehsani, V., Amirteimoury, M., Taghipour, Z., Shamsizadeh, A., Bazmandegan, G., Rahnama, A. ... Fatemi, I. (2017). Protective effect of hydroalcoholic extract of *Pistacia vera* against gentamicin-induced nephrotoxicity in rats. *Renal Failure*, 39(1), 519-525. <http://dx.doi.org/10.1080/0886022x.2017.1326384>
- [10] Ghaznavi, H., Fatemi, I., Kalantari, H., Hosseini Tabatabaei, S., Mehrabani, M., Gholamine, B. ... Goudarzi, M. (2017). Ameliorative effects of gallic acid on gentamicin-induced nephrotoxicity in rats. *J. of Asian Natural Products Research*, 1-12. <http://dx.doi.org/10.1080/10286020.2017.1384819>
- [11] Hajihashemi, S., Hamidizad, Z., Rahbari, A., Ghanbari, F., & Motealeghi, Z. (2017). Effects of Cobalamin (Vitamin B12) on Gentamicin Induced Nephrotoxicity in Rat. *Drug Research*, 67(12), 710-718. <http://dx.doi.org/10.1055/s-0043-117418>
- [12] Kakalij, R. M., Alla, C. P., Kshirsagar, R. P., Kumar, B. H., Mutha, S. S., & Diwan, P. V. (2014). Ameliorative effect of *Elaeocarpus ganitrus* on gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmacology*, 46(3), 298-302. <http://doi.org/10.4103/0253-7613.132163>
- [13] Kang, J., Koh, E., & Lee, S. (2011). Melatonin protects liver against ischemia and reperfusion injury through inhibition of toll-like receptor signaling pathway. *Journal of Pineal Research*, 50(4), 403-411. <http://dx.doi.org/10.1111/j.1600-079x.2011.00858.x>
- [14] Kapic, D., Mornjakovic, Z., Cosovic, E., Sahinovic, M. (2014). A histological study of the effect of exogenous melatonin on gentamicin induced structural alterations of proximal tubules in rats. *Bosnian J of Basic Medical Sciences*. 14(1), 30-34. doi:10.17305/bjbm.2014.2293
- [15] Kilic, U., Kilic, E., Tuzcu, Z., Tuzcu, M., Ozercan, I. H., Yilmaz, O., ... Sahin, K. (2013). Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. *Nutrition & Metabolism*, 10, 7. <http://doi.org/10.1186/1743-7075-10-7>.
- [16] Lee, I., Kim, S., Lee, S., Baek, H., Moon, C., Kim, S. ... Kim, J.C. (2012). Melatonin attenuates gentamicin-induced nephrotoxicity and oxidative stress in rats. *Archives of Toxicology*, 86(10), 1527-1536. <http://dx.doi.org/10.1007/s00204-012-0849-8>
- [17] Lopez-Novoa, J. M., Quiros, Y., Vicente, L., Morales, A., & Lopez-Hernandez, F.J. (2011). New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney Intern.*, 79(1), 33-45. <https://doi.org/10.1038/ki.2010.337>
- [18] Mahmoud, Y., & Farag, S. (2017). Kiwifruit ameliorates gentamicin induced histological and histochemical alterations in the kidney of albino mice. *Biotechnic & Histochemistry*, 92(5), 357-362. <http://dx.doi.org/10.1080/10520295.2017.1318222>
- [19] Onk, D., Onk, O. A., Turkmen, K., Erol, H. S., Ayazoglu, T. A., Keles, O. N., ... Topal, E. (2016). Melatonin Attenuates Contrast-Induced Nephropathy in Diabetic Rats: The Role of Interleukin-33 and Oxidative Stress. *Mediators of Inflammation*, 2016, 9050828. <http://doi.org/10.1155/2016/9050828>
- [20] Pierson-Marchandise, M., Gras, V., Moragny, J., Micallef, J., Gaboriau, L., Picard, S. ... Liabeuf, S. (2017). The drugs that mostly frequently induce acute kidney injury: a case - noncase study of a pharmacovigilance database. *British Journal of Clinical Pharmacology*, 83(6), 1341-1349. <http://dx.doi.org/10.1111/bcp.13216>
- [21] Randjelovic, P., Veljkovic, S., Stojiljkovic, N., Sokolovic, D., & Ilic, I. (2017). Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. *EXCLI Journal*, 16, 388-399. <http://doi.org/10.17179/excli2017-165>
- [22] Singh, A. P., Junemann, A., Muthuraman, A., Jaggi, A. S., Singh, N., Grover, K., & Dhawan, R. (2012). Animal models of acuterenal failure. *Pharmacol. Reports*, 64, 31-44.
- [23] Srisung, W., Teerakanok, J., Tantrachoti, P., Karukote, A., & Nugent, K. (2017). Surgical prophylaxis with gentamicin and acute kidney injury: a systematic review and meta-analysis. *Annals of translational medicine*, 5(5), 100-101. <http://dx.doi.org/10.21037/atm.2017.03.06>
- [24] Tavakoli, M. (2014). Kidney protective effects of melatonin. *Journal of Nephro pharmacology*, 3(1), 7-8.
- [25] Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, 15(3), 434-443. <http://doi.org/10.2174/1570159X14666161228122115>

МОРФОФУНКЦІОНАЛЬНІ ЗМІНИ НИРОК ЩУРІВ ПРИ ЗАСТОСУВАННІ МЕЛАТОНІНУ НА ТЛІ РОЗВИТКУ ГЕНТАМІЦИН-ІНДУКОВАНОГО ГОСТРОГО ПОШКОДЖЕННЯ НИРОК

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Аміноглікозиди є ефективними антибіотиками, але їх накопичення в кірковій речовині нирки викликає нефротоксичні ефекти у 20-30% пацієнтів, що значно обмежує їх застосування. З цієї причини актуальним є пошук нових профілактичних заходів, спрямованих на запобігання гострому пошкодженню нирок (ГПН), спричиненому гентаміцином. Таким чином, метою нашого дослідження було вивчення функціональних і гістопатологічних змін у нирках щурів при гентаміцин-індукованому ГПН з оцінкою нефропротекторного потенціалу пінеального гормону мелатоніну, який володіє антиоксидантними, протизапальними

та імуномодулюючими ефектами. Дослідження проводили на 24 нелінійних самцях щурів. Гентаміцин-індуковане ГПН моделювали щоденним введенням 4% гентаміцину сульфату (80 мг/кг) протягом 6 днів. Мелатонін (Sigma Aldrich, США) вводили щодня у дозі 5 мг/кг. Функціональний стан нирок оцінювали за показниками діурезу, кліренсу креатиніну, екскреції білка з сечею, фракційної екскреції іонів натрію та концентрації іонів калію в плазмі крові. Документацію патологічних процесів здійснювали за допомогою комп'ютерної морфометрії об'єктів у гістологічних препаратах. Введення гентаміцину призвело до значного погіршення функції нирок у дослідних тварин. Виявлено зниження кліренсу креатиніну в 3,1 рази, зменшення діурезу в 1,9 рази, збільшення концентрації креатиніну в плазмі в 2,6 рази. Також спостерігалось збільшення вмісту білка у сечі в 5,2 рази, підвищення фракційної екскреції іонів натрію та зниження концентрації іонів калію в плазмі. Показано, що використання мелатоніну викликало значне покращення ниркової функції порівняно з групою модельної патології. Виявлено, що функціональні розлади супроводжувалися значними гістопатологічними змінами в тканинах нирок: некроз епітеліальних клітин проксимального каналу сягав $27.0 \pm 5,2\%$, у решти клітин виявлено ознаки гідропічної вакуолізації ($7.0 \pm 2,1\%$) або оборотного гідропічного набряку ($76.0 \pm 1,5\%$), відзначався набряк або деформація деяких клубочків. У мозковій речовині просвіти каналців розширені і частково заповнені гіаліновими циліндрами, каналцеві клітини з ознаками дистрофії. Показано, що використання мелатоніну сприяло обмеженню гістопатологічних змін, що підтверджується зменшенням поширеності та виразності каналцевого некрозу (1,2%), дистрофії ($64.0 \pm 2,3\%$) та пошкодження клубочків. Отримані результати достовірно підтверджують нефропротекторний ефект пінеального гормону мелатоніну, що є підґрунтям для подальшого поглибленого вивчення його реальних ефектів, а також перспектив його застосування як нефропротектора.

Ключові слова: гентаміцинова нефропатія, мелатонін, гістопатологічні зміни, нефропротекція.

МОРФОФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ ПОЧЕК КРЫС ПРИ ПРИМЕНЕНИИ МЕЛАТОНИНА НА ФОНЕ РАЗВИТИЯ ГЕНТАМИЦИН-ИНДУЦИРОВАННОГО ОСТРОГО ПОВРЕЖДЕНИЯ ПОЧЕК

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Аминогликозиды являются эффективными антибиотиками, но их накопление в корковом веществе почек вызывает нефротоксические эффекты у 20-30% пациентов, что значительно ограничивает их использование. По этой причине актуальным является поиск новых профилактических средств, способных предотвращать гентамицин-индуцированное острое повреждение почек (ОПП). Таким образом, целью нашего исследования было изучение функциональных и гистопатологических изменений в почках крыс при гентамицин-индуцированном ОПП с оценкой нефропротекторного потенциала пинеального гормона мелатонина, обладающего антиоксидантными, противовоспалительными и иммуномодулирующими свойствами. Исследование проводили на 24 нелинейных крысах-самцах. Гентамицин-индуцированное ОПП моделировали ежедневным введением 4% гентамицина сульфата (80 мг/кг) в течение 6 дней. Мелатонин (Sigma Aldrich, США) вводили ежедневно в дозе 5 мг/кг. Функциональное состояние почек оценивали по показателям диуреза, клиренса креатинина, экскреции белка с мочой, фракционной экскреции ионов натрия и концентрации ионов калия в плазме крови. Документацию патологических процессов проводили путем компьютерной морфометрии объектов в гистологических препаратах. Введение гентамицина привело к значительному ухудшению функции почек у подопытных животных. Выявлено снижение клиренса креатинина в 3,1 раза, уменьшение диуреза в 1,9 раза, увеличение концентрации креатинина в плазме в 2,6 раза. Также наблюдалось увеличение содержания белка в моче в 5,2 раза, повышение фракционной экскреции натрия и снижение концентрации ионов калия в плазме. Показано, что использование мелатонина вызвало значительное улучшение почечной функции по сравнению с группой модельной патологии. Выявлено, что функциональные нарушения сопровождались значительными гистопатологическими изменениями в ткани почек: некроз эпителиальных клеток проксимальных каналцев достигал $27.0 \pm 5,2\%$, в других клетках выявлены признаки гидропической вакуолизации ($7.0 \pm 2,1\%$) либо обратимого гидропического набухания ($76.0 \pm 1,5\%$), отмечался отек либо деформация некоторых клубочков. В мозговом веществе просветы каналцев расширены и частично заполнены гиалиновыми цилиндрами, каналцевые клетки с признаками дистрофии. Показано, что использование мелатонина способствовало ограничению гистопатологических изменений, что подтверждается снижением распространенности и выраженности каналцевого некроза (1,2%), дистрофии ($64.0 \pm 2,3\%$) и повреждения клубочков. Полученные результаты достоверно подтверждают нефропротекторное действие пинеального гормона мелатонина, обосновывая его дальнейшее углубленное изучение реальных эффектов, а также перспективы использования как нефропротектора.

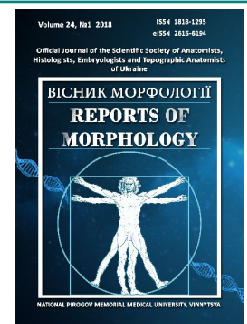
Ключевые слова: гентамицин-индуцированная нефропатия, мелатонин, гистопатологические изменения, нефропротекция.



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Variability of T-lymphocytes and macrophages composition in the urinary bladder under the influence of heavy metals salts, correction of their effect with vitamin E and in the readaptation period

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In modern urology, it is important to study and understand the features of the development of various pathologies in the organs of the urinary system since the disturbances of the urodynamics is a widespread problem all over the world. Thus, the environment pollution caused by heavy metal salts has unpredictable consequences for the functioning of the urinary bladder. That is why the object of this study is to determine the features of the expression and the quantitative composition of T-cells and macrophages in the UB wall under the intake of the HMS and correction of their effect with vitamin E, as well as during the readaptation period. According to the experimental scenario, the experiment model was implemented by using male rats that were divided into six groups. The heavy metal salts mixture consisted of the most common potentially dangerous metals-microelements (zinc, cooper, iron, manganese, lead and chrome). Vitamin E was chosen as a corrector. To determine the quantitative composition of T-cells and macrophages the immunohistochemical identification by CD3 and CD68 antibodies was used. The main results are presented as $M \pm SD$, with the assessment of the differences and dependencies between the groups by using nonparametric Mann-Whitney U test and Spearman's Rank Correlation Coefficient. We found that the number of CD3+ lymphocytes and CD68+ macrophages differed between the groups and depended on the experimental conditions. Thus, the results of our study demonstrate that the number of T-cells and macrophages in the bladder increases under the influence of heavy metals salts. The simultaneous intake of the pollutants and vitamin E is accompanied by the decrease of expression intensity of the corresponding cells. When the intake of the heavy metals is canceled, the qualitative composition of the identified CD3 and CD68 cells gradually comes to the norm, but the speed of its reduction differs depending on the group of the readaptation. Discovered that the decrease of T-cells and macrophages in the groups with the use of vitamin E was more approached to the control. Based on the results of the immunohistochemical study, a strong correlation between an increased number of the expressed T-cells and macrophages was revealed in the urinary bladder during the whole period of the study that demonstrates the dependence of their activity.

Keywords: urinary bladder, heavy metals salts, T-lymphocytes, macrophages, vitamin E, readaptation.

Introduction

In modern urology, it is important to study and understand the features of the development of various pathologies in the organs of the urinary system since the disturbances of the urodynamics is a widespread problem all over the world [3, 4]. Nowadays the mechanisms of the pathogenesis of these processes are studied insufficiently

that stipulates the awareness of the main causes of their occurrence [3].

Inflammation of the urinary bladder (UB) plays an important role in the urodynamics disturbance [10]. The pathology of this organ often accompanies various pathologies of the urogenital system and may be the first

clinical manifestation of their lesions. There are various theories of the development of the inflammation in the UB, and this complicates the understanding of the details of its pathogenesis [3, 6, 10]. It is known that the inflammation of this organ occurs when the protective barriers both in the mucous membrane and in its walls are broken [3, 5, 10]. However, the nature of the disease and the symptoms may vary, depending on the provocative factors: factors of the modern lifestyle (smoking, alcohol, chronic stress), the influence of the pollutants (anthropogenic pollution), the presence of the pathogenic microorganisms (*Escherichia coli*, *Clostridium botulinum*, etc.) and pathological processes in the UB wall (innervation disorders, cystitis and cancer) [3, 6, 7, 10, 11].

The number of the reports on the connection between the heavy metal salts (HMS) and ecologically caused diseases has increased significantly [8]. It is caused by the increased urbanization of the population and increased number of the dangerous human-made emissions followed by the environmental pollution and ecological imbalance. The effect of the heavy metals (HM) is unpredictable due to the variety of their combinations and concentrations in the polluted regions of the world [5, 8, 14].

The object of the study is to determine the features of the expression and the quantitative composition of T-cells and macrophages in the UB wall under the intake of the HMS and correction of their effect with vitamin E, as well as during the readaptation period.

Materials and methods

The experiment model was implemented by using white male rats ($n=84$) with a detailed UB research on the 30, 90, 120 and 180 day of the study. Thus, according to the experimental scenario, laboratory animals were divided into six groups: I - control group; II - rats that received water with the HMS mixture during 30 and 90 days; III - rats that received water with HM during the corrective therapy for 30 and 90 days; IV - animals on the 30 (120) and 90 (180) day after discontinuation of HM using; V - animals that received vitamin E within 30 (120) and 90 (180) days of readaptation after discontinuation of HMS using; VI - animals that received corrective therapy during 30 (120) and 90 (180) days after long-term HM+vitamin E influence. The animals were taken out of the experiment in certain periods by the decapitation after narcosis with the further UB removal. The animals were kept in the vivarium with the temperature, humidity, and day/night mode control and free access to water and food. All studies and control of the animals were conducted in accordance with the rules for the use of the animals in the experiments, adopted by the "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments", adopted by the First National Congress On Bioethics (Kyiv, 2001), "Ethical Principles and Guidelines for Experiments on Animals: 3rd Edition" (Switzerland 2005) and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (2006).

The HMS mixture consisted of the following potentially dangerous metals and microelements that circulate in the environment: zinc ($ZnSO_4 \cdot 7H_2O$) - 5 mg/l, copper ($CuSO_4 \cdot 5H_2O$) - 1 mg/l, iron ($FeSO_4$) - 10 mg/l, manganese ($MnSO_4 \cdot 5H_2O$) - 0.1 mg/l, lead ($Pb(NO_3)_2$) - 0.1 mg/l, chrome ($K_2Cr_2O_7$) - 0.1 mg/l. Vitamin E (alpha-tocopherol acetate (Vitamin E) was chosen as a corrector, with the calculated average dose for the rats (9.1 mg/kg).

After removal, the UBs were fixed in 10% formalin solution, dehydrated in graded ethanol and embedded in paraffin. The series of sections (the thickness 3-5 μm) were obtained on a rotary microtome. Visualization of the immunohistochemistry of the receptors was performed by UltraVision Quanto Detection System HRP DAB Cromogen (Thermo scientific, USA).

To determine the properties of T-cells and macrophages, as well as their quantitative composition, the immunohistochemical identification by using monoclonal antibodies of cluster differentiation CD3 (dilution 1:150) and CD68 (dilution 1:200).

The light and optical study of the UB was carried out by using "Carl Zeiss Primo Star" microscope with "Zeiss AxioCam ERs 5s" digital camera and "ZEN 2 (blue edition)" software. The obtained data was calculated in Graph Pad® 6.0. The digital results are presented as $M \pm SD$, with the assessment of the differences and dependencies between the groups by using nonparametric Mann-Whitney U test and Spearman's Rank Correlation Coefficient. $p < 0.05$ is the statistically significant value.

Results

The immunohistochemical study of the control group revealed the single CD3- and CD68-positive expression with membrane and cytoplasm patterns of the expression (Fig. 1-A). Identified T-cells (1.933 ± 0.710) and tissue-specific macrophages (2.635 ± 1.030) were localized namely in the stromal components of the UB.

In the animals from the II group that were given the HMS during 30 and 90 days, the number of positive expressed T-cells increased up to 18.42 ± 4.78 and 12.75 ± 4.12 ($p < 0.01$) (Fig. 1-B) cells in the field. The study of the macrophage generation revealed the increased activity of CD-68 positive cells due to the mixed cellular inflammatory infiltration. Herewith, the number of the macrophages increased with the extension of the experiment on the 30th day (14.55 ± 5.19 , $p < 0.05$) and 90th day (19.58 ± 6.89 , $p < 0.05$) (Fig. 1-B). Diffuse or lesion localized CD3 positive cells were localized mainly around the vessels and in the fields of the inflammatory infiltrates of the stroma, directed towards the epithelium.

At the same time, on the 30th day of the correction of the influence of the exogenous pollutants with vitamin E (III group), the number of CD3 and CD68 cells increased up to 16.38 ± 4.30 and 11.57 ± 4.06 ($p < 0.01$) in comparison with the control group. However, on the 90th day of the study the smooth reduction of the expression of the positive lymphocytes up to 10.58 ± 3.55 ($p < 0.01$) as well as increased

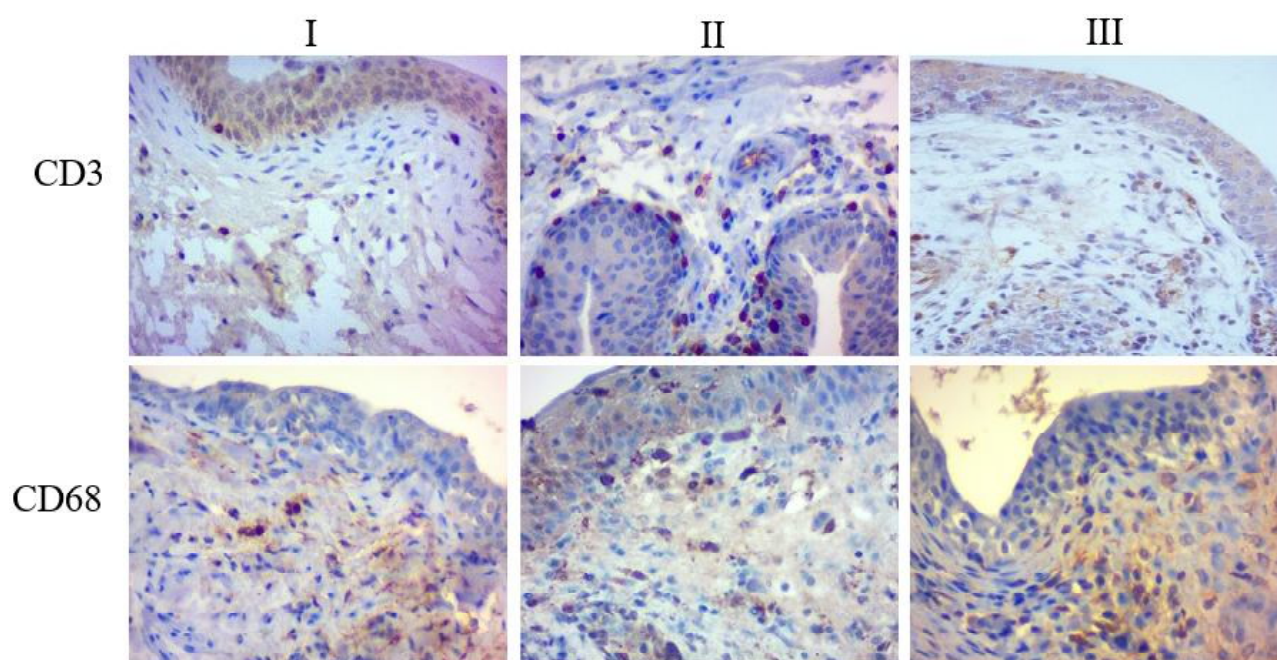


Fig. 1. The UB wall of experimental animals. I - control group; II group - laboratory animals that consumed water saturated of HMS during 90 days; III group - rats that received water with a HMS combination and vit. E within 90 days. Immunohistochemical study of CD3 and CD68 receptors. Magnification. x400.

number of macrophages up to 15.42 ± 5.35 ($p < 0.01$) was observed (Fig. 1-C).

With the extension of the experiment, on the 30th day of the readaptation the decreased number of the expressed T-cells (10.35 ± 3.49 vs 8.567 ± 3.140 vs 6.883 ± 2.510 , $p < 0.01$) in the stromal components of the UB was observed in IV, V and VI groups. It should be mentioned that the activity of T-cells markers and their numerical difference in comparison with the control group continued to decrease and accounted 6.417 ± 2.460 ($p < 0.01$) vs 3.650 ± 1.210 ($p > 0.05$) vs 2.750 ± 0.950 ($p > 0.05$), after 3 months of the rehabilitation.

The comparison of the phagocytic activity of the tissue macrophages by the identification with the antibodies to CD68+ cells on 120 and 180 days of the experiment showed the variations in their levels. Thus, the number of the macrophages in the UB wall depended on the studied group, namely: 15.80 ± 5.06 and 9.750 ± 3.430 ($p < 0.01$) - in the group after the HMS intake was canceled; 11.55 ± 3.84 and 6.217 ± 2.5 ($p < 0.01$) - with the use of the corrector during the entire rehabilitation period; 8.730 ± 3.020 and 4.283 ± 2.230 ($p < 0.01$) - with the extension of the vitamin E intake during the readaptation.

Based on the results of the immunohistochemical study, a strong correlation ($r = 0.76$, $p < 0.01$) between an increased number of the expressed T-cells and macrophages was revealed in the UB wall of all groups. These correlation indices are due to the long-term effects of HMS and shows the activity dependence of CD3 and CD68 positive cells.

Discussion

The activity of the elements of the immune system plays an important role in the pathogenesis of many diseases.

The role of the cells of the immune system in the local immune reactions, both during the inflammation and neoplasia is described in details [2, 13]. It is reported about the correlation between the composition of the immune microenvironment and the course of the disease or the development of the tumors in people who live in the polluted areas [12]. Thus, it is important to study the composition of the inflammatory infiltrate to predict the behavior of the immune system. Moreover, the modern markers of the cluster differentiation help accurately determine the cellular elements of the inflammatory infiltrate [9, 12], namely, T-cells (CD3) and macrophages (CD68).

It is known that the effect of the HMS on the body results in the deep histological and immunohistochemical changes in the UB and disorders in its structural homeostasis [11]. In this study, we revealed that the inflammatory cells of the different sub-population accumulate in the stromal components of the UB due to the long-term influence of the HMS mixture. Herewith, their activity increased when the experiment was extended and significantly exceeded the control indicators. It is considered to be the normal response of the body to the stress factor, but the composition of the inflammatory infiltration may vary depending on the conditions and period of the inflammation [2, 3, 7, 9]. On the other hand the immune reaction in the group with the corrector was less intensive that is demonstrated by the decrease of the generation of T-cells and macrophages. It can be explained by that fact that vitamin E has the effective protective effect from the HMS that is also proved by the less expressed morphological changes, demonstrated in the previous studies [1].

When the influence of the extreme factors on the body was canceled, the qualitative decrease of CD3 and CD68 positive expressed cells in the lesion was stated in all groups of the readaptation. It should be mentioned that the decrease of T-cells and macrophages activity in the groups with the use of vitamin E was more approached to the control.

The obtained results demonstrate that the immunohistochemical reaction with the selected antibodies was individual in each case. Thus, the number of CD3+ lymphocytes and CD68+ macrophages differed between the groups and depended on the experimental conditions. The long-term presence of CD68-positive cells in the lesions of the inflammatory infiltrates may be caused by the maintenance of the enhanced phagocytosis after the influence of the simulated microelementosis. Also, a strong

unidirectional correlation between the increased generation of the studied immune cells was observed. Taking into account the obtained results it is planned to study the correlation between the heavy metals accumulation and composition of the immune response.

Conclusions

The number of T-cells and macrophages in the UB wall increases under the influence of heavy metals salts. The simultaneous intake of the pollutants and vitamin E is accompanied by the decrease of expression intensity of the corresponding cells. When the intake of the heavy metals is canceled, the qualitative composition of the identified CD3 and CD68 cells gradually comes to the norm, but the speed of its reduction differs depending on the group of the readaptation.

References

- [1] Romaniuk, A., Sikora, V., Lyndin, M., Budko, H., & Piddubnyi, A. (2016). The histological changes in the urinary bladder, caused by simulated subacute influence of heavy metal salts. *Bulletin of Biology and Medicine*, 2(129), 80-83.
- [2] Abraham, S.N., & Miao, Y. (2015). The nature of immune responses to urinary tract infections. *Nat. Rev. Immunol.*, 15(10), 655-663. doi: 10.1038/nri3887.
- [3] Foxman, B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect. Dis. Clin. North Am.*, 28(1), 1-13. doi: 10.1016/j.idc.2013.09.003.
- [4] Heydari, F., Rezadoust, B., Abbaszade, S., Afrouz, E., & Ghadian, A. (2016) The Value of Urodynamic Study for Diagnosing the Causes of Lower Urinary Tract Symptoms in Male Patients: A Study From Iran. *Nephrourol. Mon.*, 8(4): e34342. doi: 10.5812/numonthly.34342.
- [5] Jaishankar, M., Tseten, T., & Anbalagan, N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.*, 7(2), 60-72.
- [6] Keay, S. K., Birder, L. A., & Chai, T. C. (2014). Evidence for Bladder Urothelial Pathophysiology in Functional Bladder Disorders. *Biomed. Res. Int.* doi: 10.1155/2014/865463
- [7] Kullmann, F. A. (2017). A new player in interstitial cystitis/ bladder pain syndrome: platelet-activating factor - PAF and its connection to smoking. *Physiol. Rep.*, 5(7): e13235. doi: 10.14814/phy2.13235
- [8] Landrigan, P. J., Sly, J.L., Ruchirawat, M., Silva, E. R., Huo, X., Diaz-Barriga, F., ... Sly, P. D. (2016). Health Consequences of Environmental Exposures: Changing Global Patterns of Exposure and Disease. *Ann. Glob. Health*, 82(1), 10-19. doi: 10.1016/j.aogh.2016.01.005.
- [9] Liu, Y., Yang, W., Zhao, L., Liang, Z., Shen, W., Hou, Q., ... Ying S. (2016). Immune analysis of expression of IL-17 relative ligands and their receptors in bladder cancer: comparison with polyp and cystitis. *BMC Immunol.*, 17, 36. doi: 10.1186/s12865-016-0174-8.
- [10] Lopez-Beltran, A., Montironi, R., & Cheng, L. (2016). *Pathology of the Urinary Bladder: An Algorithmic Approach*. Cambridge University Press.
- [11] Romaniuk, A., Sikora, V., Lyndin, M., Smiyanov, V., Sikora, V., Lyndina, Yu., ... Korobchanska, A. (2017). The features of morphological changes in the urinary bladder under combined effect of heavy metal salts. *Interv. Med. Appl. Sci.*, 9(2). doi: 10.1556/1646.9.2017.2.09.
- [12] Sjobahl, G., Lovgren, K., Lauss, M., Chebil, G., Patschan, O., Gudjonsson, S., ... Högglund, M. (2014). Infiltration of CD3+ and CD68+ cells in bladder cancer is subtype specific and affects the outcome of patients with muscle-invasive tumors. *Urol. Oncol.*, 32(6), 791-797. doi: 10.1016/j.urolonc.2014.02.007.
- [13] Sui, X., Lei, L., Chen, L., Xie, T., & Li, X. (2017). Inflammatory microenvironment in the initiation and progression of bladder cancer. *Oncotarget*, 54(8), 93279-93294. doi: 10.18632/oncotarget.21565.
- [14] Wan, D., Han, Z., Yang, J., Yang, G., & Liu, X. (2016). Heavy Metal Pollution in Settled Dust Associated with Different Urban Functional Areas in a Heavily Air-Polluted City in North China. *Int. J. Environ. Res. Public Health*, 13(11), 1119. doi: 10.3390/ijerph13111119.

ВАРІАБЕЛЬНІСТЬ СКЛАДУ Т-ЛІМФОЦИТІВ ТА МАКРОФАГІВ У СЕЧОВОМУ МІХУРІ ЗА УМОВ ВПЛИВУ СОЛЕЙ ВАЖКИХ МЕТАЛІВ, КОРЕКЦІЇ ЇХ ДІЇ ВІТАМІНОМ Е ТА У ПЕРІОД РЕАДАПТАЦІЇ

Сікора В.В.

Важливим питанням сучасної урології є вивчення та розуміння особливостей розвитку різноманітних патологій органів сечовидільної системи, так як порушення уродинаміки є широко розповсюдженою проблемою у різних країнах світі. Так, забруднення навколишнього середовища солями важких металів несе за собою непередбачувані наслідки на функціонування сечового міхура. Саме тому метою цього дослідження стало встановлення особливостей експресії та кількісного складу Т-лімфоцитів і макрофагів у стінці СМ за умов вживання солей важких металів та корекції їх дії вітаміном Е, а також у період реадaptaції. Згідно з експериментальним сценарієм модель експерименту була реалізована за допомогою самців щурів, які були розділені на шість груп. До суміші солей важких металів ввійшли найпоширеніші потенційно небезпечні метали-мікроелементи (цинк, мідь, залізо, марганець, свинець і хром). В якості коректора було обрано вітамін Е. Для визначення кількісного складу Т-клітин та макрофагів була використана імуногістохімічна ідентифікація антитілами CD3 та CD68. Основні результати представлені як $M \pm SD$, з оцінкою відмінностей та залежності між групами за допомогою непараметричного тесту Манн-Уїтні U та коефіцієнта кореляції Спірмена. Ми виявили, що кількість CD3+ -лімфоцитів та макрофагів CD68+ відрізнялася

між групами і залежала від експериментальних умов. Так, результати нашого дослідження демонструють, що за умов дії солей важких металів відбувається зростання кількості Т-лімфоцитів та макрофагів у стінці органа. Одночасне вживання поллютантів та вітаміну Е супроводжується значно меншою інтенсивністю експресії відповідних клітин. Після відміни вживання важких металів кількісний склад ідентифікованих CD3 та CD68 клітин поступово наближається до норми, однак швидкість їх зменшення відрізняється між серіями реадаптації. На підставі результатів імуногістохімічного дослідження встановлено сильні кореляційні зв'язки між зростанням кількості експресованих Т-лімфоцитів і макрофагів у сечовому міхурі протягом усього дослідження, що демонструє залежність їх активності.

Ключові слова: сечовий міхур, солі важких металів, Т-лімфоцити, макрофаги, вітамін Е, реадаптація.

ВАРИАБЕЛЬНОСТЬ СОСТАВА Т-ЛИМФОЦИТОВ И МАКРОФАГОВ В МОЧЕВОМ ПУЗЫРЕ ПРИ ВОЗДЕЙСТВИИ СОЛЕЙ ТЯЖЕЛЫХ МЕТАЛЛОВ, КОРРЕКЦИИ ИХ ДЕЙСТВИЯ ВИТАМИНОМ Е И В ПЕРИОД РЕАДАПТАЦИИ

Сикора В.В.

Важным вопросом современной урологии является изучение и понимание особенностей развития различных патологий органов мочевыделительной системы, так как нарушение уродинамики является широко распространенной проблемой в разных странах мира. Так, загрязнение окружающей среды солями тяжелых металлов влечет за собой непредсказуемые последствия на функционирование мочевого пузыря. Именно поэтому целью данного исследования стало установление особенностей экспрессии и количественного состава Т-лимфоцитов и макрофагов в стенке мочевого пузыря при условиях употребления солей тяжелых металлов и коррекции их действия витамином Е, а также в период реадaptации. Согласно экспериментальным сценарию модель эксперимента была реализована с помощью самцов крыс, которые были разделены на шесть групп. В смесь солей тяжелых металлов вошли самые распространенные потенциально опасные металлы-микроэлементы (цинк, медь, железо, марганец, свинец и хром). В качестве корректора был избран витамин Е. Для определения количественного состава Т-клеток и макрофагов была использована иммуногистохимическая идентификация антителами CD3 и CD68. Основные результаты представлены как $M \pm SD$, с оценкой различий и зависимости между группами с помощью непараметрического теста Манн-Уитни U и коэффициента корреляции Спирмена. Мы обнаружили, что количество CD3+ лимфоцитов и макрофагов CD68+ отличалось между группами и зависело от экспериментальных условий. Так, результаты нашего исследования показывают, что в условиях влияния солей тяжелых металлов происходит рост числа Т-лимфоцитов и макрофагов в стенке органа. Одновременный прием поллютантов и витамина Е сопровождается значительно меньшей интенсивностью экспрессии соответствующих клеток. После отмены употребления тяжелых металлов количественный состав идентифицированных CD3 и CD68 клеток постепенно приближается к норме, однако скорость их уменьшения отличается между сериями реадaptации. На основании результатов иммуногистохимического исследования установлено сильные корреляционные связи между ростом количества экспрессированных Т-лимфоцитов и макрофагов в мочевом пузыре в течение всего исследования, что демонстрирует зависимость их активности.

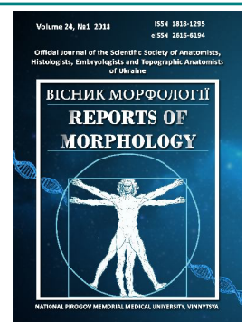
Ключевые слова: мочевой пузырь, соли тяжелых металлов, Т-лимфоциты, макрофаги, витамин Е, реадaptация.



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Morphometric analysis remodeling vessels hemomicrocirculatory bed of jejunum at resections of liver

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Liver resection is widely used in modern surgical departments. Removal of large volumes of the liver leads to postresection portal hypertension, which is complicated by bleeding from varicose veins of the esophagus, stomach, rectum, ascites, splenomegaly with secondary hypersplenism, parenchymal jaundice and portosystemic encephalopathy. The widespread prevalence of this pathology, high mortality from its complications indicates that it is an important medical and social problem. The aim of the study is quantitative morphological study of the features of remodeling the vessels of the hemomicrocirculatory bed of the jejunum at resection of different volumes of the liver. The studies were conducted on 45 sexually mature male rats, which were divided into 3 groups. The first group consisted of 15 intact virtually animals, 2- 15 rats after resection of the left lateral part - 31.5% of the parenchyma of the liver, 3- 15 animals after resection of the right and left lateral parts of the liver (58. 1%). Euthanasia of experimental animals was carried out by bloodletting in conditions of thiopental anesthesia 1 month after the beginning of the experiment. The hemomicrocirculatory bed of the jejunum was studied by injection of its vessels into the carcass-gelatinous mixture, which was injected through the abdominal aorta. From the jejunum, lightened and histologic preparates were prepared and morphometrically studied the vessels of the microhemocirculatory bed. Quantitative indicators were processed statistically, the difference between the comparative values was determined by the Student's criterion. It was established that the severity of structural transformation of the vessels of the microhemocirculatory bed of the jejunum depends on the volume of the removed parenchyma of the liver. The analysis of the obtained results revealed that one month after resection of 31.5% of liver parenchyma, the morphometric parameters of the vessels of the haemomicrocirculatory bed of the jejunum were insignificantly altered. It was determined that resection of 58. 1% of liver parenchyma caused postresection portal hypertension and marked remodeling of blood vessels of the hemomicrocirculatory bed of the jejunum, which was characterized by narrowing of arterioles by 23.4%, precapillary arterioles by 22. 8%, hemocapillaries by 22.9%, decrease in the density of microvessels - by 22. 4%, the expansion of the capillary venules - by 35.5% and venules - by 28. 7%, venous plethora, hypoxia, dystrophy, necrobiosis of cells and tissues, infiltrative processes and sclerosis. Resection of 58. 1% of liver parenchyma leads to postresection portal hypertension, pronounced narrowing of lumen of the vessels of the adnexa and exchange units hemomicrocirculatory bed, expansion of the postcapillary venules and venules, venous plethora, hypoxia, dystrophy and necrobiosis of cells and tissues, infiltrative and sclerotic processes in wall of the jejunum.

Keywords: jejunum, remodeling, hemomicrocirculatory bed, resection of liver.

Introduction

Liver resection is often performed today in modern surgical clinics [11, 14, 17, 26]. The indicated operation is performed in benign and malignant tumors, metastases, liver injuries, intrahepatic cholangiolithiasis, alveolar echinococcosis,

cholangiocarcinoma, liver transplantation [5, 9, 15, 16, 20]. Often, the removal of large amounts of liver remains the only method that allows you to rely on the healing of a patient with a malignant tumor, or significantly extend his

life [10, 12, 19, 21, 23]. Resection of large volumes of the liver can lead to various post-resection complications: bleeding from varicose veins of the esophagus, stomach, rectum, ascites, splenomegaly, secondary enlargement of the spleen, parenchymal jaundice, porto-systemic encephalopathy, liver failure, portal hypertension [7, 10, 22, 24, 26, 27]. Post-resection portal hypertension leads to structural rearrangement the organs of the basilar portal hepatic vein, as well as the remodeling of their structures [2, 7, 13, 22, 25]. Jejunum refers to the organs, venous drainage from which go through the portal hepatic vein, where hemodynamic disorders are complicated by various morphological changes in the vessels and structures of the specified organ [15, 18, 25]. It should be noted that the features of remodeling of the structures of the intestine in post-resection portal hypertension are not well understood [25].

The aim of the study is quantitative morphological study of the features of remodeling of the vessels of the hemomicrocirculatory bed of the jejunum at resection of different volumes of the liver.

Materials and methods

The studies were performed on 45 sexually mature white male rats, which were divided into 3 groups: the first group consisted of 15 intact practically healthy animals, 2 group - 15 rats after resection of the left lateral part (31.5% of the liver parenchyma), 3 group - 15 animals after the removal of the right and left side parts of the liver (58.1% of the liver parenchyma) [2]. Euthanasia of experimental animals was carried out by bloodletting under conditions of thiopental anesthesia 1 month after the beginning of the experiment. All manipulations and euthanasia of rats were conducted in compliance with the basic principles of working with experimental animals in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments" approved by the first national congress on bioethics (Kyiv, 2001), as well as the Law of Ukraine "On Protection of Animals from Cruel Treatment" of February 21, 2006 [5].

The hemomicrocirculatory channel of the jejunum was studied by injection in its vessels into the cartilaginous gelatin mixture injected through the abdominal aorta. 3-4 hours after the filling of the gastrointestinal tract of the jejunum, a sample of bits of different parts of this organ was collected, fixed in 10.0% solution of neutral formalin for 2 weeks. On a freezing microtome, sections of 30-40 μm thickness were made, which were dehydrated in ethyl alcohol of increasing concentration, clarified in methylene ether of salicylic acid and placed in polystyrene. From the observation portion of the filled vessels with the cartilaginous gelatinous mixture of the intestine, histological micropreparations were prepared, stained with hematoxylin and eosin [8]. Morphometrically determined diameters of arterioles (DA), pre-capillary

arterioles (DPCA), hemocapillaries (DH), after capillary venules (DACV), venules (DV), microvessels density (MD) of the hemomicrocirculatory bed at 1 mm^2 of the gut tissues of experimental animals [1]. The measurements were carried out in 50 fields of view of the microscope. Quantitative values were processed statistically. The results were processed in the department of systemic statistical research of I. Gorbachevsky Ternopil State Medical University in the software package Statsoft STATISTIKA. The difference between the comparative values was determined by the Student criteria [1, 6].

Results

In experimental animals one month after resection 58.1% of the liver parenchyma at the interstitial peritoneal cavity revealed expansion of the hepatic portal vein, plethora and widening of the mesenteric veins and the visible venous channel of the small and large intestines. Mucous membrane of the jejunum is full-blooded, edematous, with single cells of point hemorrhages. The above described evidence of post-resection portal hypertension [3, 8, 22]. The resulting morphometric parameters of the vessels of the microhemocirculatory duct of experimental animals are shown in Table 1.

From the data presented in Table 1, it is evident that a month after removal of 31.5% of the liver parenchyma, the hemomicrocirculatory bed changes, changes and endings were insignificant. Thus, the diameter of the arterioles in the experimental data was reduced by only 2.2%, and the diameter of the pre-capillary arterioles - by 2.8%. The diameter of the hemocapillaries (exchange link of the hemomicrocirculatory channel of the jejunum) in the experimental data significantly ($p < 0.05$) decreased from (6.10 ± 0.09) microns to (5.65 ± 0.12) microns, that is, at 7.4%. The remote link (after capillary venules and venules) of the hemomicrocirculatory channel of the jejunum at the same time tended to expand. Thus, the diameter of the capillary venules of the investigated organ a month after the resection of 31.5% of the liver parenchyma was significantly ($p < 0.01$) increased by 12.1%, and the venule diameter - by 11.7%. The density of microvessels per 1 mm^2 of the jejunum tissue in the experimental data did not change significantly,

Table 1. Morphometric characteristic of the hemomicrocirculatory channel of the gut of experimental animals ($M \pm m$).

Indicator	Surveillance group		
	1	2	3
DA, μm	17.80 \pm 0.21	17.40 \pm 0.33	13.60 \pm 0.12***
DPCA, μm	10.50 \pm 0.12	10.20 \pm 0.15	8.10 \pm 0.09***
DH, μm	6.10 \pm 0.09	5.65 \pm 0.12*	4.70 \pm 0.03
DACV, μm	12.40 \pm 0.15	13.90 \pm 0.18**	16.80 \pm 0.18***
DV, μm	26.50 \pm 0.30	28.10 \pm 0.30**	34.10 \pm 0.33***
DM	3815.6 \pm 28,2	3793.4 \pm 30.3	2960.5 \pm 24.3***

Notes: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$ compared to 1 group of observations.

providing a complete blood supply to the structures of the investigated organ [6].

One month after the resection 58.1% of the liver parenchyma of the remodeling of the blood vessels of the hemomicrocirculatory channel of the jejunum was more pronounced than the previous group of observations. Thus, the diameter of arterioles at the same time with a high significant difference ($p < 0.001$) was lower by 23.4% compared with a similar control value. Diameters of pre-capillary arterioles and hemocapillaries also with a high degree of significant difference ($p < 0.001$) decreased by 22.8% and 22.9%, respectively. The diameter of the capillary venules in the given experimental conditions with a pronounced significant difference ($p < 0.001$) increased from $(12.40 \pm 9.15) \mu\text{m}$ to $(16.80 \pm 0.18) \mu\text{m}$, that is, by 35.5%, and the venules diameter - by 28.7%. The density of microvessels per 1 mm^2 of jejunum tissue one month after the removal of 58.1% of liver parenchyma decreased from (3815.6 ± 28.2) to (2960.5 ± 24.3) . Between the given morphometric parameters, a significant ($p < 0.001$) difference was found. At the same time, the last quantitative morphological index was lower than the previous one by 22.4%, indicating deterioration of the blood supply of the investigated organ [6, 7].

Discussion

The domination of the expansion of the venous part of the hemomicrocirculatory duct of the colon within one month after resection of 58.1% of the liver parenchyma is accompanied by venous enlargement, which is complicated by hypoxia. The latter leads to dystrophy, necrobiosis of cells and tissues, and in the remote postoperative period to infiltrative and sclerotic processes in the shells of the investigated organ [2, 7]. The above was confirmed by light-optical research of micropreparations of the intestine. Microscopically, in the membranes of the indicated organ, there were unevenly enlarged, twisted, full-blooded with numerical sacculations of the venous microvessels of the hemomicrocirculatory bed. In these vessels, stasis, thrombosis,

diapedeous hemorrhage, plasmorrhagia of the wall of venous structures and para-vascular tissues were detected. In the membrane of the wall of the intestine marked places of decrease of microvessels due to their reduction and non-vascular zones. Places of reduction of microvessels in micropreparations were visualized in the form of thick-walled fibrous grafts, sometimes with signs of hyalinosis. Endothelial cells of the microvessels with edema phenomena, dystrophic and non-fibrotically altered, acquired a rounded form of cells with desquamation. Some stromal structures and smooth myocytes were also dystrophic and necrobiotic changed. The revealed morphological changes in the wall of the jejunum within a month after resection of 58.1% of the liver parenchyma adequately demonstrate that the vessels of the hemomicrocirculatory channel assume an important role not only in the trophic maintenance of tissues of organs, but also in the pathomorphogenesis of their lesions [7, 25]. The expressive decrease in the density of microvessels per 1 mm^2 of tissues of the investigated organ indicated not only the deterioration of its blood supply, but also the violation of tissue structural homeostasis [1, 7].

Comprehensive, adequate, full-fledged study of the structural transformation of the vessels of the hemomicrocirculatory channel of the jejunum in post-resection portal hypertension will allow to significantly expand the diagnosis, correction and prevention of the studied pathology.

Conclusions

Resection of 58.1% of liver parenchyma leads to post-resection portal hypertension and marked remodeling of the vessels of the hemomicrocirculatory channel of the jejunum, which is characterized by a significant narrowing of the arterial lumen (arterioles, pre-capillary arterioles), exchange (hemocapillaries) of its parts, and the expansion of the after capillary venules and venules, a decrease in the density of the microvessels, venous plethora, hypoxia, dystrophy and necrobiosis of cells and tissues, infiltrative and sclerotic processes.

References

- [1] Avtandilov, G. G. (2002). *Basic of Quantitative Pathological Anatomy*. Moscow: Medicine. ISBN: 978-5-225-04151-9
- [2] Hnatiuk, M. S., Tatarchuk, L. V., & Yasinovskyy, O. B. (2016). Morphometric evaluation of the features of remodeling of duodenal structures during resection of different volumes of the liver. *Scientific Journal of Uzhhorod University. Series "Medicine"*, 1(49), 3-5.
- [3] Kotenko, O. H., Popov, A. O., & Grynenko, A. V. (2017). Resection portal vein in treatment perychylar cholangiocarcinomy. *Ukr. J. Surgery*, 4(35), 10-17.
- [4] Lapach, S. N., Gubenko, A. V., & Babich P. N. (2001). *Statistical methods in medicobiological investigation Excell*. Kiev: Morion. ISBN: 966-7632-33-4
- [5] Reznikov, O. G. (2003). General ethical principles of experiments on animal. *Endocrinology*, 8(1), 142-145.
- [6] Sarkisov, D. S. (1987). *Structural basis adaptation and compensation damage function*. Moscow: Medicine. ISBN (B nep.) (B nep.): 2 p.
- [7] Shulgai, A. H., Tatarchuk, L. V., & Hnatiuk, M. S. (2017). Remodeling peculiarities of ileum hemomicrocirculatory bed vessels at resection of different liver size. *Scientific Journal of investigation*, 4, 145-149.
- [8] Sorochinnikov, A. G., & Dorosievich, A. Ye. (2007). *Histologikal and mikroskopik equipments*. Moscow: Medicine.
- [9] Fiodorov, V. D., Vishnievskiy, V. A., & Nazarienko, N. A. (2007). The main complication of extensive liver resection and ways to prevent them. *Bulletin of Siberian Medicine*, 4, 16-24.
- [10] Vishnievskiy, V. A., Yefanov, M. G., & Kazakov, I. V. (2012). Segmentar resection, long-term results in malignant liver tumors. *Ukr. J. Surgery*, 1(16), 5-15.
- [11] Adam, R. (2003). Chemotherapy and surgery: new perspectives on treatment of unresectable liver metastasis. *Ann. Oncol.*, 14(2), 13-16.
- [12] Are, C., Gonen, M., & Zazzali, K. (2007). The impact of

- margins on outcome after hepatic resection for colorectal metastasis. *Ann. Surg.*, 246(2), 295-390. doi: 10.1097/SLA.0b013e31811ea962
- [13] de Franchis, R., & Baveno, V. F. (2010). Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J. Hepatol.*, 53(4), 762-768. doi: 10.1016/j.jhep.2010.06.004.
- [14] Lai, H. E. I., & Lai, E. C. (2007). Hepatic resection for colorectal liver metastases. *Singapor Med. J.*, 48(7), 635-639.
- [15] Nakajima, K., Takanaishi, S., & Saito, N. (2013). Efficacy of the predicted operation time strategy for synchronous colorectal liver metastasis feasibility study for staged resection in patients in long predicted operation time. *Gastrointest. Surg.*, 17(4), 688-695.
- [16] Nanashima, A., Sumida, Y., & Abo, T. (2008). A modified grading system for post-hepatectomy metastasis liver cancer originating from colorectal carcinoma. *J. Surg. Oncol.*, 98(5), 363-370.
- [17] Neuman, U. P., Sechofer, D., & Neuhaus, D. (2010). The surgical treatment of hepatic metastasis in colorectal carcinoma. *Dtsch. Arztebl. Int.*, 107(19), 335-342. doi: 10.3238/arztebl.2010.0335
- [18] Ogata, Y., Nishi, M., & Nakayama, H. (2003). Role of bile in intestinal barrier function and its inhibitory effect on bacterial translocation in obstructive jaundice in rats. *J. Surg. Res.*, 115(1), 18-23.
- [19] Paravecino, M., Chun, I. S., Madoff, D. C., Zorzi, D., Kishi, Y., Kaseb, A. O. ... Vauthey, J. N. (2009). Major hepatic resection for hepatocellular carcinoma with or without portal vein embolization: Perioperative outcome and survival. *Surgery*, 145(4), 399-405. doi: 10.1016/j.surg.2008.10.009.
- [20] Reddy, S. S., & Civan, J. M. (2016). From Child-Pugh to Model for End-Stage Liver Disease: Deciding Who Needs a Liver Transplant. *Med. Clin. North Am.*, 100(3), 449-464.
- [21] Rialon, K. L., Murillo, R., Fevury, R. O., Kulungowski, A. M., Zurakowski, D., Liang, M. ... Fishman, S. J. (2015). Impact of screening for hepatic hemangioma in patients with multiple cutaneous infantile hemangiomas. *Pediatr. Dermatol.*, 32(6), 162-167. doi: 10.1111/pde.12656.
- [22] Sass, D. A., & Chopra, K. B. (2009). Portal hypertension and variceal hemorrhage. *Med. Clin. North Am.*, 93(4), 837-853. doi: 10.1016/j.mcna.2009.03.008.
- [23] Salloum, C., & Castaing, D. (2008). Surgical margin status in hepatectomy for liver tumor. *Bull. Cancer.*, 95(12), 1183-1191. doi: 10.1684/bdc.2008.0758
- [24] Sanyal, A. J., Bosch, J., Blei, A., & Arroyo, V. (2008). Portal hypertension and its complications. *Gastroenterology*, 134(6), 1715-1728. doi: 10.1053/j.gastro.2008.03.007.
- [25] Sawada, K., Ohtake, T., & Veno, N. (2011). Multiple portal hypertensive polyps of the jejunum accompanied by anemia of the unknown origin. *Gastrointest., Endosc.*, 73, 179-182.
- [26] Taguchi, T., Iwanaka, T., & Okamatsu, T. (2016). Operative General Surgery in Neonates and Children. *Springer Japan.*, 42, 398-399.
- [27] Tsirlina, V. B., Lau, K. N., Swan, R. Z., Montero, P. N., Sindram, D., Martinie, J. B., & Iannitti, D. A. (2013). Evaluation of an innovative Cordes ultrasonic Dissector. *Surg. Innov.*, 20(5), 524-529. doi: 10.1177/1553350612471206.

МОРФОМЕТРИЧНИЙ АНАЛІЗ РЕМОДЕЛЮВАННЯ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА ПОРОЖНЬОЇ КИШКИ ПРИ РЕЗЕКЦІЯХ ПЕЧІНКИ

Гнатюк М.С., Татарчук Л.В.

Видалення великих об'ємів печінки призводить до пострезекційної портальної гіпертензії, що ускладнюється кровотечами із варикозно розширених вен стравоходу, шлунка, прямої кишки; асцитом, спленомегалією, вторинним гіперспленізмом, паренхіматозною жовтяницею та портосистемною енцефалопатією. Широка розповсюдженість даної патології, висока смертність від її ускладнень свідчать, що вона є важливою медичною та соціальною проблемою. Мета дослідження - кількісне морфологічне вивчення особливостей ремоделювання судин гемомікроциркуляторного русла порожньої кишки при резекціях різних об'ємів печінки. Дослідження проведені на 45 статевозрілих щурах-самцях, які були розділені на 3 групи. 1 група (15 інтактних тварин), 2-15 щурів після резекції лівої бокової частки (31,5% паренхіми печінки), 3-15 тварин після резекції правої та лівої бокових часток печінки (58,1%). Евтаназію тварин здійснювали кровопусканням в умовах тіопенталового наркозу через 1 місяць від початку експерименту. Гемомікроциркуляторне русло порожньої кишки вивчали за допомогою ін'єкції її судин туш-желатиною сумішшю, котру вводили через черевну аорту. Із порожньої кишки виготовляли просвітлені та гістологічні мікропрепарати, на яких морфометрично вивчали судини мікрогемодинамічного русла. Кількісні показники обробляли статистично, різницю між порівнювальними величинами визначали за критерієм Стьюдента. Встановлено, що вираженість структурної перебудови судин мікрогемодинамічного русла порожньої кишки залежить від об'єму видаленої паренхіми печінки. Виявлено, що через місяць після резекції 31,5% паренхіми печінки досліджувані морфометричні параметри судин гемомікроциркуляторного русла порожньої кишки змінювалися незначно. Встановлено, що резекція 58,1% паренхіми печінки призводила до пострезекційної портальної гіпертензії та вираженого ремоделювання судин гемомікроциркуляторного русла порожньої кишки, яке характеризувалося звуженням артеріол на 23,4%, передкапілярних артеріол - на 22,8%, гемокапілярів - на 22,9%, зменшенням щільності мікросудин - на 22,4%, розширенням закапілярних венул - на 35,5% і венул - на 28,7%, венозним повнокров'ям, гіпоксією, дистрофією, некробіозом клітин і тканин, інфільтративними процесами та склерозуванням. Таким чином, в результаті резекції 58,1% паренхіми печінки розвивається пострезекційна портальна гіпертензія, достовірно зменшуються просвіти судин приносячої та обмінної ланок, гемомікроциркуляторного русла, розширюються закапілярні венули та венули, збільшується їх повнокров'я, гіпоксія, дистрофія та некробіоз клітин і тканин, розвиваються інфільтративні та склеротичні процеси у стінці порожньої кишки.

Ключові слова: порожня кишка, ремоделювання, гемомікроциркуляторне русло, резекція печінки.

МОРФОМЕТРИЧЕСКИЙ АНАЛИЗ РЕМОДЕЛИРОВАНИЯ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА ТОЩЕЙ КИШКИ ПРИ РЕЗЕКЦИЯХ ПЕЧЕНИ

Гнатюк М.С., Татарчук Л.В.

Удаление больших объемов печени приводит к пострезекционной портальной гипертензии, которая осложняется кровотечениями из варикозно расширенных вен пищевода, желудка, прямой кишки, асцитом, спленомегалией, вторичным

гиперспленизмом, паренхиматозной желтухой и портосистемной энцефалопатией. Широкая распространенность данной патологии, высокая смертность от ее осложнений свидетельствуют, что она является важной медицинской и социальной проблемой. Цель исследования - количественное морфологическое изучение особенностей ремоделирования сосудов гемомикроциркуляторного русла тощей кишки при резекции различных объемов печени. Исследования проведены на 45 половозрелых крысах-самцах, которые были разделены на 3 группы: 1 группа (15 интактных животных), 2-15 крыс после резекции левой боковой доли (31,5% паренхимы печени), 3-15 животных после резекции правой и левой боковых долей печени (58,1%). Эвтаназию животных осуществляли кровопусканием в условиях тиопенталового наркоза через 1 месяц после начала эксперимента. Гемомикроциркуляторное русло тощей кишки изучали с помощью инъекции ее сосудов тушь-желатиновой смесью, которую вводили через брюшную аорту. Из тощей кишки изготавливали просветленные и гистологические микропрепараты, на которых морфометрически изучали сосуды микрогемомикроциркуляторного русла. Количественные показатели обрабатывали статистически, различия между сравниваемыми показателями определяли по критерию Стьюдента. Установлено, что выраженность структурной перестройки сосудов микрогемомикроциркуляторного русла тонкой кишки зависит от объема удаленной паренхимы печени. Выявлено, что через месяц после резекции 31,5% паренхимы печени исследуемые морфометрические параметры сосудов гемомикроциркуляторного русла тощей кишки изменялись незначительно. Установлено, что резекция 58,1% паренхимы печени приводила к пострезекционной портальной гипертензии и выраженному ремоделированию сосудов гемомикроциркуляторного русла тощей кишки, которое характеризовалось сужением артериол на 23,4%, передкапиллярных артериол на 22,8%, гемокапилляров на 22,9%, уменьшением плотности микрососудов на 22,4%, расширением посткапиллярных венул на 35,5% и венул на 28,7%, венозным полнокровием, гипоксией, дистрофией, некробиозом клеток и тканей, инфильтративными процессами и склерозированием. Резекция 58,1% паренхимы печени приводит к пострезекционной портальной гипертензии, выраженному уменьшению просветов сосудов приносящего и обменного звеньев гемомикроциркуляторного русла, расширению посткапиллярных венул и венул, их полнокровию, гипоксии, дистрофии и некробиозу клеток и тканей, инфильтративным и склеротическим процессам в стенке тощей кишки.

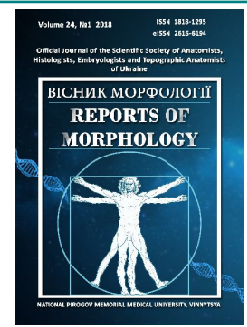
Ключевые слова: тощая кишка, ремоделирование, гемомикроциркуляторное русло, резекция печени.



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Expression of mmp-9 as a prognostic factor of uterine sarcoma

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Uterine sarcoma is a highly aggressive mesenchymal neoplasm with an extremely unfavorable prognosis. Up today there are still relevant issues concerning search for clinical-morphological and biomolecular criteria for prognosis relapse-free survival of uterine sarcoma patients. It is well-known, the increase of the expression level of MMP-9 in primary tumor or metastatic foci correlates with a low differentiation of tumor cells, high ability for invasiveness, high metastatic activity, and shortened life expectancy. It's still unknown, whether it is possible to consider the expression of MMP-9 in uterine sarcoma cells as a convincing prognostic factor. For many types of epithelial malignant neoplasms, high metastatic rate is associated with an increase level of MMP-9 both in plasma and in tumor tissue. The purpose of this study is to investigate the features of MMP-9 expression in uterine sarcoma cells for development of the model for individual prediction of the disease course. The study of the surgical material of selected 54 cases of uterine sarcoma of stage I-II (according to FIGO criteria) with a known prognosis of the disease, which were distributed depending on the morphological type done: leiomyosarcoma (LMS) - 18 cases, endometrial stromal sarcoma (ESS) - 22 cases, undifferentiated sarcoma (US) - 14 (according to the classification of tumors of the uterus of the WHO). For histological examination, pieces of tissue were cut from different parts of the tumor nodes - central, peripheral, parts of the adjacent intact tissue of myometrium (total of 6-8 bits). The tumor cell phenotype was determined using low molecular weight cytokeratins (Cytokeratin PAN, AE1 / AE3), smooth muscle actin (Smooth Muscle Actin, 1A4), myogenin (Myogenin (F5D)), CD 10 and vimentin (Vimentin, V9). The histochemical label was evaluated in two parameters: the degree of prevalence and intensity of coloration. To assess the color intensity, a qualitative scale was used: 0 - no reaction, 1+ - weak cytoplasmic coloration to 30.0% of tumor cells, 2+ - moderate reaction, 30.0 to 60.0% of stained cells, 3+ - pronounced cytoplasmic reaction in 60.0-100.0% of tumor cells. Statistical processing of the data was performed using the "STATISTICA 10.0" program package. The conducted study has showed, the negative (0) and weak (1+) expression of matrix metalloproteinase-9 were observed in the most part of ESS and only partially in US. Despite the stage of the disease, with such a status of MMP-9, there was observed no signs of relapsed disease. The moderate (2+) and high (3+) expression of MMP-9 was detected in 44.5% of uterine sarcoma, in the most part in LMS patients. However, if in LMS cases the progressive disease was observed only in one third of them (4 of 12 cases), in case of ESS and US, in all the patients with such tumors status there was observed relapsed disease. Such a reaction may be indicative for invasive and metastatic potential of ESS and US and cause of the hematogenous metastases.

Keywords: uterine sarcoma, MMP-9 expression, connection of MMP-9 expression and tumor progression, leiomyosarcoma, endometrial stromal sarcoma, undifferentiated uterine sarcoma.

Introduction

Uterine sarcoma is a highly aggressive mesenchymal neoplasm with an extremely unfavorable prognosis [24, 39]. The intraorganic location of tumor sites causes low accessibility and informative visual and instrumental research. In this regard, tumor data are still rarely found in the early stages of its development [25]. The share of sarcoma among all malignant neoplasms is only 3.0%, which is why these tumors are still one of the less well-known tumors of this localization [32, 34].

The issue of finding clinical and morphological and biomolecular prediction criteria and non-recurrent survival in tumors of the uterus is still topical. It has been established that during metastasis, tumor cells interact with the extracellular matrix (ECM), associated with its growth factors and cytokines, basal membranes, endothelial cells, circulating blood cells, and others [16]. The degradation of ECM occurs as a result of a violation of the regulation of all its components, which leads to tumor invasion [3, 20].

For many types of epithelial malignant neoplasms, high metastasis rates are associated with an increase in the level of matrix metalloproteinase-9 (MMP-9) in both plasma and tissue parenchyma.

A retrospective analysis of the studies in these patients suggests that elevation of MMP-9 expression in the primary tumor or metastases correlates with low differentiation of tumor cells, high carcinoma invasiveness, high metastatic activity, and shortening of life expectancy [22, 31].

It is still unknown whether it is possible to consider the expression of MMP-9 in uterine sarcoma cells as a convincing prognostic factor.

That is why *the purpose* of this research is to study the features of expression of MMP-9 in uterine sarcoma cells to create a model for individual prediction of the course of the disease.

Materials and methods

The study of the surgical material obtained during surgical treatment of patients in the department of oncogynecology State Institution "Grigoriev Institute for Medical Radiology of National Academy of Medical Sciences Ukraine" from 2010 to 2018, as well as fixed archival material and paraffin blocks of tissue that were obtained from patients operated at the Kherson Regional Oncology Center (on the basis of an agreement on scientific cooperation). We selected 54 cases of uterine sarcoma of stage I-II according to the FIGO criteria [23], with a known prognosis of the disease, which were distributed according to the classification of the tumors of the uterus of the WHO [29] depending on the morphological type: leiomyosarcoma (LMS) - 18 cases, endometrial stromal sarcoma (ESS) - 22 cases, undifferentiated sarcoma (US) - 14. In order to guarantee the quality of immunohistochemical studies, coded samples were processed simultaneously in the laboratories of KMAPE, Ukraine, and the University of Sweden, with subsequent decoding of the results by the leader of the research and data entry for analysis only if

there is consensus in the conclusions of both laboratories.

For histological examination, pieces of tissue were cut from different parts of the tumor nodes - central, peripheral, parts of the adjacent intact tissue of myometrium (total of 6-8 bits). Fragments of tissues were fixed in 10.0% solution of neutral formalin, buffered with phosphate buffer. Subsequently, the material was subjected to standard wiring according to the standard of increasing concentration, chloroform, after which it was poured by paraffin. From the made paraffin blocks, serial slices were made in the thickness of 3-4 microns. In all cases, standard methods of coloring with hematoxylin and eosin were used.

The tumor cell phenotype was determined using low molecular weight cytokeratins (Cytokeratin PAN, AE1 / AE3), smooth muscle actin (Smooth Muscle Actin, 1A4), myogenin (Myogenin (F5D)), CD 10 and vimentin (Vimentin, V9). The primary monoclonal antibodies (MA) of DAKO (Denmark), Ready-to-Use, were used. To study the features of the extracellular matrix of the tumor and its metastatic potential, rabbit concentrated polyclonal antibodies (PA) were used for 1:50 dilution of matrix metalloproteinase-9 (MMP-9, 92kDa Collagenase IV) from Thermo scientific (Germany). Antibody de-masking was done by boiling in sections in citrate buffer (pH 6.0). UltraVision Quanto Detection Systems HRP Polymer (Thermo scientific) detection system was used to visualize primary antibodies. DAB (diaminobenzidine) was used as a chromogen. The results were counted with Avtandilov ocular grid [2] in 10 arbitrarily selected fields of view with an increase of 400x. The evaluation of the histochemical label was carried out in two parameters: the degree of prevalence and intensity of color, taking into account the severity of the reaction and its localization. The degree of distribution of the label was taken into account for the percentage content of cells positively colored in brown color from the total number of cells in the field of vision. To assess the color intensity, a qualitative scale was used: 0 - no reaction, 1+ - weak cytoplasmic coloration to 30.0% of tumor cells, 2+ - moderate reaction, 30.0 to 60.0% of stained cells, 3+ - pronounced cytoplasmic reaction in 60.0-100.0% of tumor cells. The complex of morphological studies was performed on a microscope Primo Star (Carl Zeiss) using AxioCam (ERc 5s) programs.

Statistical processing of the data was carried out using a program package "STATISTICA 10.0".

We express our gratitude to the staff of the Institute, Kherson Regional Oncology Center and Umea University, who participated in the treatment of the above-mentioned patients, as well as scientific research.

Results

Expression of MMP-9 was detected in 30 cases (55.5%), with a quarter of all 54 uterine sarcomas (25.9%) showing a marked reaction (3+). The largest number of 3+ tumors accounted for the share of LMS - 22.2% of the total, 66.7% - within the group (Table 1).

Table 1. Level of expression of MMP-9 in tumor tissue of uterine sarcoma of different histological types.

Expression level	LMS (n=18)	ESS (n=22)	US (n=14)	Total (n=54)
0	4 (22.2%) (7.4%)	16 (72.7%) (29.6%)	4 (28.6%) (7.4%)	24 (44.4%)
1+	0	0	6 (42.8%) (11.1%)	6 (11.1%)
2+	2 (11.1%) (3.7%)	4 (18.2%) (7.4%)	4 (28.6%) (7.4%)	10 (18.6%)
3+	12 (66.7%) (22.2%)	2 (9.1%) (3.7%)	0	14 (25.9%)

Notes: the first indices in% (in brackets) is the frequency with which this level of expression occurs among different subgroups (LMS, ESS, US), the second indices in% (in brackets) is the frequency with which this expression level is encountered in the total number of cases (n=54).

In these observations, colored granules of MMP-9 were located both in the cytoplasm of the tumor cells and in the endothelial cells of the tumor itself, in the endothelium of the myometrium vessels, in the lymphocytes, macrophages, histocytes of the infiltration zone of the tumor invasion zone in myometrium (Fig. 1).

Moderate (2+) staining of cytoplasm granules showed 18.6% of uterine sarcoma, this number included 4 cases of US and ESS (by 7.4% of all observations, 28.6 and 18.2% respectively, within groups) and 2 observations with LMS (3.7 and 11.1% within the group).

The reaction was determined fairly uniformly, and if the visualization of the patient with US was limited to tumor cells, the accumulation of MMP-9 in the LMS group was also found in the extracellular matrix. ESS demonstrated focal coloration of tumor parenchyma cells, endothelial platelets of blood vessels and reactive infiltrate cells (Fig. 2).

Weak and uneven (1+) reactions were in 11.1% of the observations, all of which were related to US (42.8% within the group).

In 2 of these cases, in the cytoplasm, sometimes in endothelial cells, expression could be considered as focal moderate, however, the enhancement of coloration (2+) was detected in only 10.0% of cellular elements.

In 44.4% of all sarcomas expression of MMP-9 was not detected. ESS accounted for 29.6% of MMP-9-negative tumors (72.7% of all ESS).

The results of MMP-9 expression, depending on the stage of the disease and on the presence or absence of relapse of the tumor, are distributed as follows (Table 2).

In none of the cases, the uterine sarcoma with MMP-9-negative (0) status (24 observations) did not show the progression of the tumor, as in I and II in the stages of the disease.

Expression of MMP-9 +1 was detected only in the US group: 3 of these tumors corresponded to stage I, 3 to stage II of the disease; relapses were not observed in any of these cases.

The level of coloring corresponding to 2+ at stage I was

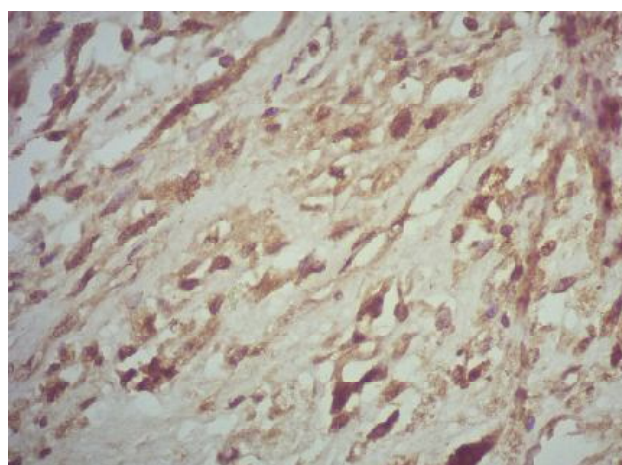


Fig. 1. The expressed cytoplasmic expression of MMP-9 in tumor cells, endothelial cells and extracellular matrix of leiomyosarcoma. Reaction with polyclonal antibodies on MMP-9, 92kDa Collagenase IV. x400.

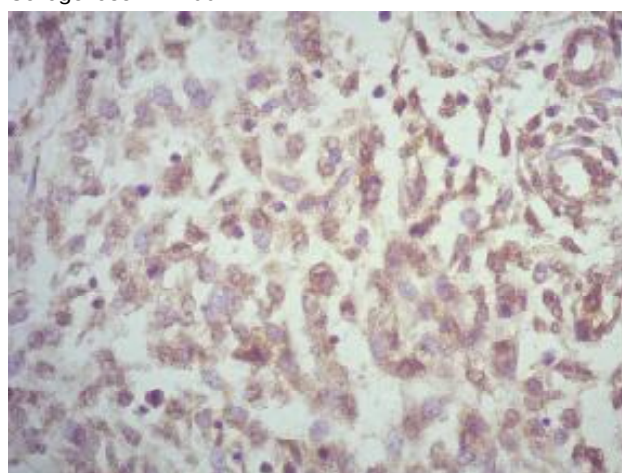


Fig. 2. Moderate cytoplasmic expression of MMP-9 in tumor cells, endothelial cells and reactive infiltrates of endometrial stromal sarcoma. Reaction with polyclonal antibodies on MMP-9, 92kDa Collagenase IV. x200.

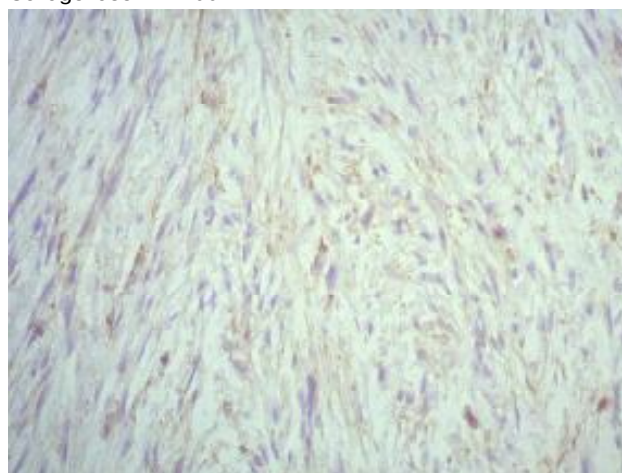


Fig. 3. Weak cytoplasmic expression of MMP-9 in tumor cells and the extracellular matrix of undifferentiated sarcoma. Reaction with polyclonal antibodies on MMP-9, 92kDa Collagenase IV. x200.

Table 2. Expression of MMP-9 in tumor tissue of the uterine sarcoma, depending on the stage of the disease and the presence of relapse.

	Stage of the disease	Presence of expression MMP-9	Presence of relapse
LMS (18)	T1 (16)	3+ - 12, 2+ - 2	4
		0 - 4	0
	T2 (2)	3+ - 2	2
ESS (22)	T1 (14)	3+ - 2, 2+ - 3	5
		0 - 9	0
	T2 (8)	2+ - 1	1
		0 - 7	0
US (14)	T1 (8)	2+ - 1, 1+ - 3	1 (2+)
		0-4	0
	T2 (6)	2+ - 3, 1+ - 3	3 (2+)
		0 - 0	0

detected in 1 case of US, 3 - ESS, and 2 - LMS, and, if the recurrence was noted at the US and ESS, then it was absent in the LMS. At stage II, moderate expression of MMP-9 was detected in ESS in 1 case, and in 3 cases with US; all of these patients had a recurrence of the disease.

Positive status (3+) MMP-9 is defined in 2 cases of ESS of stage I and in 14 LMS (12 - stage I, 2 - stage II). Recurrence of tumors is noted in two observations of the ESS. In LMS, the progression of tumors was detected in only two cases of stage I and two observations with stage II disease.

Discussion

In recent decades, many researchers have paid much attention to the study of proteolytic enzymes both in the tumor itself and in the surrounding extracellular matrix (ECM). It is known that the ability of tumor cells to invasive growth and distribution in the form of metastases depends on their properties to split the components of ECM - basal membrane, intercellular stroma, walls of blood and lymph vessels, as well as any components containing structural proteins [5, 6, 15]. The main role in the process of cleavage of structural proteins of ECM is played by the proteolytic enzymes of the matrix metalloproteinase (MMP) group, which are present both in the tumor and in the stromal cells. On the basis of currently accumulated scientific data, there is an impression that MMP are the major proteolytic enzymes that contribute to metastatic cancer cells [9, 11, 15]. Author Ganusevich I.I. established that MMP not only destroys ECM and basement membrane proteins, but also stimulates the migration of cellular tumors and plays a significant role in the survival of tumor cells that take part in the suppression of antitumor immunity and regulation of neo-angiogenesis, thereby providing additional pathways for the evacuation of primary tumor cells [7, 8].

The invasive activity of the tumor is supported by the increased enzymatic activity of the tumor or stromal cell, which secretes active MMP. Degradation and damage

facilitates the allocation of tumor cells and their spread; therefore, MMP is a positive regulator of tumor invasion and growth [27, 38]. According to the Chiang A.C. experience, solid tumors have mechanisms that increase the ability of tumor cells to invade into the extracellular matrix, which promotes the formation of distant metastatic cells. Invasion of the tumor does not always lead to the formation of metastases; only about 0.01% of tumor cells initiate a more complex process of forming distant metastases [21]. Numerous studies, analyzed by Roy R., have shown a positive correlation between elevated levels of MMP in the tumor and the degree of invasion of tumor cells or the emergence of metastases [36].

Increased activity of MMP was detected in the progression of breast cancer, stomach, lung and other malignant diseases. The authors have shown that high expression of MMP-2 and MMP-9 is a significant factor in the adverse prediction of non-recurrent and total survival in cancer [21, 28, 37].

Solovyova N.I. with co-authors have shown that the main contribution to the invasive and metastatic potential of the squamous cell carcinoma of the cervix brings about an increase in the expression of collagenases of MMP-1 and MMP-14, of MMP-9 gelatinase and a decrease in the expression of TIMP-1 and -2 inhibitors [14]. This correlates with the results of other authors - the main role in the growth of invasive neoplastic cells in cervical cancer is played by matrix metalloproteinases (MMP) -1 and -2, the level of expression of which progresses with the growth of the tumor. In invasive carcinomas of the cervix, hyperexpression of MMP-9 is observed in the initial stages, which makes it possible to consider MMP-9 as a possible marker for early tumor diagnosis and its progression [12, 30, 40]. According to Abakumova T.B. with co-authors, in the process of tumor progression, the level of expression of MMP-9 is reduced. Expression of MMP-2 in serum of patients was elevated and correlated with the prevalence of the disease [1].

Hyperexpression of MMP-7 is described in a number of malignant neoplasms, such as breast cancer, lung, stomach, pancreas, head, neck and others [4, 26]. According to Laktionov K.P. with co-authors, in ovarian cancer, the level of MMP-7 in tumor tissue was significantly higher than in benign tumors, in contrast to the MMP-2 and -9 [13].

Barinov V.V. with co-authors, on the basis of the review of literature, showed that in case of cancer of the uterus body hyperexpression of several MMP (MMP-2, -7, -8, -9, -13) [4] was detected. In this case, elevated levels of the majority of MMP in tumor preparations did not correlate with metastatic lymph node involvement. At the same time, the expression of the level of matrilysin MMP-7 and MMP-9 is directly related to the degree of tumor invasion, its propensity to metastases, and unfavorable prognosis in patients with cancer of the uterine body [4].

Studies of the role of MMP in soft tissue sarcoma are devoted few studies [17, 35]. In her study, Benassi M.S. with co-authors, studied the role of various MMP in tumors of patients with fibromatosis and sarcoma of the extremities.

It was shown that the most pronounced expression in all tumors was noted for MMP-1, while the activity of TIMP-2 was higher in fibromatosis than in sarcomas. The authors believe that the state of MMP and TIMP determines the oncogenesis of tumors in soft tissues [17]. The prognostic role of MMP in the oncogenesis of sarcoma is confirmed by Roebuck M.M. with the co-authors, where the expression of MMP-2, MMP-9 and TIMP-2 was determined on the biopsy material of several histotypes of sarcoma (liposarcoma, synovial sarcoma and malignant tumor of the peripheral nerve shell). The authors showed that the dynamics of changes in the studied indicators has a prognostic value for the overall survival and duration of the non-recurring period in patients [35].

Dolzhikov A.A. with co-authors, showed that the growth of the expression level of MMP-9 is particularly pronounced in metastatic sarcomas [10].

After conducting studies on endometrial stromal sarcoma (ESS) and leiomyosarcoma (LMS) cell lines, Ravid Y. with co-authors showed the association of transcriptional regulation of MMP-2 activity and invasive tumor cell potential [33].

Bodner-Adler B. with co-authors study the expression of MMP on preparations of leiomyomas and leiomyosarcomas of the uterus. Significant overexpression was detected in MMP-1 in 86.0% of cases, for MMP-2 in 46.0% of cases [19]. There was a positive correlation between the level of MMP-2 expression and the presence of intravascular invasion. Statistically significant dependence of MMP-2 expression on the tumor stage and recurrence was not detected, however, in the group with MMP-2 negative status, the trend of more long-term, non-recurrent

survival was found [18, 19].

Analysis of the above-mentioned works suggests that metalloproteinases-7, -9, -1, -2 are quite informative markers of soft tissue sarcomas.

In our study, expression of MMP-9 was found at 55.6%, regardless of the type of uterine sarcoma. Hyperexpression of MMP-9 is observed at 25.9% for leiomyosarcoma, in 11.1% for endometrial stromal sarcoma and 7.4% for undifferentiated sarcoma. At moderate and high levels of MMP-9 expression, recurrence of the tumor was noted in 61.5% of cases, which may indicate an invasive and metastatic tumor potential.

The study of changes in the content of MMP-9 in tumor tissue of the uterine sarcoma is promising, since its level may be useful for monitoring the course of the neoplastic process and the corresponding response to the treatment.

Conclusions

1. Negative (0) and weak positive (1+) expression of matrix metalloproteinase-9 was detected in 29.6% of ESS and 18.5% of US. In any case, the relapse of the disease is not marked.

2. Moderate (2+) and high (3+) contents of MMP-9 were detected by us in 44.5% of uterine sarcoma, of which 26.4% of LMS. However, in cases of LMS, progression was observed only in the third observation (4 out of 12 cases), then in the ESS and US - in all cases.

3. With moderate and high content of MMP-9, the recurrence of the tumor was noted in 61.5% of cases, which may indicate an invasive and metastatic potential of the tumor.

References

- [1] Abakumova, T. B., Gening, S. O., Dolgova, D. R., & Fomina, A. V. (2014). Matrix metalloproteinases in blood serum and neutrophil lysate for cervical cancer. *Siberian Cancer Journal*, 1, 18-25.
- [2] Avtandilov, G. G. (2002). *Fundamentals of quantitative pathological anatomy: [monograph]*. M.: Medicine. ISBN 5-225-04151-5: 412.00 p.
- [3] Anichkov, N. M., Kvetnoj, I. M., & Konovalov, S. S. (2004). *Biology of tumor growth (molecular medical aspects)*. SPb.: "Publishing house "Prime EVROZNAK". ISBN 5-94946-090-1
- [4] Barinov, V. V., Knjazev, R. I., Bokin, I. I., & Mustafina, E. A. (2017). Matrix metalloproteinase 7 in uterine cancer (review). *Oncogynecology*, 7, 31-36. Retrieved from <https://elibrary.ru/item.asp?id=28409047>
- [5] Berezhnaja, N. M., & Chehun, V. F. (2016). Physiological system of connective tissue and oncogenesis. I. The role of the cellular components of the stroma in tumor development. *Oncology*, 18(1), 4-14. Retrieved from http://nbuv.gov.ua/UJRN/OL_2016_18_1_3
- [6] Berezhnaja, N. M., & Chehun, V. F. (2016). Physiological system of connective tissue and oncogenesis. II. Extracellular matrix and metastasis. *Oncology*, 18(3), 164-176. Retrieved from www.oncology.kiev.ua/pdf/18_3/164.pdf
- [7] Ganusevich, I. I. (2010). The role of matrix metalloproteinases (MMP) in malignant neoplasms. I. Characteristics of MMP, regulation of their activity, prognostic value. *Oncology*, 12(1), 10-16. Retrieved from <http://dspace.nbuv.gov.ua/handle/123456789/19542>
- [8] Ganusevich, I. I. (2010). The role of matrix metalloproteinases (MMP) in malignant neoplasms. I. MMP involvement in angiogenesis, invasion and metastasis of tumors. *Oncology*, 12(2), 108-114. Retrieved from www.oncology.kiev.ua/pdf/44/108.pdf
- [9] Gershtejn, E. S., & Kushlinskij, N. E. (2013). Clinical prospects of research of tumor-associated proteases and their tissue inhibitors in cancer patients. *Bulletin of the RAMS*, 5, 16-27. Retrieved from www.fesmu.ru/elib/Article.aspx?id=279067
- [10] Dolzhikov, A. A., Churnosov, M. I., Pahomov, S. P., Orlova, V. S., Bykov, P. M., Muhina, T. S. ... Altuhova, O. B. (2012). Molecular and genetic factors predicting smooth-muscle neoplasms of the uterus: the role of matrix metalloproteinases. *Kursk Scientific and Practical Bulletin*, 2, 138-146.
- [11] Klisho, E. V., Kondakova, I. V., & Chojnizonov, E. L. (2003). Matrix metalloproteinases in oncogenesis. *Siberian Cancer Journal*, 2, 62-70. Retrieved from <https://cyberleninka.ru/article/n/matriksnye-metalloproteinazy-v-onkogeneze>
- [12] Korolenkova, L. I., Stepanova, E. V., & Baryshnikov, A. Ju. (2011). Molecular and biological markers of adhesion, loss of intercellular connections, invasion and neoangiogenesis as factors of progression of cervical neoplasia and cervical cancer.

- Russian Biotherapeutic Journal*, 10(2), 13-17. Retrieved from elibrary.ru/contents.asp?issueid=1111643
- [13] Laktionov, K. P., Levkina, N. V., Kushlinskij, D. N., & Gershtein, E. S. (2012). Study of matrix metalloproteinases and their tissue inhibitors in patients with ovarian neoplasms. *Gynecology*, 2, 48-54. Retrieved from <https://doi.org/10.17650/1994-4098-2012-0-2-48-54>
- [14] Solov'eva, N. I., Timoshenko, O. S., Gureeva, T. A., & Kugaevskaja, E. V. (2015). Matrix metalloproteinases and their endogenous regulators for squamous cell carcinoma of the cervix: an overview of our own data. *Biomedical Chemistry*, 61(6), 694-704. doi: 10.18097/PBMC20156106694
- [15] Frank, G. A., Savchenko, V. G., Parovichnikova, E. N., Zavalishina, L. Je., & Gajdamaka, N. V. (2009). Importance of matrix metalloproteinases and their inhibitors in norm, oncology and oncohematology. *Therapeutic archive*, 7, 91-96. Retrieved from www.fesmu.ru/elib/Article.aspx?id=207120
- [16] Ardi, V. C., Kupriyanova, T. A., Deryugina, E. I., & Quigley, J. P. (2007). Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. *Proc. Natl Acad. Sci. U.S.A.*, 104, 20262-20267. doi:10.1073/pnas.0706438104.
- [17] Benassi, M. S., Gamberi, G., Magagnoli, G., Molendini, L., Ragazzini, P., Merli, M. ... Picci, P. (2001). Metalloproteinase expression and prognosis in soft tissue sarcomas. *Annals of oncology*, 12, 75-78.
- [18] Bodner-Adler, B., Bodner, K., Kimberger, O., Czerwenka, K., Leodolter, S., & Mayerhofer, K. (2003). MMP-1 and MMP-2 expression in uterine leiomyosarcoma and correlation with different clinicopathologic parameters. *J. Soc. Gynecol. Investig.*, 10(7), 443-446.
- [19] Bodner-Adler, B., Bodner, K., Kimberger, O., Czerwenka, K., Leodolter, S., & Mayerhofer, K. (2004). Expression of matrix metalloproteinases in patients with uterine smooth muscle tumors: an immunohistochemical analysis of MMP-1 and MMP-2 protein expression in leiomyoma, uterine smooth muscle tumor of uncertain malignant potential, and leiomyosarcoma. *J. Soc. Gynecol. Investig.*, 11(3), 182-186. doi: 10.1016/j.jsg.2003.09.004.
- [20] Bourbouli, D., & Stetler-Stevenson, W. G. (2010). Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. *Seminars in Cancer Biology*, 20, 161-168. doi: 10.1016/j.semcancer.2010.05.002.
- [21] Chiang, A. C., Massague, J. (2008). Molecular basis of metastasis. *N. Engl. J. Med.*, 359, 2814-2823. doi: 10.1056/NEJMra0805239.
- [22] Deryugina, E. I., Quigley, J. P. (2010). Pleiotropic roles of matrix metalloproteinases in tumor angiogenesis: contrasting, overlapping and compensatory functions. *Biochim. Biophys. Acta.*, 1803(1), 103-120. doi: 10.1016/j.bbamcr.2009.09.017.
- [23] FIGO committee on gynecologic oncology. (2009). FIGO staging for uterine sarcomas. *Int. J. Gynaecol. Obstet.*, 104(3), 177-178. doi: 10.1016/j.ijgo.2008.12.008.
- [24] Gadducci, A. (2011). Prognostic factors in uterine sarcoma. *Best Prac. & Res. Clin. Obst. & Gyn.*, 25, 783-795. doi: 10.1016/j.bpobgyn.2011.06.002.
- [25] Guo, X., Jo, V. Y., Mills, A. M., Zhu, S. X., Lee, C. H., Espinosa, I. ... van de Rijn, M. (2015). Clinically Relevant Molecular Subtypes in Leiomyosarcoma. *Clinical Cancer Res.*, 21(15), 3501-3511. doi: 10.1158/1078-0432.CCR-14-3141.
- [26] Ii, M., Yamamoto, H., Adachi, Y., Maruyama, Y., & Shinomura, Y. (2006). Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Experimental Biology Medicine*, 231(1), 20-27. doi: 10.1177/153537020623100103.
- [27] Jodele, S., Blavier, L., Yoon, J. M., & DeClerck, Y. A. (2006). Modifying the soil to affect the seed: role of stromal-derived matrix metalloproteinases in cancer progression. *Cancer Metastasis Rev.*, 25, 35-43. doi: 10.1007/s10555-006-7887-8.
- [28] Kessenbrock, K., Plaks, V., & Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell.*, 141, 52-67. doi: 10.1016/j.cell.2010.03.015.
- [29] Kurman, R. J., Carcangiu, M. L., Herrington, S., & Young, R. H. (2014). *World Health Organization Classification of Tumours of the Female Reproductive Organs*. IARC, Lyon., 307. ISBN-13 9789283224358.
- [30] Minami, R., Tsunoda, H., Iijima, T., Yoshikawa, H., Nemori, R., & Noguchi, M. (2003). Early acquisition of gelatinolytic activity in carcinogenesis of the uterine. *Mod. Pathology*, 16(11), 1164-1170. doi: 10.1097/01.MP.0000094089.33691.60
- [31] Nelson, C. M., & Bissell, M. J. (2006). Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. *Ann. Rev. Cell. Dev. Biology*, 22, 287-309. doi: 10.1146/annurev.cellbio.22.010305.104315
- [32] Nucci, M. R. (2016). Practical issues related to uterine pathology: endometrial stromal tumors. *Mod. Pathol.*, 29, 92-103. doi: 10.1038/modpathol.2015.140.
- [33] Ravid, Y., Formanski, M., Smith, Y., Reich, R., & Davidson, B. (2016). Uterine leiomyosarcoma and endometrial stromal sarcoma have unique miRNA signatures. *Gynecol. Oncology*, 140(3), 512-517. doi: 10.1016/j.ygyno.2016.01.001.
- [34] Rios, I., Rovirosa, A., Morales, J., Gonzalez-Farre, B., Arenas, M., Ordi, J. ... Biete, A. (2014). Undifferentiated uterine sarcoma: a rare, not well known and aggressive disease: report of 13 cases. *Arch Gynecol Obstet.*, 290(5), 993-997. doi: 10.1007/s00404-014-3311-8.
- [35] Roebuck, M. M., Helliwell, T. R., Chaudhry, I. H., Kalogrianitis, S., Carter, S., Kemp, G. J. ... Frostick, S. P. (2005). Matrix metalloproteinase expression is related to angiogenesis and histologic grade in spindle cell soft tissue neoplasms of the extremities. *Am. J. Clin. Pathol.*, 123, 59-66. doi: 10.1309/lk1v-7r99-jl41-wvvp.
- [36] Roy, R., Yang, J., & Moses, M. A. (2009). Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. *J. Clin. Oncol.*, 27, 5287-5297. doi: 10.1200/JCO.2009.23.5556.
- [37] Stetler-Stevenson, W. G. (2008). Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. *Sci. Signal.*, 1, re6. doi: 10.1126/scisignal.127re6.
- [38] Stetler-Stevenson, W. G., & Yu, A. E. (2001). Proteases in invasion: matrix metalloproteinases. *Semin Cancer Biol.*, 11, 143-152. doi: 10.1006/scbi.2000.0365
- [39] Sukhin, V. S. (2014). Surgical treatment of uterine sarcoma patients. Review. *Women's Health*, 91(5), 46-49. Retrieved from www.med-expert.com.ua/download.php?file=http://med-expert.com.ua/wp...2014...
- [40] Zhang, Y., Qian, H., Lin, C., Lang, J., Xiang, Y., Fu, M. ... Liang, X. (2008). Adenovirus carrying TIMP-3: a potential tool for cervical cancer treatment. *Gynecol. Oncol.*, 108(1), 234-240. doi: 10.1016/j.ygyno.2007.09.033.

ЕКСПРЕСІЯ ММП-9 ЯК ФАКТОР ПРОГНОЗУ САРКОМ МАТКИ

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Саркоми тіла матки є високоагресивними мезенхімальними новоутвореннями, відмінністю яких є вкрай несприятливий прогноз. На сьогодні залишається актуальним питання пошуку клініко-морфологічних та біомолекулярних критеріїв прогнозу й безрецидивної виживаності при пухлинах матки. Відомо, що підвищення рівня експресії матриксної металопротеїнази-9 (ММП-9) у первинній пухлині чи метастазах корелює з низьким ступенем диференціювання пухлинних клітин, високим рівнем інвазивності пухлини, її високою метастатичною активністю, та скороченням тривалості життя пацієнток. Досі невідомо, чи можливо вважати експресію ММП-9 у клітинах сарком матки переконливим прогностичним фактором. Для багатьох типів епітеліальних злоякісних новоутворень високі показники метастазування пов'язують із зростанням рівня ММП-9 як в плазмі крові, так і у тканинній паренхімі. Метою даного дослідження є вивчення особливостей експресії ММП-9 у клітинах сарком матки для створення моделі індивідуального прогнозування перебігу захворювання. Проведено дослідження операційного матеріалу селекціонованих 54 випадків сарком матки I-II стадії (згідно критеріїв FIGO) з відомим прогнозом захворювання, котрі були розподілені залежно від морфологічного типу: лейоміосаркома (ЛМС) - 18 випадків, ендометріальна стромальна саркома (ЕСС) - 22 випадки, недиференційована саркома (НС) - 14 (згідно класифікації новоутворень матки ВООЗ). Для гістологічного дослідження висікали шматочки тканини з різних ділянок пухлинних вузлів - центральні, периферичні відділи, ділянки із прилеглої інтактної тканини міометрія (всього по 6-8 шматочків). Фенотип пухлинних клітин визначали за допомогою низькомолекулярних цитокератинів (Cytokeratin PAN, AE1/AE3), гладком'язового актину (Smooth Muscle Actin, 1A4), міогеніну (Myogenin (F5D)), CD 10 і виментину (Vimentin, V9). Гістохімічну мітку оцінювали за двома параметрами: ступінь розповсюдженості та інтенсивності забарвлення. Для оцінки інтенсивності забарвлення використовували якісну шкалу: 0 - відсутня реакція, 1+ - слабе цитоплазматичне забарвлення до 30,0 % пухлинних клітин, 2+ - помірна реакція, від 30,0 до 60,0 % забарвлених клітин, 3+ - виражена цитоплазматична реакція у 60,0-100,0 % клітин пухлини. Статистичну обробку отриманих даних здійснювали за допомогою пакета програм "STATISTICA 10.0". Проведене дослідження виявило, що негативна (0) та слабка позитивна (1+) експресія матриксної металопротеїнази-9 відмічалася у більшості ЕСС та частині НС. Незалежно від стадії захворювання, з таким статусом ММП-9 не відмічено розвитку рецидиву. Помірний (2+) та високий (3+) вміст ММП-9 виявлений нами у 44,5 % сарком матки із явним переважанням при ЛМС. Однак, якщо у випадках ЛМС прогресія спостерігалась лише у треті спостережень (4 з 12 випадків), то при ЕСС та НС усі пухлини із таким статусом рецидивували. Подібна реакція може свідчити про інвазивний та метастатичний потенціал ЕСС та НС і обумовлює можливість розвитку гематогенних метастазів.

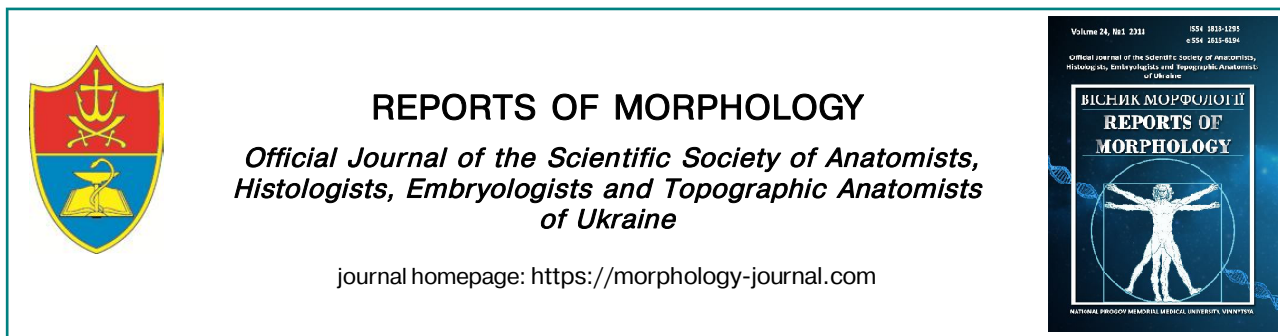
Ключові слова: саркома матки, експресія ММП-9, взаємозв'язок експресії ММП-9 та прогресії пухлини, лейоміосаркома, ендометріальна стромальна саркома, недиференційована саркома.

ЭКСПРЕССИЯ ММП-9 КАК ФАКТОР ПРОГНОЗА САРКОМ МАТКИ

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Саркомы тела матки являются высокоагрессивными мезенхимальными новообразованиями, отличием которых является крайне неблагоприятный прогноз. На сегодня остается актуальным вопрос поиска клинико-морфологических и биомолекулярных критериев прогноза и безрецидивной выживаемости при опухолях матки. Известно, что повышение уровня экспрессии металлопротеиназы-9 (ММП-9) в первичной опухоли или метастазах коррелирует с низкой степенью дифференцировки опухолевых клеток, высокой степенью инвазивности опухоли, ее высокой метастатической активностью, сокращением продолжительности жизни пациенток. До сих пор неизвестно, можно ли считать экспрессию ММП-9 в клетках сарком матки убедительным прогностическим фактором. Для многих типов эпителиальных злокачественных новообразований высокие показатели метастазирования связывают с ростом уровня ММП-9 как в плазме крови, так и в тканевой паренхиме. Целью данного исследования является изучение особенностей экспрессии ММП-9 в клетках сарком матки для создания модели индивидуального прогнозирования течения заболевания. Проведенное исследование операционного материала селекционированных 54 случаев сарком матки I-II стадии (согласно критериев FIGO) с известным прогнозом заболевания, которые были разделены в зависимости от морфологического типа: лейомиосаркома (ЛМС) - 18 случаев, эндометриальная стромальная саркома (ЭСС) - 22 случая, недифференцированная саркома (НС) - 14 (согласно классификации новообразований матки ВОЗ). Для гистологического исследования высекали кусочки ткани из разных участков опухолевых узлов - центральные, периферические отделы, участки из прилежащей интактной ткани миометрия (всего по 6-8 кусочков). Фенотип опухолевых клеток изучали при помощи низкомолекулярных цитокератинов (Cytokeratin PAN, AE1/AE3), гладкомышечного актина (Smooth Muscle Actin, 1A4), миогенина (Myogenin (F5D)), CD 10 и виментина (Vimentin, V9). Гистохимическую метку оценивали по двум параметрам: степень распространения и интенсивности окраски. Для оценки интенсивности окрашивания использовали качественную шкалу: 0 - отсутствует реакция, 1+ - слабое цитоплазматическое окрашивание до 30,0 % опухолевых клеток, 2+ - умеренная реакция, от 30,0 до 60,0 % окрашенных клеток, 3+ - выраженная цитоплазматическая реакция у 60,0-100,0 % клеток опухоли. Статистическую обработку полученных данных осуществляли с помощью пакета программы "STATISTICA 10.0". Проведенное исследование показало, что отрицательная (0) и слабая положительная (1+) экспрессия матриксной металлопротеиназы-9 отмечалась в большинстве ЭСС и части НС. Независимо от стадии заболевания, с таким статусом ММП-9 не отмечено развития рецидива. Умеренное (2+) и высокое (3+) содержание ММП-9 обнаружено нами в 44,5 % сарком матки с явным преобладанием при ЛМС. Однако, если в случаях ЛМС прогрессия наблюдалась лишь в трети наблюдений (4 из 12 случаев), то при ЭСС и НС все опухоли с таким статусом рецидивировали. Подобная реакция может свидетельствовать об инвазивном и метастатическом потенциале ЭСС и НС и обуславливает возможность развития гематогенных метастазов.

Ключевые слова: саркома матки, экспрессия ММП-9, взаимосвязь экспрессии ММП-9 и прогрессирование опухоли, лейомиосаркома, эндометриальная стромальная саркома, недифференцированная саркома.



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Structural and functional transformation of the small intestine wall in the conditions of the functioning of the artificial urinary reservoir in the experiment

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Morphological changes of the wall of the artificial urinary bladder are various and its investigation continues from the moment of the creation of the first conduit on the ileum's section. The aim of the work is to explore the structural-functional transformation of the mucous and the neobladder wall in 12 months after ileocystoplasty in the experimental conditions on mini-pigs. The results of the neobladder's experimental model, created by the execution of cystectomy and ileocystoplasty in 9 experimental mini-pigs are the material of this work. 12 months after the operation of modeling the urinary bladder, animals were removed from the experiment of nembutal overdosing, following the provisions of "The European convention for the Protection of vertebrate animals being in use for experiments or for other scientific purposes". Our morphological changes of the artificial urinary bladder, formed from the segment ileum 12 months after the orthotopic ileoplasty proved significant ultra-structural changes in the neobladder's epithelial layer. Ileum's villi within the conditions of the artificial urinary bladder are decreased and smoothed, epithelial cells are similar to the interjacent epithelium. The connective-tissue plate is thickened and becomes callous, the muscular layer is also thickened. The samples of the ileum, that remained in the normal environment without the compatibility with urine, did not undergo the significant morphological transformations and did not differ from the samples of the intact ileum. The transplantation of the ileum segment for performing new, not-programmed functions causes it to adapt to new conditions, although the urinary bladder and the ileum have different origins and structures from the very beginning. New aggressive conditions and constant contact with urine lead to the transformation of the mucous of the ileum and acquire new features necessary for performing new functions.

Keywords: radical cystectomy, ileocystoplasty, transformation of the mucous, neobladder.

Introduction

Bladder disease with loss of reservoir capacity and function, damage by the tumor process, as well as the complication of specific and non-specific inflammatory diseases requiring its complete removal or resection, led to rapid development of reconstructive surgery of bladder [4, 7, 8, 20]. The main material for restoring the functional capabilities of the bladder (accumulative and evacuator) are different sections of the gastrointestinal tract [3, 5, 15]. Many studies have been conducted on the effectiveness and safety of the use of the stomach, the area of the large and small intestines, but the best transplant was recognized area of

iliac intestine, due to the smallest electrolyte disturbances and possible adaptive abilities [9, 12, 19]. The study of morphological changes in the wall of the artificial bladder continues from the moment of the first conduit from the region of the ileum. Many researchers note the progressive atrophy of the villi and microvilli [10], and the number of glass-shaped cells decreases with time [11, 18].

The implementation of new, not programmed functions, the impact of the aggressive environment on the mucous artificial bladder, the possibility of its adaptation and transformation determined our interest in the study of structural

changes in the neobladder dynamics in the dynamics.

Aim: to study the structural and functional transformation of the mucosa and the neobladder wall 12 months after ileocystoplasty on mini-pigs under experimental conditions.

Materials and methods

The material of this work were the results of an experimental model of neobladder, created by ileocystoplasty of 9 experimental mini-pigs.

The simulation was carried out by performing cystectomy followed by ileocystoplasty in female pigs. The choice of an experimental object is due to anatomical considerations - in the female urethra is straight and 5-7 times shorter than that of males [2].

The procedure for surgical intervention was follows. Under intravenous anesthesia, pigs aged 4-5 months and weighing 8-10 kg after processing of the surgical field in the position on the back three times performed a cut. The top of the bladder was seized by forceps and tucked up. All bleeding vessels were tucked on both sides. Separated the front wall of the bladder. Crossed the urethra, the bladder separated them from the rectum. The bladder was removed. Retreating 15 cm from the ileocecal valve, the end of the isolated intestinal segment was sutured with continuous serous-muscle sutures of vicryl 4-0. During the anti-mesenteric edge, a distal part of the idiopathic segment was dissected (up to 10 cm). The cut part of the segment U-shapedly stacked adjacent edges of both knees were sewn together by a series of continuous serous-muscle sutures vicryl 4-0. The lower part of the resulting U-shaped segment was transversely upward.

Before cross-linking the free edges of the dissected segment, the urethral catheter number 3Fr was installed in the tetanus elbow, the ends of which were removed through the wall of the reservoir. In the most caudal part of the reservoir was made an opening, to which the urethra was sutured with 6 sutures of vicryl 4-0. After conducting through the urethra of Foley's catheter number 8Fr, the sutures were tied. The reservoir was drained by a cystostomy tube 12Fr, which was withdrawn along with the ureter stents through the vessel wall. The reservoir stood in place and formed an isoperistaltic knee. The ileum was dissected at the level of intersected pre-ureters - 10 cm higher than the iliac-intestinal reservoir. The ureter is obliquely cut, cut along and anastomosed "end to side" with the proximal unbranched part of the iliac-intestinal segment. Stents that are located inside the segment, are carried out in the ureter. Restore the intestinal continuity. Stents are drawn through the anterior abdominal wall, through the counter-aperture in the small pelvis install drainage. The wound is closed with vicryl stitches.

The area of the bladder wall was taken during cystectomy, at the same time, surgical intervention was removed from the area of the ileum wall. Data obtained in the study of these fragments of intact organs served as controls.

12 months after the bladder simulation, animals were drawn from the experiment by overdosing Nembutal,

following the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes" (Strasbourg, 1986).

Removed the area of the wall of the neobladder and the area of the ileum wall. The volume of each fragment of tissue taken as control and in order to evaluate the result was 1 cm³. The collected material was fixed in a 4% solution of paraformaldehyde. Then the material was carried out through the spirits of increasing concentration and poured into celloidin according to the generally accepted method [2]. The use of celloidin is associated with the need for maximum sparing effect on the tissues under study. From the received blocks, histological sections of thickness 7-9 microns were made, which were stained with hematoxylin and eosin. The obtained glasses were studied under a light microscope. When studying preparations under a microscope, the peculiarities of the structure of the layers of the organs examined were evaluated.

Results

In the macroscopic study of the ileum in intact animals, the lamination of its wall was clearly visible. The surface of the mucous membrane is velvety, transverse folds are expressed.

Microscopically, the serous wall of the intestine is represented by a single-layer cubic epithelium. Epithelial cells contain medium-sized rounded nuclei, juicily stained with hematoxylin solution. Muscle membrane with a clear division into two layers. From the submucosal basis and the serous membrane, it is separated by thin fibrous plates. Layers of the muscular membrane differ in the direction of the myocyte beams (longitudinal and circular). The bundles of myocytes are separated by thin interstitial layers containing fibrous fibers and individual fibroblasts with extracted dark nuclei. Myocytes with a homogeneous cytoplasm and juicy oval nuclei.

Submucosal base is represented by fibrous fibers, which are assembled into beams and form a mesh structure; thin-walled, moderately blood-filled vessels of small caliber; a small amount of intermediate substance of moderate eosinophilic color, in which diffused lymphoid elements. Closer to the inner surface, small lymphoid follicles of the usual structure are determined. In addition, Peyer's patch are found, representing a cluster of 2-3 follicles of almost rectangular shape with a moderate density of lymphoid elements in a rounded, hermetic center and with a dense position in the peripheral zone.

In the mucous membrane, a submucosal plate can be isolated from the beams of fibrous fibers and muscle cells, among which there are numerous lymphoid elements. On the surface mucous membrane forms numerous villi. The body of the villi is characterized by a thin-walled vessel and numerous lymphoid elements. The top of the villi is covered by the prismatic epithelium, the epithelial cells contain oval dense nuclei, which are located as "palisade". Between them, the glass-shaped cells, which are rich in mucus, are

determined. In the depth between the villi, crypts are formed (Fig. 1).

The macroscopic examination of the intact mini-pig bladder showed that the outer surface has grayish-pinkish color, shiny, smooth. By palpation wall is elastic, on the section noticeable layering, the inner surface is folded, pinkish-gray, moist.

Microscopy of the preparations of the wall of a healthy urinary bladder revealed that the external, serous membrane is formed by a single-layer cubic epithelium. The nuclei of epithelial cells are of medium size, oval, moderate in color. In some places epitheliocytes form small "influences" in which the nuclei are viewed in 2-3 rows. The thin plate of fibrous fibers and vessels of small caliber separates the serous membrane from the muscular membrane. The muscular layer is three-layered, layers separated by a layer of fibrous tissue containing vessels. The width of these layers is greater than in the intestine wall. Muscle beams are in interstitial wall, thin, containing single fibroblasts. Myocytes in tufts are densely packed, moderate in width, have juicy nuclei, and the cytoplasm is homogeneous. In bundles myocytes are unidirectional. Between the beams and in the boundary plates, numerous small vessels of moderate blood filling are observed.

The submucosal plate is represented by an intermediate substance and bundles of fibrous fibers forming a mesh structure (Fig. 2).

The submucosa plate forms numerous papillae of different sizes and heights. The top of the papilla is covered by a transitional epithelium, in which clearly stands out: the basal layer of tightly packed cells containing small rounded dark-colored nuclei, and a surface layer formed from chaotic cells with medium to large nuclei of varying density and color.

12 months after the operative formation of an artificial bladder, the study of the ileum wall revealed no discrepancy with its structure in intact animals.

During macroscopic study, the inner surface of the ileum is velvety, the folds of the mucous membrane are expressed. Wall by the touch is elastic. The outer surface is smooth, shiny.

During microscopic study, the layered organization of the intestine is unaltered. The serous membrane is covered by a single-layer cubic epithelium of the usual form. Below it is a thin plate of soft fibrous fibers, forming a grid, and small thin-walled vessels. Two-layer muscular membrane, layers of medium thickness, separated from each other by a thin connective tissue plate containing a thin-walled vessel and a small amount of intermediate tissue. The bunches of myocytes are distributed tightly, between them are thin interstitial layers, myocytes of the usual type. Submucosal basis is created by a mesh structure from fibrous beams, intermediate substance. In the last distributed lymphoid elements. Closer to the inner surface, certain small lymphatic follicles of the usual structure are determined. In the submucosal layer, Peyer's patches are defined, representing a conglomerate of 3-4 almost square follicles. The structure

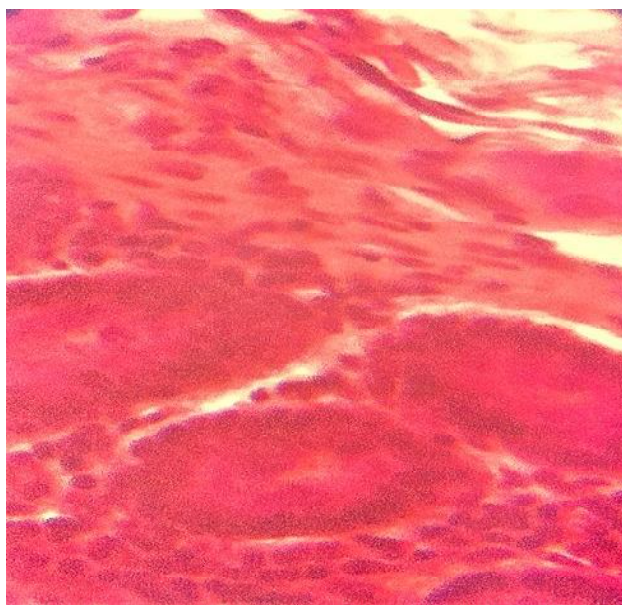


Fig. 1. Ileum of intact mini-pig. Crypts of the mucous membrane. Prismatic epithelium, lymphocytic-histiocytic elements, in the main substance fibrous bundles of the submucosal plate. Hematoxylin-eosin. x160.

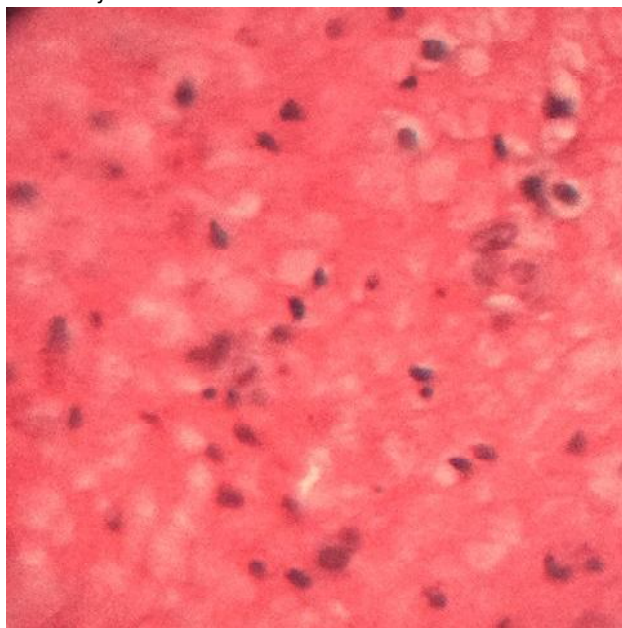


Fig. 2. Bladder of intact mini-pig. Submucosal basis. Histiocytes, fibroblasts. Homogeneous substance. Hematoxylin-eosin. x160.

of each of them without visible features (Fig. 3).

Fibrous plate of the mucous membrane has usual form, thin, in it are located in a moderate amount lymphocytes. Numerous villi of the mucous in shape, size, composition of the central part do not differ from those in control animals.

The top of the villa is covered by the prismatic epithelium with oval nucleuses located as "palisade". Among the epithelial cells there are many glass-shaped cells rich in mucus.

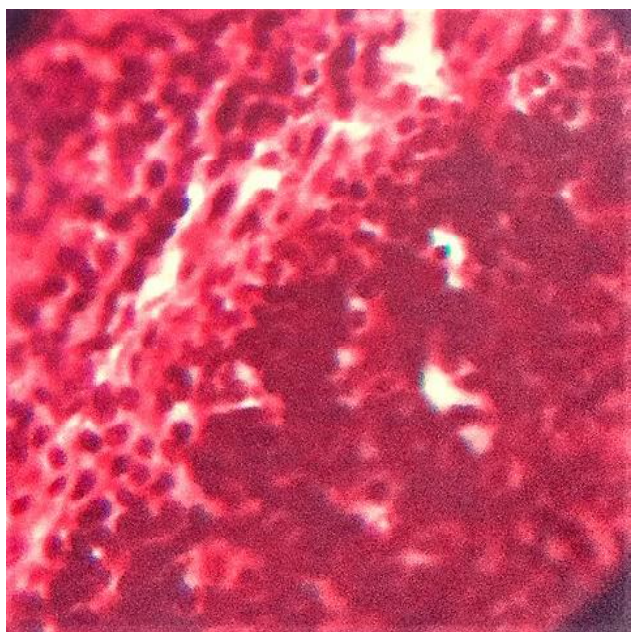


Fig. 3. Ileum of mini-pigs in 12 months after the formation of an artificial bladder. The edge of the Peyer's patch. The usual structure of the peripheral zone of the follicle. Lymphocytes in the perifollicular zone. Hematoxylin-eosin. x160.

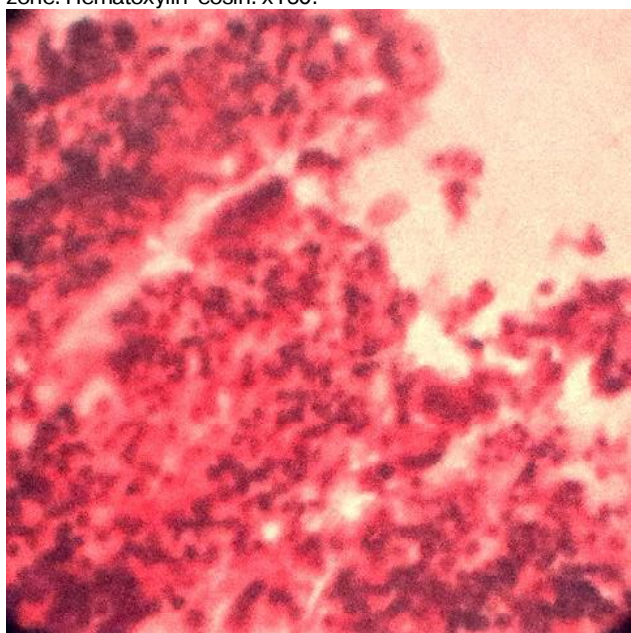


Fig. 4. The wall of the artificial bladder, 12 months after surgery. Epithelium on the surface of the papilla is similar to the intermediate. Hematoxylin-eosin. x160.

The visual assessment of the neobladder did not reveal any particular differences from the native organ. The outer surface is a grayish-pink, shine inner surface with low folds, moist, shiny.

On the touch the wall is quite elastic. Microscopy of the external (serous) membrane revealed that it was formed by a cubic single-layer epithelium of the usual form. Beneath the connective tissue, formed by fibrous fibers, fibroblasts.

Visually, it is wider than the ileum from which the neobladder is formed, with the outer plate of the muscular membrane does not merge.

The muscular membrane is visually wider than the ileum, but also a double layer. Muscle beams are thick enough due to the expansion of myocytes, the cytoplasm in them is homogeneous, the nuclei are enlarged, juicy colored. The interstitial layers between the beams are thin, but fibrous fibers that form them are coarse, many fibroblasts, and then thin-walled moderately full-blood vessels are determined. The bunches of myocytes in each layer are clearly unidirectional. Interlayer plate thicker than in the ileum, thickening is due not only to the coarsening of fibrous fibers, but also with the increase in the amount of intermediate tissue.

Submucosal base broad, as in the ileum, form its bundles of fibrous fibers, forming a mesh structure. Fiber fibers are dyed, juicy, many fibroblasts in bundles. The difference in the basis of the artificial urinary bladder from the corresponding layer in the ileum - in a very large amount of intermediate substance. If in the intestine this substance is dark eosinophilic color, then in neobladder it is brick color - obviously changes the chemical composition of this substance. In the intermediate substance, a number of lymphoid elements are scattered. Peyer's patch in the submucosal layer were not detected, nor did lymphoid follicles appear on the border of the mucous membrane. In the mucous membrane stands out a submucosa plate formed by thin beams of fibrous fibers and small inclusions of intermediate substance, fibroblasts with oval juicy nuclei, a few of them. The width of the submucosal plate is unequal, unlike the intestine, due to this formed formations, similar to the papilla or folds. The plate is covered by the epithelium, in its organization similar to the intermediate (Fig. 4).

In the basal layer of the epithelium, the cells are arranged in one row, but in some places, influences from 2-3 rows of cells are formed. Closer to the surface of the cell are sparser, their nuclei are round, medium-sized, moderately colored. It should be noted that "influences" are formed in those places where the submucosa plate is stacked like a papilla or folds, perhaps it is the remnants of degrading villi. The cryptic remains are also determined, they are located between the papillae, laced with the prismatic epithelium, glass-shaped cells in them are small, but there are desquamated epithelial cells.

Discussion

Our findings of ultrastructural changes in the neobladder mucous membrane with a decrease in microvilli, with the onset of inflammation and the processes of degeneration under the action of urine, are also noted in studies of Kojima Y. with co-authors [13]. The high degree of adaptation processes suggests the constancy of the definition of crypt and glass-shaped cells, as the main placement of Paneth cells, though in small quantities. Paneth cells play a very important role in ileum homeostasis, carrying out bactericidal and regenerative-proliferative action [6]. According to Malone

M.J. the surface epithelium changes, but without metaplasia and dysplasia [14], but some authors emphasize the high risk of malignant epithelial transformation by the action of urinary agents, since inflammatory cells can produce various growth factors [16, 17]. Since the lifetime of enterocytes is overly fast (72 hours), this is not a consequence of a genetic mutation. It is more likely that some urine solutions and low internal pH damage epithelial cells, which then lose microcirculation due to direct toxicity.

Evaluating the observed changes in the wall of the neobladder, we can assume that they have an adaptive-compensatory character, since, being in a new, unusual for themselves functional situation, the tissues of the walls of the ileum acquire the features necessary to perform the new function.

The prospect of further studies of artificial bladder is the study of changes in energy homeostasis, neurohumoral and synaptic regulation, and the search for new drug compounds that can influence the contractile activity of the neobladder

and improve the quality of life of patients with orthotopic urinary excretion.

Conclusions

1. 12 months after ileocystoplasty in the wall of the neobladder formed from the wall of the ileum, observed significant morphological changes, namely: thickening of the muscle membrane, thickening and coarsening of connective tissue in the layers of the neobladder wall, decreasing the number of glass-shaped cells and Paneth cells, from the submucosal basis, Peyer's patch and lymphoid follicles are practically disappearing, the villi are smoothed, the mucosal epithelium becomes similar to that of the intermediate.

2. The tissues of the ileum, remaining in their usual environment, will not undergo any structural transformation. Comparison of the structure of the wall of the native bladder and the neobladder showed that the structure of the latter is close to the first, but because of originally different origin and structure of complete transformation does not occur.

References

- [1] Merkulov, G. A. (1961). *Course of pathological histology*. L.: Medgiz. Retrieved from <https://www.twirpx.com/file/1352585/>
- [2] Zapadnyuk, V. I., Zakhariya, E. A., & Zapadnyuk, B. V. (1983). *Laboratory animals. Breeding, content, use in the experiment*. Kiev: High school. ISBN 5518162189, 9785518162181
- [3] Aleksic, P., Bancevic, V., Milovic, N., Kosevic, B., Stamenkovic, D.M., Karanikolas, M. ... Jovanovic, M. (2010). Short ileal segment for orthotopic neobladder: a feasibility study. *Int. J. Urol.*, 17(9):768-773. doi: 10.1111/j.1442-2042.2010.02599.x, 10.1111/j.1442-2042.2010.02600.x, 10.1111/j.1442-2042.2010.02601.x
- [4] Attalla, K., Kent, M., Waingankar, N., Mehrazin, R. (2017). Robotic-assisted radical cystectomy versus open radical cystectomy for management of bladder cancer: review of literature and randomized trials. *Future oncology*, 13(13). <https://doi.org/10.2217/fo-2017-0004>.
- [5] Baskin, L. S., Hayward, S. W., Di Sandro, M. S., Li, Y. W., & Cunha, G. R. (1999). Epithelial-mesenchymal interactions in the bladder. Implications for bladder augmentation. *Urol. Clin. North Am.*, 26, 49-59.
- [6] Beisner, J., Stange, E., Wehkamp, J. (2010). Innate Antimicrobial Immunity in Inflammatory Bowel Diseases. *Expert Rev. Clin. Immunol.*, 6(5), 809-818. doi: 10.1586/eci.10.56.
- [7] Cerruto, M., D'Elia, C., Siracusano, S., Porcaro, A., Cacciamani, G., De Marchi, D. ... Artibani, W. (2017). Is Health-Related Quality of Life after Radical Cystectomy Using Validated Questionnaires Really Better in Patients with Ileal Orthotopic Neobladder Compared to Ileal Conduit: A Meta-Analysis of Retrospective Comparative Studies. *Curr. Urol.*, 10(2), 57-68. doi: 10.1159/000447153.
- [8] Chang, D., & Lawrentschuk, N. (2015). Orthotopic neobladder reconstruction. *Urol. Ann.*, 7, 1-7. doi:10.4103/0974-7796.148553.
- [9] Dahl, D. M., & McDougal, W. S. (2007). *Use of intestinal segments in urinary diversion*; ed. A. J. Wein. 9th ed. Campbell-Walsh urology. Philadelphia: Saunders-Elsevier. ISBN: 9781416031550 1416031553
- [10] Hall, M. C., Koch, M. O., Halter, S. A., & Dahlstedt, S. M. (1993). Morphologic and functional alterations of intestinal segments following urinary diversion. *J. Urol.*, 149, 664-666. [https://doi.org/10.1016/S0022-5347\(17\)36175-X](https://doi.org/10.1016/S0022-5347(17)36175-X)
- [11] Hautmann, R. E., de Petriconi, R. C., & Volkmer, B. G. (2010). Lessons learned from 1,000 neobladders: The 90-day complication rate. *J. Urol.*, 184, 990-994. doi: 10.1016/j.juro.2010.05.037.
- [12] Koga, F., & Kihara, K. (2012). Selective bladder preservation with curative intent for muscle-invasive bladder cancer: A contemporary review. *Int. J. of Urol.*, 5(19). <https://doi.org/10.1111/j.1442-2042.2012.02974.x>
- [13] Kojima, Y., Asaka, H., Ando, Y., Takanashi, R., & Kohri, K. (1998). Mucosal morphological changes in the ileal neobladder. *Br. J. Urol.*, 82, 114-117.
- [14] Malone, M. J., Izes, J. K., & Hurley, L. J. (1997). Carcinogenesis: the fate of intestinal segments used in urinary reconstruction. *Urol. Clin. North Am.*, 24, 723-728.
- [15] Monteiro, L., & Kassouf, W. (2017). Radical Cystectomy is the best choice for most patients with muscle-invasive bladder cancer? Opinion: Yes. *Int. Braz. J. Urol.*, 43(2), 184-187. doi: 10.1590/S1677-5538.IBJU.2017.02.03
- [16] Naito, A., Kawai, T., Fujimura, T., Morikawa, T., Kume, H., & Homma, Y. (2014). Adenoma-carcinoma Sequence in the Bladder After Augmentation Cystoplasty. *Urol Case Rep*, 2(3), 73-74. doi: 10.1016/j.eucr.2014.01.009
- [17] Sallami, S. (2009). Mucinous Adenocarcinoma of the Ileal Neobladder 20 Years After Cystectomy: The First Reported Case. *UroToday/Int. J.*, 2(6). doi:10.3834/uij.1944-5784.2009.12.16
- [18] Sun, X., Song, M., Bai, R., Cheng, S., Xing, Y., Yuan, H. ... Zhou, L. (2013). Ileal interposition surgery-induced improvement of hyperglycemia and insulin resistance in Goto-Kakizaki rats by upregulation of TCF7L2 expression. *Exp. Ther. Med.*, 5, 1511-1515. doi: 10.3892/etm.2013.998
- [19] Vajda, P., Kaiser, L., Magyarlaki, T., Farkas, A., Vastyan, A. M., & Pinter, A. B. (2002). Histological findings after colocystoplasty and gastrocystoplasty. *J. Urol.*, 168(2), 698-701.
- [20] Zhang, Y., Song, Q., Song, B., Zhang, D., Zhang, W., Wang, J. (2017). Diagnosis and Treatment of Urinary Incontinence after Orthotopic Ileal Neobladder in China. *Chin. Med. J.*, 130(2), 231-235. doi: 10.4103/0366-6999.198012

СТРУКТУРНО-ФУНКЦІОНАЛЬНА ТРАНСФОРМАЦІЯ СТІНКИ ТОНКОЇ КИШКИ В УМОВАХ ФУНКЦІОНУВАННЯ ШТУЧНОГО СЕЧОВОГО РЕЗЕРВАРА В ЕКСПЕРИМЕНТІ

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Морфологічні зміни стінки артифіціального сечового міхура різноманітні та їх дослідження триває з моменту створення першого кондуїту із ділянки клубового кишечнику. Метою роботи було вивчення структурно-функціональної трансформації слизової та стінки неobladderа через 12 місяців після ілеоцистопластики в експериментальних умовах на карликових домашніх свинях. Матеріалом цієї роботи послужили результати експериментальної моделі неobladderа, створеної шляхом виконання цистектомії та ілеоцистопластики у 9 експериментальних карликових домашніх свинях. Через 12 міс. після здійснення операції моделювання сечового міхура тварин виводили з досліду передозуванням нембуталу, дотримуючись положень "Європейської конвенції про захист хребетних тварин, використовуваних для експериментів або в інших наукових цілях". Проведені нами морфологічні дослідження артифіціального сечового міхура, сформованого із сегменту клубової кишки через 12 місяців після ортотопічної ілеопластики, довели значні ультраструктурні зміни в епітеліальному шарі неobladderа. Ворсинки клубової кишки в умовах артифіціального сечового міхура зменшувались та згладжувались, епітеліоцити набували подібності із проміжним епітелієм. Сполучнотканинна пластинка стовщувалась та грубувала, м'язова оболонка, в свою чергу, також потовщувалась. Зразки клубової кишки, які залишалися в звичайному оточенні, без взаємодії із сечею, не зазнавали значних морфологічних трансформацій та не відрізнялися від зразків інтактної клубової кишки. Таким чином, трансплантація сегменту клубової кишки змушує його адаптуватися до нових умов, хоча від самого початку сечовий міхур та клубова кишка мають різне походження та будову, а нові агресивні умови та постійний контакт із сечею призводять до трансформації слизової клубової кишки та набуття нових якостей, необхідних для виконання нових, не запрограмованих функцій.

Ключові слова: радикальна цистектомія, ілеоцистопластика, трансформація слизової, неobladder.

СТРУКТУРНО-ФУНКЦИОНАЛЬНАЯ ТРАНСФОРМАЦИЯ СТЕНКИ ТОНКОЙ КИШКИ В УСЛОВИЯХ ФУНКЦИОНИРОВАНИЯ ИСКУССТВЕННОГО МОЧЕВОГО РЕЗЕРВАРА В ЭКСПЕРИМЕНТЕ

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Морфологические изменения стенки артифициального мочевого пузыря разнообразны и их исследование продолжается с момента создания первого кондуита из участка подвздошной кишки. Целью работы было изучение структурно-функциональной трансформации слизистой неobladderа через 12 месяцев после илеоцистопластики в экспериментальных условиях на карликовых домашних свиньях. Материалом этой работы послужили результаты экспериментальной модели неobladderа, созданной путем выполнения цистэктомии и илеоцистопластики у 9 экспериментальных карликовых домашних свиней. Через 12 мес. после проведения операции моделирования мочевого пузыря животных выводили из опыта передозировкой нембутала, придерживаясь положений "Европейской конвенции о защите позвоночных животных, используемых для экспериментов или в иных научных целях". Наши морфологические исследования артифициального мочевого пузыря, сформированного из сегмента подвздошной кишки через 12 месяцев после ортотопической илеопластики, доказали значительные ультраструктурные изменения в эпителиальном слое неobladderа. Ворсинки подвздошной кишки в условиях артифициального мочевого пузыря уменьшались и сглаживались, эпителиоциты приобретали сходство с промежуточным эпителием. Соединительно-тканная пластинка утолщалась и огрубевала, мышечная оболочка, в свою очередь, также утолщалась. Образцы подвздошной кишки, которые оставались в обычном окружении, без взаимодействия с мочой, не испытывали значительных морфологических трансформаций и не отличались от образцов интактной подвздошной кишки. Таким образом, трансплантация сегмента подвздошной кишки заставляет его адаптироваться к новым условиям, хотя изначально мочевой пузырь и подвздошная кишка имеют различное происхождение и строение, а новые агрессивные условия и постоянный контакт с мочой приводят к трансформации слизистой подвздошной кишки и приобретению новых качеств, необходимых для выполнения новых, не запрограммированных функций.

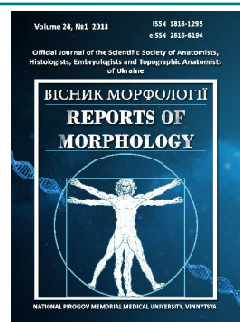
Ключевые слова: радикальная цистэктомия, илеоцистопластика, трансформация слизистой, неobladder.



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Effect of bougienage and washing of the pancreatic duct on the course of experimental acute pancreatitis

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The presence of many hypotheses of the development of acute pancreatitis such as pancreatic duct hypertension, pancreatic reflux, vascular, allergic, neuro-reflex, infectious, etc. confirm the lack of a clear understanding of the development mechanisms of this pathology, and hence inaccuracy in the treatment and negative consequences. The purpose of this study was to investigate the effect of bougienage and flushing of the pancreatic duct on the course of experimental acute pancreatitis. Experiments were carried out on 12 dogs, for which a model of pancreatitis was created by automobile administration into the pancreas duct. Animals were divided into four groups, 3 animals per each, with the term of deduce from the test in one, three, seven days and six months respectively. Before the pancreas duct perfusion, it was injected with polyvinylchloride bougie that was removed through the incision in the distal part of the pancreas duct. Such manipulation allowed to conduct duct washing with medicinal substances at a pressure of 0.49-0.6 kPa and confirmed the assumption that in acute pancreatitis, filling of pancreas duct with condensed protein masses was observed, and this, changes the approach not only to the establishment of the pathogenetic link in the process of acute pancreatitis development, but also to its treatment. The duct was washed once. The common comprehensive drug therapy was carried out in dogs within the next five, six days. At the end of the first day, out of the 12 dogs, nine stood independently, the rest - on the second day. On the third day, all animals drank water, responded to stimuli. On the fifth day they were active, taking liquid feed. On the seventh day on their behavior and feeding manner the dogs of this group did not differ from healthy ones. To study morphological changes in pancreas after duct washing, three dogs were withdrawn from the test in one day. At autopsy effusion in peritoneal cavity was not observed. The left lobe of pancreas was a little bit shorter. Place of dissection of the tissues of the pancreas and the duct is covered with a blood clot. In the area of duodenum dissection, isolated patches of steatoenecrosis retained. Microscopically, in the duct area dissection changes in pancreas tissues, in general, were the same as in duct dissection without perfusion. At the same time, the plethora for this term was great. Necrotic centers of parenchyma were isolated and with moderate neutrophilic infiltration. Distant from the dissection zone in pancreas tissues there were minor focal hemorrhages with a violation of its structure, however, hyperplasia, foci of neutrophilic infiltration of the interstitial connective tissue were less manifested. In the proximal part of the duct, the pancreas tissue retained moderate plethora. In addition, there were small foci of hemorrhages with a violation of the structure of individual acinus and slight neutrophilic infiltration in interstitial connective tissue. The latter was also marked by the accumulation of macrophages and the proliferation of fibroblasts, there were isolated areas of hemorrhages. In intact part there was an insignificant edema of interstitial connective tissue. As a result of the conducted experiments, we were convinced of the effectiveness of this method of treating acute pancreatitis.

Keywords: acute pancreatitis, bougie of the pancreatic duct.

Introduction

The presence of many hypotheses of the development of acute pancreatitis such as duct hypertension, pancreatic reflux, vascular, allergic, neuro-reflex, infectious,

pathobiochemical and others means the lack of a clear understanding of the mechanisms of development of this pathology, and hence inaccuracy in the treatment and

negative consequences [13, 14, 15, 16, 17, 18].

The leading role of duct hypertension in the pathogenetic mechanism of the development of acute pancreatitis is indicated by many researchers, as in the past century, and modern [3, 6, 8, 9, 10, 11, 12].

Significant interest in this regard are the studies conducted by Napalkov P.M. with co-authors [7]. Describing drainage operations on the pancreas duct in acute pancreatitis, they noted that hypertension develops in all forms of acute pancreatitis. In addition, in patients with progressive pancreatic necrosis, according to their data, there was a significant increase in pressure in the pancreatic duct with a sharp decrease in secretion of pancreatic juice. For the rejection of the envelope epithelium in the pancreas duct in animals that died from pancreatic necrosis in 14 days pointed out Vladimirov V.G. and co-authors [1], which, according to their data, indicates a violation of the protein synthesizing function of acinar cells.

Other authors in their studies conducted on four dogs, after induction of acute pancreatitis, who died within the first day of pancreatic necrosis, observed in its pancreas duct obturation with condensed protein mass [2, 4, 5].

Thus, if acute pancreatitis, in the first stages of its development, there is occlusion of the duct with protein masses, then there was an idea in some way to restore its patency, and to investigate how the restoration of patency will affect the course of the disease.

The purpose of this study was to investigate the effect of bougienage and duct washing of the pancreas on the course and the morphological state of the pancreas in experimental acute pancreatitis.

Material and methods

Experiments were carried out on 12 dogs, which created a model of pancreatitis by introducing automobile into the pancreas duct. Animals were divided into four groups, 3 animals per each, with the term of withdrawal from the test after one, three, seven days and six months respectively. Before perfusion of the pancreas duct, it was injected with polychlorinated bougie that was removed through the incision in the distal region of the pancreas duct. Such manipulation allowed conducting duct washing with medicinal substances at a pressure of 0.49-0.6 kPa, that is several times less than the working pressure in the duct. In addition, it confirmed the assumption that in acute pancreatitis, filling of the pancreas duct with condensed protein masses is observed, and this, as we have been sure, changes the approach not only to the establishment of the pathogenetic link in the development of acute pancreatitis, but also to its treatment. The duct was washed one time. Animals were given a common, comprehensive drug therapy within the next five to six days.

For all 12 animals pancreatic ducts were washed with a therapeutic fluid consisting of 0.25% Novocaine solution - 10 ml, Fibrinolysin - 3 ml, and 10 000 antitrypsin units of Contrykal - 2 ml. The flushing was carried out at a rate of 20-

30 drops per minute. After 14-20 minutes after duct washing, the pancreas decreased markedly in width, foci of hemorrhage took a pale coloration, areas of the gland of the usual color appeared, the number and area of foci and steatoenecrosis did not increase. After perfusion, the catheter was removed. To the place of dissection of the gland and duct was fed drainage tube. The abdominal cavity was sewn in a layer.

In the postoperative period, the withdrawal from the drainage tube was negligible - from 2 to 12 ml. In addition, in almost all animals, they were observed, mainly during the first day after the operation.

Results

Of the 12 dogs, at the end of the first day, nine stood up, the rest - on the second day. On the third day, all animals drank water, responded to stimuli. On the fifth day they were active, taking liquid feed. On the seventh day on the behavior and eating of dogs of this group did not differ from healthy ones.

To study morphological changes in the pancreas after duct washing, three dogs were withdrawn from the experiment after one day. At the intersection effusion in the peritoneal cavity was not observed. The left part of the pancreas was somewhat reduced in length. Place of dissection of the tissues of the pancreas and the duct was covered with a clot of blood. In the area of the autopsy of the duodenum, isolated patches of steatoenecrosis persisted.

Microscopically, in the region of dissection of the duct changes in the pancreas, in general, were the same as in the dissection of the duct without perfusion. At the same time, at this term was marked only large foci of plethora. Necrotic centers of parenchyma were isolated and with moderate neutrophilic infiltration. Distant from the dissection zone in the pancreas tissue there were minor focal hemorrhages with a violation of its structure, however, plethora, foci of neutrophilic infiltration of the interstitial connective tissue were less pronounced. In the proximal part of the duct, the tissue of the pancreas retained moderate plethora. In addition, there were small foci of hemorrhages with a violation of the structure of individual acinus and slight neutrophilic infiltration in the interstitial connective tissue. The latter also marked the accumulation of macrophages and the proliferation of fibroblasts. Although the wall of the ducts was not tense in their lumen, as in the dissection of the duct without rinsing, there were isolated foci of hemorrhages. In intact part there was an insignificant edema of interstitial connective tissue.

Thus, in dogs of this group perfusion of the pancreas duct by Novocaine-Fibrinolysin- Contrykal solution allowed to reduce the number of foci of hemorrhage, necrosis and neutrophil infiltration. It allowed to remove the tension of the duct wall in the proximal part of the section of the pancreas and stimulate, albeit not to a large extent, processes of productive inflammation.

At the autopsy of 3 dogs that were withdrawn from the test in three days, macroscopically observed the same

changes as in dogs, which performed only the dissection of the duct with the same term of experiment.

During histological examination of the region of dissection of the pancreas duct in its tissues, moderate plethora and edema were maintained. Exudative phenomena, when compared with the first day, were slightly expressed. The layers of the connective tissue became larger and denser due to the formation of fibrous structures and cell proliferation. When coloring tissues by Van Gieson's, intermediate connective tissue was characterized by an increase in the number of collagen fibers. At this time there was no abundant infiltration by neutrophils of the pancreas tissue. Attention was paid to the active organization of small foci of necrosis and hemorrhages. State of ducts was very special: they were semi-closed, their epithelium was swollen with hypochromic nuclei. In the lumen, there was a dense eosinophilic substrate that contained isolated detached cells. The proliferation of cells in the middle of the lacrimal ducts and interstitial connective tissue were markedly pronounced. Parenchyma in general retained the usual structure.

Similar changes were observed in the distal from the dissection pancreas duct, however, the phenomena of productive inflammation were expressed not so intensively. Gap of the duct was characterized by the expansion and presence of dense eosinophilic masses. In the proximal direction (3 cm from the dissection of the duct) there was a well-defined organization of mosaic necrosis. Characteristic was the lack of pronounced changes in the lumen of the ducts and parenchyma.

Intact part, except for insignificant plethora, was no different from the normal structure.

Thus, in dogs of this group, in comparison with the previous term of research, there were not observed the expressed phenomena of acute inflammation. Significantly more intensive was the proliferative process. Unlike dogs, where only one dissection of the duct of the pancreas was performed, the organization of small foci of necrosis and hemorrhages was more active. Gap of the duct outside the dissection zone was unobtrusive, whereas in the comparative group of animals, during this period, the eosinophilic masses and erythrocytes were partially stored there. Significantly fewer changes were noted in the proximal section of the pancreas, i.e. in the part of the gland that was washed out.

Discussion

During examination abdominal organs of dogs that had been withdrawn from the experiment after seven days, the same changes were observed in the same way as dogs in this period without flushing the duct. Results similar to ours, can be found in other authors who described the destructive forms of pancreatitis without treatment [2, 6]. However, in the literature available to us, we did not meet the description of the method of treatment similar to our [1, 7, 12, 13]. However, despite the fact that the microscopically in the pancreas of these dogs marked changes similar to those in the comparative group. Nevertheless, it should be noted

that in the group of animals being studied, the growth of connective tissue was more moderate, there was regeneration of acinus without changes in the ducts of the pancreas. In the distal from the dissection of the duct region of the gland, the process of atrophy of the parenchyma was significantly pronounced.

Thus, in dogs removed from the test after 7 days, unlike the previous term, in the proximal part from the dissection of the duct of the pancreas, a less pronounced productive inflammation of the indurational nature was observed, no changes in the parenchyma of the pancreas were observed. Consequently, bougienage with subsequent perfusion of the pancreatic duct Novocaine-Fibrinolysin-Contrykal solution, along with improving its permeability, promotes the active process of restoring pancreatic tissue and eliminating the effects of acute inflammation during the first day. In other words, the faster the conditions for the movement of pancreatic juice develop without any delay in acute pancreatitis, the sooner the restoration of pathological processes in the pancreas passes, with which other authors agree [4, 5, 15]. If there were changes, they were without significant violations of its structure.

To confirm this thesis, it was necessary to follow the state of the pancreas in the long term of treatment of acute pancreatitis. For this purpose, three dogs after bougienage followed by pancreatic duct washing with Novocaine-Fibrinolysin-Contrykal solution were withdrawn from the test six months later. At the animal's autopsy, attention was paid to preserving the form of the pancreas to the point of dissection of its tissues and ducts. The other part of the gland was presented in the form of a dense strain.

During microscopic examination of the dissection zone (between stored and atrophied parts of the pancreas), attention was paid to the preservation of the parenchyma of the gland, in which, along with the enlarged acinus, there was observed acinus with a narrow lumen. It is possible that this state reflects the functional cyclicity in the accumulation and release of pancreatic juice, which is observed in normal functioning of the pancreas. In the studied area, the phenomena of minor sclerosis between the pancreas part and in the periductal connective tissue are sometimes fixed.

In the proximal from the boundary part of the direction, a clear structure of acinus and intercellular ducts was detected. Characteristic was also the absence of intra acinus sclerosis. However, the signs of the latter were observed in periductal tissue. The area of the body and the right part of the pancreas did not differ from the normal structure.

Thus, after 6 months in animals after bougienage followed by flushing of the pancreatic duct with Novocaine-Fibrinolysin-Contrykal solution observed stabilization of the formation of connective tissue. The more pronounced growth of the latter occurred in periductal tissue.

However, despite insignificant sclerosis of the circulatory connective tissue, bougienage with subsequent duct washing is an effective measure in the complex treatment of experimental acute pancreatitis, it allows to preserve the

pancreatic tissue, which distinguishes it favorably from other methods of treating acute pancreatitis.

The prospect of further research may be the use of this technique in clinical settings.

Conclusions

Bougienage with the subsequent perfusion of the pancreas duct with a medicinal mixture has a positive effect on the course and outcome of experimental acute pancreatitis, namely:

1. Lack of death of animals;
2. Effectively relieves pressure in the duct system of the pancreas, as a result of which the spread of the pathological process is prevented;
3. Eliminated duct obturation of the pancreas with dense protein masses;
4. Structural changes in the duct, tissues, vessels of the pancreas are not developing;
5. Saved in the functional sense of the proximal part of the pancreas from the dissection of the duct.

References

- [1] Vladimirov, V. G., & Sergienko, V. I. (1986) *Acute pancreatitis (experimental and clinical study)*. M.: Medicina.
- [2] Kaliev, A. A. (2013). Macromicroscopic anatomy and internal organ histotopography of the pancreas in acute destructive pancreatitis. *Morfologicheskie vedomosti*, 2, 33-37.
- [3] Koroŭko, G. F. (2014). Secretion of the pancreas: from the Pavlov's elements to the present time (To the 110-anniversary of Nobel Prize award to I. P. Pavlov). *Russian Journal of Gastroenterology, Hepatology, Coproproctology*, 24(3), 4-12. Retrieved from www.gastro-j.ru/files/_2014_3_s1_1406994714.pdf
- [4] Kostyuk, G. Y. (2014). Consequences of high pressure in the pancreas duct. *Bulletin of the Vinnytsia National Medical University*, 18(1), 30-32.
- [5] Kostyuk, G. Y. (2017). *Perfusion of ducts of the pancreas in the treatment of acute pancreatitis*. Materials of the scientific and practical conference "Applied aspects of morphology". Vinnytsia.
- [6] Lonsky, L. Y. (2015). Morphological changes in pancreas in edema and destructive forms of acute pancreatitis. *Bulletin of the Vinnytsia National Medical University*, 19(1), 248-251.
- [7] Napalkov, P. N., & Artem'eva, N. N. (1984). *Surgical treatment of combined obstruction of the terminal section of the choledochus and duct of the pancreas*. XXX All-Union Congress of Surgeons. Minsk.
- [8] Peter A., Banks, (1982). *Pancreatitis*. Moscow: Medicine.
- [9] Savel'ev, V. S., Buyanova, V. M., & Ognev, Y. V. (1983). *Acute pancreatitis*. Moscow: Medicine.
- [10] Filiin, V. I. (1982). *Acute diseases and pancreatic injury*. Moscow: Medicine.
- [11] Aggarwal, A., Manrai, M., & Kochhar, R. (2014). Fluid resuscitation in acute pancreatitis. *World J. Gastroenterol.* 20(48), 18092-103. doi: 10.3748/wjg.v20.i48.18092.
- [12] Dua, M. M., Worhunsky, D. J., Malhotra, L., Park, W. G., Poultsides, G. A., Norton, J. A., & Visser, B. C. (2017). Transgastric pancreatic necrosectomy-expedited return to prepancreatitis health. *J. Surg. Res.*, 219, 11-17. doi: 10.1016/j.jss.2017.05.089.
- [13] Glabay, V. P., Cridnev, O. V., Arkharov, A. V., Bykov, A. N., Kaprin, I. A., & Muslimov, B. G. (2017). Complications of the 'open' surgeries for severe acute pancreatitis. *Surgery*, 10, 72-76. doi: 10.17116/hirurgia20171072-76.
- [14] Hancerli, Y., Kaplan, M., Tanoglu, A., Yesilbas, S., Kucukodaci, Z., Yildirim, M., ... Sakin, Y. S. (2017). Efficacy of tocilizumab treatment in cerulein-induced experimental acute pancreatitis model in rats. *Turk. J. Gastroenterol.*, 28(6), 485-491. doi: 10.5152/tjg.2017.16738.
- [15] Huang, Q., Shao, F., Wang, C., Qi, W., Qiu, L. J., & Liu, Z. (2018). Nasobiliary drainage can reduce the incidence of post-ERCP pancreatitis after papillary large balloon dilation plus endoscopic biliary sphincterotomy: a randomized controlled trial. *Scand. J. Gastroenterol.*, 53(1): 114-119. doi: 10.1080/00365521.2017.1391329.
- [16] Janisch, N. H., & Gardner, T. B. (2016). Advances in Management of Acute Pancreatitis. *Gastroenterol. Clin. North Am.* 45(1), 1-8. doi: 10.1016/j.gtc.2015.10.004.
- [17] Kolodecik, T. R., Reed, A. M., Date, K., Shugrue, C. A., Patel, V., Chung, S. L. ... Gorelick, F. S. (2017). The serum protein renalase reduces injury in experimental pancreatitis. *J. Biol. Chem.*, 292(51), 21047-21059. doi: 10.1074/jbc.M117.789776.
- [18] Manohar, M., Verma, A. K., Upparahalli Venkateshaiah, S., Goyal, H., & Mishra, A. (2017). Food-Induced Acute Pancreatitis. *Dig. Dis. Sci.*, 62(12), 3287-3297. doi: 10.1007/s10620-017-4817-2.

ВПЛИВ БУЖУВАННЯ ТА ПРОМИВАННЯ ПРОТОКИ ПІДШЛУНКОВОЇ ЗАЛОЗИ НА ПЕРЕБІГ ЕКСПЕРИМЕНТАЛЬНОГО ГОСТРОГО ПАНКРЕАТИТУ

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Наявність багатьох гіпотез розвитку гострого панкреатиту, таких як протокова гіпертензія, панкреатичний рефлюкс, судинна, алергічна, нервово-рефлекторна, інфекційна та інших говорить про відсутність чіткого розуміння механізмів розвитку даної патології, а, звідси, неточність у лікуванні і негативні наслідки. Метою даного дослідження було вивчити вплив бужування та промивання протоки підшлункової залози на перебіг та морфологічний стан підшлункової залози при експериментальному гострому панкреатиті. Досліди були проведені на 12 собаках, котрим створили модель панкреатиту шляхом введення аутожовчі у протоку підшлункової залози. Тварин поділили на 4 групи, по 3 тварини в кожній, з терміном виведення з досліду через 1, 3, 7 днів і через 6 місяців відповідно. Перед перфузією протоки підшлункової залози в неї вводили поліхлорвініловий буж, який через розріз в дистальному відділі протоки підшлункової залози видаляли. Така маніпуляція дозволила проводити промивання протоки лікувальними речовинами при тиску 0,49-0,6 кПа і підтвердила припущення, що при гострому панкреатиті спостерігається виповнення протоки підшлункової залози згущеними білковими масами, а це, як ми впевнились, міняє підхід не лише до встановлення патогенетичної ланки в розвитку гострого панкреатиту, а й до його лікування. Протоку промивали 1 раз. Тваринам проводили загальноприйнятну комплексну медикаментозну терапію протягом 5-6 наступних днів. Із 12 собак по закінченню першої доби самостійно підіймалося 9, решта - на другу добу. На третю добу всі тварини пили воду і реагували на подразники. На 5 добу вони були активними, приймали рідкий корм. На 7 добу по поведінці й прийому їжі

собаки даної групи не відрізнялися від здорових. Для вивчення морфологічних змін у підшлунковій залозі після промивання протоки 3 собаки були виведені із дослідження через 1 добу. Випоту в очеревинній порожнині не спостерігалось. Ліва доля підшлункової залози була дещо зменшена у довжину. Місце розсічення тканин підшлункової залози і протока прикрита згустком крові. В області розтину 12-ти палочкоподібної кишки зберігалися поодинокі плями стеатонекрозу. Мікроскопічно в області розсічення протоки зміни тканин підшлункової залози були аналогічними розсіченню протоки без перфузії. Разом із тим, повнокров'я на цей термін відмічалось тільки великовогнищеве. Некротичні вогнища паренхіми були поодинокими і з помірно нейтрофільною інфільтрацією. Дистальніше зони розсічення в тканині підшлункової залози спостерігалися незначні вогнищеві крововиливи з порушенням її структури, однак повнокров'я, вогнища нейтрофільної інфільтрації у міжчасточковій сполучній тканині були менше вираженими. У проксимальній від розсічення частині протоки тканина підшлункової залози зберігала помірно повнокров'я. Крім того, в ній зустрічались мілкі вогнища крововиливів з порушенням структури окремих ацинусів і незначною нейтрофільною інфільтрацією в міжчасточковій сполучній тканині. В останній відмічалась також скупчення макрофагів і проліферація фібробластів, зустрічались поодинокі вогнища крововиливів. В інтактній долі спостерігали незначний набряк міжчасточкової сполучної тканини. В результаті проведених експериментів ми переконалися в ефективності даного методу лікування гострого панкреатиту.

Ключові слова: гострий панкреатит, бужування протоки підшлункової залози.

ВЛИЯНИЕ БУЖИРОВАНИЯ И ПРОМЫВАНИЯ ПРОТОКА ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ НА ТЕЧЕНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ОСТРОГО ПАНКРЕАТИТА

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Наличие многих гипотез развития острого панкреатита, таких как протоковая гипертензия, панкреатический рефлюкс, сосудистая, аллергическая, нервно-рефлекторная, инфекционная и других говорит об отсутствии четкого понимания механизмов развития данной патологии, а отсюда неточность в лечении и негативные последствия. Целью данного исследования было изучить влияние бужирования и промывание протока поджелудочной железы на течение и морфологическое состояние поджелудочной железы при экспериментальном остром панкреатите. Опыты были проведены на 12 собаках, которым создали модель панкреатита путем введения аутожелчи в проток поджелудочной железы. Животных разделили на 4 группы, по 3 животных в каждой, со сроком вывода из опыта через 1, 3, 7 дней и 6 месяцев соответственно. Перед перфузией протока поджелудочной железы в нее вводили полихлорвиниловый буж, который через разрез в дистальном отделе протока поджелудочной железы удаляли. Такая манипуляция позволила проводить промывание пролива лечебными веществами при давлении 0,49-0,6 кПа и подтвердила предположение, что при остром панкреатите наблюдается заполнение протока поджелудочной железы сгущенными белковыми массами, а это, как мы убедились, меняет подход не только к установлению патогенетического звена в развитии острого панкреатита, а и к его лечению. Проток промывали 1 раз. Животным проводили общепринятую комплексную медикаментозную терапию в течение 5-6 последующих дней. Из 12 собак по окончании первых суток самостоятельно поднимались 9, остальные - на вторые сутки. На третьи сутки все животные пили воду, реагировали на раздражители. На 5 сутки они были активными, принимали жидкий корм. На 7 сутки по поведению и приему пищи собаки данной группы не отличались от здоровых. Для изучения морфологических изменений в поджелудочной железе после промывания протока, 3 собаки были выведены из опыта через 1 сутки. Випота в брюшинной полости не наблюдалась. Левая доля поджелудочной железы была несколько уменьшена в длину. Место рассечения тканей поджелудочной железы и протока прикрита сгустком крови. В области вскрытия 12-перстной кишки сохранялись редкие пятна стеатонекроза. Микроскопически в области рассечения протока изменения тканей поджелудочной железы были аналогичными рассечению протока без перфузии. Вместе с тем, полнокровие в этом сроке отмечалось только крупноочаговое. Некротические очаги паренхимы были единичными и с умеренной нейтрофильной инфильтрацией. Дистальнее зоны рассечения в ткани поджелудочной железы наблюдались незначительные очаговые кровоизлияния с нарушением ее структуры, однако полнокровие, очаги нейтрофильной инфильтрации в междольковой соединительной ткани были меньше выражены. В проксимальной от рассечения части протока ткань поджелудочной железы сохраняла умеренное полнокровие. Кроме того, в ней встречались мелкие очаги кровоизлияний с нарушением структуры отдельных ацинусов и незначительной нейтрофильной инфильтрацией в междольковой соединительной ткани. В последней отмечались также скопления макрофагов и пролиферация фибробластов, встречались единичные очаги кровоизлияний. В интактной доле наблюдали незначительный отек междольковой соединительной ткани. В результате проведенных экспериментов мы убедились в эффективности данного метода лечения острого панкреатита.

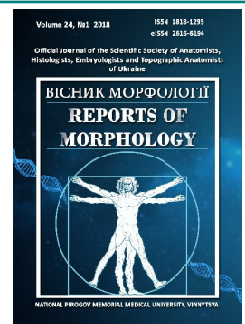
Ключевые слова: острый панкреатит, бужирование протока поджелудочной железы.



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Electron microscopic diagnostics of apoptosis processes under simulation conditions in the experiment of acute pyelonephritis and concomitant diabetes mellitus type I and II

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According to literature data, in the conditions of the balance of pro- and anti-inflammatory factors, the development of the infectious-inflammatory process tends to progress and more severe with subsequent transformation into chronic inflammation with corresponding morphofunctional effects. Under such critical conditions, an apoptosis is likely to play a crucial protective role, which can eliminate the excessive accumulation of aggressive inflammatory effects and effectively eliminate them, which in the future prevents the probable transformation into a persistent form. The aim of the work was to study the features of the early dynamic processes of apoptosis in the tissues of the kidneys under simulation conditions in the experiment of acute pyelonephritis and concomitant diabetes mellitus type I and II. The purpose of the study was to study the features of the early dynamic processes of apoptosis in the tissues of the kidneys under simulation conditions in the experiment of acute pyelonephritis and concomitant type I and type II diabetes. The work was performed on 300 adult Wistar rats, which were divided into 4 groups. Fragments of animal's kidneys were studied and photographed in an electron microscope PEM-100-01. The results showed that after modeling in the animals of pyelonephritis in the nephrons there were no significant ultrastructural changes. The structure of the podocytes of the outer sheet was almost the same as the structure of the podocytes of the control material, and in some cells there were signs of activation of their metabolic activity. In the structure of the podocytes of the inner leaf of the capsule, dystrophic changes of the internal membrane of the mitochondria were established. In kidney medulla, the structure of the glomerulus was more preserved than in the cortical. Pathological changes of the proximal and distal tubular podocytes, as well as interstitial tissue, are more pronounced than the renal glomeruli. Under the modeling of the common model of pyelonephritis and type 1 diabetes, more pronounced morphological changes occur: destructive changes in the endothelial cells of the glomerular capillaries, the homogenization of the structure of the basement membrane occurs and the mesangial tissue is significantly enlarged. In nephrons and tubules of cortex, changes are manifested to a much greater extent than in the kidney medulla. When studying in clinical conditions the pathogenetic features of acute pyelonephritis in conditions of concomitant diabetes mellitus it is expedient to carry out electron microscopic research with the aim of choosing the optimal corrective therapeutic effect and preventing the unfavorable course of infectious and inflammatory process and its transformation into persistent form. EM (electron-microscopic) studies are highly informative in the study of pathological changes and early dynamic processes of apoptosis in renal tissues in the design of acute pyelonephritis and concomitant diabetes mellitus I and II in the experimental conditions.

Keywords: modeling, pathogenesis, pyelonephritis, diabetes mellitus, electron microscopy, cortical and cerebral matter, apoptosis.

Introduction

Current scientific evidence suggests the participation of apoptosis - programmable cell death (PCD) in the

pathogenesis of a significant number of infectious and inflammatory processes that have an active course and a

tendency to chronicity [3, 7, 15, 18]. The foregoing allows to classify apoptosis as a universal general-pathological process of early development of numerous diseases and pre-nosological syndromes of infectious and inflammatory origin.

It has been established that apoptosis is capable of implementation both in structurally undamaged tissues and in the presence of signs of a pathological process in them. Apoptosis is considered as a natural and logical result of the implementation of receptor-mediated mechanisms of self-destruction of the cell. At the same time it is emphasized that the adaptive role of PCD is absolutely obvious [3, 12, 17, 18].

Literary data testify that in the conditions of a violation of the balance of pro- and anti-inflammatory factors, the development of the infectious-inflammatory process is characterized by a tendency towards progression and a more severe course of inflammation and its transformation into chronic inflammation with severe morphofunctional consequences. It is suggested that under such critical conditions, an apoptosis that can eliminate the excessive accumulation of aggressive inflammatory effects, their effective elimination, which in the future will provide a more favorable course of the inflammatory process and prevent its transformation into a persistent form, plays a decisive protective role [2, 10, 11, 13, 14]. The data of many authors underlines the transience of the process of self-destruction of cells, for completing which is enough for several minutes or hours [1, 2, 4, 10, 16].

Based on the foregoing, *the purpose* of this work was to study the features of the early dynamic processes of apoptosis in the tissues of the kidneys in terms of simulation in the experiment of acute pyelonephritis and concomitant diabetes mellitus type I and II.

Materials and methods

The work was performed on 300 adult rats of the Vistar line, which were divided into 4 groups: group A - control (30 intact animals); group B (35 animals, which were modeled acute pyelonephritis); group C (50 animals with simulations of acute pyelonephritis and type I diabetes); group D (50 animals with simulations of acute pyelonephritis and type II diabetes mellitus).

For electron microscopy, the kidney fragments were fixed in 2.5% glutaraldehyde solution in phosphate buffer at a pH=7.4, followed by fixation with 1% solution of osmic acid at the same pH of the buffer solution. The samples were then dehydrated in alcohols of ascending concentration. The material was etched and its conclusion was carried out in a mixture of epoxy resins Araldite-Epon. Subsequently, ultrathin sections were contrasted with the Reynolds technique [15].

The research objects were studied and photographed in an electron microscope PEM-100-01, 148 electronic microphotographs were obtained and analyzed. The work is performed as a part of the group of electron microscopy of the laboratory of pathoanatomical and electron microscopic research.

Results

At the electron microscopy (EM) study of the kidney cortex of animals in the control group (A), it was found that nephrons of the cortex have an electron-dense tissue of the Bowman capsule, under which on the basement membrane place epitheliocytes (podocytes) of the outer sheet of the capsule (OSC). These cells consist of a thin layer of flattened cells that also have an electron-dense cytoplasm and a nucleus (N), which contains mainly chromatin in a condensed state. Especially it should be noted its boundary location, that is, there is a margin of chromatin. The organelles of these cells are small, are defined indistinctly. Alongside the cells of this layer are located with more electron-light cytoplasm and karyoplasm. In the cytoplasm, there are isolated mitochondria (M), polysome (P), and granular endoplasmic mesh (GEM) tanks. Between the outer and inner leaves situated a cavity of the capsule (Fig. 1).

Podocytes of inner leaf occupies a large area and are located in the cavity; cytotrabecules contacting each other from different parts of the inner leaf. In the cytoplasm of the podocytes there is a lot of polysome, mitochondria, GEM elements, well-developed Golgi complex. The nucleus is predominantly circular with a convoluted karyolemma.

The glomerulus has a well-developed capillary mesh. Part of the capillaries of the body of the endothelial cells (EC) often overlap their lumen. The cells have large nuclei with invaginations and folds of karyolemma. Karyoplasm and cytoplasm electron-light. In the nucleus, there is a margin of chromatin. In the central zone chromatin is in a diffuse state. In the cytoplasm of the EC there are diffuse single mitochondria, Golgi complexes, polysomes and small foam

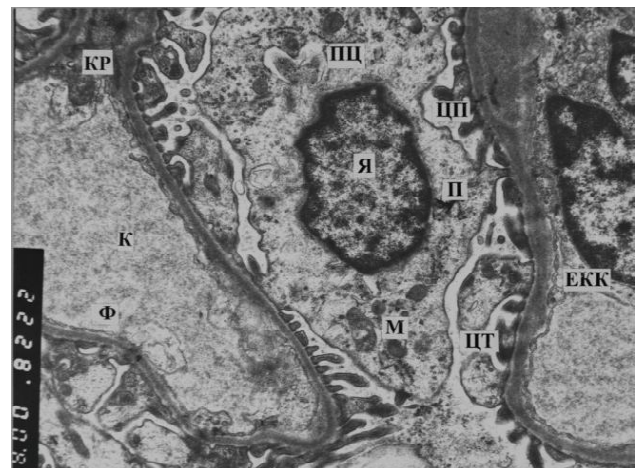


Fig. 1. Ultrasonography of the rat kidney cortex of the control group, a fragment of the capillaries and the podocyte in the normal state. EM x8000. Here and thereafter: KP - cortical substance, K - capillary, EKK - endothelial cell of the capillary, Ф - fenestrations, ЦТ - cytotrabecula, ЦП - cytopodes, ПЦ - podocyte, П - polysome, Я - nucleus, М - mitochondria, MP - renal medulla, EKK - capillary endothelial cell, МЦ - mesangiocyte, E - erythrocyte, MP - renal medulla, ЗЛК - outer layer of the capsule, ИТ - interstitial tissue, В - vacuole, ИТ - interstitial tissue, ПК - proximal tubule, БМ - basal membrane, КК - glomerular capillary mesh.

cytoplasmic vesicles. In the lumen one can see red blood cells (E). Fenestra, located on the groomed areas of the EC, are well expressed. Proximal tubules (PT) are lined with podocytes of cuboid shape, which lie on the basement membrane. They have a basal strain and apical microvilli, which form a brush rim. In the cytoplasm of these cells there is a round nucleus and a large number of mitochondria, polysomes and single elements of the GEM.

With further EM studying of the kidney medulla of animals of the control group (A), it was discovered that the podocytes of the outer sheet adhere tightly to the basement membrane. Basically, these cells have a flattened form, a spindle-shaped core and a small cytoplasmic strip. In some podocytes, cytoplasmic spinal cord and karyoplasm are electron-light. In the nucleus of these cells, there is a margin of chromatin. Euchromatin, which occupies almost the entire area of the karyoplasma, is indistinguishable, fragmented, contained in small quantities. Polysomes, small round mitochondria are well defined in the cytoplasm. To the outer sheet, the large size of the podocytes of the inner sheet are closely adjacent to each other, the slit of the cavity is very narrow, in places its larger space is determined. They have a large nucleus with a convoluted karyolemma. The chromatin of the nuclei is in a diffuse state. The cytoplasm is saturated with narrow, small tubules of GEM, polysomes and a small number of mitochondria. To the basement membrane closely adhere to polyopia (Fig. 2).

The glomerulus has a winding basal membrane. EC of capillaries have large sizes, their fenestration are well defined. The cytoplasm has a regular set of organelles. In a number of capillaries, the cytoplasm is slightly electron-light, containing a reduced number of their intracellular organelles. In the cavity of the capsule, in addition to the podocyte, there are fragments of individual cytotrabecula. In the lumen of the capillaries place a fine-grained material. PT and distal tubules (DT) by ultrastructure do not differ from such in cortex.

Electron microscopically examined kidneys of animals of group B (simulation of acute pyelonephritis). In the study of kidney cortex, it was found that the podocytes of the outer sheet of the renal corpuscles were associated with signs of activation of protein-synthetic activity in the cytoplasm: an increased number of polysomes increased in size, enlarged tubules of GEM, in profiles of which there was a fine-grained substance (Fig. 3). The cytoplasm of the podocytes of the inner leaf was somewhat compressed; part of the mitochondria was detected with complete destruction of the cristae. In the capillaries, the number of fenestration has been reduced, as well as marked breaks in the field. Occasionally there was a narrowed clearance in capillaries with cell fragments in it. In general, the structure of capillaries is close to such in control animals (Fig. 3).

The podocytes of the PC have reduced the number of mitochondria, and the existing mitochondria are closely adjacent to each other and have complete or partial destruction of cristae, which is possibly due to increased energy expenditure on intracellular processes. The nuclei

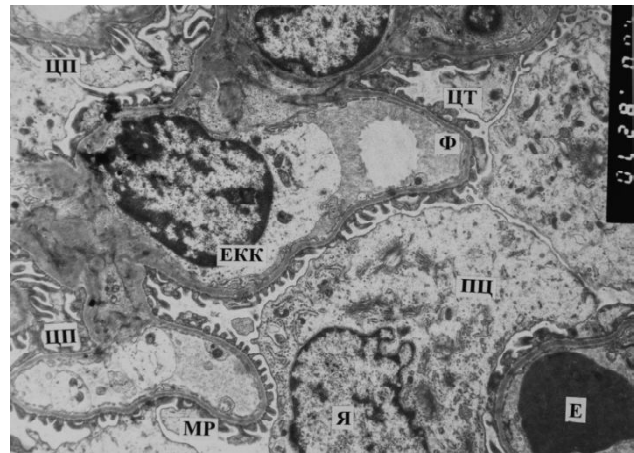


Fig. 2. Ultrastructure of the rat kidney medulla of the control group, a glomerular fragment with a normal ultrastructure. EM x4000.

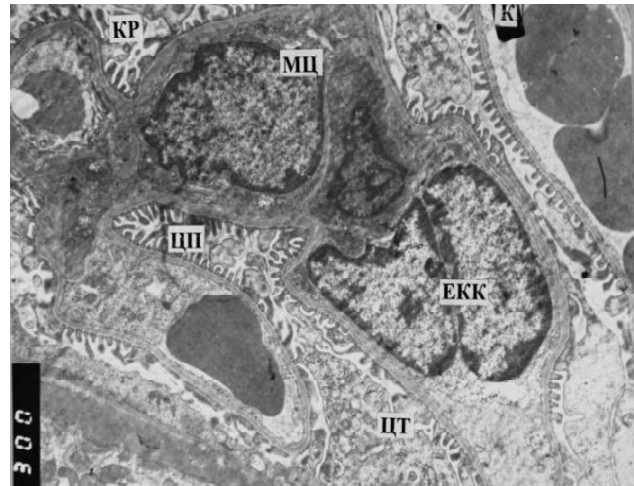


Fig. 3. Ultrastructure of rat kidney cortex after acute pyelonephritis modeling. A fragment of a glomerular capsule with signs of edema of the internal-mitochondrial matrix and destruction of mitochondria. EM x4000.

of these cells have an enlightened karyolemma, well-defined nuclear pores and a nucleolus located near the karyoplasm, indicating the activation of metabolic processes between the nucleus and the cytoplasm. In the cytoplasm, lysosomes and vacuoles also appear. On the basal surface there are shallow, rarely located basal folds.

In the electron microscopic study of medulla of group B animals, it was found that in the renal glomerulus the outer sheet under the basal membrane, consisting of flat podocytes, which contain large oval-shaped nuclei, is well defined. The chromatin of the nuclei is in diffuse state, the narrow rim under the karyolemma is the condensed chromatin. The cytoplasm of these cells is slightly enlightened, the organelles are somewhat sparse in the cytoplasm, compared with that in the control material. In a number of cells there are two nuclei.

The cytoplasm is full of organelles. The mitochondria with the destruction of cristae and the enlightened matrix

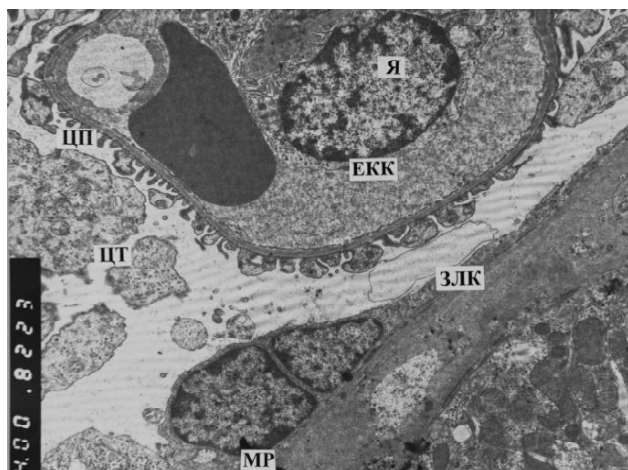


Fig. 4. Ultrastructure of the kidney medulla of the rat, model of acute pyelonephritis against the background of diabetes mellitus type I. Large cytotrabecula with an increased number of organelles, in it mitochondrias with a sign of edema and destruction of christ. EM x3000.

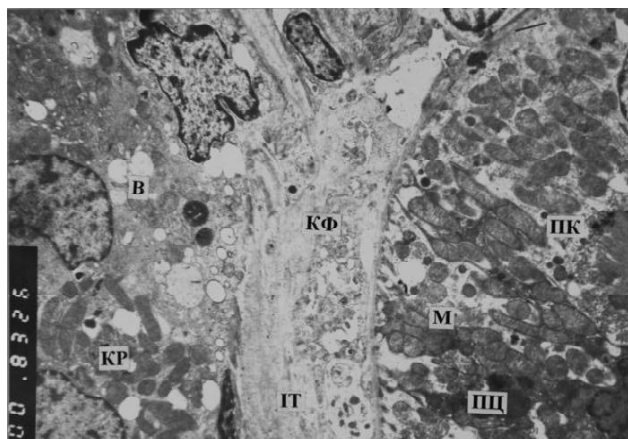


Fig. 5. Ultrasound of the rat kidney cortex after the simulation of acute pyelonephritis against the background of diabetes mellitus type I. The proximal tubule is suspected with electronically transparent cytoplasm and elements of destruction of some mitochondria's. EM x3000.

are determined, with a lot of polysome. A number of podocytes has spindle-shaped nuclei. The capillaries of the glomerulus, unlike the control material, have elevated electron-density content. EC with a large round nucleus and swollen mitochondria. Podocyte the inner leaf - large, especially their cytotrabecula. Separate cytotrabecula are located in the cleft area, along with the individual fragments of cells visible. Large bodies of podocyte act at a considerable distance to the space of the gap (Fig. 4). Sometimes cytotrabecula have the appearance of narrow strands, which extend over a large distance in the cavity of the capsule, in contact with other cytotrabecula on the opposite side of the glomerulus. Cytopedicules are sometimes thinned, in places, on the contrary, hypertrophic, that is, thickened containing a fragment of the cytoplasm.

It should be noted that in the renal corpuscles is a well-

defined area of the cavity. It is more expanded than control.

A more preserved medulla glomeruli than the kidney cortex is found. PT and DT with the phenomena of organelle destruction, especially mitochondria with the formation of a large number of vacuoles, and the edema of the cytoplasmic matrix. Signs of minor hydroponic changes are also observed in the interstitial tissue.

Thus, the results of our study showed that after simulation of pyelonephritis in animals in kidney nephrons no significant ultrastructural changes are detected. The structure of the podocytes of the outer sheet is practically similar to the control material. At the same time, in some cells there are signs of activation of their metabolic activity. In the podocytes of the inner leaf of the capsule, dystrophic changes in the internal membrane of the mitochondria section are noted, which may indicate an increased energy need for cells and is an indication of the initial mechanism of apoptosis, according to modern data. When comparing the ultrastructure of the cortical and cerebrospinal fluid of the kidney, it is found that in the medulla the structure of the glomeruli is more preserved than in the kidney cortex.

Pathological changes in the podocytes of the PT and DT and in the interstitial tissue are more pronounced than in the renal glomeruli.

The EM-study of the cortex of group C animals (simulations of acute pyelonephritis and type I diabetes mellitus) made it possible to establish that in the renal glomerulus the podocytes of the outer sheet in places, according to the ultrastructure, do not differ from such control material, there was occasional discontinuity of their plasmolemma. The structure of the basement membrane in this area has a layered composition. Podocytes the inner leaf in its greater part with pronounced signs of edema and complete absence of organelles, that is, their hydropic degeneration is observed. These cells in their swollen bodies completely overlap the cavity of the capsule. A part of the same podocyte has a structure more preserved, but they are determined by the expansion of the elements of the GEM and swelling of the mitochondria (Fig. 5). Cytopedicules are sometimes elegant and elongated, but sometimes they are thickened, but they all have a cytoplasm of elevated electron density. A number of EC capillaries are also characterized by hydropic degeneration. In their lumen, there are single or multiple erythrocytes, they are aggregated or slag. Phenesters are poorly defined in them. Other capillaries have a normal structure or with the cellular desolation of organelles and signs of hypostasis of the cytoplasmic matrix. Some of the capillaries are deformed, the lumen is narrowed.

In a number of capillaries the body of large EC overlap their lumen, the structure of these cells without visible changes. Enlightenment of all capillaries of elevated electron density.

PT and DT with the phenomena of organelle destruction and swelling of the cytoplasmic matrix, sometimes there is no basal strain in them. Signs of hydroponic changes are

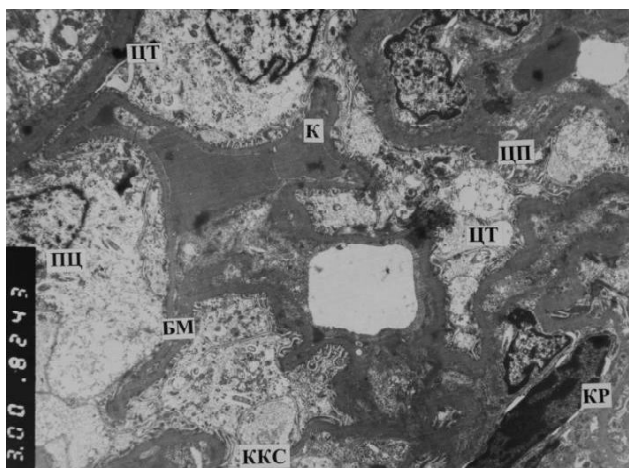


Fig. 6. Ultrastructure of the rat kidney cortex after acute pyelonephritis modeling against the background of type II diabetes mellitus. Desolation of capillaries, hypertrophied podocytes in a state of destruction. EM x3000

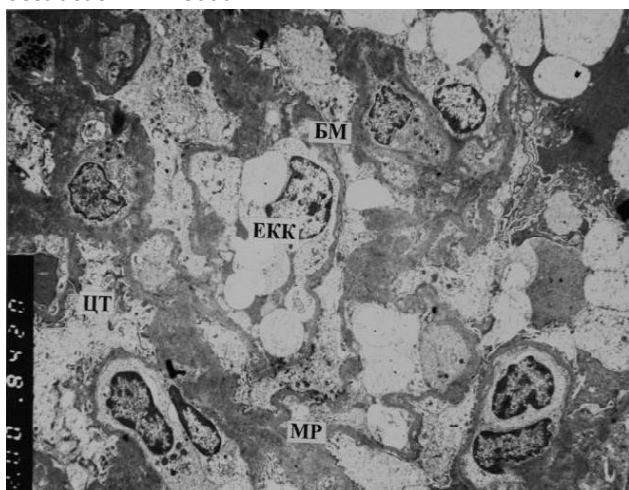


Fig. 7. Ultrastructure of the kidney medulla of the rat after the simulation of acute pyelonephritis against the background of type II diabetes mellitus. Focal narrowing of the capillaries, destructive changes in the cytoplasm of the endothelial cells of the capillaries in the podocytes glomerular capillary mesh. Homogenization of the basement membrane. EM x3000.

observed in the interstitial tissue.

Regarding the state of the medulla of the kidneys of the animals of the group C (model of AP and DM type I), the EM data indicate that the podocytes of the outer sheet are slightly flattened. Cell nuclei are hyperchromic. Single membrane structures are in a state of destruction. Capillaries are sometimes narrowed. Enlargement of most of the capillaries is densely filled with erythrocytes. Their EC have a slightly electron-transparent cytoplasm and there is a destruction of intracellular organelles, that is, there are pronounced deep hydrophilic changes. Other EC capillaries are more preserved, only destruction of mitochondria and expansion of GEM tanks is observed in them. A number of EC according to the structure does not differ from the EC of the capillaries of the control group. Most of the podocytes of the inner leaf are

somewhat large in size. They are intimate contact with each other. However, some of them have signs of hydropic changes. Intracellular structures in the state of destruction. Cytopedicles also have destructive changes, sometimes absent. The basal membrane of the glomerulus is homogenized. PT podocytes with enlightened cytoplasm and elements of destruction of organelles, especially mitochondria, and the location of a large number of vacuoles in it. Basal strain looks sparse. In DT in podocyte there is homogenization of mitochondria crust and the destruction of other organelles. A nucleus with an enlightened karyoplasm, in which there are osmiophil debris and flake-like material instead of chromosomes. In the interstitial tissue signs of edema of the main substance and cytoplasmic structures of EC of capillaries.

When comparing the ultrastructure of rat kidney nephrons after modeling of pyelonephritis and the common model of pyelonephritis and type I diabetes, it should be noted that the latter model shows more pronounced changes that are manifested by significant destructive changes in the EC of the glomerular capillaries, which lead to the complete desolation of most of the capillaries and before pronounced destructive changes in podocyte; in the homogenization of the structure of the basement membrane; expanded prolapse of mesangial tissue. The cavity of the capsule is completely absent. In podocyte, there are also signs of alteration of their ultrastructure. Podocytes of PT and DT, as well as elements of interstitial tissue with signs of deep destruction of their structures.

At the same time, we conducted the EM study of the cortical substance of the kidneys of the animals of group D (modeling of AG and DM type II), in which it was found that the basement membrane of the capsule is dense, thickened in some places. The outer sheet podocytes are compacted, others with a larger area of the nucleus, irregular shape, part of the cells in a state of collapse, are sometimes absent. The cavity of the capsule is completely absent. Many of the podocytes of the inner leaf are enlarged in size, the cytoplasm of their light, edema. The cytoplasmic organelles of the podocytes and their processes are in a state of degradation of varying degrees. Some of these cells are completely devastated, detritus is determined instead of intracellular structures (Fig. 6). The glomerular capillaries are deformed, sometimes sharply narrowed or rescued, having a nodular shape. Enlargement of the capillaries is densely filled with erythrocytes. EC in many capillaries is absent or necrotized. A part of these cells has a round nucleus, its karyolemma with invaginations and folds, chromatin is practically destroyed.

In podocytes of PT there is considerable devastation of intracellular organelles. The cells reveal a nucleus with a normal structure, near which there is a significant amount of polysome, a small number of mitochondria, whose cristae are homogenized or are in a state of dystrophy.

In DC, the organelles of the central and apical part of the cytoplasm of the podocytes are more damaged. In interstitial

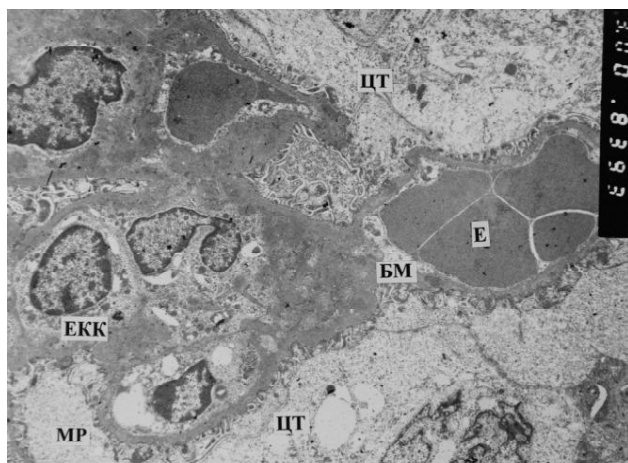


Fig. 8. Ultrastructure of the kidney medulla of the rat after the simulation of acute pyelonephritis against the background of type II diabetes mellitus. Signs of edema of endothelial cells and sludge of red blood cells in capillaries, hydrotheric changes and destruction of organelles of large cytotrabecular capsules. Reduction of part of cytopodes. EM x3000.

tissue there are elements of intracellular degeneration of EC capillaries and other cells with illumination of its basic substance.

A similar study of the medulla of the kidneys of animals of group D (model of AG and type II diabetes) has shown that polymorphic changes in EC capillaries are determined in the glomeruli. Part of the capillaries in its structure is close to normal. It is noted only the expansion of the elements of the WES and the cristae destruction of the mitochondria. Other EC with signs of varying degrees of destruction of cytoplasmic organelles. Enlightenment of capillaries is filled with homogeneous, with elevated electron-dense content. In the electron-dense lumen, there are isolated red blood cells. In the capillaries of a large caliber there is a glut of red blood cells in the lumen. Podocytes, their cytotrabecula and the cytopedicles of the inner leaf are mainly in the state of necrosis or severe degeneration of the cytoplasmic organelles, although in places there are cells with a virtually preserved structure (Fig. 7).

In podocytes of PT and DT, as well as in interstitial tissue, there are unidirectional ultrastructural changes, as in microstructures of the cortex, however, they are expressed in places to a lesser extent.

The EM differences between the model of pyelonephritis and concomitant diabetes type II from the model of pyelonephritis are: in this model, the capillaries are significantly narrowed, some narrowed, some of them formed nodes. The structure of the basement membranes is homogenized, thickened in the empty capillaries. EC in such capillaries in a state of destruction, their gap is densely filled with erythrocytes. Podocytes the outer sheet in a state of deep degeneration. Decide the inner leaf and cytotrabecula in the state of hydropic degeneration. Pedicle shortened, sometimes absent. In the PT there is a local destruction of organelles, individual mitochondria with cristae

homogenization. Elements of interstitial tissue in the state of edema. The structure of the cortex is more damaged than the medulla (Fig. 8).

The PT has a lot of vacuoles in the apical area. In the DT there is a reduced number of mitochondria. In interstitial tissue there are elements of edema of the main substance. Structure of the medulla is more preserved than cortex. Podocytes of inner leaf and cytotrabecula in the normal state, prevail over such in a state of destruction.

Discussion

The analysis of electron microscopic material related to the above-mentioned groups of experimental study showed that the leading place in the development of pathological changes in the tissues of the kidneys under the common modeling of the AP and diabetes have type I diabetes. It should be noted that in the nephrons and tubules of the cortex, the above changes appear to a much greater extent than in the kidney medulla.

According to number of authors, ischemia is one of the most important triggers of programmed cell death (PCD) initiation as a significant pathogenetic factor in the development of infectious and inflammatory processes in the kidneys [8, 12, 16, 17].

It was also established that proximal straight tubule is most prominent in the part of the nephron, which is sensitive to ischemia [12, 13]. In modern studies, this phenomenon is associated with the fact that the membrane of cells of the proximal tubule contains the largest among all segments of the nephron amount of ATP-dependent transport proteins. Ischemia causes a rapid depletion of the ATP pool in these cells, which causes their osmotic swelling, fragmentation of mitochondria, dissociation of the cytoskeleton with subsequent violation of the integrity of the cytoplasmic membrane and cell necrosis [4, 5, 6].

Contemporary literature data on ultrastructural manifestations of PCD suggest that the most pronounced changes in the Henle loop, the distal tubule and the collection tube are the blockage of the lumen of the tubules by the bodies of epithelial cells that were swollen with flake-like aggregates, cell detritus, and also the aggregation of red blood cells in peritubular blood capillaries [4, 5, 13].

Some authors [4, 6] have suggested that such obstruction of the nephrons, along with the damage to the tubular cells, is an additional barrier to the restoration of normal hemodynamics in the kidney and affects the course of the apoptotic process.

EM studies that have high informativeness in the study of ultrastructural pathological changes in the tissues of the kidneys appear to be more appropriate in clinical conditions in the study of the pathogenetic features of acute pyelonephritis in conditions of concomitant diabetes mellitus with the aim to further selecting the optimal corrective therapeutic effect and preventing the adverse flow of the infectious and inflammatory process and its transformation into a persistent form.

Conclusions

1. In conditions of reproduction of experimental models of acute pyelonephritis and concomitant diabetes mellitus types I and II, the leading role in the development of pathological changes in the tissues of the kidney plays a role diabetes type 1.

2. The pathological changes in the renal tissue revealed in the experiment have more significant manifestations, namely: in the proximal straight tubule focal local destruction

of organelles, separate mitochondria with homogenization of crista. Elements of interstitial tissue in the state of edema. The structure of the cortical substance is more damaged than the medulla.

3. EM studies have high informative character in the pathological changes and early dynamic processes of apoptosis in kidney tissues under simulation conditions in the experiment of acute pyelonephritis and concomitant diabetes mellitus I and II types.

References

- [1] Dorogoj, A. P. (2007). Life expectancy, potential labor potential losses, and death in diabetes mellitus. *International Endocrinology Journal*, 3(9), 10-12.
- [2] Kuznetsova, O. P., Vorobiev, P. A., & Yakovlev, S. V. (1997). Urinary tract infections. *Russian Medical Journal*, 1, 4-13.
- [3] Novikov, V. S. (1996). *Programmed cell death*. S.-Pb.: Science. Retrieved from humbio.ru/humbio/starenie/0001d36e.htm
- [4] Pak, L. B., Dubikov, A. I., Kabantseva, T. A., Vasilyuk, A. A., & Grigoryan, O. M. (2013). Apoptosis and kidney pathology. *Nephrology*, 17(4), 36-43. ISSN 1561-6274.
- [5] Popov, S. V., Guseinov, R. G., Gorshkov, A. N., Sivak, K. V., Yablonskiy, P. K., Skryabin, O. N., & Vinogradova T. I. (2016). Changes in the ultrastructural organization of the kidney under the conditions of experimentally modeled thermal ischemia in surgical intervention. *Bulletin of St. Petersburg University*, 11(1), 104-119. Retrieved from vestnik.spbu.ru/html16/s11/s11v1/10.
- [6] Tomilina, N. A., & Bikbov, B. T. (2005). Epidemiology of chronic renal failure and new approaches to the classification and evaluation of the severity of chronic progressive kidney disease. *Therapeutic archive*, 6, 87-89. Retrieved from onco.tnmc.ru/journal/lib-archive/terapevticheskiy-arkhiv/
- [7] Vinnichenko, L. N. (1980). *Comparative ultrastructure of nephron*. L.: Nauka, 136. Retrieved from www.cytspb.rssi.ru/books/vinnichenko_ru.htm
- [8] Becker, F., Van Poppel, H., Hakenberg, O. W., Stief, C., Gill, I., Guazzoni, G., ... Stockle, M. (2009). Assessing the impact of ischaemia time during partial nephrectomy. *Eur. Urol.*, 56, 625-635. doi: 10.1016/j.eururo.2009.07.016.
- [9] Bhayani, S. B., Rha, K. H., Pinto, P. A., Ong, A. M., Allaf, M. E., Trock, B. J. ... Kavoussi, L. R. (2004). Laparoscopic partial nephrectomy: effect of warm ischemia on serum creatinine. *J. Urol.*, 172(4), 1, 1264-1266. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/15371820>
- [10] Donohoe, J. F., Venkatachalam, M. A., Bernard, D. B. & Levinsky, N. G. (1978). Tubular leakage and obstruction after renal ischemia: structural-functional correlations. *Kidney Int.*, 13(3), 208-222. <https://doi.org/10.1038/ki.1978.31>
- [11] Gill, I. S., Colombo, J. R. Jr., Moinzadeh, A., Finelli A., Ukimura O., Tucker K., ... Desai, M. (2006). Laparoscopic partial nephrectomy in solitary kidney. *J. Urol.*, 175(2), 454-458. doi: 10.1016/S0022-5347(05)00150-3
- [12] Hansson, J., Hultenby, K., Crammert, C., Ponten, F., Jansson, H., Lindgren, D. ... Johansson, M. E. (2014). Evidence for a morphologically distinct and functionally robust cell type in the proximal tubules of human kidney. *Hum. Pathol.*, 45, 382-393. doi: 10.1016/j.humpath.2013.10.003
- [13] Humphreys, B. D., Czerniak, S., Dirocco, D. P., Hasnain, W., Cheema, R., & Bonventre, J. V. (2011). Repair of injured proximal tubule does not involve specialized progenitors. *Proc. Natl. Acad. Sci. USA*, 108(22), 9226-9231. doi: 10.1073/pnas.1100629108.
- [14] Porpiglia, F., Renard, J., Billia, M., Musso, F., Volpe, A., Burruni, R. ... Scarpa, R. M. (2007). Is renal warm ischemia over 30 minutes during laparoscopic partial nephrectomy possible? One-year results of a prospective study. *Eur. Urol.*, 52(4), 1170-1178. doi: 10.1016/j.eururo.2007.04.024
- [15] Reynoldes, E. S. (1963). The use of lead citrate at high pH an electronopaque stain in electron microscopy. *J. of Cell Biology*, 17, 208-212. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/13986422>
- [16] Thompson, R. H., Lane, B. R., Lohse, C. M., Leibovich, B. C., Fergany, A., Frank, I. ... Campbell, S. C. (2010). Every minute counts when the renal hilum is clamped during partial nephrectomy. *Eur. Urol.*, 2010, 58(3), 340-345. doi: 10.1016/j.eururo.2010.05.047
- [17] Tirapelli, L. F., Barione, D. F., Trazzi, B. F. M., Tirapelli, D. P. C., Novas, P. C., Silva, C. S. ... Martins, A. C. P. (2009). Comparison of two models for evaluation histopathology of experimental renal ischemia. *Transplant. Proc.*, 41, 4083-4087.
- [18] Verlander, J. W. (1998). Normal ultrastructure of the kidney and lower urinary tract. *Toxicol. Pathol.*, 26(1), 1-17. doi: 10.1177/019262339802600101

ЕЛЕКТРОННО-МІКРОСКОПІЧНА ДІАГНОСТИКА ПРОЦЕСІВ АПОПТОЗА ЗА УМОВ МОДЕЛЮВАННЯ В ЕКСПЕРИМЕНТІ ГОСТРОГО ПІЕЛОНЕФРИТУ ТА СУПУТНОГО ЦУКРОВОГО ДІАБЕТУ I ТА II ТИПІВ Борисов С.О. Костєв Ф.І. Борисов О.В. Молчанюк Н.І.

Як свідчать літературні дані, в умовах порушення балансу про- та протизапальних факторів розвиток інфекційно-запального процесу набуває тенденції до прогресування і більш важкого перебігу з подальшою трансформацією у хронічне запалення з відповідними морфофункціональними наслідками. За таких критичних умов вирішальну захисну роль, імовірно, відіграє апоптоз, котрий здатний усунути надмірне скупчення агресивних ефекторів запалення, їх ефективно елімінувати, що в подальшому попередить імовірну трансформацію у персистуючу форму. Метою роботи стало вивчення особливостей ранніх динамічних процесів апоптоза в тканинах нирок за умов моделювання в експерименті гострого піелонефриту та супутнього цукрового діабету I та II типів. Робота виконана на 300 дорослих щурах лінії Вістар, що були розподілені на 4 групи. Фрагменти нирок тварин вивчали і фотографували в електронному мікроскопі ПЕМ-100-01. Результати показали, що після моделювання у тварин піелонефриту в нефронах не було значних ультраструктурних змін. Структура подоцитів зовнішнього листка практично була аналогічною структурі подоцитів контрольного матеріалу, а в деяких клітинах спостерігались ознаки активації їх

метаболической активности. У структуре подоцитов внутреннего листка капсулы восстановлены дистрофические изменения внутренней мембраны части митохондрий. У мозговой речовині нирки структура клубочка была больше сохранена, чем у корковой. Патологические изменения подоцитов проксимальных и дистальных канальцев, а также интерстициальной ткани больше выражены, чем ниркового клубочка. За умов моделювання співдружньої моделі пієлонефриту і діабету I типу відбуваються більш виражені морфологічні зміни: деструктивно значно змінюються ендотеліальні клітини капілярів клубочка, відбувається гомогенізація структури базальної мембрани та значно розростається мезангіальна тканина. У нефронах і канальцях кіркової речовини зміни проявляються у значно більшій мірі, ніж у мозковій речовині нирки. При вивченні в клінічних умовах патогенетичних особливостей гострого пієлонефриту за умов супутнього цукрового діабету доцільним є проведення електронно-мікроскопічних досліджень з метою обрання оптимального коригуючого лікувального впливу та запобігання несприятливого перебігу інфекційно-запального процесу та його трансформації у персистуючу форму. ЕМ-дослідження є високоінформативним при дослідженні патологічних змін та ранніх динамічних процесів апоптозу в тканинах нирки при моделюванні гострого пієлонефриту та супутнього цукрового діабету I та II типів в умовах експерименту.

Ключові слова: моделювання, патогенез, пієлонефрит, цукровий діабет, електронна мікроскопія, кіркова та мозкова речовина, апоптоз.

ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКАЯ ДИАГНОСТИКА ПРОЦЕССОВ АПОПТОЗА В УСЛОВИЯХ МОДЕЛИРОВАНИЯ В ЭКСПЕРИМЕНТЕ ОСТРОГО ПИЕЛОНЕФРИТА И СОПУТСТВУЮЩЕГО САХАРНОГО ДИАБЕТА I И II ТИПОВ

Борисов С.А., Костев Ф.И., Борисов А.В. Молчанюк Н.И.

Согласно литературным данным, в условиях нарушения баланса про- и противовоспалительных факторов развитие инфекционно-воспалительного процесса приобретает тенденцию к прогрессированию и более тяжелого течения с дальнейшей трансформацией в хроническое воспаление с соответствующими морфофункциональными последствиями. В таких критических условиях решающую защитную роль, вероятно, сыграет апоптоз, который способен убрать чрезмерную скупченность агрессивных эффекторов воспаления, их эффективно элиминировать, что предупредит в дальнейшем возможную трансформацию в персистирующую форму. Целью работы стало изучение особенностей ранних динамических процессов апоптоза в тканях почек в условиях моделирования в эксперименте острого пиелонефрита и сопутствующего сахарного диабета I и II типов. Работа выполнена на 300 взрослых крысах линии Вистар, которые были распределены на 4 группы. Фрагменты почек животных изучали и фотографировали в электронном микроскопе ПЭМ-100-01. Результаты показали, что после моделирования у животных пиелонефрита в нефронах не было значительных ультраструктурных изменений. Структура подоцитов внешнего листа практически была аналогичной структуре подоцитов контрольного материала, а в некоторых клетках наблюдались признаки активации их метаболической активности. В структуре подоцитов внутреннего листка капсулы установлены дистрофические изменения внутренней мембраны части митохондрий. В мозговом веществе почки структура клубочка была более сохранной, чем в корковом. Патологические изменения подоцитов проксимальных и дистальных канальцев, а также интерстициальной ткани более выражены, чем почечных клубочков. В условиях моделирования содружественной модели пиелонефрита и диабета I типа происходят более выраженные морфологические изменения: деструктивно значительно изменяются эндотелиальные клетки капилляров клубочка, происходит гомогенизация структуры базальной мембраны и значительно разрастается мезангиальная ткань. В нефронах и канальцах коры изменения проявляются в значительно большей степени, чем в мозговом веществе почки. При изучении в клинических условиях патогенетических особенностей острого пиелонефрита в условиях сопутствующего сахарного диабета целесообразным является проведение электронно-микроскопических исследований с целью избрания оптимального корректирующего лечебного воздействия и предотвращения неблагоприятного течения инфекционно-воспалительного процесса и его трансформации в персистирующую форму. Электронно-микроскопическое исследование является высокоинформативным при исследовании патологических изменений и ранних динамических процессов апоптоза в тканях почки при моделировании острого пиелонефрита и сопутствующего сахарного диабета I и II типов в условиях эксперимента.

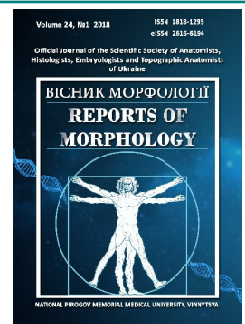
Ключевые слова: моделирование, патогенез, пиелонефрит, сахарный диабет, электронная микроскопия, корковое и мозговое вещество, апоптоз.



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Effect of the Forkal on histological changes of the rat's mandible bone tissue in the area of the traumatic defect at the pathology of the hepatobiliary system

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The leading place in the structure of general injuries and among facial injuries occupy fractures of the upper and lower jaws. An important factor in the occurrence of complications is the microbial factor, but the concomitant pathology, such as hepatobiliary diseases, is commonly associated with about 70% of the population. The purpose of the work was to study the features of reparative regeneration of the defect of the mandible in the pathology of the hepatobiliary system on the background of the administration of Forkal in the animal experiment. An experimental study was conducted on 100 white male Wistar rats weighing 240-270 g, aged from 5 to 6 months. During the work, the rats were divided into 5 groups: control (20 rats, in which histological changes of the bone tissue of the mandible of healthy rats with mandibular injury at the site of defect were investigated); experimental No. 1 (20 rats in which histological changes of mandibular bone tissue were studied at the site of defect at obstructive hepatitis, which was obtained by ligation and intersection of the common bile duct); experimental No. 2 (20 rats in which histological changes of bone tissue of the mandible were examined at the site of the defect in toxic hepatitis, which was obtained by administering per os four carbon monoxide); experimental No. 3 (20 rats in which histological changes of the bone tissue of the mandible were examined at the site of defect in obstructive hepatitis, which were obtained by ligation and intersection of the common bile duct, and then added Forkal); experimental No. 4 (20 rats in which histological changes in the bone tissue of the mandible of rats at the site of the defect in toxic hepatitis were studied, which was obtained by introducing per os four carbon monoxide, and subsequently added to Forkal). All rats were injured in the mandible with a standardized defect. In the future, we observed the histological signs of healing the area of the perforation defect. It was established that regeneration of the area of the defect of the mandible is worsening in the conditions of the experimental pathology of the hepatobiliary system: the rate of recovery of the specific volume of fibroreticular tissue during obstructive hepatitis is slowed down by 2.6 times and 3.4 times in the course of toxic hepatitis. With the use of Forkal in conditions of experimental pathology of the hepatobiliary system, the rate of regeneration of the defect site of the mandible improves and accelerates. Thus, in order to accelerate the rate of recovery of the specific volume of fibroreticular tissue for patients with mandible trauma and associated pathology of the hepatobiliary system, it is advisable to use Forkal.

Keywords: rat, maxillofacial area, perforated defect of mandible, regeneration, morphometric analysis, Forkal.

Introduction

An increase in the number of fractures of the mandible and facial injuries remains one of the topical issues of jaw-facial traumatology the problem of the etiopathogenesis of complications. Fractures of the upper and lower jaw occupy

a leading place among facial injuries and in the structure of general injury. Among lesions of the jaw-facial area they occur in 75-87% of cases [2, 9, 10, 15, 16, 17, 19, 20]. Injuries of jaw-facial area make up about 15-38% among all

hospitalized [2, 9, 20]. Along with the increase in the total number of injuries, an increase in the frequency of jaw-facial injuries and their complications is observed, which is especially relevant with a steady increase in the number of patients with concomitant pathology [1, 3, 5, 6, 7, 8, 11, 12, 19, 20]. An important factor in the occurrence of complications, of course, is the microbial factor, but the concomitant pathology plays an important role. Infection, above all, leads to the development of pathological local and general changes, which in the future causes the development of complications. Most commonly observed: bone marrow suppuration, posttraumatic osteomyelitis, false joint, pathological motion of bone fragments, secondary hemorrhage, post-traumatic sinusitis or neuritis, bite violation [2, 17, 9, 12, 15, 20].

Analyzing this problem one of the neglected factors should be considered - the presence of diseases of the hepatobiliary system, the pathology of which occurs in about 70% of the population [1, 3, 13, 17]. The marked problem, with a certain coincidence, may have a major impact on the healing conditions of soft tissue of the jaw-facial area and bones of the facial skeleton, especially in the case of posttraumatic complications.

Post traumatic regeneration of the bone wound occurs due to the ability of the living organism to restore the tissue, primarily through the formation of connective tissue matrix, resulting in the ossification of the previous tissue structure is restored [3, 4, 8, 18]. An important task of jaw-facial surgery to improve the conditions for regurgitation of jaw fractures is to prevent or reduce the risk of developing complications that may occur with fractures of the bones of the facial skeleton.

Healing of fractures of the mandible on the background of concomitant pathology depends on the functional state of the bone itself and the body as a whole. The trauma of the lower jaw triggers a mechanism that requires two components: the first one is cells that can proliferate and differentiate in the osteogenic direction, and secondly, cells that survive trauma and synthesize osteoinductive products. No reports of experimental studies of the features of regeneration of the tissues of the maxillofacial area in the background of the pathology of the liver were found.

The purpose of the study is an experimental study of the features of reparative regeneration of the defect of the mandible in the pathology of the hepatobiliary system on the background of receiving Forkal.

Materials and methods

An experimental study was carried out on 100 white male rats of the Vistar line aged from 5 to 6 months and weights in the range of 240-270 g. The animals were in the general diet, had free access to water, food and standard conditions of stay in cages of vivarium of National Pirogov Memorial Medical University, Vinnytsya.

A large number of models of fracture of the mandible in laboratory animals are known, but not all of these techniques

can satisfy the necessary objectification and standardization of the fracture, which will not allow an objective assessment [3, 8, 17, 18, 19]. In the experiment, we used the technique of creating a defect of the mandible of rats by surgical boron with a diameter of 1 mm, with a speed of rotation of up to 10000 turnovers per minute, which allowed to obtain a standard post-traumatic defect and objectively monitor the regeneration processes [17].

In the study, all the rats were divided into 5 groups:

control (20 rats) - examined the histological changes of the bone tissue of the mandible of healthy rats with trauma of the mandible at the site of defect;

experimental number 1 (20 rats) - examined the histological changes of the bone tissue of the mandible of rats with trauma of the mandible at the site of the defect in obstructive hepatitis, which were obtained by ligation and intersection of the common bile duct;

experimental number 2 (20 rats) - examined the histological changes in the mandibular bone tissue of rats with trauma of the mandible at the site of the defect in toxic hepatitis, which was obtained by introducing per os four carbon monoxide (CCl₄);

experimental number 3 (20 rats) - examined the histological changes of the bone tissue of the mandible of rats with trauma of the mandible at the site of the defect in obstructive hepatitis, obtained by ligation and intersection of the common bile duct, which were added on the day of jaw trauma and subsequent 2 weeks before with food Forkal (200 mg/kg of rat mass);

experimental number 4 (20 rats) - examined the histological changes of the bone tissue of the mandible of rats with mandibular trauma at the site of the defect in toxic hepatitis that was obtained by administering per os CCl₄, which were added on the day of jaw trauma and subsequent 2 weeks before with food Forkal (200 mg/kg of rat mass).

Forkal is a drug containing the active ingredient of calcitriol, which is an active metabolite of vitamin D₃, which affects the metabolism of calcium and stimulates the activity of osteoblasts of the bones of the skeleton. It is formed in the kidneys from its predecessor, 25-hydroxycholecalciferol. With normal functioning of the body 0.5-1 micrograms of calcitriol per day is produced. Forkal promotes the absorption of calcium in the distal intestinal tract, increases its reabsorption in the kidneys and enhances the mineralization of bones. Forkal suppresses parathyroid hormone secretion, reduces pain in the bones and muscles.

In the course of an experimental study, we established the features of regeneration of bone defects. Quantitative evaluation of histological changes was carried out using morphometric techniques. After the manufacture of histological preparations, 5 characteristics were taken into account: the specific volume of fibroreticular tissue in the center of bone regeneration (%), specific volume of blood vessels (%), specific volume of bone beams in the bone regeneration center (%), the number of osteoblasts in a certain area in the bone regeneration cell, specific volume

of the bone marrow in the bone regeneration cell (%). The research was conducted on 7, 14, 30, 60 days of the study.

The results of the experiment were processed using commonly used methods to determine the validity of differences by Student. The average arithmetic values of the studied parameters (M) and their relative average statistical errors ($\pm m$) were calculated using the package "Statistica 10".

Results

The data obtained in a result of the study is shown in Table 1. It was found that the specific volume of fibroreticular tissue at the center of bone regeneration on 7 day is dominant over other elements and is about 91.41%. The number of osteoblasts per unit area of bone beams is increased. The specific volume of blood vessels on the 7 day of the experiment in the center of regeneration of the bone reaches 3.502%, moreover these blood vessels located almost exclusively in the area of fibroreticular tissue. It is noteworthy that on 7 day there are no formed bone marrow elements, although in fibroreticular tissue there is a significant presence of cells, which according to morphology should be called lymphoid cells. They have circular shape, round nuclei and a narrow rim of the cytoplasm. Such cells can either be lymphocytes or stem (polyotent) cells, from which later elements of the bone marrow are formed.

In the analysis of the data presented in Table 1, it should be noted that the specific volume of fibroreticular tissue at the center of bone regeneration in the dynamics of the experiment without external influence decreases and for 60 day is 12.40 \pm 0.22%.

Bone beams become well visible when stained with hematoxylin and eosin, their specific volume up to 14 day increases by more than 5 times compared to 7 day of the experiment. By 30 day, the specific volume of beams is still increasing and at 60 day of the experiment is more than 72.0%, that is, bone beams in this period are already the bigger part of the elements of the zone of bone regeneration.

Data on the specific volume (%) of fibroreticular tissue in the center of bone regeneration of experimental animals in the ligation of the common bile duct in the dynamics of the experiment without the use of correctional agents are given in Table 1.

The analysis of the data showed that the specific volume of fibroreticular tissue in the bone regeneration center for 7 day is dominant over other elements and is more than 90%. It should be noted that the increased number of osteoblasts per unit area of bone beams. The analysis of the data presented in Table 1 shows that on the 60 day of the experiment, the specific volume of fibroreticular tissue in the bone regeneration center at the ligation of the common bile duct decreases, compared with 7 day, almost threefold. At the same time in the center of bone regeneration at the ligation of the common bile duct decreases the specific volume of blood vessels. At the 60 day of the experiment, the blood vessels were localized not only in the fibroreticular tissue, but also in the bone marrow that was already formed, although at the 30 day of the experiment, the formed bone marrow elements in the cell of regeneration of the bone were not found in the animals of the experimental group number 1.

Specific volume of bone beams up to 14 day of

Table 1. Morphometric indices of regenerative tissues of the mandible at the defect site in the dynamics of the experiment at the ligation of the common bile duct without correction and under conditions of correction by Forkal (n=60).

Morphometric indices	Research groups	Day of experiment			
		7	14	30	60
Specific volume (%) of fibroreticular tissue	Control	91.41 \pm 0.940	56.30 \pm 0.711	48.22 \pm 0.510	12.40 \pm 0.220
	experimental group №1	92.10 \pm 0.880	84.50 \pm 0.731*	74.63 \pm 0.580*	32.80 \pm 0.241*
	experimental group №3	92.21 \pm 0.831	76.40 \pm 0.701	64.00 \pm 0.511**	22.21 \pm 0.211**
Specific volume (%) of blood vessels	Control	3.211 \pm 0.051	2.012 \pm 0.051	1.613 \pm 0.051	0.521 \pm 0.011
	experimental group №1	3.712 \pm 0.081	3.411 \pm 0.090*	3.202 \pm 0.051*	1.701 \pm 0.021*
	experimental group №3	3.801 \pm 0.061	3.202 \pm 0.081	3.101 \pm 0.061	1.201 \pm 0.021**
Specific volume (%) of bone beams	Control	1.801 \pm 0.042	12.92 \pm 0.84	48.01 \pm 0.191	72.20 \pm 0.680
	experimental group №1	1.431 \pm 0.051	5.601 \pm 0.141*	14.81 \pm 0.160*	53.40 \pm 0.641*
	experimental group №3	1.521 \pm 0.061	6.912 \pm 0.511**	28.21 \pm 0.151**	59.82 \pm 0.611**
The average number of osteoblasts of bone beams in the bone regeneration center on an area of 100 μ m ²	Control	3.502 \pm 0.041	5.702 \pm 0.151	2.001 \pm 0.061	1.001 \pm 0.050
	experimental group №1	3.801 \pm 0.081	3.502 \pm 0.101*	3.301 \pm 0.061*	2.802 \pm 0.041*
	experimental group №3	3.701 \pm 0.091	4.501 \pm 0.121**	2.603 \pm 0.051**	1.101 \pm 0.021**
Specific volume (%) of bone marrow	Control	0	0	1.602 \pm 0.041	3.801 \pm 0.050
	experimental group №1	0	0	0	2.301 \pm 0.061*
	experimental group №3	0	0	0	2.703 \pm 0.041

Note: * - significantly relative to the control group ($p < 0.05$); ** - significantly relative to the experimental group №1 ($p < 0.05$).

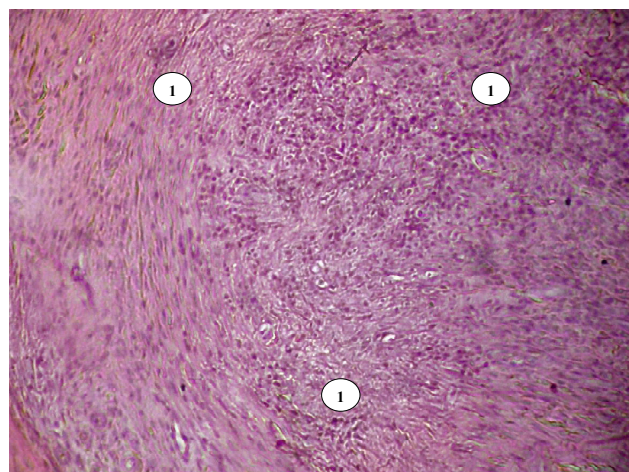


Fig. 1. Replacement by fibroreticular tissue (1) solid tissues of the rat's mandible at the site of its perforation when the ligation of the common bile duct done at the 7 day of the experiment. Experimental group №1. Hematoxylin-eosin. Lens x10, eyepiece x20.

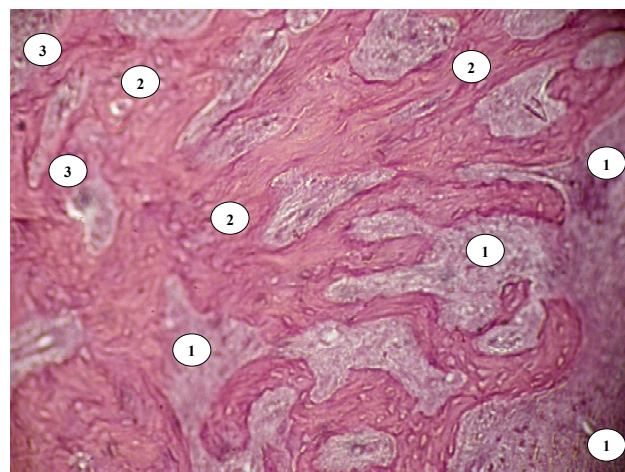


Fig. 2. Solid tissues of the rat's mandible at the site of its perforation when the ligation of the common bile duct done at the 60 day of the experiment. Experimental group №1. 1 - fibroreticular tissue. 2 - bone beams. 3- bone marrow. Hematoxylin-eosin. Lens x10, eyepiece x20.

experiment in rats of experimental group number 1, increases by more than 4 times, compared to 7 day, and up to 30 day of the experiment continues to increase and on 60 day is more than 50%, that is, bone beams in this period are already the bulk of the elements of the zone of bone regeneration (Fig. 1, 2).

The average data of morphometric indices of solid tissues of the mandible at the site of its defect in animals of the experimental group number 2 with toxic hepatitis in the dynamics of the experiment without drug correction are given in Table 2 and Fig. 3, 4.

Under conditions of correction of bone regeneration using a Forkal in the ligation of the common bile duct (experimental group 3), beginning from the 7 day of the experiment, including up to 60 day (Fig. 5, 6), the following changes, which in general can be regarded as acceleration of the rate of bone regeneration are noted (Table 1).

The use of Forkal in animals of the experimental group number 4 for the purpose of correction of bone regeneration in the zone of its defect in toxic hepatitis significantly changes the picture, compared with the indicators of experimental group number 2. Dynamics of healing at 30, 60 day is

Table 2. Morphometric indices of regenerative tissues of the mandible at the defect site in the dynamics of the experiment at toxic hepatitis without correction and under conditions of correction by Forkal (n=60).

Morphometric indices	Research groups	Day of experiment			
		7	14	30	60
Specific volume (%) of fibroreticular tissue	Control	91.43±0.941	56.32±0.711	48.21±0.511	12.40±0.220
	experimental group №2	92.01±0.842	88.70±0.761*	79.90±0.590*	41.01±0.500*
	experimental group №4	92.11±0.671	73.21±0.731**	61.21±0.521**	24.80±0.241**
Specific volume (%) of blood vessels	Control	3.201±0.050	2.001±0.050	1.603±0.051	0.501±0.011
	experimental group №2	3.201±0.081	3.003±0.080*	2.701±0.04*	2.001±0.030*
	experimental group №4	3.302±0.081	3.101±0.060	3.001±0.051	1.002±0.011**
Specific volume (%) of bone beams	Control	1.801±0.041	12.91±0.841	48.01±0.190	72.21±0.681
	experimental group №2	1.401±0.071	3.801±0.161*	11.32±0.121*	48.41±0.652*
	experimental group №4	1.402±0.061	6.402±0.560**	28.91±0.161**	62.81±0.620**
The average number of osteoblasts of bone beams in the bone regeneration center on an area of 100 μm ²	Control	3.511±0.041	5.711±0.151	2.001±0.0602	1.001±0.051
	experimental group №2	3.703±0.051	3.602±0.101*	3.502±0.072*	2.802±0.050*
	experimental group №4	3.501±0.080	4.603±0.140**	2.411±0.041**	1.001±0.020**
Specific volume (%) of bone marrow	Control	0	0	1.601±0.041	3.802±0.051
	experimental group №2	0	0	0	1.602±0.021*
	experimental group №4	0	0	0	2.411±0.051**

Note: * - significantly relative to the control group (p<0.05); ** - significantly relative to the experimental group №2 (p<0.05).

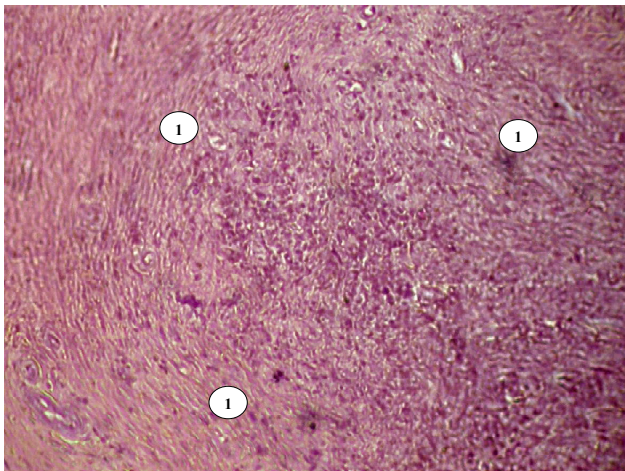


Fig. 3. Solid tissues of the rat's mandible at the site of its perforation with toxic hepatitis at the 7 day of the experiment. Experimental group №2. 1 - fibroreticular tissue. Hematoxylin-eosin. Lens x10, eyepiece x20.

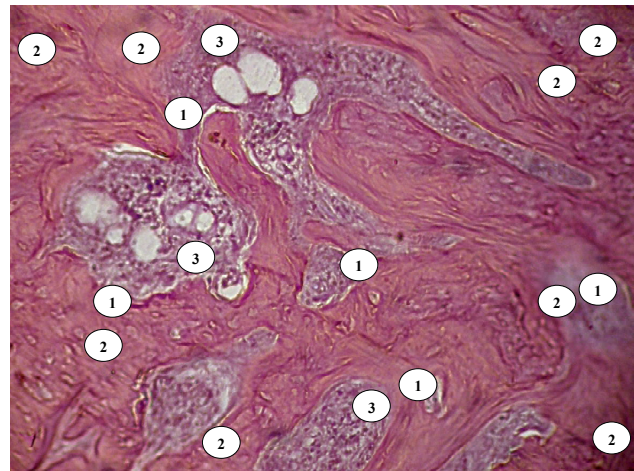


Fig. 4. Solid tissue of the rat's mandible at the site of its perforation with toxic hepatitis at the 60 day of the experiment. Experimental group №1. 1 - fibroreticular tissue. 2 - bone beams. 3- bone marrow. Hematoxylin-eosin. Lens x10, eyepiece x20.

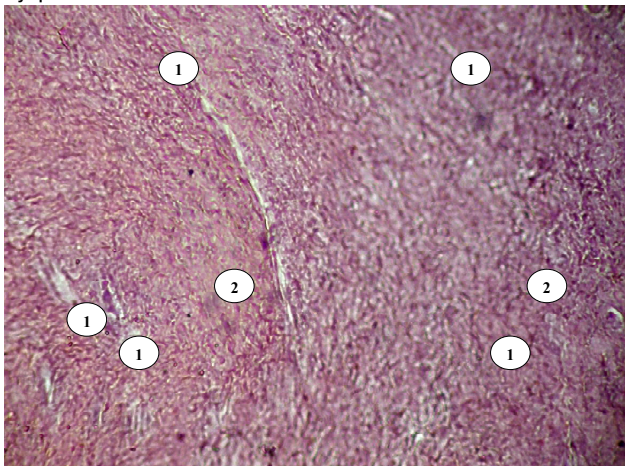


Fig. 5. Solid tissues of the rat's mandible at the site of its perforation during the ligation of the common bile duct on the 7 day of the experiment, under the conditions of the correction of Forkal, replaced by fibroreticular tissue - 1. Hematoxylin-eosin. Lens x10, eyepiece x20.

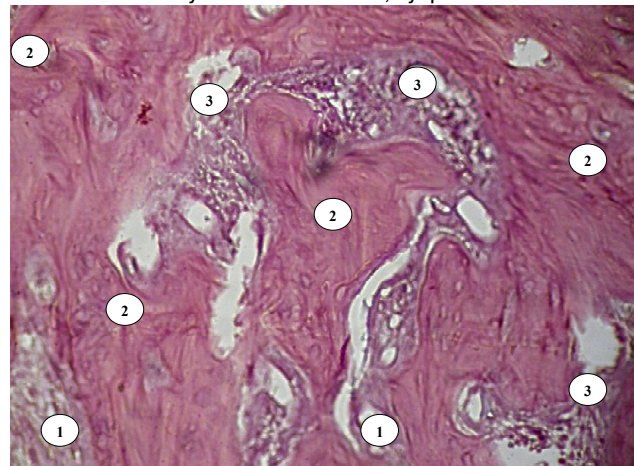


Fig. 6. Solid tissue of the rat's mandible at the site of its perforation when the bile duct is ligated on 60 day of the experiment under the conditions of the Forkal correction. 1 - fibroreticular tissue. 2 - bone beams. 3- bone marrow. Hematoxylin-eosin. Lens x10, eyepiece x20.

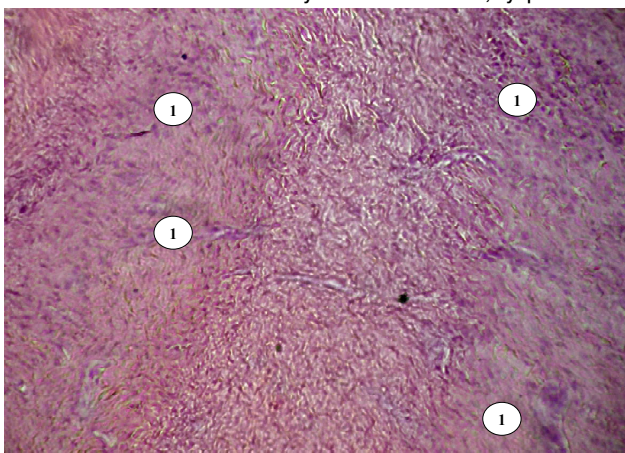


Fig. 7. Replacement by fibroreticular tissue (1) solid tissues of the rat's mandible at the site of its perforation with toxic hepatitis at the 7 day of the experiment under Forkal correction conditions. Hematoxylin-eosin. Lens x10, eyepiece x20.

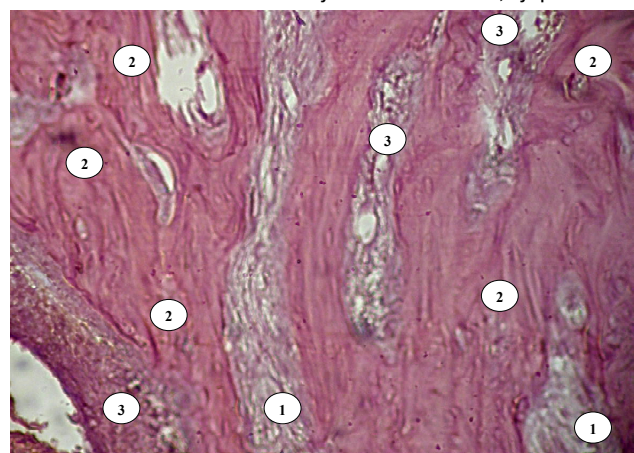


Fig. 8. Solid tissues of the rat's mandible at the site of its perforation with toxic hepatitis at the 60 day of the experiment under the conditions of Forkal correction. 1 - fibroreticular tissue. 2 - bone beams. 3- bone marrow. Hematoxylin-eosin. Lens x10, eyepiece x20

depicted in Fig. 7, 8. Morphometric indices obtained during the study of animals of the experimental group number 4 are given in Table. 2.

Discussion

Taking into account the obtained indicators, the presence of individual bone beams on the 7 day of the study is due, of course, not to their new creation, but to the remnants of the former bone beams that were previously located on the site of the traumatic defect before the injury. A large number of osteoblasts in the bone beams indicates that the old bone beams also participate in bone regeneration processes at the defect site, which coincides with the point of view of Davydenko I.S. [4].

Along with this, in the dynamics of the experiment in the cell of regeneration of the bone decreases the specific volume of blood vessels. It should be noted that in the control group rats up to 60 day these blood vessels are located not only in the fibroreticulum layer, but also in the bone marrow, which is already present up to 30 day and is even more pronounced and formed at 60 day.

The number of osteoblasts of bone beams in the bone regeneration center at an area of $100 \mu\text{m}^2$ decreases at a relatively slow pace. It should be noted that at 14 day (compared to the 7 day of the experiment) there is no probable decrease in the average number of osteoblasts of bone beams in the center of bone regeneration per unit area ($p > 0.05$), but there is only a tendency to decrease, but for 30 day, compared to 7 day, changes are already statistically significant ($p < 0.05$). A similar picture can be noticed for 60 day of the experiment, when the average number of osteoblasts of bone beams in the center of bone regeneration in the area has the smallest value. In animals of the control group, explicit bone marrow elements are present only in the histological materials of the fracture site of the mandible in rats at 30 and 60 day of the experiment. It should be noted that in the bone marrow dominate hematopoietic elements at different stages of development with domination of lymphoid-type cells, single thin-walled slit-like blood vessels and individual lymphocytes. Analyzing the obtained parameters, the high activity of the regenerative elements involved in the construction of a new bone tissue and the replacement of the bone defect was found to be quite high.

The increased concentration of osteoblasts per unit area of bone beams in bone beams shows that old bone beams also participate in bone regeneration processes at the defect site, similar to data of Tashchyan A.Ye. with co-authors [19]. The specific volume of blood vessels on the 7 day of the experiment in the center of regeneration of the bone does not reach even 4%, and these blood vessels are located almost exclusively in the area of fibroreticular tissue. It is noteworthy that at 7 day, in rats of the first experimental group, with obstructive hepatitis, which was obtained by ligation and intersection of the common bile duct, there are no observed bone marrow elements, although the

fibroreticular tissue is marked by the presence of cells that, according to morphology, should called lymphoid cells. It is precisely from them that the elements of the bone marrow are formed in the future.

In rats of experimental group number 1, the average number of osteoblasts of bone beams in the center of bone regeneration on an area of $100 \mu\text{m}^2$ decreases at a slow pace. At the 14 day, compared to the 7 day of the experiment, there was no probable decrease in the average number of osteoblasts of bone beams in the center of bone regeneration per unit area ($p > 0.05$), except that there was only a tendency to decrease, but at 30 day, compared with 7 day, changes, though not expressed, but were statistically significant ($p < 0.05$). At the 60 day of the experiment, the average number of osteoblasts of bone beams in the bone regeneration center per unit area has the smallest value among all periods of the experiment. In the conditions of ligation of the common bile duct in the experiment, the presence of bone marrow elements was determined only in histological materials of rats for 60 day. It is noteworthy that in the bone marrow the hematopoietic elements were dominated at different stages of development with the dominance of lymphoid-type cells, isolated thin-walled slit-like blood vessels and individual lymphocytes.

In toxic hepatitis, in the dynamics of the experiment without medication correction, the indicators reflect the dynamics of changes characterizing regenerative processes in the bone, and the dynamics in general is very similar to that described for the experiment with the ligation of the common bile duct. The difference is that in the course of time the specific volume of fibroreticular tissue and blood vessels decreases gradually, the average number of osteoblasts of bone beams decreases in the bone regeneration center per unit area, but, at the same time, the specific volume of bone beams increases. In toxic hepatitis, the rate of decrease in the specific volume of fibroreticular tissue is less than in the ligation of the common bile duct, while at the end point of the experiment (60 day), this figure remains significantly higher. Concerning the specific volume of blood vessels, the situation was similar. The above changes confirm Levitsky A.P. viewpoint [6] on the importance of the influence of the hepatobiliary system on the functioning of the organism as a whole and Levitsky A.P. with co-authors [8] on the teeth-jaw system.

In the analysis of the obtained indicators, it is noticeable that the specific volume of bone beams, on the contrary, is greater in the presence of toxic hepatitis than when the ligation of the common bile duct at each point of the experiment, including 60 day. However, with toxic hepatitis, the average number of osteoblasts of bone beams in the center of bone regeneration per unit area shows a similar dynamics, as in the ligation of the common bile duct. Statistically significant differences have not been established.

It is important to note that the specific volume of bone marrow at the 60 day of the experiment with toxic hepatitis is on average less than that of the common bile duct ligation.

The dynamics of regenerative processes in rats' bones, both in toxic and in obstructive hepatitis, were similar, but differed in the rate of decrease in the specific volume of fibroreticular tissue in toxic hepatitis, which confirms the negative influence of hepatobiliary system disorders on the exchange of calcium and vitamin D and coincides with the data of Moroz L.V. with co-authors [14].

In particular, on the 14 day of the experiment, the specific volume of fibroreticular tissue in the bone regeneration center, when corrected by the Forkal, in the rats of experimental group 3, on the average, is significantly lower ($p < 0.05$) than in the experiment without the use of correctional drugs. This pattern persists until the end of the experiment, that is, up to 60 day. That is, with the use of a Forkal, a more rapid decrease in the specific volume of bone precursor tissue is observed.

At the same time, regarding the specific volume of blood vessels, it cannot be asserted about the rate of growth and decrease of this indicator, up to the 60 day of the experiment with the use of Forkal. But the specific volume of bone beams in the application of Forkal compared to the experiment without regeneration correction, although it begins to grow from the 14 day, but the greatest effect from the action of Forkal is marked on the 30 day of the experiment and lasted until 60 day of the experiment.

It should be noted that the use of Forkal at the ligation of the common bile duct did not lead to earlier formation of bone marrow elements, although the specific volume of bone marrow at 60 day of the experiment with the use of a Forkal is slightly higher than without the use of correctional agents.

So, in general, it can be stated that on the 60 day of the experiment, when the ligation of the common bile duct was applied, the use of a Forkal led to a more profound maturation of the bone tissue at the place of perforation than without correction.

A clearly expressed regularity is noted with respect to the number of osteoblasts of bone beams in the bone regeneration center with the use of a Forkal at the ligation of the common bile duct. In particular, the number of these cells on the 14 day does not decrease, but on the contrary increases, and only later stimulation of osteoblast growth (at 30 and 60 day) is slowing down. This may mean that the use of a Forkal in the ligation of the common bile duct can stimulate the proliferation of osteoblasts of bone beams in the center of bone regeneration, which is likely to largely explain the increase in the specific volume of bone beams themselves at the 14 day of the experiment, although the number of osteoblasts at 30 and 60 days of the experiment is reduced, which positively characterizes the processes of maturation of the bone beams themselves.

References

- [1] Avrunin, A. S., Kornilov, N. V., & Ioffe, I. D. (2001). Adaptive mechanisms of bone tissue and the regulatory and metabolic profile of the body. *Morphology*, 6, 7-12.
 [2] Bernadsky, Yu. I. (1999). *Traumatology and reconstructive*

Comparing with the data of the control group, the specific volume of bone beams and bone marrow at 60 day is significantly higher in the experimental group 3 animals at the correction of the Forkal.

When comparing bone regeneration in the model of obstructive and toxic hepatitis without treatment, it was found that up to 60 day, the proportion of bone beams and bone marrow is significantly higher in the group of obstructive hepatitis.

In particular, starting from the 14 day, at toxic hepatitis more rapidly decreases the specific volume of fibroreticular tissue and this pattern is maintained until the end of the experiment, moreover on the 60 day the effect becoming particularly pronounced. At the same time, the effect of Forkal on the specific volume of blood vessels in toxic hepatitis becomes noticeable only on 60 day.

But the effect of Forkal on increasing the specific volume of bone beams in toxic hepatitis is already observed on 14 day. At the same time, this effect is felt until the end of the experiment.

The average number of osteoblasts of bone beams in the center of bone regeneration per unit area during toxic hepatitis temporarily increases by 14 day, and subsequently decreases, similarly to the ligation of the common bile duct. It is important that Forkal at toxic hepatitis on 60 day increases the specific volume of bone marrow, which accelerates healing.

Thus, the use of a Forkal for correction of regenerative processes in toxic hepatitis is sufficiently effective, the rate of regeneration is increasing, and at the end of the experiment (at 60 day), the bone tissue in the place of the former defect looks more mature than in the experiment without correction.

In the future, we consider it expedient to continue the study of the effect of therapeutic agents on regeneration of the defect of the mandible in the pathology of the hepatobiliary system.

Conclusions

1. Experimental pathology of the hepatobiliary system negatively affects the regeneration of the segment of the defect of the mandible, which is manifested by a decrease in the rate of recovery of the specific volume (%) of fibroreticular tissue by 2.6 times at obstructive hepatitis and toxic hepatitis - by 3.4 times.
2. With the experimental pathology of the hepatobiliary system, the regeneration of the area of the defect of the mandible is improved and accelerated with the use of Forkal.
3. In patients with injuries of the mandible when identifying the pathology of the hepatobiliary system, it is advisable to use Forkal.

surgery of the maxillofacial region. M.: Medical literature.

- [3] Volozhin, A. I., Gemonov, V. V., Kabaloeva, D. V., & Surazhev, B. Yu. (2010). Experimental substantiation of the effectiveness of the use of recombinant epidermal growth factor

- in the treatment of wounds of the oral mucosa in the immunodeficient state. *Dentistry*, 89(4), 4-6.
- [4] Davydenko, I. S. (2000). Semi-automatic quantitative computer analysis of microscopic image in histopathology. *Bukovinsky medical bulletin*, 4(2), 165-169.
- [5] Korzh, N. A., & Dedukh, N. V. (2006). Reparative bone regeneration: a modern view of the problem. The stages of regeneration. *Orthopedics, traumatology and prosthetics*, 1, 76-84.
- [6] Levitsky, A. P. (2003). Adaptation-trophic systems of the body and their role in pathology. *Herald of Dentistry*, 1, 91-95.
- [7] Levitsky, A. P. (2007). Functional classification of adaptogens. *Bulletin of Pharmacology and Pharmacy*, 2, 32-36.
- [8] Levitsky, A. P., Demyanenko, S. A., Skidan, M. I. & Pustovoi, P. I. (2013). Efficiency of treatment of chronic catarrhal gingivitis in patients with hepatobiliary pathology using hepatoprotector and prebiotic. *Innovations in dentistry*, 2, 5-9.
- [9] Malanchuk, V. O., Volovar, O. S., & Garliauskaite, I. Yu. (2011). *Surgical dentistry and maxillofacial surgery*, 1. K.: Logos.
- [10] Malanchuk, V. O., Logvinenko, I. P., Malanchuk, T. O., & Tsilenko, O. L. (2011). *Surgical dentistry and maxillofacial surgery*, 2. K.: Logos.
- [11] Meerson, F. Z. (1993). *Adaptive Medicine: Mechanisms and protective effects of adaptation*. M.: Hyp. Med. Ltd.
- [12] Mirchuk, B. M., Dragomiretskaya, M. S., Money, O. V., & Levitsky, A. P. (2007). Application of photoadaptogens in dentistry (review of literature). *Herald of Dentistry*, 2, 62-66.
- [13] Moroz, L. V. (2002). *Chronic viral hepatitis B and C. Prevalence, clinical morphological parallels*. (Dissertation medical doctor.) Institute of Epidemiology and Infectious Diseases L.V.Gromashevsky AMS of Ukraine, Kyiv.
- [14] Moroz, L. V., Musayev, Y. Yongibar Ogly, Zaichko, N. V., Androsova, O. S., & Gaiduk, O. A. (2016). Influence of vitamin D on the results of treatment of chronic hepatitis C. *Hepatology*, 4, 42-50.
- [15] Polishchuk, S. S. (2005). Correction of psychoemotional condition in patients with injuries of maxillofacial area. *Herald of Dentistry*, 1, 50-56.
- [16] Polishchuk, S. S. (2016). Experimental study of the effect of quartulin on the healing of traumatic damages of the mandible. *Herald of Dentistry*, 3, 17-22.
- [17] Polishchuk, S. S., Skiba, V. Ya., Levytsky, A. P., & Shuvalov, S. M. (2015). Experimental study of the effect of quartulin on the healing of traumatic lesions of the mucous membrane of the rat's cavity during violation of the function of the hepatobiliary tract. *Innovations in dentistry*, 4, 9-16.
- [18] Skiba, V. Ya., Kosenko, K. M., Levitsky, A. P., & Skyba, O. I. (2002). *Pre-clinical study of means for the treatment and prevention of mucosal diseases of the oral cavity*. Kyiv: State Pharmacological Center of the Ministry of Health of Ukraine.]
- [19] Tashchyan, A. E., Gulyuk, A. G., & Makarenko, O. A. (2012). Stimulation of mineralization and collagen formation in the bone tissue of rats with fractures of the mandible with the drug "osteovitis". *Herald of Dentistry*, 3, 5-8.
- [20] Timofeev, O. O. (2011). *Maxillofacial surgery*. K.: VSV "Medicine". ISBN: 978-617-505-575-5
- [21] Huttunen, T., Kannus, P., Pihlajamki, H. & Mattila, V.M. (2014). Pertrochanteric fracture of the femur in the Finnish National Hospital Discharge Register: validity of procedural coding, external cause for injury and diagnosis. *BMC Musculoskeletal Disorders*, 15(98), 98-102. doi:10.1186/1471-2474-15-98.
- [22] Liu, D., Jiang, F., Shan, Z., Wang, B., Wang, J., Lai, Y. ... Teng, W. (2010). A cross-sectional survey of relationship between serum OOA level and blood pressure. *J. Hum. Hypertens.*, 24(2), 134-138. doi: 10.1038/jhh.2009.44.
- [23] Monzani, F., Caraccio, N., Kozakowa, M., Dardano, A., Vittone, F., Viridis, A., ... & Ferrannini, E. (2004). Effect of levothyroxine replacement on lipid profile and intima-media thickness in subclinical hypothyroidism: a doubleblind, placebo-controlled study. *J. Clin. Endocrinol. Metab.*, 89(5), 2099-106. doi: 10.1210/jc.2003-031669.
- [24] O'Malley, N. T., Deeb, A. & Bingham, K. W. (2012). Outcome of the dynamic helical hip screw system for intertrochanteric hip fractures in the elderly patients. *Geriatric Orthopaedic Surgery & Rehabilitation*, 3(2), 68-73. doi:10.1177/2151458512450707.
- [25] Radosavljevic, N., Nikolic, D., Lazovic, M., & Jeremic, A. (2014). Hip fractures in a geriatric population - rehabilitation based on patients needs. *Aging and Disease*, 5(3), 177-182. doi:10.14336/AD.2014.050017.
- [26] Razvi, S., Ingoe, L., Keeka, G., Oates, C., McMillan, C. & Weaver, J.U. (2007). The beneficial effect of Lthyroxine on cardiovascular risk factors, endothelial function, and quality of life in subclinical hypothyroidism: randomized, crossover trial. *J. Clin. Endocrinol. Metab.*, 92(5), 1715-1723. doi: 10.1210/jc.2006-1869.
- [27] Razvi, S., Weaver, J. U., Butler, T. J. & Pearce, S. H. (2012). Levothyroxine treatment of subclinical hypothyroidism, fatal and nonfatal cardiovascular events, and mortality. *Arch. Intern. Med.*, 172(10), 811-817. doi: 10.1001/archinternmed.2012.1159.
- [28] Rodondi, N., den Elzen, W. P., Bauer, D. C., Cappola, A. R., Razvi, S., Walsh, J. P. ... Gusssekloo, J. (2010). Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *JAMA*, 304, 2998-3007. doi: 10.1001/jama.2010.1361.
- [29] Zhou, Z., Tian, S., Zhang, X. & Wu, Y. (2012). Minimally invasive versus conventional dynamic hip screw for the treatment to intertrochanteric fractures in older patients. *Orthopedics*, 35(2), 244-249. doi:10.3928/01477447-20120123-42.

ВПЛИВ ФОРКАЛУ НА ГІСТОЛОГІЧНІ ЗМІНИ КІСТКОВОЇ ТКАНИНИ НИЖНЬОЇ ЩЕЛЕПИ ЩУРІВ У ДІЛЯНЦІ ТРАВМАТИЧНОГО ДЕФЕКТУ ПРИ ПАТОЛОГІЇ ГЕПАТОБІЛІРНОЇ СИСТЕМИ

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Провідне місце в структурі загального травматизму та серед травм обличчя займають переломи верхньої та нижньої щелеп. Важливим чинником виникнення ускладнень є мікробний фактор, але при цьому важливе місце займає супутня патологія, наприклад захворювання гепатобілірної системи, патологія котрої зустрічається близько у 70% населення. Метою роботи стало вивчення в експерименті на тваринах особливостей репаративної регенерації дефекту нижньої щелепи при патології гепатобілірної системи на фоні прийому Форкалу. Експериментальне дослідження було проведено на 100 білих щурах-самцях лінії Вістар масою 240-270 г, віком від 5 до 6 місяців. У процесі роботи щурі були поділені на 5 груп: контрольна (20 щурів, у котрих досліджували гістологічні зміни кісткової тканини нижньої щелепи здорових щурів з травмою

нижньої щелепи у місці нанесення дефекту; дослідна №1 (20 щурів, у яких досліджували гістологічні зміни кісткової тканини нижньої щелепи щурів у місці нанесення дефекту при obturаційному гепатиті, котрий отримували шляхом перев'язки та пересічення загального жовчного протоку; дослідна №2 (20 щурів, у яких досліджували гістологічні зміни кісткової тканини нижньої щелепи щурів у місці нанесення дефекту при токсичному гепатиті, котрий отримували шляхом введення *per os* чотирьох хлористого вуглецю; дослідна №3 (20 щурів, у яких досліджували гістологічні зміни кісткової тканини нижньої щелепи щурів у місці нанесення дефекту при obturаційному гепатиті, котрий отримували шляхом перев'язки та пересічення загального жовчного протоку, а потім додавали Форкал; дослідна №4 (20 щурів, у яких досліджували гістологічні зміни кісткової тканини нижньої щелепи щурів у місці нанесення дефекту при токсичному гепатиті, котрий отримували шляхом введення *per os* чотирьох хлористого вуглецю, а у подальшому додавали Форкал. Всім щурам наносили травму нижньої щелепи з утворенням стандартизованого дефекту. У подальшому спостерігали за гістологічними ознаками загоєнням ділянки перфораційного дефекту. Встановлено, що регенерація ділянки дефекту нижньої щелепи погіршується в умовах експериментальної патології гепатобіліарної системи: швидкість відновлення питомого об'єму фібронетикулярної тканини при obturаційному гепатиті уповільнюється в 2,6 рази та в 3,4 рази в умовах токсичного гепатиту. При застосуванні Форкалу в умовах експериментальної патології гепатобіліарної системи швидкість регенерації ділянки дефекту нижньої щелепи покращується та прискорюється. Таким чином, з метою прискорення швидкості відновлення питомого об'єму фібронетикулярної тканини для пацієнтів із травмою нижньої щелепи та супутньою патологією гепатобіліарної системи доцільно використовувати Форкал.

Ключові слова: щур, щелепно-лицева ділянка, перфораційний дефект нижньої щелепи, регенерація, морфометричний аналіз, Форкал.

ВЛИЯНИЕ ФОРКАЛА НА ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ КОСТНОЙ ТКАНИ НИЖНЕЙ ЧЕЛЮСТИ КРЫС В ОБЛАСТИ ТРАВМАТИЧЕСКОГО ДЕФЕКТА ПРИ ПАТОЛОГИИ ГЕПАТОБИЛИАРНОЙ СИСТЕМЫ

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Ведущее место в структуре общего травматизма и среди травм лица занимают переломы верхней и нижней челюстей. Важным фактором возникновения осложнений является микробный фактор, но при этом важное место занимает сопутствующая патология, например заболевания гепато-билиарной системы, патология которой встречается около 70% населения. Целью работы стало изучение в эксперименте на животных особенностей репаративной регенерации дефекта нижней челюсти при патологии пищеварительной системы на фоне приема Форкала. Экспериментальное исследование было проведено на 100 белых крысах-самцах линии Вистар массой 240-270 г, в возрасте от 5 до 6 месяцев. В процессе работы крысы были разделены на 5 групп: контрольная (20 крыс, у которых исследовали гистологические изменения костной ткани нижней челюсти здоровых крыс с травмой нижней челюсти в месте нанесения дефекта; исследовательская №1 (20 крыс, у которых исследовали гистологические изменения костной ткани нижней челюсти крыс в месте нанесения дефекта при obturационном гепатите, который получали путем перевязки и пересечения общего желчного протока; исследовательская №2 (20 крыс, у которых исследовали гистологические изменения костной ткани нижней челюсти крыс в месте нанесения дефекта при токсическом гепатите, который получали путем введения *per os* четырех хлористого углерода; исследовательская №3 (20 крыс, у которых исследовали гистологические изменения костной ткани нижней челюсти крыс в месте нанесения дефекта при obturационном гепатите, который получали путем перевязки и пересечения общего желчного протока, а затем добавляли Форкал; исследовательская №4 (20 крыс, у которых исследовали гистологические изменения костной ткани нижней челюсти крыс в месте нанесения дефекта при токсическом гепатите, который получали путем введения *per os* четыреххлористого углерода, а в дальнейшем добавляли Форкал. Всем крысам наносили травмы нижней челюсти с образованием стандартизованного дефекта. В дальнейшем наблюдали за гистологическими признаками заживлением участка перфорационного дефекта. Установлено, что регенерация участка дефекта нижней челюсти ухудшается в условиях экспериментальной патологии гепато-билиарной системы: скорость восстановления удельного объема фибронетикулярной ткани при obturационном гепатите замедляется в 2,6 раза и в 3,4 раза в условиях токсического гепатита. При применении Форкала в условиях экспериментальной патологии гепато-билиарной системы скорость регенерации участка дефекта нижней челюсти улучшается и ускоряется. Таким образом, с целью ускорения скорости восстановления удельного объема фибронетикулярной ткани у пациентов с травмой нижней челюсти и сопутствующей патологией гепато-билиарной системы целесообразно использовать Форкал.

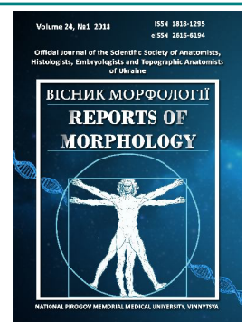
Ключевые слова: крыса, челюстно-лицевая область, перфорационный дефект нижней челюсти, регенерація, морфометрический анализ, Форкал.



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Regression models of individual linear sizes of molars depending on the features of cephalometric indices in practically healthy men of the Western and Eastern regions of Ukraine

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Restoration of missing teeth should be done as close as possible to the natural anatomical features of the person's tooth-jaw apparatus. That is why the problem of aesthetics in dentistry has ceased to be only orthopedic and has shifted towards a constitutional and ethnic odontology. The purpose of the study is to construct and carry out analyzes obtained by regression analysis, models of computed-tomographic size of molars, depending on the features of cephalometric indices of practically healthy men of the Western and Eastern regions of Ukraine. A computed-tomographic study of molars with the following odontometry and cephalometry of 36 practically healthy men of the Western region of Ukraine - residents of Volyn, Rivne, Lviv, Chernivtsi, Ternopil, Khmelnytskyi, Transcarpathian and Ivano-Frankivsk regions and 35 men of the Eastern region of Ukraine - residents of Kharkiv, Donetsk and Luhansk regions was performed with the help of the Dental Cone-ray Tomography Verviewepocs-3D (Morita, Japan). Regression models of linear sizes of molars, depending on cephalometric indices, were conducted using the statistical software package Statistica 6. 1. As a result of the conducted research, reliable models of computed-tomographic size of the upper and lower jaws molars were constructed in practically healthy men of the Western [7 models of mesio-distal dimensions ($R^2 = 0.532-0.646$), most of which include sagittal arc (12.5%), outer-eye width, nose depth, distance between nasion and inter-cutter point, body length of the lower jaw on the left, largest head circumference and maximum head length (by 7.5%); 6 models of vestibular-tongue dimensions ($R^2 = 0.527-0.646$), which most often include nasal depth (20.0%), body length of the lower jaw to the left (16.0%), physiological face length and morphological face length (by 12.0%); 1 model of crown height ($R^2 = 0.579$)] as well as Eastern [3 models of crown height ($R^2 = 0.538-0.682$), which most often include nose depth (15.0%), face type, physiological face length, morphological face length, smallest width head and sagittal arc (by 10.0%); 2 models of mesio-distal sizes ($R^2 = 0.572$ and 0.556), which most often include inter eye fossa width (15.4%)] regions of Ukraine. Thus, in practically healthy men from the Western and Eastern administrative-territorial regions of Ukraine, based on the specifics of cephalometric indicators and face type, reliable regression models (with determination coefficient R^2 greater than 0.5) of computed-tomographic size of molars of the upper and lower jaws (out of 40 possible 14 for the western and 5 for the eastern regions of Ukraine).

Keywords: regression analysis, dimensions of molars, computed-tomography, cephalometry, practically healthy men, Western and Eastern regions of Ukraine.

Introduction

One of the main issues of orthodontics is the prevention and correction of the wrong bite, as well as other tooth-jaw anomalies. The current level of orthodontic development allows to restore the form and function of the tooth-jaw system by various methods [9, 10, 21]. The progress of dentistry,

the emergence of new technologies, materials, methods of treatment gradually formed a new direction - aesthetic dentistry. When working, you must strive not only to close the defect and deprive the patient of pain, but also to reproduce the natural beauty of the teeth [3, 7, 8, 26].

In order to achieve stable and guaranteed clinical success, a comprehensive approach is needed. That is, the problem of returning the natural form of teeth does not belong to the category of exclusively aesthetic. Restoration of missing teeth should be done as close as possible to the natural anatomical features of the person's tooth-jaw apparatus. That is why the problem of aesthetics in dentistry has ceased to be only orthopedic and has shifted towards the constitutional and ethnic odontology [13, 15, 17, 19, 22].

The process of competent modeling of anatomical forms leads to the fact that newly created designs from restoration materials are harmoniously combined with craniofacial structures. The work on predicting the size of the teeth - this is a daily task facing the dentist, but it was solved earlier, as a rule, intuitive [5, 14, 27, 28, 30].

Previously, methods for modeling the teeth parameters were based mainly on simplified models with numerous assumptions, but now it is important to simulate the size of the teeth, which requires deep knowledge of anatomy and, in particular, the consideration of individual dimensional features of the tooth-jaw and craniofacial system [2, 4, 6, 11, 12].

The purpose of the study is to construct and carry out analyzes obtained by regression analysis, models of computed-tomographic size of molars, depending on the features of cephalometric indices of practically healthy men of the Western and Eastern regions of Ukraine.

Materials and methods

On the basis of the medical center "WinIntermed LTD", for 200 somatologically healthy men aged from 19 to 35 years from different administrative regions of the regions of Ukraine done a cone-ray computer tomography using the Veraviewepocs-3D Dental Cone-ray Tomography (Morita, Japan). Among them: the western region - 36 inhabitants of Volyn, Rivne, Lviv, Chernivtsi, Ternopil, Khmelnytsky, Transcarpathian and Ivano-Frankivsk regions; Eastern region - 35 inhabitants of Kharkiv, Donetsk and Luhansk regions. Bioethics Committee of National Pirogov Memorial Medical University, Vinnitsya found that the studies fully met ethical and moral-legal requirements in accordance with the order of the Ministry of Health of Ukraine No. 281 of November 1, 2000 and do not contradict the basic bioethical norms of the Helsinki Declaration, the Council of Europe Convention Human Rights and Biomedicine (1977).

On cone-ray computed-tomograms of molars of the upper and lower jaws, measurements were made: height of the crown of the corresponding tooth; vestibular-tongue dimensions of the crown and neck of the tooth; mesio-distal dimensions of the crown and neck of the corresponding tooth [20].

A cephalometric study was conducted taking into account the generally accepted recommendations and anatomical points [1] with the help of a large sliding compass with a scale of the real size of the Martin system and a soft centimeter ribbon. The shape of the head was determined according to the following formula [30]: $EU_EU / G_OP \times 100$. Up to 75.9,

men belonged to dolichocephals; 76.0-80.9 - to mesocephals; 81.0-85.4 - for brachycephals; 85.5 and more - to hyperbrachycephals. The value of the face index (Garson morphological index) was obtained by the corresponding formula [18]: $N_GN / ZY_ZY \times 100$. Up to 78.9 men belonged to the group with a very wide face; 79.0-83.9 - with a wide face; 84.0-87.9 - with middle face; 88.0-92.9 - with a narrow face; 93.0 and more - with a very narrow face.

For the determination of computed-tomographic linear dimensions of molars, according to the features of cephalometric indices, craniotype and the type of the face of practically healthy men from the Western and Eastern regions of Ukraine, a direct stepwise regression analysis was conducted using the licensed statistical software package "Statistica 6.1". For the analysis of the obtained results, only models in which the determination coefficient (R^2) was not less than 0.50 and the value of the F-criterion was not less than 2.5 were taken into account.

Results

As a result of the regression analysis, the following reliable models of computed-tomographic size of molars were constructed, depending on the features of cephalometric indices, craniotype and the face type of practically healthy men from the Western and Eastern regions of Ukraine:

mesio-distal size of the crown of the upper right second molar (western region) = $4.492 + 0.368 \times FMT_FMT + 0.909 \times N_PRN - 0.197 \times DUGS_GOP + 0.603 \times EK_EK - 0.137 \times TIP_LICA - 0.800 \times N_SN$ ($R^2=0.646$; $F_{(6,29)}=8.82$; $p<0.001$; Error of estimate=0.468);

height of the crown of the upper right first molar (western region) = $-0.540 + 2.101 \times N_STO + 0.317 \times DUG_AUAU - 1.628 \times AL_AL + 1.233 \times CHI_CHI - 0.455 \times EU_EU - 0.082 \times N_PRN$ ($R^2=0.579$; $F_{(6,29)}=6.65$; $p<0.001$; Error of estimate=0.874);

mesio-distal size of the crown of the upper left second molar (western region) = $2.934 + 0.308 \times RGO_GN + 0.930 \times SN_PRN - 0.254 \times DUGS_GOP + 0.216 \times TR_GN + 0.093 \times N_SN + 0.485 \times EK_EK$ ($R^2=0.615$; $F_{(6,29)}=7.72$; $p<0.001$; Error of estimate=0.479);

mesio-distal crown size of the lower left second molar (western region) = $7.787 + 0.445 \times N_STO + 0.607 \times SN_PRN + 0.341 \times TIP_LICA - 0.297 \times ZM_ZM + 0.466 \times TR_N + 0.604 \times N_I + 0.819 \times AL_AL - 0.125 \times DUG_GOP$ ($R^2=0.646$; $F_{(6,17)}=8.27$; $p<0.001$; Error of estimate=0.413);

mesio-distal size of the neck of the lower left second molar (western region) = $5.612 + 1.466 \times N_I + 0.789 \times LGO_GN - 0.257 \times DUG_GOP - 0.474 \times N_GN + 0.162 \times DUGS_GOP$ ($R^2=0.581$; $F_{(5,30)}=8.31$; $p<0.001$; Error of estimate=0.387);

vestibular-tongue size of the neck of the lower left first molar (western region) = $-0.859 + 1.641 \times SN_PRN + 0.504 \times LGO_GN + 0.444 \times TR_GN - 0.371 \times N_GN - 0.278 \times TR_N$ ($R^2=0.646$; $F_{(5,30)}=10.95$; $p<0.001$; Error of estimate=0.441);

vestibular-tongue crown size of the lower left first molar (western region) = $-4.197 + 1.752 \times SN_PRN + 0.176 \times DUG_AUAU + 0.489 \times LGO_GN + 0.442 \times N_STO$ ($R^2=0.614$;

$F_{(4,31)}=12.34$; $p<0.001$; Error of estimate=0,566);

mesio-distal crown size of the lower left first molar (western region) = $-9.286 + 0.443 \times EU_EU - 1.010 \times MF_MF + 0.418 \times G_OP + 0.527 \times LGO_GN + 0.856 \times SN_PRN + 0.146 \times ZM_ZM$ ($R^2=0.575$; $F_{(6,29)}=6.54$; $p<0.001$; Error of estimate=0.585);

vestibular-tongue size of the neck of the lower right first molar (western region) = $-0.399 + 1.567 \times SN_PRN + 0.473 \times LGO_GN + 0.252 \times TR_GN - 0.228 \times N_GN$ ($R^2=0.582$; $F_{(4,31)}=10.80$; $p<0.001$; Error of estimate=0.463);

vestibular-tongue crown size of the lower right first molar (western region) = $-5.950 + 1.570 \times SN_PRN + 0.282 \times TR_GN + 0.326 \times RGO_GN + 0.377 \times EK_EK$ ($R^2=0.571$; $F_{(4,31)}=10.32$; $p<0.001$; Error of estimate=0.560);

mesio-distal cervical size of the lower right first molar (western region) = $-0.053 + 0.105 \times DUGS_GOP - 0.261 \times DUG_AUUU + 0.434 \times EU_EU + 0.343 \times EK_EK + 0.239 \times G_OP$ ($R^2=0.544$; $F_{(5,30)}=7.16$; $p<0.001$; Error of estimate=0.484);

vestibular-tongue dimension of the cervix of the lower right second molar (western region) = $-7.589 + 1.019 \times LGO_GN + 1.129 \times N_I + 0.710 \times SN_PRN - 0.318 \times N_GN$ ($R^2=0.527$; $F_{(4,31)}=8.62$; $p<0.001$; Error of estimate=0.570);

vestibular-tongue crown size of the lower right second molar (western region) = $-8.965 + 0.437 \times N_STO + 0.919 \times AL_AL + 0.970 \times N_I + 0.757 \times RGO_GN$ ($R^2=0.539$; $F_{(4,31)}=9.05$; $p<0.001$; Error of estimate=0.570);

mesio-distal size of the neck of the lower right second molar (western region) = $6.668 + 1.897 \times N_I - 0.209 \times DUG_GOP + 0.251 \times DUGS_GOP + 0.626 \times LGO_GN - 0.603 \times N_GN - 0.374 \times G_OP$ ($R^2=0.532$; $F_{(5,50)}=6.29$; $p<0.001$; Error of estimate=0.487);

height of the crown of the upper right second molar (eastern region) = $14.12 + 0.740 \times N_GN - 0.205 \times DUGS_GOP - 1.216 \times SN_PRN - 0.146 \times FMT_FMT - 0.583 \times EK_EK + 0.668 \times MF_MF - 0.466 \times CHI_CHI$ ($R^2=0.682$; $F_{(7,27)}=8.27$; $p<0.001$; Error of estimate=0.442);

height of the crown of the upper right first molar (eastern region) = $12.21 - 0.382 \times TIP_LICA - 1.096 \times SN_PRN + 0.264 \times TR_GN - 0.619 \times N_I - 0.428 \times FMT_FMT - 0.102 \times N_PRN + 0.456 \times LGO_GN$ ($R^2=0.538$; $F_{(6,49)}=7.27$; $p<0.01$; Error of estimate=0.862);

mesio-distal crown size of the upper left first molar (eastern region) = $11.90 - 0.276 \times DUGS_GOP + 0.235 \times N_SN + 1.046 \times AL_AL - 0.592 \times MF_MF + 0.312 \times EU_EU - 0.153 \times N_PRN - 0.147 \times TIP_LICA$ ($R^2=0.572$; $F_{(6,15)}=7.27$; $p<0.001$; Error of estimate=0.498);

height of the crown of the upper left second molar (eastern region) = $7.681 + 0.635 \times N_GN - 1.088 \times SN_PRN - 0.208 \times DUGS_GOP - 0.638 \times N_STO - 0.262 \times TIP_LICA + 0.105 \times TR_GN$ ($R^2=0.639$; $F_{(6,28)}=8.27$; $p<0.001$; Error of estimate=0.520);

mesio-distal size of the neck of the lower left first molar (eastern region) = $15.56 - 0.415 \times N_GN + 0.763 \times EK_EK - 0.371 \times ZM_ZM - 0.277 \times ZY_ZY - 0.333 \times TR_N + 0.578 \times MF_MF$ ($R^2=0.556$; $F_{(5,84)}=6.28$; $p<0.001$; Error of estimate=0.519);

where, R^2 - coefficient of determination; $F_{(t,!!)}$ = $!!$, $!!$ - critical ($t,!!$) and got ($!!$, $!!$) value of Fisher's criterion; St. Error of estimate - standard error of the standardized regression coefficient; FMT_FMT - the smallest width of the head (sm); N_PRN - nose length (sm); DUGS_GOP - sagittal arc (sm); EK_EK - exterior eye width (sm); TIP_LICA - face type (1 - wide, 2 - medium, 3 - narrow, 4 - very narrow); N_SN - nose height (sm); N_STO - height of the upper face part (sm); DUG_AUUU - transverse arc (sm); AL_AL - width of the base of the nose (sm); CHI_CHI - mouth width (sm); EU_EU - maximum head width (sm); RGO_GN - the length of the body of the mandible on the right (sm); SN_PRN - depth of the nose (sm); TR_GN - physiological length of the face (sm); ZM_ZM - average width of the face (sm); TR_N - height of the forehead (sm); N_I - the distance between the nasion and the inter-cutter point (sm); DUG_GOP - the largest girth of the head (sm); LGO_GN - the length of the body of the mandible on the left (sm); N_GN - morphological length of the face (sm); MF_MF - inter-orbital width (sm); G_OP - the largest length of the head (sm); ZY_ZY - face width (sm).

Models of all other linear dimensions of molars in practically healthy men of the Western and Eastern regions of Ukraine have a determination coefficient less than 0.5 and therefore have no significance for practical dentistry.

Discussion

Knowledge of odontology of different groups of the population allows to develop an individual approach in the course of activities aimed at the treatment and restoration of teeth and dental-jaw system in general, extends the importance of other specialties, including forensic medicine and anthropology [12, 20, 23, 29]. Specialists engaged in this branch of odontology, seek to identify general patterns of structure and development of the dental system, individual and sexual variability of teeth, the laws of their morphogenesis, the relationship between different elements of the system, the correlation of the size and structure of individual teeth between themselves and the skull. As a result, with a number of known morphometric parameters of the patient's teeth, by calculating on the basis of regression equations, we obtain the individual parameters of missing teeth and their tissues [11, 16, 24, 25].

Using regression analysis, based on the characteristics of cephalometric indices, craniotype and face type, reliable models (with determination coefficient R^2 greater than 0.5) of linear computed-tomographic sizes of molars of the upper and lower jaws in practically healthy men of the Western (4 models of mesio-distal sizes of the crown of the teeth of the upper and lower jaws, $R^2 = 0.575-0.646$; 3 models of mesio-distal dimensions of the neck of the teeth of the mandible, $R^2 = 0.532-0.581$; 3 models of vestibular-tongue crowns of the lower teeth $R^2 = 0.527-0.646$; 1 model of the height of the crown of the tooth of the upper jaw, $R^2 = 0.579$) and the Eastern (3 models of the height of crowns of the teeth of the upper jaw, $R^2 = 0.538-0.682$; 1 model of mesio-distal size of crown of tooth of upper jaw, $R^2 = 0.572$; 1 model of

mesio-distal size of the neck of the mandible tooth, $R^2 = 0.556$) of the regions of Ukraine.

Constructed models of computed-tomographic sizes of molars of the upper and lower jaws with a determination coefficient of more than 0,5 most often include:

in men of the Western region of Ukraine - parameters of the facial part of the head 72.6% (nose depth - 11.0%, the length of the body of the mandible on the left - 9.6%, the distance between the nasion and the inter-cutter point and the morphological length of the face - by 6.8%, exterior eye width, the height of the upper face and physiological face length - by 5.5%) and the parameters of the cerebral head were 24.7% (sagittal arc - 6.8%; transverse arc, greatest head width, largest head girth and largest head length - by 4.1%); individually for models of mesio-distal teeth size - sagittal arc (12.5%), exterior eye width, nasal depth, distance between nasion and inter-cutter point, length of the body of the mandible on the left, largest head circumference and maximum head length (by 7.5%); to the models of vestibular-tongue sized teeth - the depth of the nose (20.0%), the length of the body of the lower jaw to the left (16.0%), physiological face length and morphological face length (by 12.0%);

in men of the Eastern region of Ukraine - parameters of the facial part of the head 72.7% (nose depth, morphological length of the face and inter-orbital width - by 9.1%, nose length, exterior eye width and physiological facial length - by 6.1%), parameters of the cerebral head 18.2% (sagittal arc - 9.1%; lowest head width - 6.1%) and face type 9.1%; separate to the models of the height of crowns of teeth - nose depth (15.0%), face type, physiological face length, morphological length of face, smallest head width and sagittal arc (by 10.0%); to models of mesio-distal dimensions of teeth - inter-orbital width (15.4%).

When comparing our results with the results of modeling

the linear dimensions of premolars in similar regions [16] it is necessary to note: for men of the Western region of Ukraine constructed 4 models of mesio-distal sizes of premolars ($R^2 = 0.535-0.659$), which most often include the largest girth the head and distance between the nasion and the inter-cutter point (by 14.8%), the transverse arc, the width of the mouth gap and the length of the body of the mandible on the right (by 11.1%); 1 model of the height of crowns of premolars ($R^2 = 0.522$); men of the Eastern region of Ukraine had 6 models of mesio-distal premolars sizes ($R^2 = 0.505-0.641$), most of which included sagittal arc, mouth width and forehead height (by 12.8%), average facial width (10.3%), and length of the body of the mandible on the left (7.7%); 2 models of the vestibular-tongue dimensions of premolars ($R^2 = 0.519$ and 0.559), which most often include the transverse arc, the width of the mouth, exterior eye width, the width of the mandible, and the length of the nose (by 14.3%); 1 model of height of crown of premolars ($R^2 = 0.603$).

Constructed regression models of linear dimensions of molars, depending on the features of cephalometric indices and the type of face of men of the Western and Eastern regions of Ukraine, will allow dentists to more correctly carry out treatment and diagnostic measures of various tooth-jaw anomalies.

Conclusion

In practically healthy men from the Western and Eastern administrative-territorial regions of Ukraine, based on the specifics of cephalometric indices and face type, reliable regression models (with determination coefficient R^2 greater than 0.5) of computed-tomographic size of molars of the upper and lower jaws (out of 40 possible 14 for the Western and 5 for the eastern regions of Ukraine) have been constructed.

References

- [1] Alekseev, V. P., & Debets, G. F. (1964). *Craniometry: anthropological research methodology*. M.: Science.
- [2] Allen, D., Rebellato, J., Sheats, R., Ceron, A. M. (2003). Skeletal and Dental Contributions to Posterior Cross-bites. *Angle Orthodontist.*, 5, 515-524. doi: 10.1043/0003-3219(2003)073<0515:SADCTP>2.0.CO;2
- [3] Anari, S. (2017). Aesthetic dentistry Changing public perceptions. *British dental journal*, 223(6), 390. <https://doi.org/10.1038/sj.bdj.2017.791>
- [4] Anderson, G., Fields, H. W., Beck, M., Chacon, G., Vig, K. W. (2006). Development of Cephalometric Norms Using a Unified Facial and Dental Approach. *The Angle Orthodontist*, 76(4), 612-618. doi: 10.1043/0003-3219(2006)076[0612:DOCNUA]2.0.CO;2
- [5] Bamber, M. A., Harris, M., Natcher, C. (2001). A validation of two orthognathic model surgery techniques. *J. Orthod.*, 28, 135-142. doi: 10.1093/ortho/28.2.135
- [6] Bedoya, A., Osorio, J. C., Tamayo, J. A. (2015). Dental Arch Size, Biting Force, Bizygomatic Width and Face Height in Three Colombian Ethnic Groups. *International Journal of Morphology*, 33(1), 55-61. <http://dx.doi.org/10.4067/S0717-95022015000100009>
- [7] Bennet, M. E., & Tulloch, J. F. C. (1999). Understanding orthodontic treatment satisfaction from the patient's perspective: a qualitative approach. *Clinical Orthod. and Research.*, 2(2), 53-61. PMID: 10534980
- [8] Chernyak, V. V., Gasyuk, P. A., & Nikiforov, A. G. (2013). Method of determination of individual odontoglyphic status. *Bukovinsky Medical Bulletin*, 3, 181-182.
- [9] Cracel-Nogueira, F., & Pinho, T. (2013). Assessment of the perception of smile esthetics by laypersons, dental students and dental practitioners. *Int. Orthod.*, 11(4), 432-444. PMID: 24427802
- [10] Flis, P. S., Omelchuk, N. A., & Rashchenko, N. B. (2008). *Orthodontics*. Kiev: Medicine. ISBN 978-966-10-0026-0
- [11] Gayvoronskiy, I. V., Gayvoronskiy, A. I., Gayvoronskaya, M. G., Dubovik, E. I., Kevorkova, S. A., Koltunov, A. V., ... Schanikova, A. S. (2008). Craniometric correlations of the dentoalveolar system, temporomandibular joint and facial skull in an adult. *Morphology*, 133(2), 29.
- [12] Glushak, A. A. (2015). Vestibular-oral size of teeth in boys and girls with orthognathic bite depending on the shape of head and type of face. *Reports of Vinnytsia National Medical University*, 19(1), 37-43.

- [13] Hieu, L. C., Zlatov, N., Vander Sloten, J., Bohez, E., Khanh, L., Binh, P. H., ... Toshev, Y. (2005). Medical rapid prototyping applications and methods. *Assembly Automation*, 25(4), 284-292. <https://doi.org/10.1108/01445150510626415>
- [14] Mavili, M. E., Canter, H. I., Saglam-Aydinatay, B., Kamaci, S., & Kocadereli, I. (2007). Use of three-dimensional medical modeling methods for precise planning of orthognathic surgery. *The Journal of Craniofacial Surgery*, 18(4), 740-747. doi: 10.1097/scs.0b013e318069014f
- [15] Muzurova, L. V., & Rezugin, A. M. (2008). Correlation relations of linear cranio-cephalometric parameters in persons with orthognathic occlusion. *Morphology*, 2, 92.
- [16] Orlovskiy, V. O., Shinkaruk-Dykovytska, M. M., & Gunas, I. V. (2018). Modeling using regression analysis of individual linear dimensions of premolars depending on cephalometric indicators of practically healthy men from different regions of Ukraine. *Bulletin of scientific research*, 1, 90-95. doi: 10.11603/2415-8798.2018.1.8737
- [17] Pacheco-Pereira, C., Canto, G. D. L., Major, P. W., & Flores-Mir, C. (2014). Variation of orthodontic treatment decision-making based on dental model type: A systematic review. *The Angle Orthodontist*, 85(3), 501-509. <https://doi.org/10.2319/051214-343.1>
- [18] Proffit, U. R. (translation from English; Ed. L. S. Persina) (2015). *Modern orthodontics*. M.: Medpress-inform. ISBN: 978-5-00030-236-1
- [19] Rasulov, I. M. (2011). Odontological features of the structure of the central incisors of the upper jaw (anthropological aspects). *Bulletin of the Dagestan Scientific Center. Makhachkala*, 3, 45-47.
- [20] Samusev, R. P., Krayushkin, A. I., & Dmitrienko, S. V. (Ed. M. R. Sapin) (2002). *Basics of clinical morphology of teeth: a tutorial*. M.: OOO "Publishing House "ONIX 21 vek": OOO "World and Education". ISBN: 5-329-00426-8
- [21] Santoroa, M., Jarjourab, K., & Cangialosic, T. J. (2006). Accuracy of digital and analogue cephalometric measurements assessed with the sandwich technique. *American J. of Orthodontics add Dentofacial Orthopedics*, 3, 345-351. doi: 10.1016/j.ajodo.2005.12.010
- [22] Scherbakova, L. V. (2004). *Anthropometric parameters of cranio-facial complex of females and males 18-20 years old*. The theses are presented in the materials of the conference dedicated to the 65th anniversary of the Department of Operative Surgery and Topographic Anatomy of the Volgograd State Medical University. Volgograd, 60(3), 93-94.
- [23] Slavicek, G. (2011). *Cephalometrie*. Berlin: Steinbeis-Ed. ISBN: 9783941417724
- [24] Smerdina, L. N., & Smerdina, Yu. G. (2010). Biological norm of orthognathic occlusion (monograph). *The successes of modern natural science*, 2, 48-49.
- [25] Swennen, G. R. J., Barth, E., Eulzer, C., & Schutyser, F. (2007). The use of a new 3D splint and double CT scan procedure to obtain an accurate anatomic virtual augmented model of the skull. *International Journal of Oral & Maxillofacial Surgery*, 36(2), 146-152. doi: 10.1016/j.ijom.2006.09.019
- [26] Swennen, G. R. J., Mommaerts, M. Y., Abeloos, J., De Clercq, C., Lamoral, P., Neyt, N., ... Schutyser, F. (2009). A cone-beam CT based technique to augment the 3D virtual skull model with a detailed dental surface. *International Journal of Oral and Maxillofacial Surgery*, 38(1), 48-57. doi: 10.1016/j.ijom.2008.11.006
- [27] Trezubov, V. N., Bulycheva, E. A., Chikunov, S. O., Trezubov, V. V., & Alpatyeva, U. V. (2015). The cephalometric study of the facial skeleton during the preparation to eliminating the occlusal surface deformations of dentition. *Institute of Stomatology*, 4(69), 102-104.
- [28] Walker, F., Ayoub, A. F., Moos, K. F., & Barbenel, J. (2008). Face bow and articulator for planning orthognathic surgery: 1 face bow. *British Journal of Oral & Maxillofacial Surgery*, 46(7), 567-572. doi: 10.1016/j.bjoms.2008.02.015
- [29] Yadav, A. O., Walia, C. S., Borle, R. M., Chaoji, K. H., Rajan, R., Datarkar, A. N. (2011). Cephalometric norms for Central Indian population using Burstone and Legan analysis. *Indian J. Dent. Res.*, 22(1), 28-33. doi: 10.4103/0970-9290.79970
- [30] Zubov, A. A. (1968). *Odontology. Methodology of anthropological research*. M.: Science.

РЕГРЕСІЙНІ МОДЕЛІ ІНДИВІДУАЛЬНИХ ЛІНІЙНИХ РОЗМІРІВ ВЕЛИКИХ КУТНІХ ЗУБІВ В ЗАЛЕЖНОСТІ ВІД ОСОБЛИВОСТЕЙ ЦЕФАЛОМЕТРИЧНИХ ПОКАЗНИКІВ ПРАКТИЧНО ЗДОРОВИХ ЧОЛОВІКІВ ЗАХІДНОГО І СХІДНОГО РЕГІОНІВ УКРАЇНИ

Коцюра О. О.

Відновлення відсутніх зубів необхідно здійснювати максимально наближуючись до природних анатомічних особливостей зубощелепного апарату індивідуума. Саме тому проблема естетики в стоматології перестала бути лише ортопедичною і змістилась в напрямку конституціональної та етнічної одонтології. Мета дослідження - побудувати та провести аналіз, отриманих за допомогою регресійного аналізу, моделей комп'ютерно-томографічних розмірів великих кутніх зубів в залежності від особливостей цефалометричних показників практично здорових чоловіків Західного і Східного регіонів України. За допомогою денального конусно-променевого томографа Veraviewerocs-3D (Morita, Японія) проведено комп'ютерно-томографічне дослідження великих кутніх зубів із наступними одонтометриєю і цефалометрією 36 практично здорових чоловіків Західного регіону України - мешканці Волинської, Рівненської, Львівської, Чернівецької, Тернопільської, Хмельницької, Закарпатської та Івано-Франківської областей та 35 чоловіків Східного регіону України - мешканці Харківської, Донецької та Луганської областей. Регресійні моделі лінійних розмірів великих кутніх зубів залежно від цефалометричних показників, проводили за допомогою ліцензійного статистичного програмного пакета Statistica 6.1. В результаті проведених досліджень побудовані достовірні моделі комп'ютерно-томографічних розмірів великих кутніх зубів верхньої і нижньої щелепи у практично здорових чоловіків Західного [7 моделей мезіо-дистальних розмірів ($R^2=0.532-0.646$) до яких найчастіше входять сагітальна дуга (12.5%), зовнішньоочна ширина, глибина носа, відстань між навіон та міжкріцевою точкою, довжина тіла нижньої щелепи зліва, найбільший обхват голови та найбільша довжина голови (по 7.5%); 6 моделей присінково-язикових розмірів ($R^2=0.527-0.646$) до яких найчастіше входять глибина носа (20.0%), довжина тіла нижньої щелепи зліва (16.0%), фізіологічна довжина обличчя та морфологічна довжина обличчя (по 12.0%); 1 модель висоти коронки ($R^2=0.579$)], а також Східного [3 моделі висоти коронок ($R^2=0.538-0.682$) до яких найчастіше входять глибина носа (15.0%), тип обличчя, фізіологічна довжина обличчя, морфологічна довжина обличчя, найменша ширина голови та сагітальна дуга (по 10.0%); 2 моделі мезіо-дистальних

розмірів ($R^2=0.572$ і 0.556) до яких найчастіше входить міжчоньямова ширина (15.4%)] регіонів України. Таким чином у практично здорових чоловіків із Західного та Східного адміністративно-територіальних регіонів України на основі особливостей цефалометричних показників і типу обличчя розроблені достовірні регресійні моделі (з коефіцієнтом детермінації R^2 більшим ніж 0,5) комп'ютерно-томографічних розмірів великих кутніх зубів верхньої і нижньої щелеп (із 40 можливих 14 для Західного та 5 для Східного регіонів України).

Ключові слова: регресійний аналіз, розміри великих кутніх зубів, комп'ютерна томографія, цефалометрія, практично здорові чоловіки, Західний і Східний регіони України.

РЕГРЕССИОННЫЕ МОДЕЛИ ИНДИВИДУАЛЬНЫХ ЛИНЕЙНЫХ РАЗМЕРОВ БОЛЬШИХ КОРЕННЫХ ЗУБОВ В ЗАВИСИМОСТИ ОТ ОСОБЕННОСТЕЙ ЦЕФАЛОМЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ ПРАКТИЧЕСКИ ЗДОРОВЫХ МУЖЧИН ЗАПАДНОГО И ВОСТОЧНОГО РЕГИОНОВ УКРАИНЫ

Коцюра О. А.

Восстановление отсутствующих зубов необходимо проводить максимально приближаясь к естественным анатомическим особенностям зубочелюстного аппарата индивидуума. Именно поэтому, проблема эстетики в стоматологии перестала быть только ортопедической и сместилась в направлении конституциональной и этнической одонтологии. Цель исследования - построить и провести анализ, полученных с помощью регрессионного анализа, моделей компьютерно-томографических размеров больших коренных зубов в зависимости от особенностей цефалометрических показателей практически здоровых мужчин Западного и Восточного регионов Украины. С помощью денального конусно-лучевого томографа Veravieweross-3D (Morita, Япония) проведено компьютерно-томографическое исследование больших коренных зубов с последующей одонтометрией и цефалометрией 36 практически здоровых мужчин Западного региона Украины - жителей Волынской, Ровенской, Львовской, Черновицкой, Тернопольской, Хмельницкой, Закарпатской и Ивано-Франковской областей и 35 мужчин Восточного региона Украины - жителей Харьковской, Донецкой и Луганской областей. Регрессионные модели линейных размеров больших коренных зубов в зависимости от цефалометрических показателей, проводили с помощью лицензионного статистического программного пакета Statistica 6.1. В результате проведенных исследований построены достоверные модели компьютерно-томографических размеров больших коренных зубов верхней и нижней челюстей у практически здоровых мужчин Западного [7 моделей мезио-дистальных размеров ($R^2 = 0.532-0.646$) к которым чаще всего входят сагиттальная дуга (12.5%), внешнеглазная ширина, глубина носа, расстояние между назион и между-резцовой точкой, длина тела нижней челюсти слева, самый обхват головы и наибольшая длина головы (по 7.5%); 6 моделей преддверно-языковых размеров ($R^2 = 0.527-0.646$) к которым чаще всего входят глубина носа (20.0%), длина тела нижней челюсти слева (16.0%), физиологическая длина лица и морфологическая длина лица (по 12,0%); 1 модель высоты коронки ($R^2 = 0.579$)], а также Восточного [3 модели высоты коронок ($R^2 = 0.538-0.682$), к которым чаще всего входят глубина носа (15.0%), тип лица, физиологическая длина лица, морфологическая длина лица, наименьшая ширина головы и сагиттальная дуга (по 10.0%); 2 модели мезио-дистальных размеров ($R^2 = 0.572$ и 0.556), к которым чаще всего входит междуглазничная ширина (15.4%)] регионов Украины. Таким образом, у практически здоровых мужчин из Западного и Восточного административно-территориальных регионов Украины на основе особенностей цефалометрических показателей и типа лица разработаны достоверные регрессионные модели (с коэффициентом детерминации R^2 большим 0,5) компьютерно-томографических размеров больших коренных зубов верхней и нижней челюстей (с 40 возможных 14 для Западного и 5 для Восточного регионов Украины).

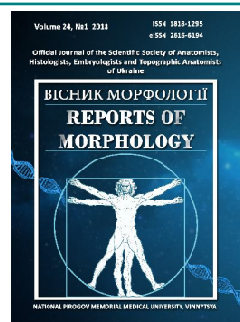
Ключевые слова: регрессионный анализ, размеры больших коренных зубов, компьютерная томография, цефалометрия, практически здоровые мужчины, Западный и Восточный регионы Украины.



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INDICATORS OF THE CARDIOMYOCYTES' CELLS CYCLE UNDER INFUSION OF BLOOD SUBSTITUTES AND IN THE CORRECTION OF EXPERIMENTAL BURN INJURY BY 0.9% NaCl SOLUTION

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According to the WHO, the thermal trauma is on the third place among other injuries. Burned injury is not only damage to the skin, but also the traumatization of all organs and systems of the body as a result of the stress response of the vascular system and the effects of toxic products coming from the area of burn injury. Firstly, such damages affect cardiomyocytes and the microcirculation vessels of the heart. The purpose of our study was to evaluate the changes in the cell cycle of myocardial cells in the left ventricle of rats under conditions of blood substitutes infusion and in the correction of experimental burn injury with a 0.9% solution of NaCl. The burn trauma was modeled using the Regas' method and placed a catheter into the lower vena cava for intravenous infusion. The following solutions were used for infusion: 0.9% NaCl solution, lactoproteinum with sorbitol (Lactoproteinum-C) and colloidal-hyperosmolar HAES-LX-5% solution. Flow cytometry of the nuclear suspension of left ventricular cardiomyocytes was performed on the 1st, 3rd, and 7th days of the experiment. The statistical analysis of the results was carried out using the "STATISTICA 6.1" program package. The results of the performed study show a fairly stable picture of cell cycle parameters in myocardial cells of animals without burn injury with a predominance, on the one hand, of cells present in the G0G1 phase and the presence of a certain balance between the processes of creation of nuclear DNA synthesis and apoptosis. Changes in the phase of cardiac myocyte cell cycle against the background of the thermal injury of the skin throughout the observation time indicate a prolonged, uncorrected cell cycle disorder and a lack of effective normalization on the background of the physiological solution usage in the first 7 days after burning trauma of the skin. The protective effect of HAES-LX-5% prevents over-strain of cells, as evidenced by the lower synthetic activity of nuclei of cardiomyocytes at all times of the experiment.

Keywords: burn trauma, myocardium, cell cycle, rats, cardiomyocytes, 0,9% NaCl solution, lactoproteinum with sorbitol, HAES-LX-5%.

Introduction

A large burn injury causes significant hemodynamic and cardiodynamic changes that support the development of sepsis, multiple organ failure, and leads to death [8]. Cardiogenic stress is a sign of the acute phase of response, and the negative results of the treatment of thermal damage are associated, in particular, with severe cardiac dysfunction [11, 12, 19]. Compromised cardiac function leads to hypoperfusion of organs, disturbance of peripheral microcirculation, increase of burn area and decrease of resistance to bacterial infection in the wound area [10, 11, 13]. Infusion therapy for burn injury is designed to compensate for the amount of fluid lost, with subsequent

maintenance of the volume of circulating blood at a constant level, reduction of edema syndrome, normalization of the acid-base balance, electrolyte balance, and also the enhancement of perfusion of organs and tissues [2, 14, 15, 16, 18]. Literary data indicate that the problem of adequate use of infusion-transfusion solutions in the case of burn injury is far from the solution [2, 5, 14, 15, 18]. First of all, it concerns the changes in the parameters of the cardiomyocyte cell cycle, which are almost not covered in modern literature.

The aim of our study was to evaluate changes in the cell cycle of myocardial cells in the left ventricle of rats under conditions of infusion of blood substitutes and correction of

experimental burn injury by a 0.9% solution of NaCl.

Materials and methods

Experimental research was carried out on the basis of vivarium, research laboratory of functional morphology and genetics of the development of the research center (certificate of State Pharmacological Center of Ministry of Health of Ukraine No. 003/10 dated January 11, 2010) and the chemical scientific laboratory of the Department of Pharmacology (certificate of the State Pharmacological Center of Ministry of Health of Ukraine No. 000679 from January 11, 2008) National Pirogov Memorial Medical University, Vinnytsya.

All manipulations with animals and their keeping were conducted in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), and also guided by the recommendations of the "European Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific goals" (Strasbourg, 1985) and the provisions of the "Rules of preclinical safety assessment of pharmacological agents (GLP)", fully complied with the rules of humane treatment of experimental animals that cough Department of Bioethics, National Pirogov Memorial Medical University, Vinnytsya (Minutes No. 1 dated January 14, 2010).

Experiments were performed on 77 white male rats weighing 160-180 g obtained from the vivarium of the State Institution "Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine".

The control group consisted of intact rats, which throughout the study period were infusion therapy with physiological solution.

All other rats under the general anesthesia of propofol (60 mg/kg of animal weight) were placed into the lower vena cava of the intravenous infusion catheter and modeled the burns of II-III degrees using the Regas method (fourth group of experimental animals) [17].

By the nature of infusion therapy, all experimental animals were randomly assigned to four groups: to group 1 - rats, which were given a 0.9% NaCl solution in a dose of 10 ml/kg; group 2 - rats, which were injected with a solution of lactoproteinum with sorbitol (Lactoprotein-C, issued by Kiev Closed Joint-Stock Company "Biopharm", Certificate of state registration of the Ministry of Health of Ukraine No. 464/09-300200000 dated March 12, 2009) at a dose of 10 ml/kg; group 3 - rats, which were injected with a colloid-hyperosmolar HAES-LX-5% solution (developed at the SI "Institute of Blood Pathology and Transfusion Medicine of the National Academy of Medical Sciences of Ukraine", Lviv) at a dose of 10 ml/kg; group 4 - rats that were injected by 0.9% NaCl solution at a dose of 10 ml/kg against a burn injury.

The first injection was carried out 1 hour after the simulation of burn injury, the subsequent infusions were performed 1 time per day during the first 7 days of the experiment.

Animals were withdrawn from the experiment on 1, 3, and 7 days by overdose of propofol anesthesia in accordance with the basic requirements for euthanasia (Annex 4 "Rules for carrying out work using experimental animals", approved by order number 755 dated August 12, 1977, Ministry of Health of the USSR "On measures to further improve organizational forms of work using experimental animals", Helsinki Declaration of the World Medical Association (2000).

To detect the peculiarities of changes, cell cycle indices, and determination of DNA content in the nuclei of myocardial cells of rats, we used a flow-through DNA-cytofluorometry method.

After the heart was removed from the body of the rat, suspensions of the nuclei from the left ventricular myocardial cells of rats were prepared. The suspension was prepared using a CyStain DNA Step 1 for DNA dilution solution from Partec, Germany, in accordance with the manufacturer's protocol. This solution allows for the extraction of nuclei and the labeling of nuclear DNA by 4.6-Diamino-phenylindole (DAPI). In the manufacture of nucleic suspensions, we used CellTrics 50 μ m disposable filters (Partec, Germany).

Flow analysis was performed on a multi-functional flow-through flow cytometric analyzer "Partec PAS" from Partec (Germany), at the CRC of National Pirogov Memorial Medical University, Vinnytsya. We used UV radiation to stimulate DAPI fluorescence. From each sample of a nuclear suspension, 10,000 events were subject to analysis. The distribution of DNA reflecting the cell cycle and fragmentation of DNA is presented on a page with one histogram using a linear scale. Calculation, plotting, cyclic analysis of cells were performed using FloMax software application (Partec, Germany), which was provided by the manufacturer to the equipment, in full digital equivalence according to the mathematical model, which determined:

G0G1 (G1%) - percentage ratio of G0G1 phase cells to all cells in the cell cycle (DNA content = 2c);

S (S%) - percentage of cells in the phase of DNA synthesis to all cells of the cell cycle (DNA content > 2c and < 4c);

G2 + M (G2M%) - percentage of cells in the G2 + M phase to all cells in the cell cycle (DNA = 4c), or cells containing DNA = 4c;

Determination of DNA fragmentation is accomplished by isolating the SUB-G0G1 site on the DNA histograms-RN1 before the peak G0G1, which indicates the nuclei of the cells containing DNA < 2c. This is the percentage of cell nuclei in the state of apoptosis.

IP is an index of proliferation (a proliferative index), which is determined by the sum of the indices S + G2 + M. The larger its value, the more intensive proliferation and vice versa - the smaller the value, the less proliferative activity.

BP - block of proliferation. An increase in the number of cells in the G2 + M phase at low values of the S-phase indicates a delay (cell proliferation) of the cell cycle in the G2 + M stage. This indicator is rated by the ratio: S / (G2 + M).

The statistical analysis of the results was carried out using

the "STATISTICA 6.1" program package (license number BXXR901E246022FA).

Results

In the study of DNA histograms of a nuclear suspension of rat myocardial cells without skin burn on 1 day after application of 0.9% NaCl solution, lactoproteinum with sorbitol or HAES-LX-5%, we did not find any significant difference between the cell cycle and fragmentation of myocardial cell DNA rats.

In all groups, most of the cells were in the G0G1 and G2 + M-phase, phase S parameters were different in the HAES-LX-5% group, while in groups with administration of lactoproteinum with sorbitol and 0.9% NaCl solution, we did not find any reliable differences, the SUB-G0G1 interval was the highest in the group of animals that were infused with lactoproteinum with sorbitol (Fig. 1).

For the third day of the experiment, cell cycle parameters, such as the number of cells in the G0G1 and G2 + M-phases, were almost identical for the experimental groups of animals 1, 2 and 3, with the lowest data being when using HAES-LX-5%.

At the same time, with the application of HAES-LX-5%, the S-phase and SUB-G0G1 intervals were the highest.

On the seventh day, we observed that when infusions of blood substitutes in groups 1, 2 and 3, the bulk of cardiomyocytes were also in the phases G0G1 and G2 + M.

Infusion of HAES-LX-5% resulted in a decrease in the parameters of the SUB-G0G1 and S-phase (Fig. 2) compared to similar groups in which 0.9% NaCl solution ($p < 0.01$) and lactoproteinum with sorbitol ($p < 0.05$) were used.

Therefore, as a control, we selected a group of rats that we injected a 0.9% NaCl solution without burn injury (group 1) and compared with those obtained in a group of rats, which injected a 0.9% NaCl solution for 7 days at the background of burn injury (group 4).

It was established that the difference in the number of nuclei of cardiomyocytes located in the resting phase and the presynthetic phase of the CC (G0-G1 interval) was higher in animals with burn injury at the 1 and 7 day of the experiment and amounted to 2%, on 3 day the difference in the rate in 1 and 4 groups was 0.5% and was not reliable (Fig. 3).

We considered this trend as a mobilization of cardiomyocytes reserves (G0 phase) to provide regeneration. At the same time, the percentage of nuclei that were in the phase of DNA synthesis (S-phase) was

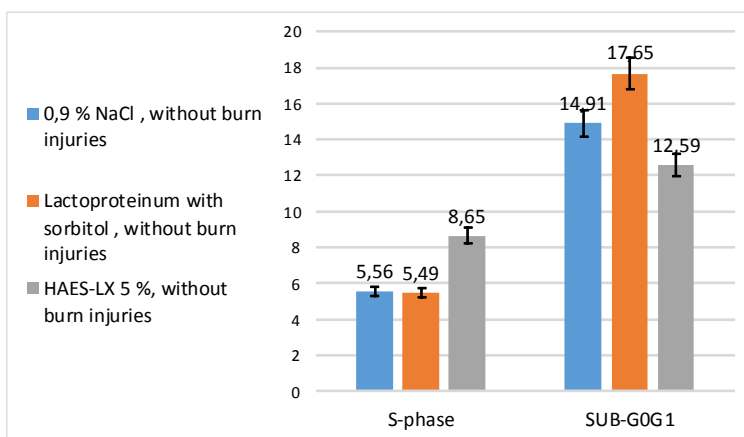


Fig. 1. S-phase indices and SUB-G0G1 intervals with 0.9% NaCl, lactoproteinum with sorbitol and HAES-LX-5% in rats without burn injuries on 1 day.

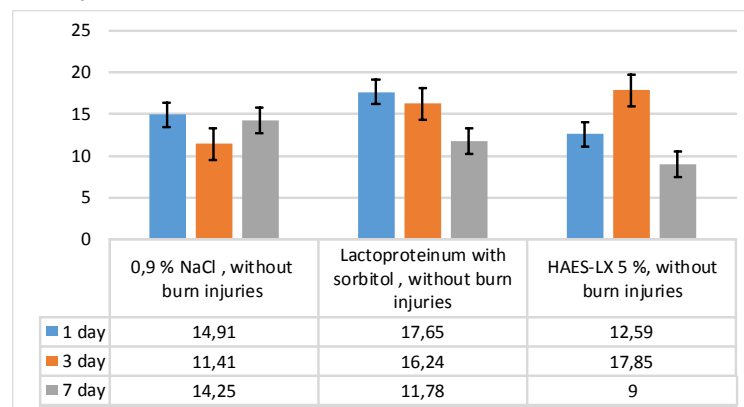


Fig. 2. Indicators of the interval SUB-G0G1 in the application of physiological solution of 0.9% NaCl, lactoproteinum with sorbitol and HAES-LX-5% in rats without burn injury at 1, 3 and 7 days.

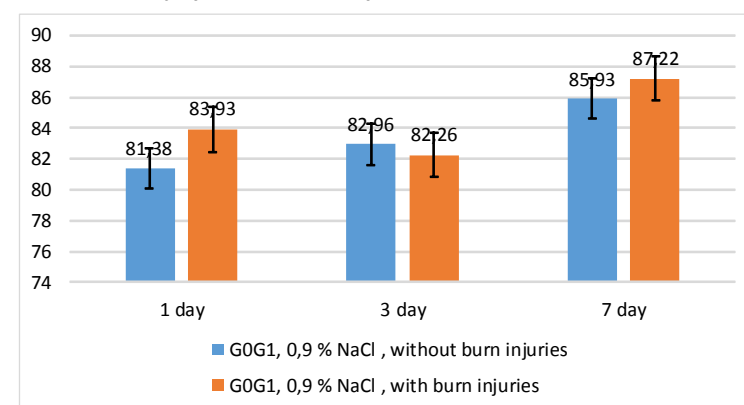


Fig. 3. Indicators of interval G0-G1 during using 0.9% NaCl physiological solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

larger by almost 2 times ($p < 0.05$) on 3 day in rats with burn injury compared with group 1, which indicated an increase in synthetic processes in the nuclei of cardiomyocytes against the background of dystrophic changes that were detected histologically with a burn injury (Fig. 4).

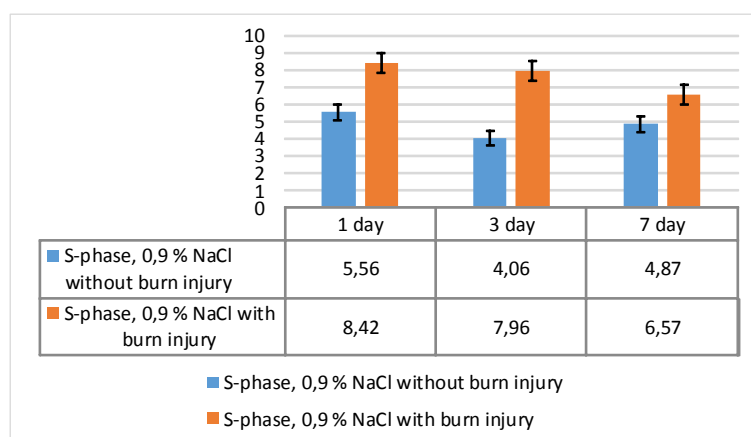


Fig. 4. S-phase indices with the use of 0.9% NaCl solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

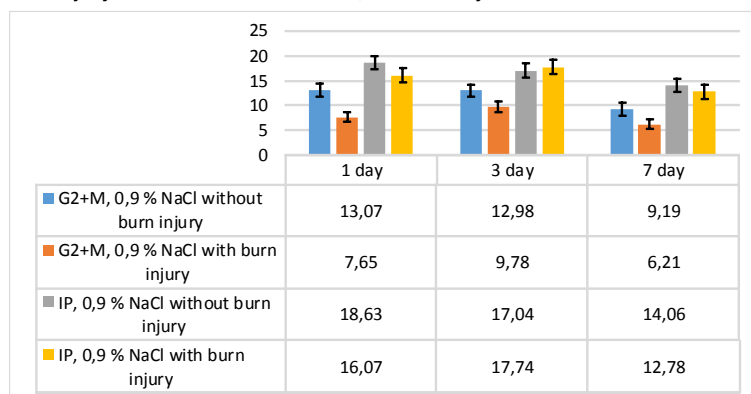


Fig. 5. Indicators of the interval G2M and the index of proliferation using 0.9% NaCl physiological solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

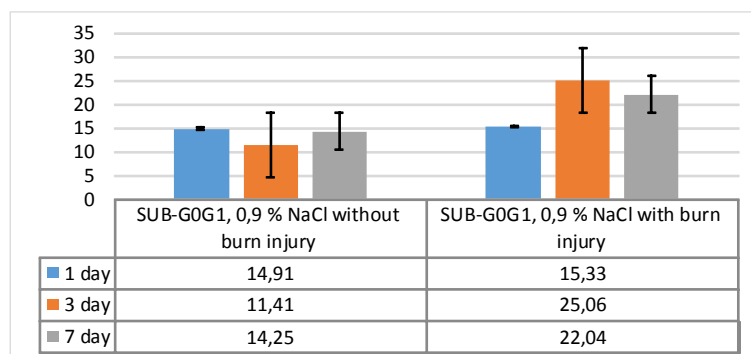


Fig. 6. Indicators of the interval SUB-G0G1 with the use of a 0.9% NaCl solution in rats without burn injury and after burning of skin on 1, 3 and 7 days.

We observed a significantly lower percentage of nuclei in the post-synthetic and mitotic phases (G2M interval) in group 4, with the largest difference in group 1 (in 1.71 times) found on the first day of the experiment. At the same time, the index of proliferation (IP - the sum of the indicators of phases S and G2M) at that time was also lower by 13.74% ($p < 0.05$), on the 7 day - by 9.1%, which we considered as a decrease the ability for reparative regeneration of cardiomyocytes in response to their damage by burn injury

and use of a physiological solution (Fig. 5).

In the case of a burn injury in group 4, we established a significantly higher percentage of nuclei of cardiomyocytes in the S-phase: in 2 times on 3 day of the experiment, in 1.5 and 1.35 times on 1 and 7 days of the experiment, respectively, compared to the animals in group 1. The foregoing indicated an increase in the synthesis of DNA, which we considered as a compensatory-adaptive reaction, aimed at restoring the mass of the damaged organ. In animals of group 4 with burn injury, the interval SUB-G0G1 (apoptosis score) on the third and seventh day of the experiment was in 2.19 and 1.55 times higher than that of animals of group 2 ($p < 0.05$) (Fig. 6). Such a significant increase in the DNA fragmentation of the nuclei of cardiomyocytes, in our opinion, is the main indicator of pathogenically induced apoptosis, which, in the future, can lead to the development of irreversible damage to cells.

In rats with burn injury, which were given with a physiological solution, the percentage of diploid cells with a set of chromosomes 2c was only 2.45% higher in the first day of the experiment, almost the same - on 3 day and 1.29% higher - on the seventh day of the experiment comparatively with group 1.

This indicates slight fluctuations in the percentage of nuclei that are in the range of G0-G1 and pass into the following phases of the CC, which prepare the cell for division to implement reparative regeneration. Larger values of the index of the interval of SUB-G0G1 of cardiomyocytes against the background of thermal damage to the skin during the entire observation time indicate a violation of the time values of the cell cycle.

A higher percentage of diploid nuclei in animals with burn injury and the introduction of the physiological solution was combined with a lower percentage of tetraploid - almost twice (Fig. 7), which was reflected in an increase of the ratio 2c/4c, which was 10.97 against 6.22 in animals of group 1. The change in the ratio between percentage 2c and 4c nuclei in this case indicates an increase in the ability to regenerate a damaged organ.

Discussion

Summarizing the results of the study, analyzing the results and comparing them with the results of researchers who studied post-mortem changes in other organs, we can conclude that there is a possibility of violations of the phases of the cell cycle of cardiomyocytes in later time (14, 21 and 30 days) after burn injuries of skin in rats [1, 3,

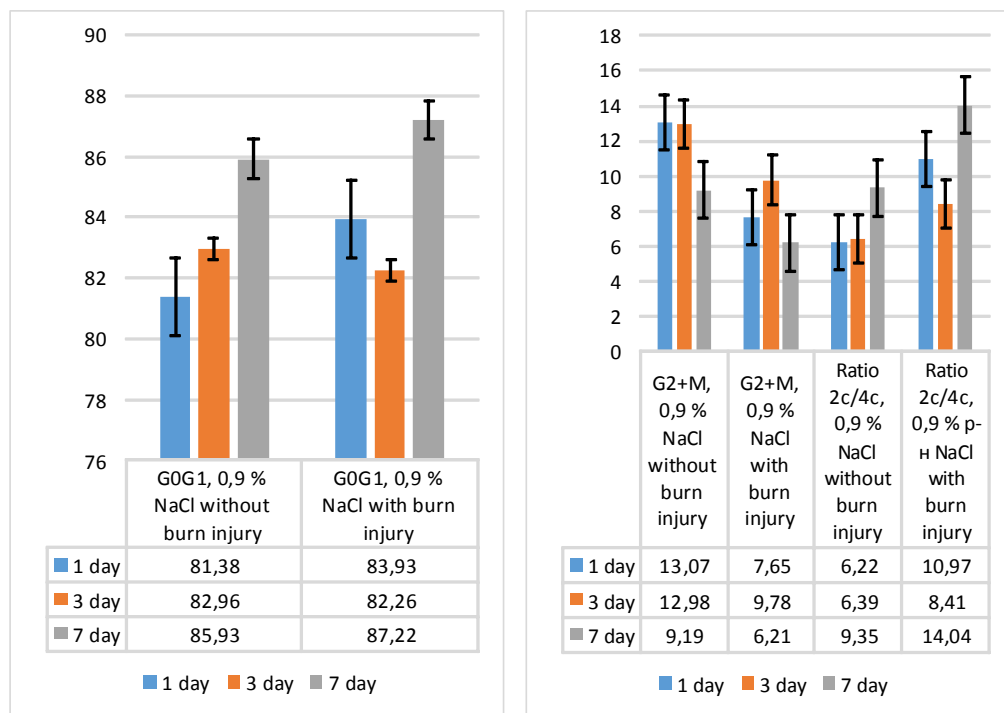


Fig. 7. Indicators of the intervals G0G1, G2M and the ratio 2c/4c in the use of a 0.9% NaCl solution in rats without burn injury and after skin burn on 1, 3 and 7 days.

6, 7, 9]. We did not find literary data on analogous studies of the cardiomyocyte cell cycle in the long term after burn infections of the skin. We can assume that in the remote post-mortem period, the processes of myocardial rehabilitation are carried out by increasing the synthesis of cellular material against the background of amplification of apoptosis of damaged cells. Despite the presence of views on the protective role of apoptosis after thermal damage, comparing the clinical data of other researchers and the results obtained by us suggests that heart damage can occur precisely on the background of amplification of apoptosis [4, 20, 21]. This may also indicate an increase in the S-phase, set 7 days after the thermal trauma, which, in turn, indicates a lack of cardiomyocyte reparation processes in the early

stages of the burn injury. It is also important to note that a comparable increase in the population of cells with fragmented nuclear DNA may indicate an imbalance of reparative processes in the cardiac muscle 7 days after the thermal loss. In favor of the hypothesis of the violation of reparative processes in the myocardium indicates the dynamics of indexes and the block of proliferation recorded during the study. The foregoing suggests that the normalization of the leading indicators of the cellular cycle of cardiomyocytes against the background of burn injury can occur only in the long term after burn injury.

In our opinion, a promising further study of morphophysiological indicators of cardiac activity subject to a thermal trauma and its adjustment with various infusion drugs.

Conclusions

1. Infusion of 0.9% solution of NaCl, lactoproteinum with sorbitol or HAES-LX-5% for 7 days in rats without skin burns does not cause significant changes in cell cycle and fragmentation of myocardial cell DNA.

2. The long-term, unregulated changes in the cell cycle and its insufficiently effective normalization against the background of the use of saline solution for 7 days after burn injury to the skin have been determined.

References

- [1] Dzevulska, I. V., Kovalchuk, O. I., Cherkasov, E. V., Majewskiy, O. Ye., Shevchuk, Yu. G., Pastukhova, V. A., & Kyselova, T. M. (2018). Influence of lactoproteinum solution with sorbitol on dna content of cells of endocrine glands on the background of skin burn in rats. *World of medicine and biology*, 2(64), 033-039. doi:10.26724/2079-8334-2018-2-64-33-39
- [2] Osadchaya, O. I., Boyarskaya A. M. & Sheyman, B. S. (2008). Effect of enterosorption on the content of pro- and anti-inflammatory mediators in severe thermal trauma. *Internal Medicine*, 3(16), 76-78.
- [3] Ocheretna, N. P., Guminskiy, Yu. I., & Gunas, I. V. (2018). Indicators of cell cycle and dna fragmentation of spleen cells in early terms after thermal burns of skin on the background of using "lactoprotein with sorbitol" or HAES-LX-5%. *Bulletin of scientific research*, 1, 141-146. doi:10.11603/2415-8798.2018.1.8627
- [4] Archana, M., Bastian, Yogesh, T. L., & Kumaraswamy K.L. (2013). Various methods available for detection of apoptotic cells - a review. *Indian Journal of Cancer*, 50(3), 274-283. doi:10.4103/0019-509X.118720
- [5] Eick, B. G., & Denke, N. J. (2018). Resuscitative Strategies in the Trauma Patient: The Past, the Present, and the Future. *J Trauma Nurs.*, 25(4), 254-263. doi:10.1097/JTN.0000000000000383
- [6] Gavryluk, A. O., Galunko, G. M., Cheresniuk, I. L., Tikholaz, V. O., Cherkasov, E. V., Dzevulska, I. V., & Kovalchuk O. I. (2018). Indicators cell cycle and DNA fragmentation in cells of small intestine mucosa 14, 21 and 30 days after skin burns on the background of preliminary infusion of solution lactoprotein with sorbitol or HAES-LX5%. *World of Medicine and Biology*, 1(63), 104-108 doi:10.26724/2079-8334-2017-4-62-104-108

- [7] Gavryluk, A. O., Gunas, I. V., Galunko, G. M., Chereshniuk, I. L., & Lysenko, D. A. (2017). Indicators of the cell cycle and fragmentation of DNA of cells of small intestinal mucosa through 14, 21 and 30 days after burn skin damage on the background of infusion of 0.9% NaCl solution. *Biomedical and Biosocial Anthropology*, 29, 104-108. Retrieved from <https://bba-journal.com/index.php/journal/article/view/295>
- [8] Guillory, A. N., Clayton, R. P., Herndon, D. N., & Finnerty, C. C. (2016). Cardiovascular Dysfunction Following Burn Injury: What We Have Learned from Rat and Mouse Models. *International journal of molecular sciences*, 17(1), 53. doi:10.3390/ijms17010053
- [9] Gunas, I. V., Guminskiy, Yu. I., Ocheretna, N. P., Lysenko, D. A., Kovalchuk, O. I., Dzevulska, I. V., & Cherkasov, E. V. (2018). Indicators cell cycle and dna fragmentation of spleen cells in early terms after thermal burns of skin at the background of introduction 0.9% NaCl solution. *World of Medicine and Biology*, 1(63), 116-120. doi:10.26.724/2079-8334-2018-1-63-116-120
- [10] Hernekamp, J. F., Neubrech, F., Cordts, T., Schmidt, V. J., Kneser, U., & Kremer, T. (2016). Influences of Macrohemodynamic Conditions on Systemic Microhemodynamic Changes in Burns. *Annals of Plastic Surgery*, 77(5), 523-528. doi:10.1097/SAP.0000000000000868
- [11] Hoesel, L. M., Niederbichler, A. D., Schaefer, J., Ipaktchi, K. R., Gao, H., Rittirsch, D., ... Ward, P. A. (2007). C5a-blockade improves burn-induced cardiac dysfunction. *J Immunol.*, 178(12), 7902-7910. doi: 10.4049/jimmunol.178.12.7902
- [12] Jeschke, M. G., Chinkes, D. L., Finnerty, C. C., Kulp, G., Suman, O. E., Norbury, W. B., ... Herndon, D. N. (2008). Pathophysiologic response to severe burn injury. *Ann. Surg.*, 248(3), 387-401. doi:10.1097/SLA.0b013e3181856241
- [13] Korkmaz, H. I., Ulrich, M. M. W., van Wieringen, W. N., Vlieg, M., Emmens, R. W., Meyer, K. W., ... Niessen, H. W. M. (2017). The Local and Systemic Inflammatory Response in a Pig Burn Wound Model With a Pivotal Role for Complement. *J Burn Care Res.*, 38(5), 796-806. doi:10.1097/BCR.0000000000000486
- [14] Laxenaire, M. C., Charpentier, C., & Feldman L. (1994). Anaphylactoid reaction to colloid plasma substitutes: incidence, risk factors, mechanism. A French multicenter prospective study. *Ann. Fr. Anesth. Reanim.*, 13(3), 301-310. PMID:7992937
- [15] Ljunstrom, K. (2007). Colloid safety: fact and fiction. *Ballieres Clin. Anaesthesiol.* 11(1). 163-177. doi:[https://doi.org/10.1016/S0950-3501\(97\)80010-4](https://doi.org/10.1016/S0950-3501(97)80010-4)
- [16] Nalos, M., Kholodniak, E., Smith, L., Orde, S., Ting, I., Slama, M., ... Huang, S. (2018). The comparative effects of 3% saline and 0.5M sodium lactate on cardiac function: a randomised, crossover study in volunteers. *Critical Care and Resuscitation*, 20(2), 124-130. PubMed PMID: 29852851
- [17] Regas, F. C., & Ehrlich, H. P. (1992). Elucidating the vascular response to burns with a new rat model. *J. Trauma*, 32(5), 557-563. PMID:1588642
- [18] Ring, J., & Messmer, K. (1977). Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet.*, 1(8009), 446-469. PMID:65572
- [19] Williams, F. N., Herndon, D. N., Suman, O. E., Lee, J. O., Norbury, W. B., Branski, L. K., ... Jeschke, M. G. (2011). Changes in cardiac physiology after severe burn injury. *J. Burn Care Res.*, 32(2), 269-274. doi:10.1097/BCR.0b013e31820aafcf
- [20] Xie, Q., Ye, Z., Chen, L., Zhao, C., Ruan, Q., & Xie, W. (2015). Expression of microRNA-126 in myocardial tissue of rats in the early stage of severe burn injury and its relation with myocardial damage. *Chinese journal of burns*, 31(5), 367-371. PMID:26714406
- [21] Zhang, J. P., Ying, X., Liang, W. Y., Luo, Z. H., Yang, Z. C., Huang, Y. S., & Wang, W. C. (2008). Apoptosis in cardiac myocytes during the early stage after severe burn. *J. Trauma*, 65(2), 401-408. doi:10.1097/TA.0b013e31817cf732

ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ КАРДІОМІОЦИТІВ В УМОВАХ ІНФУЗІЇ КРОВОЗАМІННИКІВ ТА ПРИ КОРЕКЦІЇ ЕКСПЕРИМЕНТАЛЬНОЇ ОПІКОВОЇ ТРАВМИ 0,9% РОЗЧИНОМ NaCl

Радьога Р.В.

Згідно даних ВООЗ, опікова травма посідає третє місце серед інших видів травм. Термічна травма - це не тільки ушкодження шкірних покривів, але й травматизація всіх органів і систем організму внаслідок стресової реакції судинної системи та впливу токсичних продуктів, які надходять із ділянки опікової травми. У першу чергу такі ушкодження впливають на кардіоміоцити та судини мікроциркуляторного русла серця. Метою нашого дослідження ми обрали оцінку змін показників клітинного циклу клітин міокарда лівого шлуночка щурів в умовах інфузії кровозамінників та при корекції експериментальної опікової травми 0,9% розчином NaCl. Опікову травму моделювали за методикою Regas та катетеризували нижню порожнисту вену для внутрішньовенної інфузії. Для інфузії використовували наступні розчини: 0,9% розчин NaCl, лактопротеїн з сорбітолом (Лактопротеїн-С) та колоїдно-гіперосмолярний розчин HAES-LX-5%. Проточну цитометрію ядерної суспензії кардіоміоцитів лівого шлуночка виконували на 1, 3, та 7 добу експерименту. Статистичний аналіз отриманих результатів проводили за допомогою пакету програм "STATISTICA 6.1". Результати здійсненого дослідження вказують на досить стабільну картину показників клітинного циклу у кардіоміоцитах тварин без термічної травми з переважанням, з однієї сторони, клітин, що перебувають у фазі G0G1, і присутністю певного балансу між процесами утворення ядерної ДНК і апоптозу. Зміни показників фаз клітинного циклу кардіоміоцитів на фоні опікової травми шкіри протягом усього часу спостереження вказують на некореговане, стійке порушення та недостатність ефективної нормалізації клітинного циклу на фоні застосування фізіологічного розчину протягом 7 діб після опікової травми шкіри.

Ключові слова: опікова травма, клітинний цикл, щурі, кардіоміоцити, 0,9% розчин NaCl, лактопротеїн з сорбітолом, HAES-LX-5%.

ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА КАРДИОМИОЦИТОВ В УСЛОВИЯХ ИНФУЗИИ КРОВЕЗАМЕНТЕЛЕЙ И ПРИ КОРЕКЦИИ ЭКСПЕРИМЕНТАЛЬНОЙ ОЖОГОВОЙ ТРАВМЫ 0,9% РАСТВОРОМ NaCl

Радёга Р.В.

Согласно данным ВОЗ, ожоговая травма занимает третье место среди других видов травм. Термическая травма - это не только повреждения кожных покровов, но и травматизация всех органов и систем организма в результате стрессовой реакции сосудистой системы и воздействия токсических продуктов, поступающих из участка ожогового повреждения. В

первую очередь такие повреждения влияют на кардиомиоциты и сосуды микроциркуляторного русла сердца. Целью нашего исследования мы выбрали оценку изменений показателей клеточного цикла клеток миокарда левого желудочка крыс в условиях инфузии кровезаменителей и при коррекции экспериментальной ожоговой травмы 0,9% раствором NaCl. Ожоговую травму моделировали по методике Regas и катетеризировали нижнюю полую вену для внутривенной инфузии. Для инфузии использовали следующие растворы: 0,9% раствор NaCl, лактопротеин с сорбитолом (Лактопротеин-С) и коллоидно-гиперосмолярный раствор HAES-LX-5%. Проточную цитометрию ядерной суспензии кардиомиоцитов левого желудочка выполняли на 1, 3, и 7 сутки эксперимента. Статистический анализ полученных результатов проводили с помощью пакета программ "STATISTICA 6.1". Результаты проведенного исследования указывают на достаточно стабильную картину показателей клеточного цикла в кардиомиоцитах животных без термической травмы с преобладанием, с одной стороны, клеток, находящихся в фазе G0G1, и присутствием определенного баланса между процессами образования ядерной ДНК и апоптоза. Изменения показателей фаз клеточного цикла кардиомиоцитов на фоне ожоговой травмы кожи в течение всего времени наблюдения указывают на некорректированное, стойкое нарушение и недостаточность эффективной нормализации клеточного цикла на фоне применения физиологического раствора в течение 7 дней после ожоговой травмы кожи.

Ключевые слова: ожоговая травма, клеточный цикл, крысы, кардиомиоциты, 0,9% раствор NaCl, лактопротеин с сорбитолом, HAES-LX-5%.

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For publication, scientific articles are accepted only in English only with translation on Ukrainian or Russian, which contain the following necessary elements: UDC code; title of the article (in English, Ukrainian and Russian); surname, name and patronymic of the authors (in English, Ukrainian and Russian); the official name of the organization (institution) (in English, Ukrainian and Russian); city, country (in English, Ukrainian and Russian); structured annotations (in English, Ukrainian and Russian); keywords (in English, Ukrainian and Russian); introduction; purpose; materials and methods of research; research results; discussion; conclusions; bibliographic references.

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Keywords: 4-6 words (or phrases).

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The introduction reflects the state of research and the relevance of the problem according to the world scientific literature (at least 15 references to English articles in international journals over the past 5 years). At the end of the entry, the purpose of the article is formulated (contains no more than 2-3 sentences, in which the problem or hypothesis is addressed, which is solved by the author).

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The section should allow other researchers to perform similar studies and check the results obtained by the author. If necessary, this section may be divided into subdivisions. Depending on the research objects, the ethical principles of the European Convention for the protection of vertebrate animals must be observed; Helsinki Declaration; informed consent of the surveyed, etc. (for more details, see "Public Ethics and its Conflict"). At the end of this section, a "statistical processing of results" section is required, which specifies the program and methods for processing the results obtained by the automobile.

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Requirements for writing this section are general, as well as for all international scientific publications. The data is presented clearly, in the form of short descriptions, and must be illustrated by color graphics (no more than 4) or drawings (no more than 8) and tables (no more than 4), the information is not duplicated.

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In the discussion, it is necessary to summarize and analyze the results, as possible, compare them with the data of other researchers. It is necessary to highlight the novelty and possible theoretical or practical significance of the results of the research. You should not repeat the information already listed in the "Introduction" section. At the end of the discussion, a separate paragraph should reflect the prospects for using the results obtained by the author.

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