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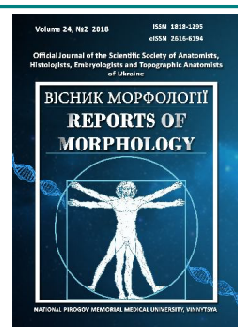
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Morphological analysis of interstitial Cajal cells and mast cells in experimental hyperactivity bladder and stress incontinence under influence of pharmacocorrection

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The existing data indicate the multifactorial mechanisms of development of the overactive bladder (OAB), but the issue of OAB pathogenesis remains unclear. In more recent times, the neurogenic theory of OAB genesis has been accompanied by the increasing attention to the study of morphological changes that occur in the smooth myocytes of the detrusor and their interaction with the extracellular matrix. Therefore, the objective of our study became the evaluation of distribution of interstitial Cajal cells (ICC) and basophilic granulocytes (BG) in the structural elements of the bladder wall under stress urinary incontinence and its overactivity before and after treatment with Mirabegron, Spasmex, Quercetin and combination thereof with testosterone and estradiol, using histochemical and immunohistochemical methods. The experimental models of OAB and stress urinary incontinence (SUI) presented the increase in the amount and functional activity of BG revealed by histological and immunohistochemical methods, as well as ICC at all terms of OAB monitoring, while the SUI presented with high concentration and functional activity of BG only after 14 days of the experiment. After 28 days, we observed a sharp decrease of the parameters, indicating decompensation and depletion of the functional activity. The number of ICC decreased under SUI after both 14 days and 28 days of the experiment. The group of experimental animals receiving Spasmex and its combination with hormones, presented no significant effect on the quantitative and qualitative composition of BG and ICC at OAB and SUI on Day 14 of the experiment, but the combination with testosterone demonstrated statistically reliable ($p < 0.001$) reduction of BG and ICC expression in the muscle layer of the bladder after 14 days, and unreliable after 28 days of the experiment ($p > 0.05$). The administration of Mirabegron alone and especially its combination with testosterone and estradiol presented positive trends in histochemical and immunohistochemical expression of BG and ICC. The experiment proved high efficacy of Quercetin in combination with testosterone and estradiol under OAB and SUI, confirmed by stabilization of the functional activity of BG and ICC quantitative composition.

Keywords: overactive urinary bladder, stress urinary inconsistency, immunohistochemical evaluation, interstitial Cajal cells, mast cells, pharmacocorrection.

Introduction

Urinary incontinence (UI) is a disease that affects the quality of life of more than 200 million patients worldwide. The sharp annual increase in the number of reviews on this issue indicates a huge scope of research in this medical problem [18]. Despite the increasing number of scientific papers dedicated to this issue, the mechanisms of urinary incontinence under overactive urinary bladder (OAB) and stress urinary incontinence (SUI) remain

unrevealed. We are still unaware of the exact causes of OAB development, which can reliably lead to formation of a mechanism of urgent and accelerated urination. Most authors tend to associate the emergence of OAB symptoms with ischemia of the bladder-emptying muscle (detrusor) most often caused by infravesical obstruction or arteriosclerosis. The disturbance of blood supply to the bladder muscle-emptying muscle results in the post-

synaptic denervation with subsequent structural changes of intercellular junctions in the myocytes. The difference in the physiological characteristics of the detrusor-sphincter apparatus and the ureter gives us the reason to differentiate three smooth muscle functional systems of the lower urinary tract - the bladder muscle-emptying muscle, the muscle system of the urinary bladder trigone, and the ureter [6]. The muscle cover of the bladder is a complex apparatus, consisting of many types of cells: smooth myocytes, nerve cells, and the population of interstitial cells (IC). According to some authors, the spontaneous rhythmic electrical activity is a universal mechanism for regulating the tone of smooth muscle cells (SMC) of visceral organs [3], including the organs of the urinary system.

Just like in the gastrointestinal tract (GIT), IC of the urinary bladder (UB) coordinate the electrical and contractive activity of large SMC populations by synchronizing their individual oscillators. Such synchronization is achieved by combining the activity of individual oscillators into a unified local driver due to a long depolarizing signal that goes through the gap junctions [8]. In UB, they were identified by co-authors as Smet in 1996 [25]. Due to their origin, IC carry a neuronal marker, the receptor tyrosine kinase (c-Kit) (CD117+) and a marker of cells of mesenchymal origin (vimentin), used to identify these cells in tissues of various organs. IC are spindle- or star-like cells with processes that contact on the one side with neurons (nerve fibers), and with smooth myocytes on the other side [4, 24]. Since the full identity of these cells with GIT and CNS Cajal cells has not been proved yet, the term "interstitial Cajal cell-like cells", abbreviated as ICC-LC, or interstitial cells of Cajal (ICC) are used for their differentiation.

According to the results of the study by Vahabi et al. (2011), ICC have a close relationship with cholinergic nerve fibers and play a role of mediator in transmission of cholinergic signals to SMC [26]. The recent data show that ICC can participate not only in transmitting signals between muscle fiber bundles, regulating the bladder-emptying muscle tone, but also in transmitting signals from the afferent nerves to the SMC with involvement of ICC located in the bladder-emptying muscle, and from the urothelia to the afferent nerves with involvement of ICC, located in the suburothelial zone. The disturbance in transmission of these signals may lead to formation of OAB [15].

As for involvement of basophilic granulocytes (BG, granule cells, tissue basophils, mast cells, labrocytes) in the formation of OAB and SUI, most authors point to an increase in their number in the UB wall, but the mechanisms of their involvement in the development of OAB and SUI still remain unclear [20, 27]. Due to this fact, the role of ICC and BG in formation of urodynamic disorders of the lower urinary tract has been actively studied in recent years.

That is why the *objective* of this study was to evaluate the immunohistochemical evaluation of distribution of ICC and BG in the structural elements of the bladder wall under stress urinary incontinence and overactivity of the bladder

before and after treatment with Mirabegron, Spasmex, Quercetin and combination thereof with testosterone and estradiol.

Material and methods

The experiments on reproduction of OAB model were conducted on 300 g sexually mature white laboratory female rats. For this purpose, the animals were divided into two groups. In the first, control group of rats, 0.3 ml sterile physiological solution was injected OD intraperitoneally for 14 days. In order to reproduce the OAB model, animals from the second study group were intraperitoneally administered 0.3 ml OD Homviotensin® (Homviora, Germany) solution containing 0.45 mg of Reserpinum for 14 days. The solution was prepared by grinding tablets in sterile conditions with further dissolving the powder in a physiological solution. We initiated SUI by cutting the pudendal nerve (n. pudendus). The reproduction of the models was confirmed by histological studies. In the OAB group, Mirabegron 1 ml (Astellas Pharma Europe B.V.) solution was administered OD through the gastric probe from Day 14 for 14 and 28 days (8 mg, 1/6 tablet dissolved in 1 ml of distilled water); 1 ml Kvertin solution (PAT NVTs Borshchahivskiy KhFZ) OD via a gastric probe for 14 and 28 days, containing Quercetin 10 mg (1/4 tablet dissolved in 1 ml of distilled water); Spasmex 1 ml (Dr. R.Pfleger GmbH) OD intraperitoneally, containing trospium chloride 0.4 mg (1/4 tablets per 10 ml of physiological saline); Testosterone Propionate solution 0.05 ml (PAT Farmak) OD for 14 and 28 days intramuscularly, containing 1 mg testosterone, and Divigel (Orion Corporation, Finland) OD for 14 and 28 days by applying gel to shaved portion of the back, which contained 0.2 mg of estradiol; the doses did not change in medicine combinations. In the SUI group, Quercetin and its combination with hormones at the aforementioned doses were used. In total, we used 460 rats, 20 experimental animals in each group.

When working with animal models, we adhered to the requirements of the "Scientific and practical recommendations for keeping and using laboratory animals" issued by the State Pharmacological Center of MoH of Ukraine (Minutes No.8 dated 22 June 2012).

For histological studies, on days 14 and 28, the animals were withdrawn from the experiment by overdose of 10% sodium thiopental solution, followed by removal of the bladder and fixing it in a 10% neutral formalin solution for 24 hours, dehydration in alcohol solutions of growing concentration, clarification in chloroform and sealing in paraffin. 5-7 micron sections were stained with hematoxylin and eosin, and by van Gieson's picrofuchsin and Weigert's resorcin-fuchsin methods [1]; for visualization of BG, we used the basic brown.

The immunohistochemistry assay was performed using DAKO and ThermoFisher Scientific paraffin blocks and reagents with monoclonal antibodies CD 117 (c-kit) for identification of ICC by EnVisionTMFLEX and Invitrogen

Histostain®-SP visualization systems. The immunohistochemical response was evaluated in 10 view fields at 200 and 400x magnification. The expression intensity was evaluated by a semi-quantitative method based on the expressiveness and integrity of basal membrane coloration, the nuclear and cytoplasmic staining of cells according to the following scheme: low, moderate and high, taking into account localization of pathological changes and percentage of positively stained cells by phase analysis using Quick PHOTO MICRO 2.3 computer program. The density of BG and ICC was determined using an optical microscope with magnification of x400. The cells were counted and typed in 10 fields with subsequent recount per unit area ($S = 0.01 \text{ mm}^2$). The functional activity of BG (degranulation) was evaluated as the ratio of the number of completely degranulated cells to the total number of analyzed cells, expressed as a percentage [2].

The Microsoft Office Excel 2007 program was used for statistical processing of the data. The differences were considered statistically significant at $p < 0.05$. UB sites taken from animals before treatment were used as control. The histological preparations were microscoped and photographed using OLIMPUS BX 41 optical microscope at 40, 100, 200 and 400x magnification.

Results

The experimental animals in the control group had UB lining formed by the transitional epithelium (TE), which consisted of 5-8 layers in a relaxed and 3-4 layers in the contracted state of UB. TE was represented by surface, intermediate and basal cells. The surface cells were large, light, with a free dome-like surface, often containing two nuclei with large nucleoli. The intermediate cells were of polygonal shape, while the basal were of cubic or low-cylindrical shape. TE was placed on its own mucous membrane plate, which separated TE and muscle cover, with collagen fibers dominated in the latter and few isolated elastic fibers.

Its deepest layer consisted of a more loose tissue and contained more elastic fibers, which was typical for submucosal UB base. Collagen and elastin were the two main proteins we registered, which were part of the extracellular matrix (ECM) of the connective tissue. Collagen is known to be responsible for breaking strength and integrity, while elastin endures elasticity and tensility of tissues.

Due to the fact that BG is also positively stained with CD 117, the cuts were pre-treated with the basic brown to determine BG localization for ICC identification. Cells with rounded nuclei and cytoplasm with granular inclusions were recorded as BG, while cells with positively stained spindle-shaped nuclei and cytoplasm were defined as ICC.

The histochemical study of UB wall in the control group using the basic brown revealed BG located predominantly around the vascular walls, both in the submucosal and in muscular and serous membranes (Fig. 1).

In this case, in most of them, the cytoplasmic membrane preserved a moderate, sometimes weak functional activity,

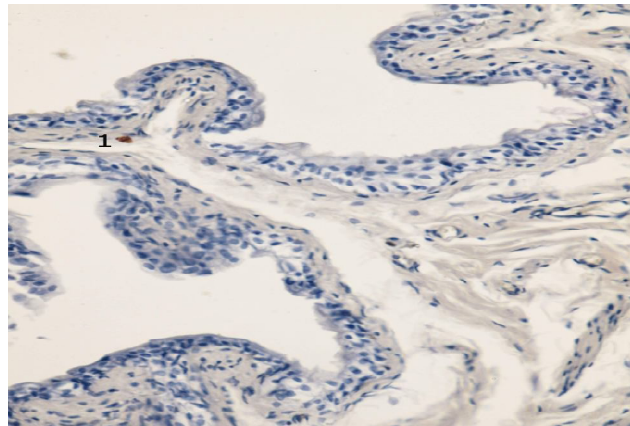


Fig. 1. Isolated basophilic granulocytes with weak functional activity in the submucosal wall of the bladder. Day 14, control group. Basic brown. x200. 1 - basophil granulocyte.

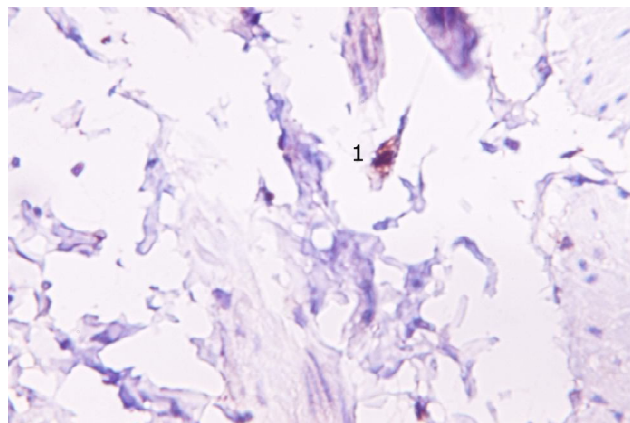


Fig. 2. Interstitial cell of Cajal with characteristic processes between smooth muscle bladder myocytes. 14 Day, control group. Immunohistochemical marking CD 117, x1000. 1 - interstitial cell of Cajal.

which was characterized by the ratio of degranulated cells to all BG (percent). The morphometric analysis revealed an average of 3.200 ± 0.489 BG per 0.01 mm^2 , of which $10.00 \pm 4.08\%$ were functionally active (Table 1).

The immunohistochemical study of the ICC of UB wall in animals of the control group revealed that ICC were mainly localized in the suburothelial layer, in the own plate of the mucous membrane, along the longitudinal bundles of detrusor SMC and between them, and less frequently in the subserous sections, predominantly in the bottom and neck of the bladder. It was possible to differentiate the cells with typical processes (processed) and those resembled fibroblasts (fibroblast-like) by the structure. It's worthy to note that processed ICC (Fig. 2) were more common in SMC, fibroblast-like cells, in sub-epithelial and subserous sections of UB wall.

Morphological characteristics of the bladder-emptying muscle under experimental overactive urinary bladder. The group of rats with OAB administered Homviotensin for 2 weeks was characterized by an increase in the number of BG and ICC (Tables 1, 2, Fig. 3, 4) with a definite prevalence in the neck section (UB transition to the ureter), associated

Table 1. Quantitative composition of basophilic granulocytes and their functional activity in % in the bladder wall under disorders of urodynamics of the lower urinary tract on Days 14 and 28 of the experiment, calculated as for 0.01 mm² surface.

| Animal group \ Pharmacocorrection type | | Basophilic granulocytes, count | | Basophilic granulocytes, functional activity | |
|--|-----------------------------------|--------------------------------|------------------------------|--|-----------------------------|
| | | Day 14 | Day 28 | Day 14 | Day 28 |
| Control group | | 3.200±0.489 | 4.000±0.365 | 10.00±4.08 | 8.500±1.979 |
| OAB | | 18.60±0.84 [†] | 15.90±0.71 | 42.00±5.12 [†] | 38.00±5.12 [†] |
| SUI | | 7.600±0.426 [†] | 2.000±0.381 [†] | 27.00±3.67 [†] | 3.630±1.363 [†] |
| OAB pharmacocorrection | Mirabegron | 15.30±0.39 [*] | 13.90±0.69 [*] | 30.00±2.69 [*] | 26.50±5.73 [*] |
| | Spasmex | 17.80±0.63 [#] | 15.60±0.56 [#] | 39.00±5.26 [*] | 36.00±3.40 |
| | Quercetin | 15.80±0.78 [*] | 11.50±0.82 [*] | 29.50±2.93 [*] | 20.50±5.04 [*] |
| | Mirabegron+Testosterone | 13.00±0.74 [*] | 10.80±0.42 [*] | 28.50±2.24 [*] | 23.00±3.59 [*] |
| | Mirabegron+Estradiol | 13.70±0.45 [*] | 10.90±0.48 [*] | 29.00±2.67 [*] | 25.50±2.93 [*] |
| | Mirabegron+Testosterone+Estradiol | 12.80±0.51 [*] | 9.500±0.619 [*] | 27.50±2.39 [*] | 21.50±3.17 [*] |
| | Spasmex+Testosterone | 15.70±0.66 [°] | 14.80±0.49 [#] | 36.50±4.08 [#] | 32.00±4.16 [#] |
| | Spasmex+Estradiol | 16.00±0.60 [°] | 14.90±0.60 [#] | 37.50±4.73 [#] | 34.00±3.23 [#] |
| | Spasmex+ Testosterone+Estradiol | 15.10±0.67 [°] | 14.10±0.50 [#] | 33.50±3.65 [#] | 30.00±3.65 [#] |
| | Quercetin+Testosterone | 13.90±0.64 [*] | 10.40±0.58 [*] | 26.50±2.89 [*] | 17.00±4.23 [*] |
| | Quercetin+ Estradiol | 14.30±0.39 [*] | 10.60±0.67 [*] | 28.50±1.50 [*] | 20.50±3.61 [*] |
| | Quercetin+ Testosterone+Estradiol | 13.30±0.58 [*] | 8.30±0.39 [*] | 26.00±3.64 [*] | 12.00±3.26 [*] |
| | Testosterone | 15.10±0.41 [*] | 11.00±0.49 [*] | 29.50±2.52 [*] | 27.50±4.17 [*] |
| | Estradiol | 15.60±0.50 [*] | 11.20±0.39 [*] | 33.00±2.00 [*] | 28.00±4.90 [*] |
| Testosterone+Estradiol | 14.10±0.52 [*] | 9.600±0.476 [*] | 27.00±2.49 [*] | 22.00±4.16 [*] | |
| SUI pharmacocorrection | Quercetin | 5.900±0.378 ^{&} | 5.400±0.400 | 17.50±2.14 | 11.50±2.99 |
| | Testosterone | 5.700±0.472 ^{&} | 5.200±0.359 ^{&} | 18.00±1.85 ^{&} | 13.00±3.35 ^{&} |
| | Estradiol | 6.000±0.494 ^{&} | 5.600±0.426 ^{&} | 19.0±2.67 ^{&} | 15.00±3.16 ^{&} |
| | Testosterone+Estradiol | 5.600±0.339 ^{&} | 5.500±0.401 ^{&} | 16.50±2.36 ^{&} | 11.00±3.78 ^{&} |
| | Quercetin+Testosterone+ Estradiol | 5.300±0.495 ^{&} | 4.400±0.476 ^{&} | 16.00±2.21 ^{&} | 10.00±2.98 ^{&} |

Note: OAB - overactive bladder, SUI - stress urinary incontinence, ICC - interstitial cells of Cajal; * - p<0.001 compared to OAB; † - p<0.001 compared to control; & - p<0.001 compared to SUI; ° - p<0.05 compared to OAB; # - p>0.05 compared to OAB.

with hypertrophic and degenerative changes in smooth myocytes and the interstitium swelling phenomena. We observed SMC polymorphism, varied in size, degree of staining and size of nuclei. The cells had a different saturation with contractile proteins and looked either darker or lighter; most of smooth myocytes were hypertrophied.

The vessels of the microcirculatory stream were dilated, some of them with a thinned wall, dystrophic changes in the endothelium, and erythrocytic stasis, other with thickened walls due to edema, hyperplasia, hypertrophy of collagen fibrils, dilated perivascular space and accumulation of BG in it.

As Table 1 shows, the average number of cells in the degranulation state was 42.00±5.12% and statistically significantly (p<0.001) greater compared to the control group (10.00±4.08%). In the event of degranulation, BG granules that came out to the extracellular space, acquired a variety of optical densities, lost their content, the BG cytoplasm illuminated, with some of them becoming hollow. The granules were of different sizes and vacuolized.

Polymorphism and different size of BG and their granules reflect the processes of cellular degranulation. The predominantly vascular location of BG creates conditions when degranulation products are located in the wall of microvessels, leading to stasis and contributing to ischemia and hypoxia of the bladder-emptying muscle, the important components of OAB pathogenesis.

In a group of experimental animals that received Spasmex separately from hormonal preparations at a morphological analysis on Day 14 of the study, hypertrophic and dystrophic changes in muscle fibers with minor areas of their vacuolization and disorganization were detected, the number of BG and ICC decreased somewhat, but this decrease was not statistically reliable (p>0.05, Table 1, 2).

However, combinations of Spasmex with testosterone, estradiol, and especially combinations of these medicines at this phase of the study, presented with a significant decrease in the amount of BG, despite keeping their functional activity at high level (Table 1). As for ICC,

Table 2. Quantitative composition of ICC in the bladder wall under disorders of urodynamics of the lower urinary tract on Days 14 and 28 of the experiment, calculated as for 0.01 mm² surface.

| Animal group \ Pharmacocorrection type | | Interstitial cells of Cajal | |
|--|-----------------------------------|------------------------------|------------------------------|
| | | Day 14 | Day 28 |
| Control group | | 5.700±0.422 | 5.000±0.421 |
| OAB | | 18.10±0.52 [†] | 16.60±0.63 [†] |
| SUI | | 2.200±0.416 [†] | 1.900±0.284 [†] |
| OAB pharmacocorrection | Mirabegron | 14.80±0.42* | 13.90±0.69* |
| | Spasmex | 17.90±0.57 [#] | 15.60±0.56 [#] |
| | Quercetin | 15.40±0.63* | 11.50±0.82* |
| | Mirabegron+Testosterone | 12.50±0.58* | 10.80±0.42* |
| | Mirabegron+Estradiol | 12.90±0.58* | 10.90±0.48* |
| | Mirabegron+Testosterone+Estradiol | 12.20±0.51* | 9.500±0.619* |
| | Spasmex+Testosterone | 16.40±0.50* | 14.80±0.49* |
| | Spasmex+Estradiol | 16.50±0.60 [#] | 14.90±0.60 [#] |
| | Spasmex+ Testosterone+Estradiol | 16.10±0.66* | 14.10±0.50* |
| | Quercetin+Testosterone | 13.10±0.67* | 10.40±0.58* |
| | Quercetin+ Estradiol | 13.50±0.60* | 10.60±0.67* |
| | Quercetin+ Testosterone+Estradiol | 12.40±0.70* | 8.300±0.395* |
| | Testosterone | 14.80±0.39* | 11.00±0.49* |
| | Estradiol | 14.90±0.64* | 10.90±0.43* |
| Testosterone+Estradiol | 13.80±0.55* | 9.600±0.476* | |
| SUI pharmacocorrection | Quercetin | 4.100±0.276 ^{&} | 4.100±0.316 ^{&} |
| | Testosterone | 3.600±0.426 ^{&} | 3.900±0.423 ^{&} |
| | Estradiol | 3.500±0.268 ^{&} | 3.800±0.359 ^{&} |
| | Testosterone+Estradiol | 4.000±0.516 ^{&} | 4.200±0.290 ^{&} |
| | Quercetin+Testosterone+ Estradiol | 4.300±0.334 ^{&} | 4.700±0.300 ^{&} |

Note: OAB - overactive bladder, SUI - stress urinary incontinence, ICC - interstitial cells of Cajal; * - p<0.001 compared to OAB; † - p<0.001 compared to control; & - p<0.001 compared to SUI; # - p>0.05 compared to OAB.

combination of Spasmex with estradiol demonstrated statistically unreliable ICC reduction (p<0.05, Table 2) after 14 days of the study.

In the groups of rats administered Mirabegron and Quercetin and combinations thereof with hormonal preparations after 14 days, a reduction in hypertrophic and dystrophic changes in smooth myocytes was observed. The amount of BG and their functional activity was statistically significantly (p<0.001) lowered compared to the one in the OAB group; the same changes were observed under the morphometric analysis of ICC (Tables 1, 2).

Testosterone and estradiol administered alone and combinations thereof demonstrated a decrease in the amount of BG and ICC; however the positive effect of hormonal medicines was lower than the accumulated effect thereof on BG and ICC compared to Mirabegron and Quercetin.

After 28 days of Homviotensin administration, the experimental animals from OAB group demonstrated progressive dystrophic changes in smooth myocytes represented by fibrosis of UB wall muscular layer with

patches of sclerosis as a growth of connective tissue between the UB wall smooth muscle fibers and their replacement by fibrous ones. The number of BG and ICC in the degranulation state somewhat decreased compared with the previous period (Table 1, 2), which may be associated with decompensation (depletion) of their functional activity.

The experimental animals administered Mirabegron and its combination with testosterone and estradiol showed a statistically significant (p<0.001) decrease in both BG with their functional activity and ICC (Table 1, 2).

After 28 days of the experiment, no significant changes were recorded in the group of Spasmex and its combination with hormonal medicines in the quantitative composition and functional activity of BG compared to OAB (p>0.05). The number of ICC decreased statistically significantly when administered a combination with testosterone and the combination of testosterone and estradiol with Spasmex (p<0.05). However, the results in Spasmex+Estradiol group were not statistically reliable for ICC count (p>0.05).

Separate administration of testosterone, estradiol and

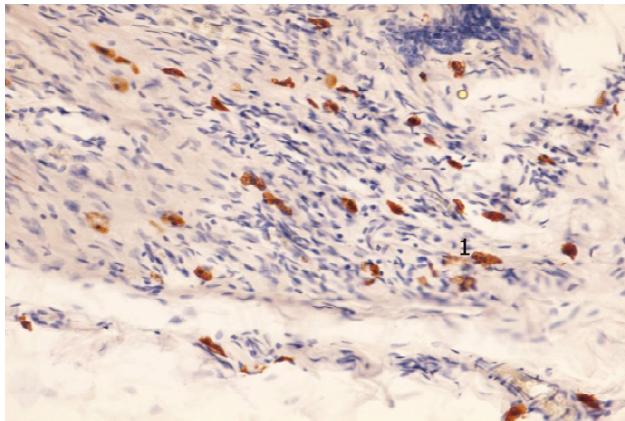


Fig. 3. The increase in the number of basophilic granulocytes with high functional activity in subserous parts of bladder wall. Day 14, OAB. Basic brown, x200. 1 - basophilic granulocytes.

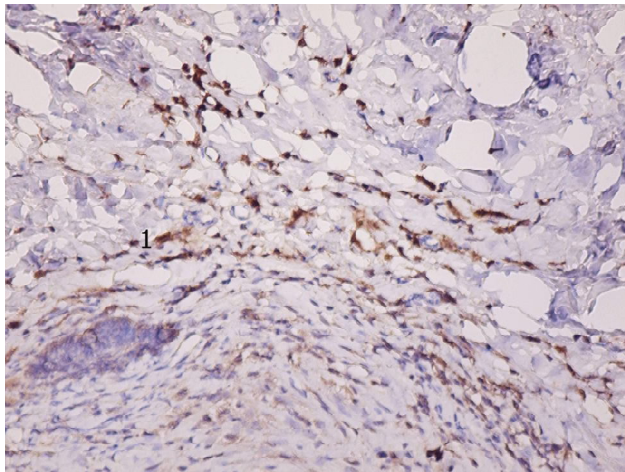


Fig. 4. The increase in the number of interstitial cells of Cajal between the muscle fibers of bladder wall. Day 14, OAB. Immunohistochemical marking CD 117, x400. 1 - interstitial cells of Cajal.

combinations thereof statistically significantly reduced BG count, their functional activity and ICC count ($p < 0.001$), but the efficacy was inferior compared to Quercetin in combination with hormonal medicines (Tables 1, 2).

As for ICC quantitative changes in this monitoring period, the best results were obtained for Quercetin in combination with estradiol and testosterone - 8.300 ± 0.395 , $p < 0.001$ (Table 2).

Morphological characteristic of the bladder-emptying muscle under experimental stress urine incontinence after ligation of n. pudendus.

The morphological examination of the bladder wall in experimental animals with ligated n. pudendus presented with the opposite results compared with OAB group. After 14 days, mostly atrophic changes in the bladder wall were observed, which were manifested by a significant thinning of the muscular layer. As we can see from Table 1, the BG count (7.600 ± 0.426) and the level of their functional activity ($27.00 \pm 3.67\%$) were significantly increasing ($p < 0.001$) compared with the control group of rats - 3.200 ± 0.489 and 10.00 ± 4.08 , respectively.

The ICC count, on the contrary, decreased (2.200 ± 0.416 , $p < 0.001$) by almost twice compared with the control group at this phase of the study - 5.700 ± 0.422 (Table 2).

Pharmacocorrection of SUI using Quercetin, testosterone, estradiol and combinations thereof resulted in a decrease in the amount and functional activity of both BG and ICC on Day 14 of the study (Table 2).

On Day 28 of the experiment, we observed the progression of dystrophic and sclerotic changes in smooth myocytes, blood vessel walls and submucosal UB base in SUI group after ligation of n. pudendus. The BG count (2.000 ± 0.381) and their functional activity ($3.630 \pm 1.363\%$) decreased sharply ($p < 0.001$) compared to the control group of rats - 4.000 ± 0.365 and $8.500 \pm 1.979\%$, respectively.

Quantitative ICC changes after ligation of n. pudendus in SUI rats deteriorated at this phase of the study (1.900 ± 0.284) versus 5.000 ± 0.421 in the control group ($p < 0.001$).

Correction of SUI with Quercetin, testosterone, estradiol and combinations thereof stabilized the quantitative composition and functional activity of BG and ICC and their morphometric parameters approached the ones in the control group (Tables 1, 2).

Consequently, the results of the morphological analysis of BG and ICC in OAB model were characterized by an increase in the amount and functional activity of BG as well as ICC at all phases of the study, while in SUI model the amount and functional activity of BG were high only after 14 days of the experiment, which could be explained by compensatory response to reduction of the total volume of nerve elements in the wall of the bladder caused by atrophy and fibrotic changes in the UB wall. After 28 days, we observed a sharp decrease of the volume, indicating decompensation and depletion of their functional activity. The ICC count decreased under SUI after both Day 14 and Day 28 of the experiment.

The administration of Spasmex alone did not statistically significantly influence the functional activity and quantitative composition of BGs under OAB, but its combinations with hormonal medicines and combinations of testosterone and estradiol demonstrated positive changes only after 14 days of the experiment, whilst the decrease in BG count and their functional activity were not statistically reliable ($p < 0.05$) on Day 28.

The pharmacokinetic correction with Mirabegron, Quercetin and combinations thereof with testosterone, estradiol and combination of the latter, as well as the separate administration of hormonal medicines presented with a positive effect, but the best results were obtained with the combination of Quercetin with testosterone and estradiol at all phases of the study in both OAB and SUI groups.

Discussion

The results of our study established that experimental animals with OAB had increased rhythmic activity of the bladder-emptying muscle, associated with an increase in the number of c-kit-positive cells (ICC) at all phases of the

study, which coincides with the findings of other authors [16, 17]. The high level of BG degranulation along with an increase in their count in OAB group indicates an increase in their functional activity under OAB. The analysis of literature and our data suggest that BG products influence on the contractility and relaxation of UB smooth muscle. As for BG, the ability to influence the muscles of the detrusor and the nervous elements is quite large and preconditioned by production of various substances by these cells: biogenic amines (histamine, serotonin, dopamine), proteoglycans, neutral peptidases (chymase, tryptase), acid hydrolase, leukotrienes, prostaglandins, platelet activation factors, some cytokines (IL-3, IL-4, IL-5, IL-6, IL-10, and IL-13), fibroblast growth factor (FGF), stem cell factor (GM-CSF), vascular permeability and vessel endothelium growth factor [9, 22, 27]. In addition to production of a large group of cytokines, the synthesis of FGF indicates the involvement of these cells in the sclerotic changes in UB wall; the fact established in our work, which is in agreement with the data of other researchers [22]. Unlike other studies, in our experiment we practically did not observe inflammatory infiltration (white blood cells, lymphocytes, macrophages) of UB wall, noted by other authors [20]. In some animals (2%), we observed only isolated lymphocytes that went beyond the boundaries of the vascular walls in the interstitium, which offset the role of these cells as pathogenetic links in the development of OAB and SUI. In our opinion, the contractile activity of UB SMC is brought into effect largely due to the hypoxic state of structural elements of the wall, as evidenced by the numerous stasis in vessels of the microcirculatory stream.

The morphological analysis of smooth myocytes under OAB and SUI confirms the effect of BG and ICC on the contraction state of SMC, as in OAB and SUI models, we observed a polymorphism of smooth myocytes by size, degree of staining and size of the nuclei. The cells had different saturation with contractile proteins and looked either darker or lighter; part of smooth myocytes was hypertrophied or atrophied. Our data on SMC morphological changes coincide with the previous studies [17].

According to our study, the animals in Mirabegron group and combinations thereof with hormonal medicines presented with a significant decrease in the functional BG activity, which may indicate the presence of a stabilizing effect on BG cell membranes in Mirabegron in addition to the stimulating effect on beta3-adrenergic receptors.

The effect of hormonal preparations administered alone on ICC quantitative composition is explained by the presence of receptors in ICC nuclei that are sensitive to both androgens and estrogens, which has also been shown in the studies by other authors [11].

According to McHale et al. (2006) and Hashitani H., Lang R.J. (2010), the cyclic discharge of Ca^{2+} from intracellular depot of ICC causes opening Ca-activating Cl-channels, which leads to depolarization of the cytoplasmic membrane spreading to surrounding smooth myocytes, which are closely bound with ICC by electric signal [13, 19]. Placed in

different locations, ICC generate the potential not simultaneously, resulting in asynchronous contractions of individual SMC groups. Possible involvement of ICC in the pathogenesis of UB overactivity was confirmed by Okada et al. (2011). He showed that chemical cystitis model rats (intravenous administration of cyclophosphamide or protamine sulfate) presented with statistically significantly more pronounced increase in the frequency of spontaneous UB contractions in the WsRC+/+ rats, as UB of the latter contained c-kit-positive cells and were characteristic of the expression of kit-protein, absent in WsRCWs-/Ws- rats [21]. In our previous studies, we found that paxillin, the selective blocker of calcium-sensitive potassium channels of high conductivity (BKCa), inhibited the potassium streams by about twice, which confirmed the significant role of these channels in regulation of activity of the bladder and their possible involvement in the development of pathology, whereas the liposomal Quercetin increased the inhibited potassium channel [10]. One of the mechanisms for restoring potassium channels in administration of Quercetin may be an increased endothelial NO-synthase expression (eNOS) followed by activation of protein kinase G, which leads to a decrease in Ca^{2+} by inhibition of phospholipase C and formation of inositol 1,4,5-triphosphate. These transmitters also stimulate relaxation by activating potassium channels (K^{+} channels), which coincides with the data of other authors, for example, hydrogen sulfide activates protein kinase G, which results in relaxation of muscle fibers by activating ATP-dependent K^{+} channels [12]. The relaxing effect of NO-synthase is closely related to activation of soluble guanylate cyclase and accumulation of cyclic guanosine monophosphate (cGMP). The increased concentration of GMP activates cGMP-dependent protein kinase and ATPase involved in dephosphorylation of light myosin chains, which leads to the release of calcium from muscle cells and, as a result, to vasodilation [5]. It should be noted that in addition to the effect on calcium and potassium channels, in our previous studies we also obtained an antioxidant effect both at separate administration of Quercetin and Mirabegron and in combination thereof with hormones, especially with testosterone, associated with an increase in the synthesis of endothelial fraction of NO-synthase [14].

Therefore, the consideration of complex pathogenetic links in interaction of ICC and BG with smooth myocytes, hormonal and metabolic factors in disorders of urodynamics of the lower urinary tract would allow in the future to use this combination of medicines for treatment of OAB and SUI.

Given the leading role of ICC in regulating the contractile activity of smooth myocytes under AB and SUI, further study of ICC distribution and functional activity of BG in the structural elements of the bladder wall opens the prospects for changing existing perceptions about the mechanism of urodynamic disorders of the lower urinary tract and development of new medicines for correction and optimizing the therapeutic regimens.

Conclusion

1. BG histochemical studies and immunohistochemical analysis of ICC expression in OAB and SUI models confirm their involvement in the pathogenetic mechanisms of urodynamic disorders of the lower urinary tract. According to our research, ICC play the prominent role in the contractile activity of smooth myocytes.

2. The main reaction of BG in modelled OAB was an increase in their count in 14 days and a slight decrease in 28 days of the experiment followed by degranulation, while the models with ligated nerve presented mostly with their degranulation and a significant decrease in the count after 28 days of SUI ($p < 0.001$).

3. In a group of experimental animals receiving Spasmex and its combination with hormones, there was no reliable evidence of their effect on the quantitative and qualitative composition of BG and ICC under OAB and SUI

on Day 14 of the experiment, but a significantly ($p < 0.001$) decreased expression of BG and ICC in the UB muscle layer after 14 days was observed in combination with testosterone, unreliable after 28 days ($p > 0.05$).

4. Administration of Mirabegron alone and especially its combination with testosterone and estradiol demonstrated positive trends in histochemical and immunohistochemical expression of BG and ICC. Stabilization of functional activity of BG was characterized by a decrease in the number of degranulated and vacuolized cells.

5. The results of the study proved the positive effect of Quercetin for treatment of experimental OAB and CMC, and its high efficacy in combination with testosterone and estradiol, which was confirmed by stabilization of the functional activity of BG and ICC quantitative composition at all monitoring phases.

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МОРФОЛОГІЧНИЙ АНАЛІЗ ІНТЕРСТИЦІАЛЬНИХ КЛІТИН КАХАЛЯ І БАЗОФІЛЬНИХ ГРАНУЛОЦИТІВ ПРИ ГІПЕРАКТИВНОМУ СЕЧОВОМУ МІХУРІ ТА СТРЕСОВОМУ НЕТРИМАННІ СЕЧІ В ЕКСПЕРИМЕНТІ ПІД ВПЛИВОМ ФАРМАКОКОРЕКЦІЇ
Яцина О.І., Вернигородський С.В., Костєв Ф.І.

Сучасні уявлення вказують на багатофакторні механізми розвитку гіперактивного сечового міхура (ГАСМ), однак до кінця питання патогенезу ГАСМ залишаються не з'ясованими. Останнім часом поряд з нейрогенною теорією виникнення ГАСМ все більша увага приділяється вивченню морфологічних змін, що виникають в гладких міоцитах детрузора і взаємодії їх з екстрацелюлярним матриксом. Тому метою нашого дослідження стало за допомогою гістохімічних та імуногістохімічних методів оцінити розподіл інтерстиціальних клітин Кахаля (ІКК) і базофільних гранулоцитів (БГ) в структурних елементах стінки сечового міхура при стресовому нетриманні сечі і його гіперактивності до і після лікування мірабегроном, спазмексом, кверцетином і їх комбінації з тестостероном і естрадіолом. На експериментальних моделях ГАСМ і стресового нетримання сечі (СНМ) за допомогою гісто- і імуногістохімічного методу виявлено підвищення кількості та функціональної активності БГ, а також ІКК на всіх термінах спостереження при ГАСМ, в той час як при СНМ кількість і функціональна активність БГ були високими тільки після 14 днів експерименту. Після 28 днів спостерігали різке їх зменшення, що свідчило про декомпенсацію і виснаження їх функціональної активності. Кількість ІКК зменшується при СНМ як після 14 днів, так і через 28 днів експерименту. У групі експериментальних тварин, які отримували спазмекс і комбінацію його з гормонами не виявлено достовірної ефективності їх впливу на кількісний і якісний склад БГ та ІКК при ГАСМ і СНМ на 14 добу експерименту, проте в поєднанні з тестостероном виявлено достовірне ($p < 0,001$) зниження експресії БГ і ІКК у м'язовому шару сечового міхура після 14 днів, і недостовірне через 28 днів ($p > 0,05$). При окремому застосуванні мірабегрона і особливо при його комбінації з тестостероном і естрадіолом встановлена позитивна динаміка гістохімічних та імуногістохімічних показників експресії БГ і ІКК. З'ясовано високу ефективність кверцетину в поєднанні з тестостероном і естрадіолом при ГАСМ та СНС, що підтверджується стабілізацією функціональної активності БГ і кількісного складу ІКК.

Ключові слова: гіперактивний сечовий міхур, стресове нетримання сечі, імуногістохімічний аналіз, інтерстиціальні клітини Кахаля, базофільні гранулоцити, фармацевтична корекція.

МОРФОЛОГИЧЕСКИЙ АНАЛИЗ ИНТЕРСТИЦИАЛЬНЫХ КЛЕТОК КАХАЛЯ И БАЗОФИЛЬНЫХ ГРАНУЛОЦИТОВ ПРИ ГИПЕРАКТИВНОМ МОЧЕВОМ ПУЗЫРЕ И СТРЕССОВОМ НЕДЕРЖАНИИ МОЧИ В ЭКСПЕРИМЕНТЕ И ПРИ ФАРМАКОКОРРЕКЦИИ
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Современные представления указывают на многофакторные механизмы развития гиперактивного мочевого пузыря (ГАМП), однако окончательно вопросы патогенеза ГАМП остаются не выясненными. Последнее время наряду с нейрогенной теорией возникновения ГАМП, все большее внимание уделяется изучению морфологических изменений, возникающих в гладких миоцитах мышцы, выталкивающей мочу и взаимодействия их с экстрацеллюлярным матриксом. Поэтому целью нашего исследования стало с помощью гистохимических и иммуногистохимических методов оценить распределение интерстициальных клеток Кахаля (ИКК) и базофильных гранулоцитов (БГ) в структурных элементах стенки мочевого пузыря при стрессовом недержании мочи и его гиперактивности до и после лечения мирабегроном, спазмексом, кверцетином и их комбинации с тестостероном и эстрадиолом. На экспериментальных моделях гиперактивного мочевого пузыря и стрессового недержания мочи (СНМ) с помощью гисто- и иммуногистохимического метода обнаружено повышение количества и функциональной активности БГ, а также ИКК на всех сроках наблюдения при ГАМП, в то время как при СНМ количество и функциональная активность БГ были высокими только после 14 суток эксперимента. После 28 суток наблюдали резкое их уменьшение, что свидетельствовало о декомпенсации и истощении их функциональной активности. Количество ИКК снижалась при СНМ как после 14 суток, так и после 28 суток эксперимента. В группе экспериментальных животных, получавших спазмекс и комбинацию его с гормонами, не обнаружено достоверной эффективности их влияния на количественный и качественный состав БГ и ИКК при ГАМП и СНМ на 14 сутки эксперимента, однако в сочетании с тестостероном отмечено достоверное ($p < 0,001$) снижение экспрессии БГ и ИКК в мышечном слое мочевого пузыря после 14 суток и недостоверное после 28 суток ($p > 0,05$). При отдельном применении мирабегрона и, особенно, при его комбинации с тестостероном и эстрадиолом установлена положительная динамика гистохимических и иммуногистохимических показателей экспрессии БГ и ИКК. Установлена высокая эффективность кверцетина в сочетании с тестостероном и эстрадиолом при ГАМП и СНС, что подтверждалось стабилизацией функциональной активности БГ и количественного состава ИКК.

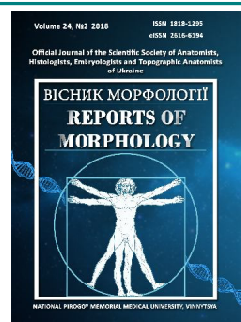
Ключевые слова: гиперактивный мочевой пузырь, стрессовое недержание мочи, иммуногистохимический анализ, интерстициальные клетки Кахаля, базофильные гранулоциты, фармацевтическая коррекция.



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Galectin-3 as the marker of hypertrophy and myocardial dysfunction in males with essential hypertension, carriers of polymorphic genes of angiotensin II type 1 receptor

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One of the main etiological causes of development of heart failure is essential hypertension. The diagnosis of heart failure is usually made on the basis of comprehensive analysis of medical history, sonographic and biochemical examination. Normal ejection fraction does not exclude dyspnea of cardiac origin. Objective: to determine the role of galectin-3 as a marker of structural and functional changes of the myocardium in males with essential hypertension and CHF, carriers of polymorphic AT1R genes, residents of Podillya region of Ukraine. In this contingent, the surveyed were studied concentrations of galectin-3 and brain natriuretic peptide (BNP), parameters of central and systemic hemodynamics in carriers of polymorphic variants of angiotensin II type 1 receptor gene (AT1R) - individuals with no cardiovascular pathology (n=79), male patients with II-III degree essential hypertension (EH) and hypertrophy of the myocardium (n=62), and essential hypertension (n=50) complicated by chronic heart failure (CHF), residents of Podillya region of Ukraine, were studied. Genotyping of AT1R gene was performed using polymerase chain reaction. Galectin-3 and brain natriuretic peptide levels were determined by enzyme immunoassay. Structural and functional parameters of myocardium were assessed by ultrasound using the apparatus "RADMIR ULTIMARA". Statistical analysis of the results obtained was done on personal computer using standard statistical package Statistica 10.0. The data are represented as mean values (M) and standard deviations ($\pm\sigma$). Carriers of C allele of angiotensin II type 1 receptor gene were found to be dominating among the males with essential hypertension and resultant myocardial hypertrophy. Concentrations of galectin-3 and brain natriuretic peptide were significantly higher in men with essential hypertension and essential hypertension associated with chronic heart failure, as compared to those with no cardiovascular diseases, as well as the carriers of C allele of angiotensin II type 1 receptor gene. It was found that concentrations of study biomarkers were higher in individuals with severe and eccentric left ventricular hypertrophy, as well as in those with decreased ejection fraction of the left ventricle. Therefore, those biomarkers can be used in complex diagnosis of left ventricular hypertrophy in essential hypertension and the development of chronic heart failure in such patients.

Keywords: polymorphism of angiotensin II type 1 receptor gene (AT1R), galectin-3, brain natriuretic peptide, structural and functional changes in the myocardium, essential hypertension, chronic heart failure.

Introduction

Over the last decades, heart failure (HF) has become not only the most significant and rapidly progressive medical, but also an important social problem in many economically developed countries, as it leads to early disability of patients, reduced quality of life and its duration [29]. According to the European Society of Cardiology (ESC) [22], essential

hypertension is a major cause of CHF. Traditionally, CHF and its severity are associated with decreased systolic function of the left ventricle (LV), which is usually evaluated by its ejection fraction (EF). However, in some patients, the signs of inadequate blood supply are observed even in slightly modified or normal LV systolic function. The

percentage of this group of patients is quite high - 35%-50% [15]. Asymptomatic diastolic dysfunction (DD) occurs in 28% of American population (age 67 years), and in 27% of European population (age 58 years). 17-25% of patients diagnosed with CHF die within 12 months after hospitalization [13]. Because of that, success in timely diagnosis, prevention and treatment of EH and CHF on its background, are closely related to study of mechanisms of their development and progression [2, 22].

During the last 20 years the role of AT1R polymorphism in the development of EH and CHF is emphasized. A number of such studies were carried out among the residents of Podillya region. In studies conducted by Sakovich O.O. [24], the inheritance of genotypes of AT1R gene with the presence of allele C-AC and CC - by female patients with EH was proved to be associated with primary identification of severe and eccentric left ventricular hypertrophy (LVH), DD by pseudonormal and restrictive types, systolic dysfunction of the LV myocardium. Inheritance of CC genotype by EH female patients with the signs of heart failure stage IIA was associated with higher levels of blood pressure (BP): systolic BP (SBP) - irrespective of the state of LV systolic function, and diastolic blood pressure (DBP) - in those with left ventricular systolic dysfunction. In the study of Starzhynska O.L., the carriage of C allele of AT1R gene was found to be associated with significantly high risk of developing hypertensive disease in males, residents of Podillya region, and the presence of C allele significantly correlated with pronounced pathological changes in the state of the heart and vascular endothelium [25].

In the study by Chandra S. et al. (2014), carriership of allele C of AT1R gene was found to be associated with high levels of blood pressure and development of essential hypertension, as well as high cardiovascular risk [10].

Two phenotypic responses in the form of CHF with different values of LV EF may be associated with the same initial reasons. Several studies have demonstrated that formation of any CHF phenotype can be associated with certain genetic factors, such as polymorphism of cytokine axis genes, NO-synthetase, angiotensin converting enzyme and AT1 receptors, fibroblast growth factor superfamilies, tumor necrosis factor- α , receptors to endogenous catecholamines [8, 9, 20].

Taking into consideration the fact that intensity of heart and vessels remodeling is associated with anti-inflammatory reaction of low intensity, being realized by involvement of promitotic, prooxidant, fibroplastic and neurohumoral factors, one of the methods to assess the contribution of these factors in evolution of HF is the use of molecular biological indicators [5]. To improve the prognosis and to determine the risk of cardiovascular mortality in patients with HF, the search is made for biomarkers that would increase the probability of accurate diagnosis in the presence of a small number of clinical symptoms.

The reference marker for the diagnosis of acute CHF and its exacerbation is brain natriuretic peptide (BNP) [1].

One of the main advantages of BNP use is the possibility to distinguish dyspnea of cardiac origin from dyspnea caused by respiratory system pathology [22]. One of relatively new biomarkers is galectin-3. Its expression is minimal or practically absent in healthy individuals, as well as in patients with cardiovascular diseases in remission or compensatory stage [16]. At the same time, it is maximal at the peak of development of cardiac muscle fibrosis. Galectin-3 practically is not found in cardiomyocytes, whereas its high levels are found in myocardial fibroblasts [21]. In the myocardium galectin-3 stimulates rapid increase of myofibroblast number and release of procollagen 1 in extracellular matrix through paracrine effect, leading to cardiac fibrosis [26]. When there are no clinical manifestations of HF but the ejection fraction (EF) is reduced, galectin-3 level can increase [11]. The probability of 1.5 times increase in galectin-3 level in Ukrainian patients with CHF and EH were noted in the study conducted by Tseluyko V.I. et al. [27]. However, no additional factors were considered, namely the etiology of EH, presence of left ventricular hypertrophy (LVH) and its variants, the carriership of polymorphic variants of AT1R gene, etc. The question arises of whether it is possible to consider galectin-3 a marker not only of the fibrosis processes in the myocardium, which clearly can be marked in overt CHF, but also of their beginning in the development of either type of hypertrophy. On the other hand, to what extent are those processes influenced by the carriership of AT1R gene, which can be a factor causing the development of EH and CHF? Understanding these processes can contribute to more effective primary and secondary prevention of chronic heart failure.

It is galectin-3 that is the marker of the same fibrotic processes underlying CHF. So another question arises of whether it is possible to use galectin-3 levels for screening of candidates for the development of CHF in EH.

Objective: to determine the role of galectin-3 as a marker of structural and functional changes of the myocardium in males with essential hypertension and CHF, carriers of polymorphic AT1R genes, residents of Podillya region of Ukraine.

Materials and methods

The study was performed according to the standards of proper clinical practice and the principles of Helsinki Declaration. A written informed consent was received before enrollment of participants in the study.

The following males - residents of Podillya region were studied: 79 men aged 40-60, average age 57.06 ± 0.50 years, with no signs of cardiovascular disease, 62 men, average age 49.19 ± 0.66 years, with stage II hypertensive disease and 50 men, average age 50.14 ± 0.99 years, with EH complicated by CHF. During enrollment of males in the groups of patients with stage II EH and EH complicated by CHF, the following factors were considered: verified diagnosis of EH (with obligatory exclusion of symptomatic hypertension), the presence of LVH, confirmed by clinical

and instrumental examinations. Exclusion criteria were symptomatic arterial hypertension, impaired renal function and liver, coronary heart disease developed prior to EH, endocrine, hematological, neoplastic and autoimmune disorders, complications of EH - myocardial infarction, acute cerebrovascular accident. In study patients with EH, the diagnosis of concomitant coronary artery disease was excluded after assessment of pre-test probability of the disease on the basis of simple clinical indicators - complaints, anamnesis of the disease, detailed data analysis of outpatient cards of patients, results of ECG at rest and ultrasound examination of the heart at rest.

All the males were inpatients at Vinnytsia Regional Specialized Clinical Hospital of Radiation Protection of Ministry of Health of Ukraine and Military Medical Clinical Center of Central Region of Air Forces of Ukraine, and were also observed on outpatient basis from December 2013 to July 2014.

To determine alleles of polymorphic site (A1166C) of angiotensin II type 1 receptor (AT1R) gene, genomic DNA was isolated from venous blood leukocytes using "Set for separation of DNA/RNA from blood serum or blood plasma" (Private enterprise "Scientific Production Company "LitTech", Russia).

Blood sampling was done in fasting state from cubital vein in the amount of 4ml whole blood into cooled polypropylene tubes, containing EDTA (1mg/1ml of blood). The collected material was stored at -20°C for no more than 6 months before the analysis initiation. Polymerase chain reaction technique was used. Genotyping of AT1R gene was carried out in cooperation with Scientific and Research Institute of Genetic and Immunological Foundations of Pathology and Pharmacogenetics at Higher State Educational Institution "Ukrainian Medical Academy of Dentistry" (Poltava, chief - prof. Kaidashev I.P). To determine alleles of polymorphic site (A1166C) of AT1R gene, genomic DNA was isolated from venous blood leukocytes.

To determine plasma concentration of BNP in study patients, strip immunoassay analyzer "HumaReader single" (Germany) at wavelength of 450 nm and differential filter 620 nm was used, supplied with standard set of Peninsula laboratories Inc. (USA). Blood sampling for analysis was done in fasting state at 8 a.m. from cubital vein in the amount of 3ml whole blood by cooled syringe into cooled polypropylene tubes, containing EDTA (1mg/1ml of blood) and aprotinin (500U per 1 ml of blood). Immediately after sampling, the whole blood was centrifuged for 15 minutes at 0°C (1600 rpm). The resulting plasma was transferred to Ependori type polypropylene tube. Maximum period of sampling material preservation at -32°C before initiation of enzyme immunoassay did not exceed 6 months.

Plasma galectin-3 concentration of study patients was determined by enzyme-linked immunoassay using standard set of reagents produced by Bender MedSystems GmbH (Austria), and "Stat Fact 330" apparatus.

The analysis was carried out in clinical diagnostic

laboratory of private laboratory center "Center B" (date of laboratory certification - March 5, 2014). Laboratory director S.O. Stepanets was executive in charge. Detection of galectin-3 level is indicated in attestation certificate of the laboratory. Reference value of plasma galectin-3 concentration - 0.0-2.28 ng/ml, average level - 0.54 ng/ml.

Blood sampling was done in fasting state from cubital vein in the amount of 2ml whole blood at 8 a.m. Then 0.5 ml of plasma was separated, time between sampling of whole blood and plasma separation was less than 30 min. The separated plasma was frozen at -25°C.

Frequency distribution of polymorphic genes in the population was tested according to Hardy-Weinberg equilibrium law using GeneXpert calculator for calculation of a number of statistical parameters in case-control studies which use SNP (gen-exp.ru). ECG registration was done according to conventional method using 12 standard leads. Blood pressure was measured according to recommendations of WHO experts. Evaluation of parameters of systemic and intracardiac hemodynamics was performed using echocardiography on echogram RADMIR ULTIMARA (Kharkiv, Ukraine). Left ventricular muscle mass index (LVMMI) was calculated using Penn Convention formula: $LVMMI = 1.04[(EDS + PWT + IVST)^3 - EDS^3] - 13.6$, where EDS - end diastolic size, PWT - posterior wall thickness, IVST - intraventricular septum thickness. $LVMMI \geq 115 \text{ g/m}^2$ was considered the criterion of LVH for males according to clinical recommendations of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) to treatment of arterial hypertension (2016) [2].

LVMMI below 170 g/m² denoted moderate LVH, while that over 170 g/m² was indicative of severe LVH. Calculation of relative wall thickness (RWT) of the left ventricle by the formula $(2 \times PWd) / EDS$, where PWd - posterior wall thickness in diastole, EDS - end diastolic size) allows to distinguish increased mass of the LV to concentric ($LV \text{ RWT} \geq 0.42$) and eccentric ($LV \text{ RWT} \leq 0.42$) hypertrophy and allows to distinguish concentric left ventricular remodeling (normal LVMMI with increasing $LV \text{ RWT} \geq 0.42$). The following transmitral flow parameters were studied in pulsed wave Doppler regime: maximum velocity of rapid early diastolic filling (E) and maximum speed (A) of left ventricular filling during systole of the left atrium (cm/c), their ratio (VE/VA). Diastolic LV function was assessed according to current recommendations [22]. Systolic function of the LV myocardium was evaluated by ejection fraction (EF). $EF > 40\%$ was indicative of preserved systolic function.

Mathematical processing was performed on a personal computer using standard statistical package "Statistica 10.0".

For initial preparation of tables and intermediate calculations Microsoft Excel package was used.

Calculated the primary statistical indicators, identified the differences between the groups by statistical characteristics. All data is presented in the form of average (M) and standard deviation ($\pm\sigma$). Differences between the

samples distributed according to the law of normal distribution, were evaluated for Student's t-criterion (t) for unbound measurements.

Results

Considering vital importance of C allele carriership as one of the prognostic signs of EH, as has been emphasized earlier, we conducted frequency distribution of gene variants and alleles of AT1R gene among males, residents of Podillya region of Ukraine, ill with EH and resultant CHF. According to the data obtained, the distribution was found to correspond to Hardy-Weinberg equilibrium. Predominance of genotype frequency which include C allele of AT1R gene was found in males with EH and CHF, included in study population, (Fig. 1), this being consistent with other authors.

To make further analysis easier both in patients with EH, and EH and CHF, as well as in the group of individuals with no cardiovascular disease, - those with genotypes which include C allele (A1166C and C1166C), it was decided to combine them in one group - the carriers of AT1R gene C allele.

According to results obtained in the study of biomarkers concentrations, plasma level of galectin-3 and BNP in patients with EH of various stages, men of 40-60 years, residents of Podillya region of Ukraine, are different depending on the constitutional characteristics, severity of the disease and inheritance of different genotypes of AT1R gene. It is noteworthy, that among the patients with EH, regardless of the stage of disease, higher plasma concentrations of galectin-3 and brain peptide were identified in the carriers of C allele than in homozygotes A1166A of AT1R gene. Galectin-3 level was found to be was significantly higher in male patients with EH and CHF as compared to those with stage II EH ($p < 0.05$) (Table 1).

Pathophysiology of CHF is directly related to remodeling of the LV, fibrosis of the myocardium being one of its characteristic features [17]. Galectin-3 induces the

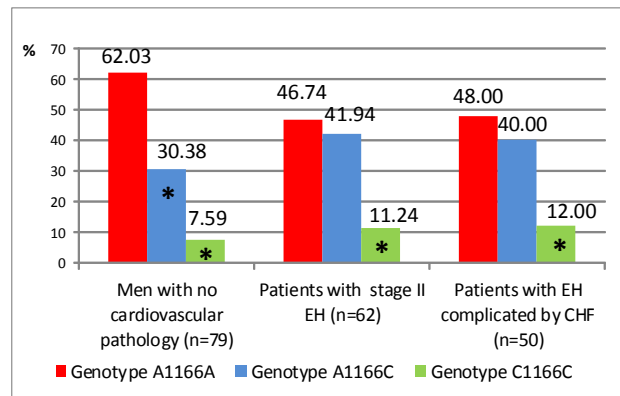


Fig. 1. Frequency distribution of genotypes of AT1R gene among males, residents of Podillya region of Ukraine, with stage II EH and EH complicated by CHF, (%). * - difference between the indices is significant ($p \leq 0.05$) when compared with A1166A genotype within the group.

processes of proliferation of fibroblasts, collagen synthesis and expression of cycline D1. Table 2 presents the levels galectin-3 and BNP in male patients with EH at different stages of LV hypertrophy.

According to our data, galectin-3 and BNP levels were significantly higher in the carriers of AT1R gene C allele and in the patients with severe and eccentric LV hypertrophy.

The next step was to determine the changes of biomarkers in the development of different variants of myocardial dysfunction.

Among male patients with stage II EH, diastolic dysfunction was registered in 8 patients (40.00%) - homozygotes of A1166A genotype, and 12 patients (60.00%) - carriers of AT1R gene C allele ($p > 0.05$). It should be noted that no systolic dysfunction was registered in patients with stage II EH.

Normal transmitral flow (TMF) and preserved diastolic function was observed in 21 individuals (72.41%) - homozygotes of A1166A genotype, and 22 patients (66.67%)

Table 1. Blood plasma galectin-3 and BNP levels in males with no cardiovascular pathology, patients with EH and EH complicated by CHF, carriers of different variants of AT1R gene (ng/ml, pcg/ml).

| Groups | Blood plasma galectin-3 level | Blood plasma BNP level |
|--|--|--|
| Males with no cardiovascular pathology (n=79) | 7.031±0.127 (n=79) (1) | 21.74±0.5 (n=79) (1) |
| Homozygotes of A1166A genotype | 6.824±0.251 (n=49) (2) | 20.05±2.02 (n=49) (2) |
| C allele carriers | 7.363±0.405 (n=30) (3) | 21.88±0.62 (n=30) (3) |
| Patients with stage II EH (n=62) | 21.31±0.22 (n=62) (4) | 77.40±2.85 (n=62) (4) |
| Homozygotes of A1166A genotype | 20.82±0.51 (n=29) (5) | 56.56±0.90 (n=29) (5) |
| C allele carriers | 22.87±0.56 (n=33) (6) | 88.79±2.97 (n=33) (6) |
| Patients with EH and CHF (n=50) | 46.65±2.18 (n=50) (7) | 185.9±5.7 (n=50) (7) |
| Homozygotes of A1166A genotype | 44.27±2.77 (n=24) (8) | 163.5±6.8 (n=24) (8) |
| C allele carriers | 59.23±3.39 (n=26) (9) | 216.2±5.3 (n=26) (9) |
| P | $p_{3-2} > 0.05; p_{4-1} < 0.0001; p_{5-2} < 0.0001; p_{6-3} < 0.0001; p_{6-5} < 0.05; p_{7-1} < 0.0001; p_{7-4} < 0.0001; p_{8-2} < 0.0001; p_{8-5} < 0.0001; p_{9-3} < 0.0001; p_{9-6} < 0.0001; p_{9-8} < 0.05$ | $p_{3-2} > 0.05; p_{4-1} < 0.0001; p_{5-2} < 0.0001; p_{6-3} < 0.0001; p_{6-5} < 0.05; p_{7-1} < 0.0001; p_{7-4} < 0.0001; p_{8-2} < 0.0001; p_{8-5} < 0.0001; p_{9-3} < 0.0001; p_{9-6} < 0.0001; p_{9-8} < 0.05$ |

Table 2. Blood plasma galectin-3 and BNP levels in male patients with stage II EH and various types of LV hypertrophy, carriers of polymorphic variants of AT1R gene (ng/ml, pcg/ml).

| Patient group | 1. Galectin-3 level in AA homozygotes | 2. Galectin-3 level in carriers of C alleles | 3. BNP level in AA homozygotes | 4. BNP level in carriers of C alleles | p<0.05 |
|---|--|--|--|--|-------------------------------------|
| 1. Patients with stage II EH, CLVH (n=44) | 20.77±0.56 (n=18) | 25.82±0.67 (n=26) | 49.21±1.39 (n=18) | 82.31±1.17 (n=26) | p ₂₋₁ , p ₃₋₄ |
| 2. Patients with stage II EH, ELVH (n=18) | 21.99±1.34 (n=7) | 29.15±1,00 (n=11) | 53.59±1.01 (n=7) | 97.73±1.20 (n=11) | p ₂₋₁ , p ₃₋₄ |
| 3. Patients with stage II EH, MLVH (n=53) | 25.33±1.75 (n=25) | 31.29±0.57 (n=28) | 50.78±1.23 (n=25) | 93.03±1.48 (n=28) | p ₂₋₁ , p ₃₋₄ |
| 4. Patients with stage II EH, SLVH (n=9) | 32.91±0.54 (n=4) | 38.50±0.38 (n=5) | 49.14±1.86 (n=4) | 93.44±1.87 (n=5) | p ₂₋₁ , p ₃₋₄ |
| p<0.05 | p ₂₋₁ >0.05; p ₃₋₄ >0.05 | p ₂₋₁ >0.05; p ₃₋₄ >0.05 | p ₂₋₁ >0.05; p ₃₋₄ >0.05 | p ₂₋₁ >0.05; p ₃₋₄ >0.05 | |

Note: CLVH - concentric left ventricular hypertrophy; ELVH - eccentric left ventricular hypertrophy; MLVH - moderate left ventricular hypertrophy; SLVH - severe left ventricular hypertrophy.

- carriers of C allele (p>0.05). Impaired TMF, its hypertrophic type, was registered in 7 individuals (24.14%) - homozygotes of A1166A genotype, and 10 patients (30.30%) - carriers of AT1R gene C allele (p>0.05) (Fig. 2).

Plasma levels of galectin-3 and BNP in patients with stage II EH and EH and CHF, at different TMF types, were found to be significantly higher in carriers of AT1R gene C allele than in A1166A homozygotes (p<0.05). In addition, concentrations of galectin-3 and BNP in patients with EH and CHF with hypertrophic type of TMF, were higher than in those with stage II EH, both in carriers of A1166A genotype and in carriers of AT1R gene C allele at the appropriate TMF type (p<0.05).

Thus, males with stage II EH - A1166A homozygotes, and carriers of AT1R gene C allele, regardless the state of diastolic function, had higher plasma levels of galectin-3 and BNP than those with no cardiovascular pathology (p<0.05), but significantly lower ones than patients with EH and CHF - carriers of corresponding genotypes (p<0.05). The highest concentrations of galectin-3 and BNP were observed in patients with EH and CHF with diastolic dysfunction of LV and FV<40% - carriers of AT1R gene C allele.

Analysis of frequency distribution of AT1R gene genotypes in males with EH and CHF at EF>40% and EF<40% demonstrated that the first group included 36 patients - homozygotes of A1166A genotype, and the second one - 14 men - carriers of C allele - patients with EH and CHF. Carriers of AT1R gene C allele significantly dominated in the group of patients with EF<40% - 85.71% (n=12) (p<0.05) (Fig. 3).

Galectin-3 and BNP levels in patients with EH and CHF with different ejection fraction were calculated (Fig. 4). The higher values of these biomarkers were found in patients with decreased ejection fraction. However, the degree of increase of biomarkers was different in carriers of polymorphic AT1R genes.

Significantly higher values of galectin-3, BNP, LVMMI, LP were detected in patients with EH and CHF with EF>40%, carriers of AT1R gene C allele, than in A1166A homozygotes.

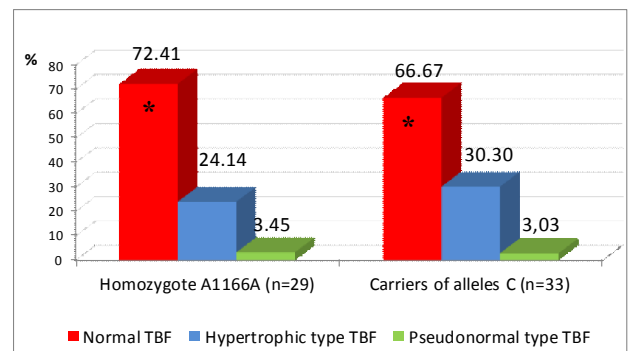


Fig. 2. Types of transmittal blood flow in male patients with stage II EH, carriers of different variants of AT1R gene, (%). * - difference of indicators is significant at (p<0.05) as compared to pseudonormal type of TMF within each group.

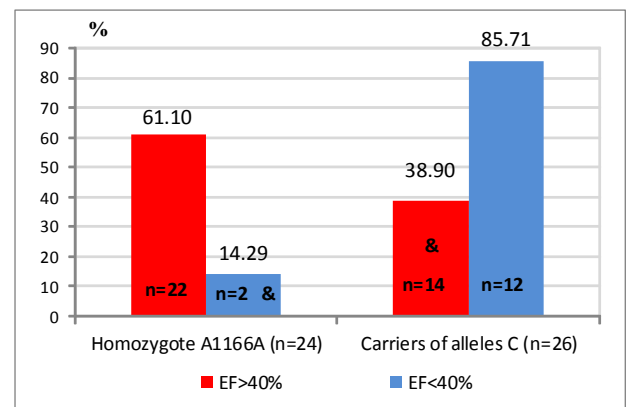


Fig. 3. Frequency distribution of AT1R gene genotypes in male patients, with EH and CHF at EF>40% and EF<40%, residents of Podillya region of Ukraine, (%). & - the difference of indicators is statistically significant (p<0.05) when compared between genotypes of AT1R gene.

Hemodynamic parameters (SAP and DAP) proved to be higher in carriers of allele C, corresponding to EH of II degree, and A1166A homozygotes of AT1R gene, corresponding to EH of I degree (p<0.05).

Among patients with CHF and EF<40%, carriers of AT1R

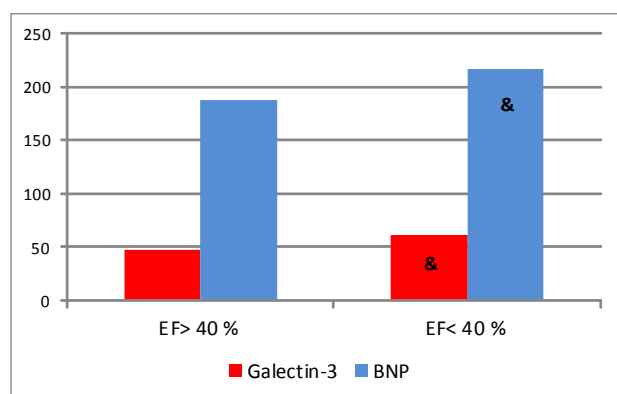


Fig. 4. Frequency distribution of AT1R gene genotypes in male patients with EH and CHF at EF>40% and EF<40%, residents of Podillya region of Ukraine, (ng/ml; pg/ml). & - difference of indicators is statistically significant ($p<0.05$) when compared between the groups of patients with different EF.

gene C allele, the highest indices of galectin-3, BNP, LVMMI, LP were registered. BP was also higher in carriers of allele C, corresponding to III degree of EH, and in A1166 homozygotes of AT1R gene, corresponding to II degree of EH.

The question rose, to what extent the levels of biomarkers reflect total changes in the myocardium, leading to the development of CHF, and whether their concentrations are influenced by myocardial hypertrophy type.

To answer those questions, first the levels of galectin-3 and BNP in eccentric left ventricular hypertrophy (ELVH) and concentric left ventricular hypertrophy (CLVH) were analyzed. Patients with EH and CHF, with ELVH, carriers of AT1R gene C allele, had higher levels of plasma concentrations of galectin-3 (29.15 ng/ml in EH versus 55.16 ng/ml in EH and CHF) and peptide (97.73 pg/ml in EH versus 208.9 pg/ml in EH and CHF) as compared to those with CLVH. Galectin-3 level was 25.82 ng/ml in patients with EH and 61.6 ng/ml in EH complicated by CHF, respectively, and peptide level was 82.31 pg/ml in uncomplicated EH and 198.08 pg/ml in EH and CHF.

The next step was the analysis of galectin-3 and BNP concentrations at EF 40%> and <40%. According to the data obtained, levels of galectin-3 (83.89 pg/ml) and BNP (229.2 pg/ml) were significantly higher in individuals with EH and CHF, CLVH and ELVH and FV<40%, carriers of allele C.

Discussion

AT1R gene is mapped in the 3rd chromosome (3q21-3q25), it contains 5 exons, and today 16 structural polymorphisms of this gene have been described. However, it is polymorphism-change of adenine (A) to cytosine (C) [23] at position 1166, that is associated with essential hypertension [14, 19, 23], coronary artery disease [12] and myocardial infarction [14]. According to our data, the carriers of AT1R gene C allele dominated in study population of 40-60 year old males with EH and EH complicated by CHF, residents of Podillya region. They had higher BP levels, and

there is relationship between ELVH and impaired diastolic function of the left ventricle by pseudonormal and restrictive types. These data are similar to those received by Sakovich O.O. (2011): carriership of genotypes of AT1R gene with C allele is associated not only with higher degrees of hypertension, but also with the presence of eccentric LVH and dysfunction of the left ventricle in the form of pseudonormal and restrictive types of transmitral flow, LV systolic dysfunction. Those disturbances were especially evident in patients with EH complicated by CHF [24]. In the study of Blonar O.L. and Zhebel V.M. [7] the carriership of AC genotype in male patients with EH was found to determine 77% probability for the development of HF with decreased systolic left ventricular function ($\chi^2=8.86$, $p<0.05$). There is significant correlation between genotype AC and burdened inheritance of EH and early CHF signs. According to A.M. Bilovol et al. (2015), significantly higher levels of arterial pressure, more evident disturbances in structural and functional parameters of the heart and blood vessels are more typical of genotypes AC and CC of AT1R gene than AA genotype [6].

According to our data, levels of plasma concentrations of galectin-3 and peptide were significantly higher in patients with ELVH than those with CLVH. In the study of Beltrami M. (2016) positive correlation between increased galectin-3 level and increased LVMMI, LA sizes and severity of diastolic dysfunction were found [4]. According to Qiu-Sheng Yin et al. (2014), concentrations of galectin-3 and BNP were significantly higher in the patients with EH as compared to individuals with no cardiovascular diseases. Those indices were also significantly higher in patients with CHF, developed as a complication of EH with reduced EF than in those with preserved ejection fraction [28].

In the works of Nikonova E.S. et al. (2013), positive correlation between the level of BNP and end systolic volume (ESV) was found only in patients with decreased EF, as well as a marked increase in the level of BNP with the progression of diastolic and systolic dysfunction [18]. Positive correlation relationship between BNP level and systolic arterial pressure, stroke volume (SV), end systolic volume (ESV) and end diastolic volume (EDV) was found by D.A. Andreev et al. (2007), that can be explained by increased intraventricular pressure and distention of the left ventricle [3].

All patients with EH and EH complicated by CHF had significantly higher level of galectin-3 and BNP than individuals with no cardiovascular disease, and it was significantly higher in those with EH and CHF in FV<40%, thus, those biomarkers can be used for screening of individuals with hypertrophy and myocardial dysfunction to direct them for follow-up examination (Echocardiography).

The results of the study allow further study of galectin-3 as a biomarker of the structural and functional state of the myocardium in essential hypertension and chronic heart failure in the presence of various comorbid conditions (diseases).

Conclusions

1. Blood concentration of galectin-3 and BNP in male patients with stage II EH and EH and CHF, regardless of the level of LV EF, was significantly higher in C allele carriers than in carriers of A1166A genotype of AT1R gene ($p < 0.05$).

2. Carriership of C allele in the structure of AT1R gene in patients with EH and CHF was associated with ELVH and impaired diastolic function by pseudonormal and restrictive types, which can be indicative of more severe disturbances

of the processes of relaxation and LV filling because of increased stiffness and decreased compliance of LV myocardium.

3. Determination of galectin-3 level in the study of morphological changes of the myocardium in patients with EH and CHF was as informative as the study of concentration of "reference" biomarker BNP, thus providing the use of this biological factor for bio-labeling of myocardial hypertrophy and associated functional disorders.

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ГАЛЕКТИН-3 ЯК МАРКЕР ГІПЕРТРОФІЇ І ДИСФУНКЦІЇ МІОКАРДА У ЧОЛОВІКІВ ХВОРИХ НА ЕСЕНЦІАЛЬНУ ГІПЕРТЕНЗІЮ, НОСІЇВ ПОЛІМОРФНИХ ГЕНІВ РЕЦЕПТОРУ АНГІОТЕНЗИНУ II ТИПУ 1

Ружанська В.О., Сівак В.Г., Сакович О.О., Жебель В.М.

Однією з основних етіологічних причин виникнення серцевої недостатності є есенціальна гіпертензія. Діагноз серцевої недостатності зазвичай встановлюють на основі аналізу історії хвороби, результатів ехографічного та біохімічного обстеження хворого. Наявність нормальної фракції викиду не виключає задишку кардіального генезу. Мета - визначити роль галектину-3 як маркера структурно-функціональних змін міокарда у чоловіків, хворих на есенціальну гіпертензію та ХСН носіїв поліморфних генів AT1R, мешканців Подільського регіону України. У зазначеного контингенту обстежених вивчали концентрацію галектину-3 та мозкового натрійуретичного пептиду, показники центральної та системної гемодинаміки у носіїв поліморфних варіантів гену рецептору ангіотензину II типу 1 (AT1R) в осіб без серцево-судинної патології (n=79), чоловіків хворих на есенціальну гіпертензію з гіпертрофією міокарда II-III ступеня (n=62) та з есенціальною гіпертензією (n=50), що ускладнилась хронічною серцевою недостатністю. Генотипування гену AT1R проводили за допомогою полімеразної ланцюгової реакції, рівень галектину-3 та мозкового натрійуретичного пептиду - імуноферментним аналізом. Структурно-функціональні показники міокарда оцінювали за допомогою ультразвукового дослідження на апараті "РАДМИР ULTIMARA". Отримані дані обробляли математично на персональному комп'ютері з використанням стандартного статистичного пакету Statistica 10,0. Усі дані представлені у вигляді середнього значення (M) та стандартного відхилення ($\pm\sigma$). Виявлено, що у чоловіків з есенціальною гіпертензією II стадії і з есенціальною гіпертензією та хронічною серцевою недостатністю, що виникла на її тлі, домінують носії алелі С гену рецептору ангіотензину II типу 1. Концентрація галектину-3 та мозкового натрійуретичного пептиду достовірно вища у чоловіків з есенціальною гіпертензією та есенціальною гіпертензією і хронічною серцевою недостатністю порівняно з особами без серцево-судинних захворювань, а також у носіїв алелі С в гені рецептору ангіотензину II типу 1. З'ясовано, що концентрація біомаркерів, що вивчали, була вища в осіб з вираженою та ексцентричною гіпертрофією лівого шлуночка та при його пониженні фракції викиду. Дані біомаркери можуть бути використані в комплексній діагностиці гіпертрофії лівого шлуночка серця при есенціальній гіпертензії та при розвитку у пацієнтів хронічної серцевої недостатності.

Ключові слова: поліморфізм гену рецептору ангіотензину II типу 1 (AT1R), галектин-3, мозковий натрійуретичний пептид, структурно-функціональні зміни міокарда, есенціальна гіпертензія, хронічна серцева недостатність.

ГАЛЕКТИН-3 КАК МАРКЕР ГИПЕРТРОФИИ И ДИСФУНКЦИИ МИОКАРДА У МУЖЧИН С ЭССЕНЦИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ, НОСИТЕЛЕЙ ПОЛИМОРФНЫХ ГЕНОВ РЕЦЕПТОРА АНГИОТЕНЗИНА II ТИПА 1

Ружанская В.А., Сивак В.Г., Сакович Е.А., Жебель В.Н.

Одной из основных этиологических причин возникновения сердечной недостаточности является эссенциальная гипертензия. Диагноз сердечной недостаточности обычно устанавливают на основе анализа истории болезни, эхографического и биохимического обследования больного. Наличие нормальной фракции выброса не исключает одышку кардиального генеза. Цель - определить роль галектина-3 как маркера структурно-функциональных изменений миокарда у мужчин, больных эссенциальной гипертонией и ХСН носителей полиморфных генотипов AT1R, жителей Подольского региона Украины. В обозначенном контингенте обследуемых лиц изучали концентрацию галектина-3 и мозгового натрийуретического пептида, показатели центральной и системной гемодинамики у носителей полиморфных вариантов гену рецептора ангиотензина II типа 1 (AT1R) у лиц без сердечно-сосудистой патологии (n=79), мужчин больных эссенциальной гипертонией с гипертрофией миокарда II-III степени (n=62) и с эссенциальной гипертонией (n=50), которая усложнилась хронической сердечной недостаточностью. Генотипирование гену AT1R проводили с помощью полимеразной цепной реакции, уровень галектина-3 и мозгового натрийуретического пептида - иммуноферментным анализом. Структурно-функциональные показатели миокарда оценивали с помощью ультразвукового исследования на аппарате "РАДМИР ULTIMARA". Полученные данные обрабатывали математически на персональном компьютере с использованием стандартного статистического пакета Statistica 10,0. Все данные представлены в виде среднего значения (M) и стандартного отклонения ($\pm\sigma$). Выведено, что у мужчин с эссенциальной гипертонией II стадии и с эссенциальной гипертонией и хронической сердечной недостаточностью, которая возникла на ее фоне, доминируют носители алели С гену рецептора ангиотензина II типа 1. Концентрация галектина-3 и мозгового натрийуретического пептида достоверно более высокая у мужчин с эссенциальной гипертонией и эссенциальной гипертонией и хронической сердечной недостаточностью по сравнению с лицами без сердечно-сосудистых заболеваний, а также у носителей алели С в гене рецептора ангиотензина II типа 1. Выяснено, что концентрация изучаемых биомаркеров была более высокой у лиц с выраженной и эксцентрической гипертрофией левого желудочка и при его снижении фракции выброса. Данные биомаркеры могут быть использованы в комплексной диагностике гипертрофии левого желудочка сердца при эссенциальной гипертонии и при развитии у пациентов хронической сердечной недостаточности.

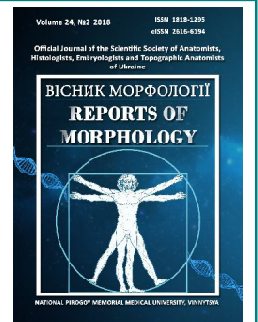
Ключевые слова: полиморфизм гену рецептора ангиотензина II типа 1 (AT1R), галектин-3, мозговой натрийуретический пептид, структурно-функциональные изменения миокарда, эссенциальная гипертония, хроническая сердечная недостаточность.



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Determination of standard cephalometric parameters using the Downs method for Ukrainian adolescents

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The difference in the characteristics of craniofacial structures in different races and ethnicities established in many studies requires the creation of a normative basis for telerontgenographic indices and indices characteristic for a particular ethnic group. The purpose of the work is to set the cephalometric parameters by W. Downs for young men and women from Podillia region of Ukraine with orthognathic bite and compare the results with the data proposed by the author of the technique. With the device Veraviewepocs 3D device, Morita (Japan), 38 young men (17 to 21 years of age) and 55 young women (aged from 16 to 20 years) with orthognathic bite (normal occlusion close to orthognathic bite) received and analyzed lateral telerontgenograms. Cephalometric points and measurements were performed in accordance with the recommendations of W. Downs (1948). Anatomical points were determined taking into account recommendations A.E. Athanasiou (1997), S.I. Doroshenko and Y.A. Kulginsky (2007). The statistical processing of the obtained results was carried out in the licensed package "Statistica 6.0" using nonparametric methods for evaluating the obtained results. As a result of the research, virtually no sex differences in cephalometric parameters by W. Downs between adolescents from Podillia with orthognathic bite were found. When comparing the cephalometric parameters obtained by W. Downs from similar figures obtained in Ukrainian young men and women with orthognathic bite, regardless of gender, established significantly lower values of Cant Occlusal Plane (angle PO_r-DOP) and angle II, as well as significantly higher values were established for angle 1I-DOP, angle 1I-MeGo and distance 1u-APog in Ukrainian young men and women of Podillia. The obtained results indicate the need to create a normative base for cephalometric standards by W. Downs for the population of different regions of Ukraine, taking into account gender and age belonging.

Keywords: skull, cephalometry, Downs analysis.

Introduction

One of the first in 1948, William Downs outlined a technique for cephalometric analysis and recommendations for its clinical application as a result of the study of 10 boys and 10 girls aged from 12 to 17 who had "perfect occlusion" [10]. The proposed method consists of ten indicators that are equally divided into skeletal and dental ones. And for a more easy perception and interpretation of the meanings, a graphic polygon [11] with average values for each indicator and the limits of its deviations was developed. According to R. G. Keim et al. [16] method has gained great popularity and was used from 11.1 to 26.3% of dental practitioners from 1986 to 2008. The Center for the Study of Human Growth and Development, at the University of Michigan in 1974, for all indicators based on the study of 83 people aged from 6 to

16 years, were defined average standards for each child's age [18]. But it should be borne in mind that the understanding of true occlusion relates to the harmonious features of the person and takes into account the individual ethnic, age and sexual characteristics, which according to various studies vary considerably [1, 4, 12, 19, 20].

Since in different races and ethnicities the characteristics of craniofacial structures vary considerably, this situation is not caused by any curiosity of researchers with regard to the study and creation of a normative basis for telerontgenographic indices and indices characteristic of a particular ethnic group [3, 4, 13, 17, 21]. As these studies indicate significant variations in the various cephalometric indices, the question arises as to the correctness of the application of normative data obtained in

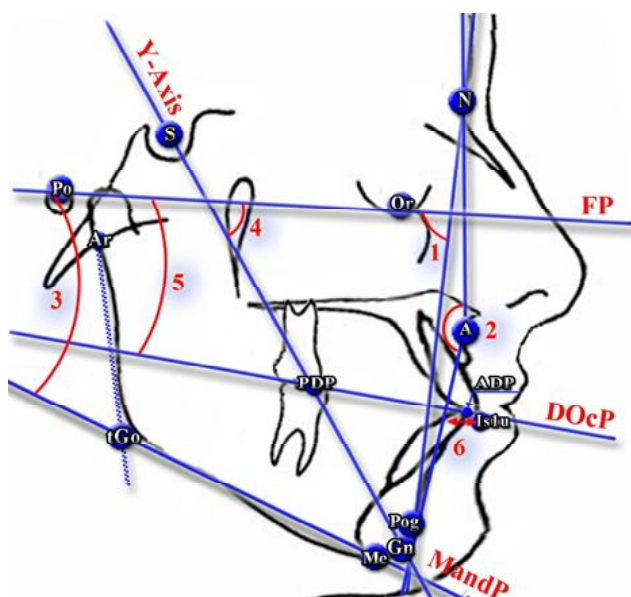


Fig. 1. *A* (subspinale) - point A by Downs, the most posteriorly located point of the anterior contour of the upper jaw; ADP (front locking point by Downs) - middle of vertical incisal overlap between the cutting edges of the middle incisors of the upper and lower jaws, the middle of the vertical and arched gaps between the medial incisors; Ar (articulare) - intersection of the anterior surface of the main part of the occipital bone with the back surface of the cervix of the mandible; Gn (gnation) - the place of connection of the lower edge of the mandible and the outer contour of the ossified symphysis, the anterior point on the lower contour of the body of the mandible; Is1u (incision superior) - point located on the cutting edge of the upper middle cutter; Me (menton) - the lowest point on the ossified symphysis of the mandible; N (nasion) - the foremost point of the fronto-nasal seam, joints of the frontal and nasal bones in the medial-arterial plane; Or (orbitale) - the lowest part of the infraorbital region, is located on the orbital edges of the caudal bone; PDP (posterior Downs point) - middle of the surface of the closure of the near edge of the first upper and lower molar teeth; Po (porion) - placed on the upper edge of the external auditory hole; Pog (pogonion) - the most forward point of the bony pectoral projection; S (sella) - constructive point in the center of the Turkish saddle; tGo - the projection point on the angle of the mandible, is formed at the intersection of the lines, one of which is the tangent to the posterior margin of the branch of the mandible from the point Ar, second is the tangent to the lower edge of the body of the mandible from the point Me (usually a few millimeters lower and more distant than a point Go); 1 - angle PO_r_NPog; 2 - angle of Convexity; 3 - Mandibular Plane Angle; 4 - Y-Axis; 5 - Cant of Occlusal Plane; 6 - distance 1_u_APog.

the study of another population, for the residents of Ukraine. This calls for further researches.

The purpose of the work - to establish cephalometric parameters by W. Downs in young men and women from Podillia region of Ukraine with orthognathic bite and compare results with the data suggested by the author of the technique.

Materials and methods

With the device Veraviewepocs 3D device, Morita (Japan), 38 young men (17 to 21 years of age) and 55 young women (aged from 16 to 20 years) with orthognathic occlusion

(normal occlusion close to orthognathic bite) received and analyzed lateral teleroentgenograms.

Cephalometric points and measurements were carried out in accordance with the recommendations of W. Downs [10]. Anatomical points were determined taking into account the recommendations of A. E. Athanasiou [2] and S. I. Doroshenko and Y. A. Kulginsky [9].

According to the method of W. B. Downs studied the following indicators (Fig. 1, Fig. 2):

- angle PO_r_NPog - Facial Depth, angle formed by the reference lines Po-Or and N-Pog;
- angle NAPog - Angle of Convexity, formed by the reference lines N-A and A-Pog;
- angle AB_NPog - AB Plane Angle, formed by the reference lines A-B and N-Pog (angle of the plane A-B, defines the position of the plane A-B in relation to the N-Pog);
- angle PO_r_MeGo - Mandibular Plane Angle, angle between the mandibular plane and the Frankfort Horizontal, formed by the reference lines Po-Or and Me-tGo;
- angle PO_r_GnS - Y-Axis, determines the cant of the y-axis in relationship to the Frankfort Horizontal, formed by the reference lines P-Or and S-Gn;
- angle PO_r_DOP - Cant of Occlusal Plane, determines the angle of the occlusal plane in relationship to the Frankfort Horizontal, formed by the reference lines Po-Or and ADP-PDP (occlusal plane);
- angle II - II, angulation of axes of upper to lower incisor, formed by the reference lines Ap1u-Is1L (central axes of the

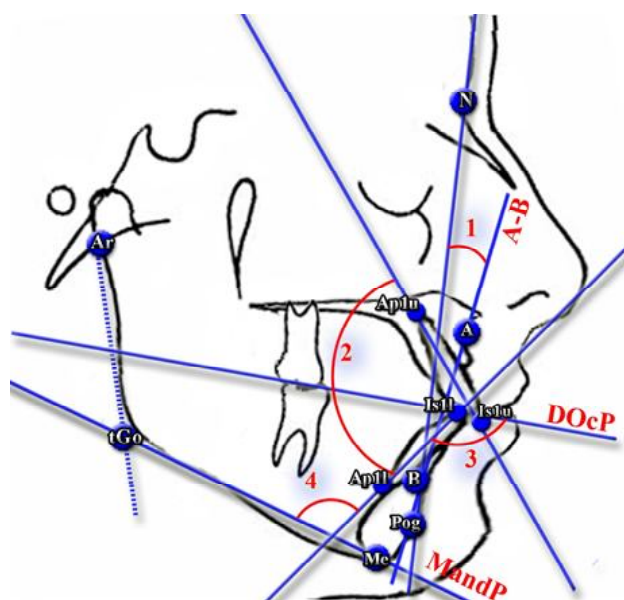


Fig. 2. *A* (subspinale); Ap1L (apex first inferior incisor); Ap1u (apex first upper incisor); Ar (articulare) - intersection of the anterior surface of the main part of the occipital bone with the back surface of the cervix of the mandible; B (submentale) - point B by Downs, the deepest point on the anterior contour of the mandible; Is1L (incision inferior) - point located on the cutting edge of the lower middle cutter; Is1u (incision superior); Me (menton); N (nasion); Pog (pogonion); tGo; 1 - AB Plane Angle; 2 - II angle; 3 - Angle 1_l_DOP; 4 - Angle 1_l_MeGo.

upper central incisor) and Ap1L-Is1L (central axes of the lower incisor);

- **angle 1I_DOP** - formed by the reference lines Ap1L-Is1L (central axes of the lower incisor) and ADP-PDP (occlusal plane), measures of deviation from the right angle with a positive value in the direction of the clockwise, with a negative if counterclockwise;

- **angle 1I_MeGo** - Determines the position of the axes of the mandibular incisors (Ap1L-Is1L) in relationship to the mandibular plane (Me-tGo);

- **distance 1u_APog** - distance from the incisal edge of the most prominent upper incisor (Is1u) to the reference line A-Pog.

The statistical processing of the obtained results was carried out in the licensed package "Statistica 6.0" using nonparametric methods for evaluating the obtained results.

Results

The cephalometric parameters by W. Downs (mean with standard deviation and percentile velocity) in young men and women from Podillia region of Ukraine with orthognathic bite are presented in Table 1.

When comparing cephalometric parameters by W. Downs between young men and women from Podillia region of Ukraine with orthognathic bite, only significantly higher ($p < 0.05$) values of Cant of Occlusal Plane (POr-DOP) were found in young women (see Table 1).

When comparing the cephalometric parameters obtained by W. Downs with the indices obtained in young men and women from Podillia with orthognathic bite, established significantly lower ($p < 0.05-0.001$) values of Cant of Occlusal Plane (angle POr-DOP) and angle II, as well as the tendency ($p = 0.064$ and 0.072) to the lower values of the Y-axis (angle POr-GnS) in Ukrainian young men and women

with orthognathic bite (Table 2). Conversely, the value of angle 1I-DOP, angle 1I-MeGo and distance 1u-APog in Ukrainian young men and women with orthognathic bite is significantly ($p < 0.01-0.001$) greater than the cephalometric parameters obtained by W. Downs (see Table 2).

Discussion

When comparing cephalometric parameters used in the Downs analysis between young men and women from Podillia with orthognathic bite, there is practically no gender discrepancy between the values of these indices. Only the value of the indicator of Cant Occlusal Plane (POr-DOP) in young men is significantly higher than that of young women. Since persons with second-degree pathology according to Engle have a more vertically arranged closure plane, and patients with third-degree pathology by Engle have a more horizontal locating plane, this indicator helps to orientate towards the development of the face and determine the person's belonging to one or another groups of anomalies [10]. In previous studies, in the analysis of cephalometric parameters by other methods, we, in contrast to the cephalometric parameters by W. Downs, revealed pronounced sexual differences [5-8, 14, 15].

Comparing the cephalometric parameters obtained by W. Downs with the magnitude of the data obtained in the young men and women of Podillia with orthognathic bite, we established the pronounced differences only in the dental parameters, namely: significantly lower values of Cant Occlusal Plane (angle POr-DOP) and angle II (which describes both the vertical and horizontal parameters of the closing of the incisors and in clinic, is very important in the consideration of the stable position of the incisors and their operation [10]), and also significantly higher values of the angle 1I-DOP (which allows to estimate the location of the lower incisor to

Table 1. Limits of percentile scope (25p-I -75p-I) teleroentgenographic indicators by the Downs method in Ukrainian boys and girls with orthognathic bite.

| Indicators | Young men | | Young women | | p |
|--------------------------|--------------|---------------|--------------|---------------|-------|
| | M±σ | 25p-I, 75p-I | M±σ | 25p-I, 75p-I | |
| Skeleton analysis | | | | | |
| POr-NPog (°) | 89.25±3.26 | 86.6 - 91.0 | 88.57±2.91 | 86.5 - 90.6 | >0,05 |
| NAPog (°) | 1.388±4.786 | -2.3 - 5.3 | 0.996±5.077 | -2.6 - 4.3 | >0,05 |
| AB-NPog (°) | -4.772±2.979 | -6.3 - -2.6 | -4.135±2.912 | -5.9 - -2.3 | >0,05 |
| POr-MeGo (°) | 19.95±6.23 | 16.1 - 24.1 | 21.89±4.44 | 18.4 - 24.1 | >0,05 |
| POr-GnS (°) | 57.64±3.85 | 56.2 - 59.9 | 58.02±3.06 | 56.5 - 60.4 | >0,05 |
| Dental analysis | | | | | |
| POr-DOP (°) | 5.350±3.779 | 2.6 - 7.5 | 7.264±3.727 | 4.8 - 9.9 | <0,05 |
| II (°) | 130.6±7.1 | 126.0 - 134.9 | 130.5±8.0 | 125.4 - 135.5 | >0,05 |
| 1I-DOP (°) | 21.67±5.69 | 18.1 - 25.8 | 19.69±6.52 | 15.5 - 24.5 | >0,05 |
| 1I-MeGo (°) | 7.063±8.082 | 0.5 - 13.2 | 5.073±6.492 | -1.1 - 9.6 | >0,05 |
| 1u-APog (mm) | 4.889±1.609 | 4.0 - 5.8 | 4.553±1.902 | 3.2 - 5.8 | >0,05 |

Notes: M±σ - average ± standard deviation; p - the reliability of sizes differences between young men and women.

Table 2. Comparison of teleroentgenographic indicators obtained by W. Downs with Ukrainian young men and women with orthognathic bite ($M \pm \sigma$).

| Indicators | Downs value | Young men | Young women |
|--------------------------|-------------|-----------------|-----------------|
| Skeleton analysis | | | |
| POr-NPog (°) | 87.8±3.57 | 89.25±3.26 | 88.57±2.91 |
| NAPog (°) | 0.0±5.09 | 1.388±4.786 | 0.996±5.077 |
| AB-NPog (°) | -4.6±3.67 | -4.772±2.979 | -4.135±2.912 |
| POr-MeGo (°) | 21.9±3.24 | 19.95±6.23 | 21.89±4.44 |
| POr-GnS (°) | 59.4±3.82 | 57.64±3.85 t | 58.02±3.06 t |
| Dental analysis | | | |
| POr-DOP (°) | 9.3±3.83 | 5.350±3.779 *** | 7.264±3.727 * |
| II (°) | 135.4±5.76 | 130.6±7.1 ** | 130.5±8.0 ** |
| 1I-DOP (°) | 14.5±3.48 | 21.67±5.69 *** | 19.69±6.52 *** |
| 1I-MeGo (°) | 1.4±3.78 | 7.063±8.082 *** | 5.073±6.492 ** |
| 1u-APog (mm) | 2.7±3.05 | 4.889±1.609 *** | 4.553±1.902 *** |

Notes: *, **, *** - significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$) of corresponding indicators obtained by W. Downs method with young men and women from Podillia region of Ukraine with orthognathic bite; t - trends differences of corresponding indicators obtained by W. Downs method with young men and women from Podillia region of Ukraine with orthognathic bite.

the functional plane, and since the mandibular plane has large fluctuations, especially in terminal skeletal profiles, the definition of this indicator is very useful in treating and evaluating the index of the position of the lower incisor in relation to the mandibular plane [10]), angle 1I-MeGo (which clinically helps to determine the position of the lower median incisors not in relation to the facial planes, to which the lower jaw may be located in different positions, namely to the anatomical structure in which it is located [10]) and distance 1u-APog (which is used as a measure of evaluation of maxillary denture protrusion and allows in millimeters to define and interpret the position of the incisors in the sagittal plane [10]) in Ukrainian young men and women with orthognathic bite. Among the skeletal indicators, only the tendency towards

the lower values of the Y-Axis (angle POr-GnS) in Ukrainian young men and women with orthognathic bite is established.

It should be noted that according to our research, most of the cephalometric parameters obtained in Podillia young men and women with orthognathic bite didn't have significant differences with the data of the parameters obtained by G. Shmut [5], C. Stiner [8] and A.M. Schwartz [6]. Numerous differences in the cephalometric parameters used in Charles H. Tweed International Foundation analysis with the results obtained in Ukrainian young men and women with orthognathic bite have been set for angles (regardless of sex, the smaller angles of FMA and POr_OcP and larger IMRA angles values), so and for linear dimensions (larger PFH distances for young men and smaller AFH distances for young women and AFH/PFH ratios for young men and women) [15], as well as almost half of the cephalometric parameters obtained in young men and women from Podillia with orthognathic bite with marked differences in the magnitude of these parameters derived by J. McNamara [14].

The results obtained by us indicate the need to establish a normative base for cephalometric norms by W. Downs for the population of different regions of Ukraine, taking into account gender and age belongings.

Conclusion

1. In the analysis of sexual differences of cephalometric parameters by W. Downs between young men and women of Podillia with orthognathic bite, only significantly higher values of the indicator of Cant of Occlusal Plane (POr-DOP) in young men were established.

2. Differences in the cephalometric parameters obtained by W. Downs from the indices obtained in young men and women from Podillia with orthognathic bite are set independently from the gender mainly for dental indicators: significantly less values of Cant Occlusal Plane (angle POr-DOP) and angle II, as well as the values of angle 1I-DOP, angle 1I-MeGo and distance 1u-APog are significantly higher in Podillia young men and women.

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ВИЗНАЧЕННЯ НОРМАТИВНИХ ЦЕФАЛОМЕТРИЧНИХ ПАРАМЕТРІВ МЕТОДОМ W.DOWNS ДЛЯ УКРАЇНСЬКИХ ЮНАКІВ І ДІВЧАТ

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Встановлена в багатьох дослідженнях відмінність характеристик краніофациальних структур у різних рас та етносів вимагає створення нормативної бази для телерентгенографічних показників та індексів, характерних для певної етнічної групи. Ціль роботи - встановити цефалометричні параметри за W. Downs у юнаків і дівчат Подільського регіону України з ортогнатичним прикусом і порівняти результати з даними, що запропоновані автором методики. За допомогою пристрою Veraviewerocs 3D, Morita (Японія), у 38 юнаків (віком від 17 до 21 року) та 55 дівчат (віком від 16 до 20 років) з ортогнатичним прикусом (нормальною оклюзією наближеною до ортогнатичного прикусу) були отримані та проаналізовані бокові телерентгенограми. Цефалометричні точки і вимірювання були проведені згідно з рекомендаціями W. Downs (1948). Анатомічні точки визначали з урахуванням рекомендацій А.Е. Athanasiou (1997), С.І. Дорошенко та Є.А. Кульгінського (2007). Статистична обробка отриманих результатів проведена в ліцензійному пакеті "Statistica 6,0" з використанням непараметричних методів оцінки отриманих результатів. В результаті проведених досліджень практично не виявлено статевих розбіжностей цефалометричних параметрів за W. Downs між юнаками та дівчатами Поділля з ортогнатичним прикусом. При порівнянні цефалометричних параметрів, що отримані W. Downs від аналогічних показників, що отримані в українських юнаків і дівчат з ортогнатичним прикусом незалежно від статі, встановлені достовірно менші значення нахилу оклюзійної площини (кут PO-GOP) та кута II, а також достовірно більші значення величини кута 1-DOP, кута 1-MeGo та відстані 1u-APog в юнаків і дівчат Поділля. Отримані результати вказують на необхідність створення нормативної бази цефалометричних нормативів за W. Downs для населення різних регіонів України з урахуванням статевої та вікової належності.

Ключові слова: череп, цефалометрія, аналіз Downs.

ОПРЕДЕЛЕНИЕ НОРМАТИВНЫХ ЦЕФАЛОМЕТРИЧЕСКИХ ПАРАМЕТРОВ МЕТОДОМ W.DOWNS ДЛЯ УКРАИНСКИХ ЮНОШЕЙ И ДЕВУШЕК

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Установленное во многих исследованиях различие характеристик краніофациальных структур в различных рас и этносов требует создания нормативной базы для телерентгенографических показателей и индексов характерных для определенной этнической группы. Цель работы - установить цефалометрические параметры по W. Downs у юношей и девушек Подольского региона Украины с ортогнатическим прикусом и сравнить результаты с данными, предложенные автором методики. С помощью устройства Veraviewerocs 3D, Morita (Япония), у 38 юношей (в возрасте от 17 до 21 года) и 55 девочек (в возрасте от 16 до 20 лет) с ортогнатическим прикусом (нормальной окклюзией приближенной к ортогнатическому прикусу) были получены и проанализированы боковые телерентгенограммы. Цефалометрические точки и измерения были проведены в соответствии с рекомендациями W. Downs (1948). Анатомические точки определяли с учетом рекомендаций А.Е. Athanasiou (1997), С.И. Дорошенко и Е.А. Кульгінського (2007). Статистическая обработка полученных результатов проведена в лицензионном пакете "Statistica 6,0" с использованием непараметрических методов оценки полученных результатов. В результате проведенных исследований практически не обнаружено половых различий цефалометрических параметров по W. Downs между юношами и девушками Подолья с ортогнатическим прикусом. При сравнении цефалометрических параметров, полученные W. Downs от аналогичных показателей, полученных от украинских юношей и девушек с ортогнатическим прикусом независимо от пола, установлены достоверно меньшие значения наклона окклюзионной плоскости (угол PO-GOP) и угла II, а также достоверно большие значения величины угла 1-DOP, угла 1-MeGo и расстояния 1u-APog у юношей и девушек Подолья. Полученные результаты указывают на необходимость создания нормативной базы цефалометрических нормативов по W. Downs для населения различных регионов Украины с учетом половой и возрастной принадлежности.

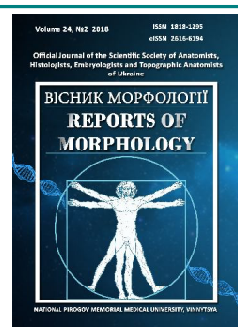
Ключевые слова: череп, цефалометрия, анализ Downs.



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Character and features of morphofunctional reactions of renal vessels in rats at chronic poisoning with acetylsalicylic acid

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The prevalence and availability of aspirin-containing analgesic products makes them sources of unintentional overdose, and even suicidal admission. Therefore, the toxic effect of salicylates is an important cause of morbidity and mortality. In this case, as a rule, the target body is the kidneys. The purpose of the study was to identify the structural changes in the kidneys in the process of chronic poisoning with acetylsalicylic acid. In an experiment on rats using the histological methods of the study, it was found that chronic poisoning with acetylsalicylic acid leads to severe organ disorders in the kidneys, which are manifested in the form of congestive venous plethora with reactive ascending vasoconstriction of the arterial part of the blood route of the organ with a decrease in its throughput. At the same time there was a thickening of the walls of the vessels, which occurred both at the expense of increasing their tone, and due to edema, which was confirmed by the enlargement of the cytoplasm of the leiomyocytes of the medial cover and an increase in the distance between the nuclei of adjacent layers of the smooth muscle cells. The endothelium of these arteries looked swollen as evidenced by the protrusion of its nuclei into the lumen of the vessels in the form of a "palisade". Such changes can be considered as a protective reaction that partially prevents hemodynamic overload of the hemomicrocirculatory channel, known in the scientific literature as the reflex of Kitaev. However, in spite of this, in the process of chronic poisoning with acetylsalicylic acid, the complete compensation of blood circulation in the kidneys does not occur. Prolonged vasoconstriction leads to the tissue ischemia, which may be the cause of the development and progression of functional and dystrophic changes in the structural components of the organ parenchyma as a morphological substrate of renal insufficiency. Confirmation of this can be considered the changes in the glomerular apparatus found during the study, which consisted in the gradual reduction of the total area of nephrons with the simultaneous decrease in the area of capillary glomeruli. Moreover, the decrease in the area of capillary glomeruli occurred more intensively, which led to the expansion of urinary spaces. In the final stage of the experiment, the capillary glomeruli were often in collaptoid condition, their size significantly decreased, and the contours became unevenly. Thus, because of the chronic poisoning by acetylsalicylic acid in the kidneys appears stagnant disturbances of the organ circulation, which cause ascending vasoconstriction in the arterial part of the blood stream of the organ, which subsequently leads to ischemia and dystrophic changes in its parenchyma.

Keywords: acetylsalicylic acid, arteries, plethora, nephrons, capillary glomeruli.

Introduction

Salicylates are the most commonly used pharmacologically active substances found in hundreds of prescription and over-the-counter forms of medicines. They are used with an analgesic purpose for the treatment of mild to moderate pain. As an antipyretic drug and as an anti-inflammatory agent, they are used in the treatment of

inflammatory processes of soft tissues, joints, and also in vasculitis. In low doses, acetylsalicylic acid preparations are also used to prevent thrombosis. At the same time, the prevalence of aspirin-containing analgesic products makes these agents available in almost every household, the public sources of unintentional and suicidal admission [5,

12, 14, 15, 16].

Drugs containing acetylsalicylic acid, as the main active compound, are considered safe at low therapeutic doses, but at the same time have life-threatening side-effects when applied at high doses. Long-term administration of therapeutic doses of the drug is associated with the risk of hepatotoxicity, nephrotoxicity, the appearance of ulcerative processes in the gastrointestinal tract and the development of malignant tumors in the kidneys [7, 10]. Also reported is the negative effects of acetylsalicylic acid in pregnancy [4]. In vitro and in vivo studies have shown that aspirin at high doses has a destructive effect on the tissues of the blood vessels [18].

That is why the toxic effect of salicylate is an important cause of morbidity and mortality. In this case, often the target organ is the kidney as it is known that acetylsalicylic acid is rapidly absorbed from the stomach and small intestine, primarily through passive diffusion and rapidly hydrolyzed to salicylic acid with esterase in the mucosa and blood plasma. The salicylic acid itself is metabolized by conjugation in the liver to form salicylic acid and several other metabolites. The drug is spread throughout the body, with its highest concentration contained in plasma, kidney cortex, liver, heart and lung [13].

The *purpose* of the study: to establish the features of structural changes in the structural components of rat's kidney in the process of chronic poisoning with acetylsalicylic acid.

Materials and methods

Experiments were performed on 42 white laboratory mature male rats weighing 160-180 g and age 2.5-3 months. Of these, 12 animals were a control group; another 30 rats received pre-gastric acetylsalicylic acid every day at a rate of 150 mg per 1 kg of body weight for the simulation of chronic intoxication [3, 19]. Slaughter of animals was carried out at 1, 3, 7, 14 and 28 days from the beginning of the experiment by rapid decapitation under ketamine anesthesia. All experimental studies were carried out in accordance with the principles of bioethics set forth in the Helsinki Declaration and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (№1759-VI of 15.12.2009).

The material for the histological examination (kidneys slices) was fixed after collection in the alcohol and in 10% neutral formalin. Histological sections were stained by H&E, Weigert's and Van Gieson's.

Morphometric studies included determination of: area of glomeruli with Bowman's capsule (μm^2), the area of the capillary loops of the glomeruli (μm^2), and area of the urinary space in the renal corpuscle (μm^2). Morphometry was performed using an eyepiece micrometer MOB-1-15x, with measurements performed in 5 visual fields on each histological cut.

The digital material obtained during the research was statistically processed using Microsoft Excel for Windows 98 with the definition of averages and their standard errors.

Reliability was estimated by the Student's coefficient (t) at $p < 0.05$.

Results

According to the results of the study, it was found that 1 day after pre-gastric administration of acetylsalicylic acid in the structural components of the kidney of rats there were certain morphofunctional changes, the basis of which were disorders of organ blood circulation. The latter manifested itself in the form of moderate venous and partly arterial hypertrophy, capillary stasis. On the background of congestive plethora there was an increase in the tone of the walls of small arteries, which was confirmed by the strengthening of the tortuosity of their internal elastic membranes with the simultaneous decrease in the throughput of arterioles due to the narrowing of their lumen (Fig. 1).

At the same time, there was a slight increase in the area of the section of the glomeruli capillary loops with a slight narrowing of the area of the urinary spaces of the renal corpuscles, which was also a consequence and manifestation of stagnant phenomena in the bloodstream of the organ (Table 1).

Such hemodynamic changes were accompanied by moderate edema of the epithelium of the renal tubules, especially in the subcapsular regions of the kidneys, which was manifested by the enlightenment of the epithelial cytoplasm with a simultaneous increase in their cross-sectional area.

After 3 days of the experiment there was partial compensation for the detected pre-changes with signs of normalization of organ blood flow. Slightly decreased the tone of the walls of small arteries and arterioles, signs of hyperhydration of the epithelial cells of the walls of the renal tubules disappeared. However, in the lumen as the arteries and especially the veins, various concentrations of

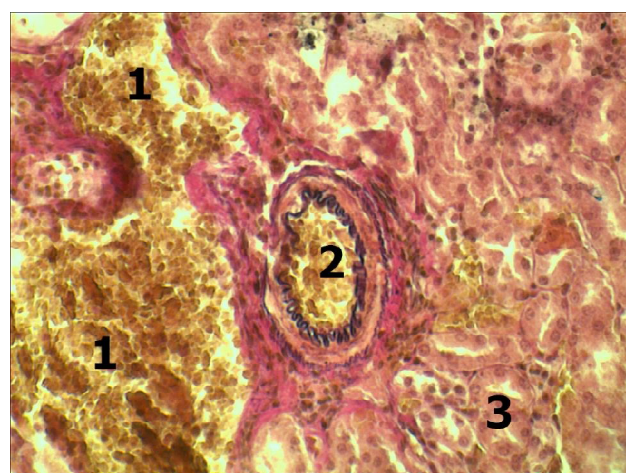


Fig. 1. The structural organization of the rat's kidney 1 day after the introduction of acetylsalicylic acid. Weigert's elastic stain. x140. Full-blooded veins - 1, erythrocytes in the lumen of the artery with increased wall tone and folded internal elastic membrane - 2, renal tubules with enlightened cytoplasm of epithelial cells - 3.

Table 1. Quantitative characteristic of changes in parameters of structural components of rat's renal corpuscle in chronic poisoning with acetylsalicylic acid (M±m).

| Indicator | | Area of glomerulus with Bowman's capsule (µm ²) | Area glomerulus capillary loops (µm ²) | Area of urinary space in the renal corpuscle (µm ²) |
|---|---------|---|--|---|
| Control | | 5596±215 | 3961±253 | 1635±73 |
| Chronic poisoning with acetylsalicylic acid | 1 day | 5641±217 | 4095±272 | 1546±62 |
| | 3 days | 5485±193 | 3872±271 | 1612±86 |
| | 7 days | 5290±197 | 3583±250 | 1707±60 |
| | 14 days | 5187±234 | 3179±177 | 2008±67* |
| | 28 days | 5250±218 | 2892±203* | 2357±32** |

Note: * - p<0.05; ** - p<0.01 in comparison with control.

erythrocytes continued to manifest themselves.

However, 7 days after experimental observation, the recovery and progression of detected pre-vascular reactions, which consisted in the repeated increase in the tone of arterioles and small arteries, with the simultaneous thickening of their walls and narrowing of lumen and decrease in bandwidth, was characteristic. Moreover, thickening of the walls of the vessels occurred both by increasing their tone, and due to edema, which was confirmed by the enlightenment of the leiomyocytes cytoplasm of the middle mucosa and increasing the distance between the nuclei of adjacent layers of the smooth muscle cells. The endothelium of these arteries looked swollen as evidenced by the protrusion of its nuclei into the lumen of the vessels in the form of "palisade" (Fig. 2).

The arteries of the middle and larger caliber, as well as the veins, were expanded and full-blooded. At the same time, enlightenment of the urinary spaces of the kidney cells became moderately enlarged. In the epithelium of the renal tubules, signs of hydropic dystrophy were renewed.

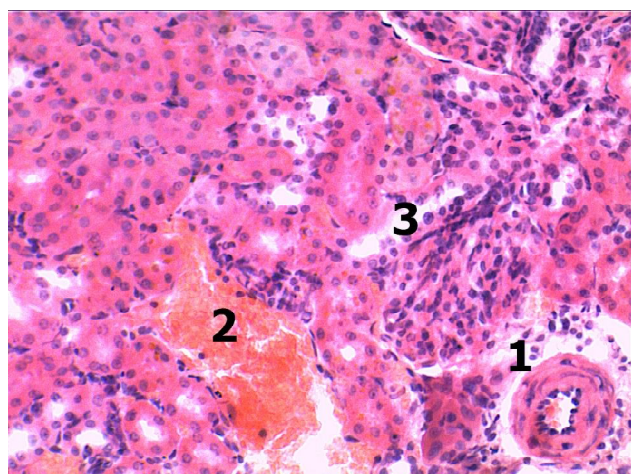


Fig. 2. The structural organization of the rat's kidney 7 days after the introduction of acetylsalicylic acid. H&E stain. x140. Artery with thickened wall, narrowed lumen and swollen endothelium - 1, erythrocytes in the lumen of the vein - 2, renal corpuscles - 3.

After 14 days from the beginning of the experiment, changes that were detected in the 7-day period, progressively increased. As a result of ascending vasoconstriction, the tone increased not only small but also arteries of medium caliber (Fig. 3).

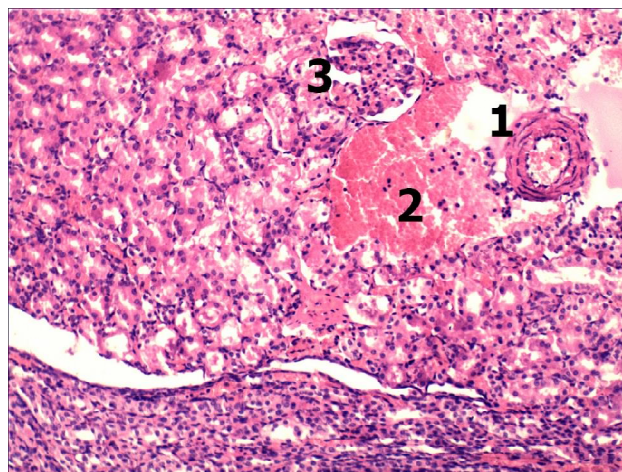


Fig. 3. The structural organization of the rat's kidney 14 days after the introduction of acetylsalicylic acid. H&E stain. x100. Intra-organ artery with thickened wall - 1, full-blooded vein - 2, renal corpuscle - 3.

The veins and arteries of large caliber remained elevated and full-blooded. As a result of violation of hemomicrocirculation developed perivascular and interstitial edema.

Changes in the glomerular apparatus during this period of observation were to reduce the total area of nephrons with a simultaneous decrease in the area of capillary glomeruli. Moreover, the decrease in the area of capillary glomeruli occurred more intensively, which led to a reliable (p<0.05) expansion of the Bowman's capsule urinary spaces (Fig. 4).

The epithelium of the proximal and distal tubules was in

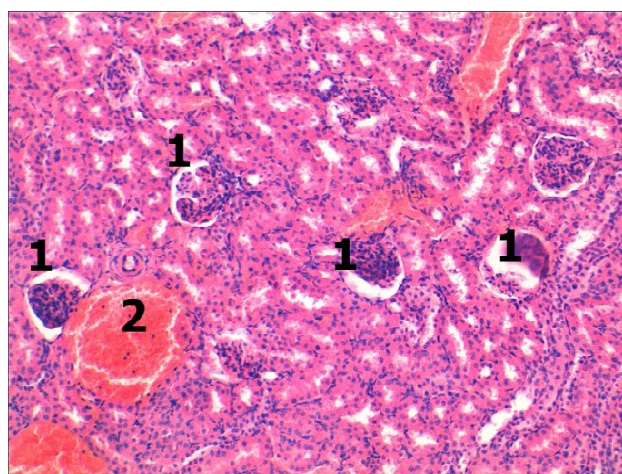


Fig. 4. The structural organization of the rat's kidney 14 days after the introduction of acetylsalicylic acid. H&E stain. x100. Reduction of the area of capillary glomeruli with enlargement of urinary spaces - 1, full-blooded vein - 2.

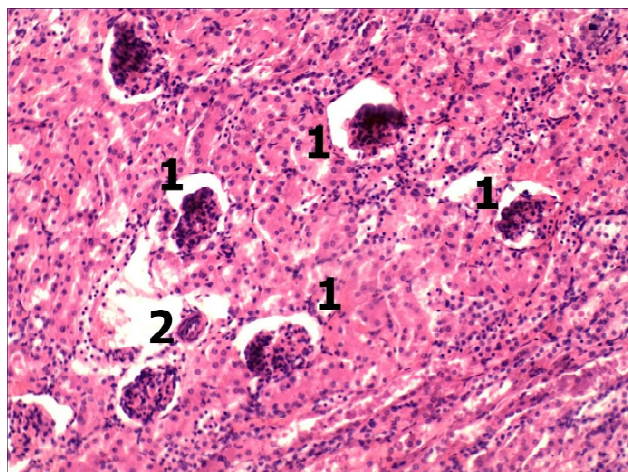


Fig. 5. The structural organization of the rat's kidney 28 days after the introduction of acetylsalicylic acid. H&E stain. x100. Expanded urinary space in the renal corpuscle - 1, arteriola - 2.

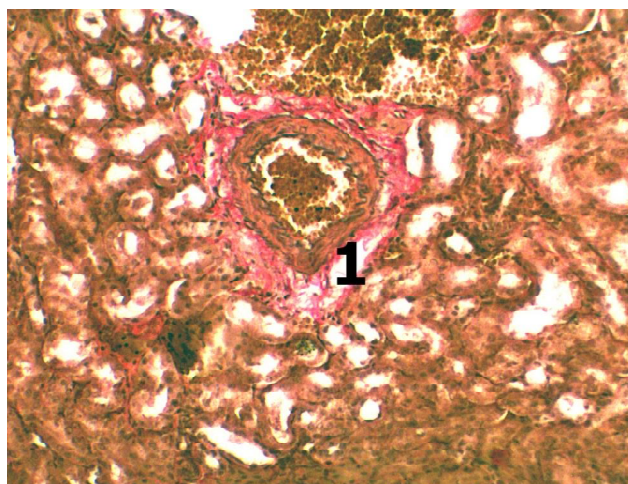


Fig. 6. The structural organization of the rat's kidney 28 days after the introduction of acetylsalicylic acid. Van Gieson's stain. x100. Perivascular growth of connective tissue - 1.

a state of hydropic dystrophy, the intensity of which increased. Tubular lumen was unevenly enlarged, some of them containing cell detritus and cylinders. In the renal medulla, there were also focuses of dystrophy, somewhere with polymorphic-cellular infiltrates.

The 28-day observation period was characterized by signs of further progression of organ-hemodynamics disorders of ischemic orientation. The tonus increased, the wall thickness increased and the throughput of the intraorganic arteries was already decreasing in all investigated types. The veins remained expressed full-blooded.

In the renal corpuscle, due to the decrease in the blood flow to the capillary glomerular loops, there was a pronounced ($p < 0,05$) increase in the Bowman's capsule urinary space, while the capillary glomeruli were often collaptoid: their size decreased significantly (by 25-30%), and contours became uneven-hilly (Fig. 5).

At the same time, for this observation period, signs of

perivascular and interstitial sclerosis that could have been due to ischemia, as well as the result of tissue infiltration in the pre-term period (Fig. 6).

Discussion

Thus, the results of our study suggest that chronic poisoning with acetylsalicylic acid leads to severe organ disorders in the kidneys that manifest themselves in the form of congestive venous hypertension with the development of reactive plethora of the arterial department of the blood stream of the organ and the decrease in its throughput, the morphological manifestation of which is the thickening of the arterial walls, the foldiness of their internal elastic membranes and the narrowing of the lumen.

Objective confirmation of morphological changes in the structural components of kidney parenchyma was the results of morphometric studies according to which a significant decrease in the area of capillary loops of glomeruli in the long term (28 days of experiment) was established with a simultaneous significant increase in the area of urinary spaces in the renal corpuscles (14-28 days of experiment).

The results obtained agree with other studies carried out in this area, according to which the main link in the pathogenesis of acute renal failure due to acute poisoning by substances that cause the state of toxic shock and accompanied by changes in tissue metabolism, is precisely the violation of renal blood circulation [9, 20, 21].

The structural changes we have established in the renal vessels can be considered as a protective reaction that partially prevents hemodynamic overload of the hemomicrocirculatory bed during venous congestion, known in the scientific literature as the veno-arterial reaction, or the reflex of Kitaev [6].

However, in the process of chronic poisoning with acetylsalicylic acid, this mechanism does not provide complete compensation for organ renal blood circulation. In addition, prolonged vasoconstriction leads to tissue ischemia, which may be the cause of the development and progression of functional and dystrophic changes in the structural components of the organ parenchyma as a morphological substrate of renal failure, which is also fully consistent with modern notions [17, 21].

One of the links in the morphogenesis of structural changes in the kidneys may be the development of acidosis, which is also common in overdose, in particular, salicylic preparations [1, 11]. Products of metabolism, causing acidosis, are a real danger, as they are able not only to break the functions, but also lead to morphological changes in various organs and tissues [2]. The negative effect of acidosis on the state of the vessels is manifested by an increase in the permeability of the capillary wall and a change in the reaction of vascular sphincters, arterioles, and venules, which also contributes to the development of tissue hypoxia and their degenerative transformation [8, 22]. Therefore, further detailed study of structural changes in the kidneys and their bloodstream after the application of toxic doses of

acetylsalicylic acid and its preparations can contribute to an understanding of the pathogenesis of poisoning and to be important in the development of new complex methods for their correction.

Further research in this direction will allow the development of correction methods for chronic poisoning with acetylsalicylic acid and give them a morpho-functional justification.

Conclusions

1. Chronic poisoning with acetylsalicylic acid leads to stagnant organ-hemodynamic disorders in the kidneys, which is manifested by capillary stasis, venous and arterial plethora.

2. At the venous stasis, the arterial section of the bloodstream of the kidneys reacts with ascending vasoconstriction with increased tone (increased folding of

the internal elastic membranes), thickening of the walls (due to swelling and hypertrophy of the smooth muscle cells of the middle membrane), and narrowing of the lumen initially of the arterioles and small arteries, and in the subsequent - intraorganic arteries medium and large diameter.

3. As a result of prolonged vasoconstriction, ischemia of the organ develops, which leads to dystrophic changes in the structural components of the parenchyma: hydropic dystrophy of the epithelium of the proximal and distal tubules, plasma permeation of tissues with the subsequent development of perivascular and interstitial sclerosis.

4. A quantitative confirmation of the reorganization of the renal parenchyma in the long term is a significant decrease in the area of the capillary loops of the glomeruli by 25-30% with a simultaneous significant increase in the area of the urinary space in the renal corpuscles to 44%.

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ХАРАКТЕР ТА ОСОБЛИВОСТІ МОРФОФУНКЦІОНАЛЬНИХ РЕАКЦІЙ НИРКОВИХ СУДИН У ЩУРІВ ПРИ ХРОНІЧНОМУ ОТРУЄННІ АЦЕТИЛСАЛІЦИЛОВОЮ КИСЛОТОЮ

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Поширеність і доступність аспіриновмісних знеболюючих продуктів робить їх джерелами ненавмисного передозування, а то і суїцидального прийому. Тому токсична дія саліцилатів є важливою причиною захворюваності і смертності. При цьому, як правило, органом-мішенню бувають нирки. Метою дослідження було встановлення особливостей структурних змін у нирках в процесі хронічного отруєння ацетилсаліциловою кислотою. В експерименті на щурах з використанням гістологічних методів дослідження було встановлено, що хронічне отруєння ацетилсаліциловою кислотою приводить до виражених розладів органного кровообігу у нирках, які проявляються у вигляді застійного венозного повнокров'я з реактивною висхідною вазоконстрикцією артеріального відділу кровоносного русла органу із зменшенням його пропускної здатності. При цьому спостерігалось потовщення стінок судин, яке відбувалося як за рахунок підвищення їх тонусу, так і за рахунок набряку, що підтверджувалось просвітленням цитоплазми лейомиоцитів середньої оболонки і збільшенням відстані між ядрами сусідніх шарів гладком'язових клітин. Ендотелій таких артерій виглядав набряклим про що свідчило випинання його ядер у просвіт судин у вигляді "частоколу". Такі зміни можуть розглядатися, як захисна реакція, яка дозволяє частково запобігати гемодинамічному перевантаженню гемомікроциркуляторного русла, що у науковій літературі відомо як рефлекс Китаєва. Однак, не зважаючи на це, у процесі хронічного отруєння ацетилсаліциловою кислотою, повної компенсації кровообігу у нирках не відбувається. Тривала вазоконстрикція веде до ішемії тканин, що може бути причиною розвитку та прогресування функціональних і дистрофічних змін в структурних компонентах паренхіми органу як морфологічного підґрунтя ниркової недостатності. Підтвердженням цього можна вважати виявлені у процесі дослідження зміни клубочкового апарату, котрі полягали у поступовому зменшенні загальної площі нефронів з одночасним зменшенням площі капілярних клубочків. Причому, зменшення площі капілярних клубочків відбувалося більш інтенсивно, що приводило до розширення сечових просторів. У завершальній стадії експерименту капілярні клубочки нерідко перебували у колаптоїдному стані, їх розміри значно зменшувалися, а контури ставали нерівномірно-горбистими. Таким чином, при хронічному отруєнні ацетилсаліциловою кислотою у нирках виникають розлади органного кровообігу застійного характеру, які викликають висхідну вазоконстрикцію в артеріальному відділі кровоносного русла органу, що у подальшому приводить до ішемії та дистрофічних змін у паренхімі.

Ключові слова: ацетилсаліцилова кислота, артерії, повнокров'я, нефрони, капілярні клубочки.

ХАРАКТЕР И ОСОБЕННОСТИ МОРФОФУНКЦИОНАЛЬНЫХ РЕАКЦИЙ ПОЧЕЧНЫХ СОСУДОВ У КРЫС ПРИ ХРОНИЧЕСКОМ ОТРАВЛЕНИИ АЦЕТИЛСАЛИЦИЛОВОЙ КИСЛОТОЙ

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Распространенность и доступность аспириносодержащих обезболивающих продуктов делает их источниками непреднамеренной передозировки, а то и суицидального приема. Поэтому токсическое действие саллицилатов является важной причиной заболеваемости и смертности. При этом, как правило, органом-мишенью бывают почки. Целью исследования было установление особенностей структурных изменений в почках в процессе хронического отравления ацетилсаллициловой кислотой. В эксперименте на крысах с использованием гистологических методов исследования было установлено, что хроническое отравление ацетилсаллициловой кислотой приводит к выраженным расстройствам органного кровообращения в почках, которые проявляются в виде застойного венозного полнокровия с реактивной восходящей вазоконстрикцией артериального отдела кровеносного русла органа с уменьшением его пропускной способности. При этом наблюдалось утолщение стенок сосудов, которое происходило как за счет повышения их тонуса, так и за счет отека, что подтверждалось просветлением цитоплазмы лейомиоцитов средней оболочки и увеличением расстояния между ядрами соседних слоев гладкомышечных клеток. Эндотелий таких артерий выглядел набухшим о чем свидетельствовало выпячивание его ядер в просвет сосудов в виде "частокола". Такие изменения могут рассматриваться как защитная реакция, которая позволяет частично предотвратить гемодинамическую перегрузку гемомикроциркуляторного русла и которая в научной литературе описана как рефлекс Китаева. Однако, несмотря на это, в процессе хронического отравления ацетилсаллициловой кислотой, полной компенсации кровообращения в почках не происходит. Длительная вазоконстрикция приводит к ишемии тканей, что может быть причиной развития и прогрессирования функциональных и дистрофических изменений в структурных компонентах паренхимы органа как морфологического основания почечной недостаточности. Подтверждением этого можно считать обнаруженные в процессе исследования изменения клубочкового аппарата, которые заключались в постепенном уменьшении общей площади нефронов с одновременным уменьшением площади капиллярных клубочков. Причем, уменьшение площади капиллярных клубочков происходило более интенсивно, что приводило к расширению мочевых пространств. В завершающей стадии эксперимента капиллярные клубочки нередко находились в колаптоидном состоянии, их размеры значительно уменьшались, а контуры становились неравномерно-бугристыми. Таким образом, при хроническом отравлении ацетилсаллициловой кислотой в почках возникают расстройства органного кровообращения застойного характера, которые вызывают восходящую вазоконстрикцию в артериальном отделе кровеносного русла органа, что в дальнейшем приводит к ишемии и дистрофическим изменениям в паренхиме.

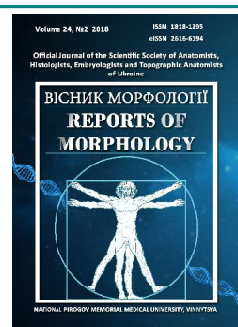
Ключевые слова: ацетилсаллициловая кислота, артерии, полнокровие, нефроны, капиллярные клубочки.



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Histomorphometric assessment of changes in the acinus and islets of Langerhans of the pancreas under conditions of general dehydration of the body

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Dehydration accompanies a number of pathological conditions and causes alteration of the organs and systems of an organism. Research aim: to study histomorphometric changes in pancreas under the conditions of general dehydration of an organism, to identify correlation between the studied groups. The experiment was carried out on 35 white male rats. Pancreas was stained with haematoxylin and eosin and also Van Gieson's stain was used. The size of acini and islets of Langerhans and other parenchyma formations were assessed. The acini area of has decreased by 6.5% ($p>0.05$) in comparison with the intact group on the 3rd day. The area of islets of Langerhans decreased by 2% ($p>0.05$) in comparison with the intact group. The area of islets of Langerhans has increased by 0.2% ($p>0.05$) on the 7th day of experiment in comparison with the control group and by 2.2% ($p>0.05$) in comparison with the 3rd day. Acini area has increased by 7.2% ($p>0.05$) on the 7th day in comparison with the control group and by 13.2% ($p>0.05$) in comparison with the 3rd day. The acini area has increased by 25.8% ($p<0.05$) on the 10th day in comparison with the control group; by 30.6% ($p<0.01$) in comparison with the 3rd day; by 20.2% ($p>0.05$) in comparison with the 7th day. The area of islets of Langerhans has increased by 55.5% ($p<0.001$) in comparison with the control group, by 56.3% ($p<0.001$) in comparison with the 3rd day; by 55.4% ($p<0.001$) in comparison with the 7th day. The results analysis found the correlation between the area of islets of Langerhans and acini $r=0.407$, $p<0.05$; between the area of islets of Langerhans and dehydration degree $r=0.708$, $p<0.001$; between acini area and dehydration degree $r=0.534$, $p<0.001$. It was found by morphologically that the presence of destructive changes in the pancreatic parenchyma, which progress as the degree of severity of dehydration.

Keywords: pancreas, islets of Langerhans, acini, dehydration.

Introduction

Water is the basis of internal environment of an organism [4, 5, 9, 22, 23]. The violation of water-electrolytic balance accompanies a number of pathological conditions and causes alteration of the organs and systems of an organism [7, 10, 21]. WHO informs that about 1.5 million people die annually from dehydration in the world [15, 18]. Understanding the vital role of water in a human body is the basis for comprehending the development mechanisms of pathological conditions [1, 2, 8, 25]. Endocrine system plays a key role in the system of water exchange control, providing an optimal volume of liquid in a body [14, 19]. All biochemical reactions, which occur in a human body and are associated with digestion, take place in the aquatic environment [3, 11,

24]. Pancreas pathology takes the lead in the structure of morbidity, disability and mortality among Ukrainian population [6, 12, 17, 20]. According to the research, the incidence of pancreas pathology is about 226 cases per 100.000 of Ukrainian population [17, 26].

Research *aim*: to carry out experimental research on mature laboratory rodents in order to identify histomorphometric changes in pancreas under the conditions of general dehydration of an organism and correlation of the studied groups.

Materials and methods

The experiment was carried out on 35 sexually mature

white male rats, which were kept in inpatient conditions of vivarium. During the experiment, the experimental animals were kept in accordance with the regulations of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the principles of the Declaration of Helsinki adopted by the General Assembly of World Medical Association (1964-2000) "General ethical rules of experiments on animals", approved by the National Congress of Bioethics (Kyiv, 2001).

The animals were divided into two batches: control (5 rats) and experimental (30 rats). The experimental batch was divided into 3 groups with 10 rats in each group depending on dehydration degree. After reaching the correspondent degree of dehydration, the animals were released from the experiment under anesthesia on the 3rd day with mild dehydration, on the 7th day with moderate dehydration and on the 10th day severe dehydration.

For histological examination, pancreas was exposed by midline laparotomy and has been fixed in 10% neutral formalin solution for 24 hours. The experiment results were entered in the sample protocol. The passing of microtome knife and manufacture of paraffin blocks were carried out according to the established procedure [13, 16].

The sledge microtome MC-2 was used to produce paraffin serial sections of acini center with the thickness of 5-9 microns, they were stained with Haematoxylin and Eosin and also Van Gieson's stain was used. The obtained preparations were studied and photographed with the help of image viewing system "SEO Scan Lab 2.0" (Ukraine). The size of acini and islets of Langerhans, the structure of endocrine cells and other gland parenchyma formations and gland stroma were assessed. The results of morphometric measurements were processed using the statistical methods and the statistical software IBM SPSS Statistic 21. The identification of differences probability between the mean values of several groups was performed using One-Way ANOVA together with F-test and Bonferroni correction. As a correlation coefficient between the values of the interval scale, the Pearson correlation coefficient was used.

Results

The average area of acini and islets of Langerhans are

shown in Table 1.

While studying the pancreas histostructure of the control series, it was microscopically determined that the gland is represented predominantly by the exocrine tissue - cone shaped acini, apical parts are turned into secretory duct from which the insertion sections of the pancreatic duct originate. The average acini size was 983.4±63.5 microns. In the acinous parenchyma, there were endocrine cells, islets of Langerhans of various shapes and sizes, sharply separated from the exocrine cells by a thin layer of connective tissue, consisting of collagen, reticular and elastic fibers. There are cells which are firmly against each other in the center of the islets. These are β-cells of oval, sometimes round shape with light cytoplasm. There is an insignificant amount of α- and D-cells on the periphery of an islet, which are smaller in size. The average size of islets of Langerhans was 13934±688 microns.

Under the conditions of general dehydration in pancreas parenchyma of laboratory rodents, the structural changes occurred in all experimental groups. On the 3rd day of study, histological structure showed that the exocrine part of pancreas retains its lobulation. The center acinous cells are of irregular cone shape, the oval nucleus is located on the basal pole. There are some binucleate acinar cells. Loose fibrous connective tissue appears around the vessels. Capillaries and arterioles are somewhat enlarged with the signs of moderate edema of the endothelium without signs of haemorheologic disorders (Fig. 1).

The acini area of has decreased by 6.5% (p>0.05) in comparison with the intact group on the 3rd day. The area of islets of Langerhans decreased by 2% (p>0.05) in comparison with the intact group.

The 7th day of experiment has shown the growth of connective tissue fibers around the vessels. Haemocapillaries are significantly enlarged, venules are dilated, arterioles lumen is uneven. There are single infiltrates around the vessels represented mainly by lymphocytes and macrophages. The excretory duct are with singular leukocytic infiltrates and inhomogenous secretion in their lumen (Fig. 2).

The area of islets of Langerhans on the 7th day has increased by 0.2% (p>0.05) in comparison with the control

Table 1. The average area of acini and islets of Langerhans.

| Experimental series | Control group/condition 0 (n=5) | 1 group, mild dehydration (n=10) | 2 group, moderate dehydration (n=10) | 3 group, severe dehydration (n=10) | P ₀ | P ₁ | P ₂ | P ₃ | P ₄ | P ₅ | F |
|------------------------------------|---------------------------------|----------------------------------|--------------------------------------|------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
| S of islets of Langerhans, microns | 13934±688 | 1365±1434 | 13960±1571 | 31281±2858 | >0.05 | >0.05 | <0.01 | >0.05 | <0.01 | <0.01 | 23.78 |
| S of acini, microns | 983.4±63.5 | 920.2±121.3 | 1059±63 | 1326±70 | >0.05 | >0.05 | <0.05 | >0.05 | <0.01 | >0.05 | 4.600 |

Note: P₀ - statistical significance of differences between groups 0 and 1; P₁ - statistical significance of differences between groups 0 and 2; P₂ - statistical significance of differences between groups 0 and 3; P₃ - statistical significance of differences between groups 1 and 2; P₄ - statistical significance of differences between groups 1 and 3; P₅ - statistical significance of differences between groups 2 and 3.

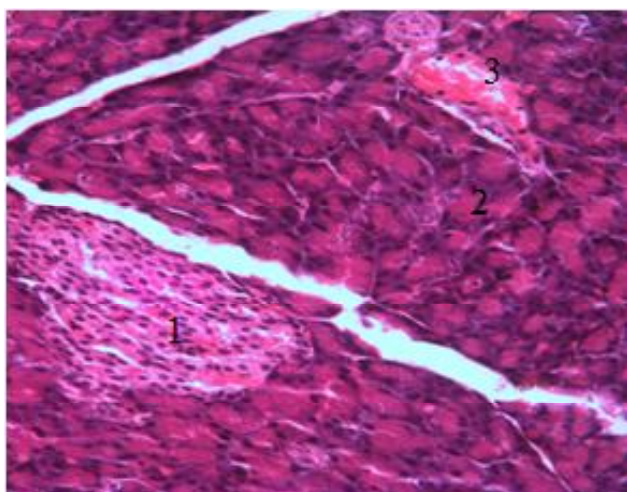


Fig. 1. Pancreas of a rat. General dehydration. 3rd day. Anislet of Langerhans, lobulation of pancreas parenchyma is preserved. 1 - Anislet of Langerhans, 2 - pancreatic acinus, 3 - blood vessel. Haematoxylin and eosin stain. x200.

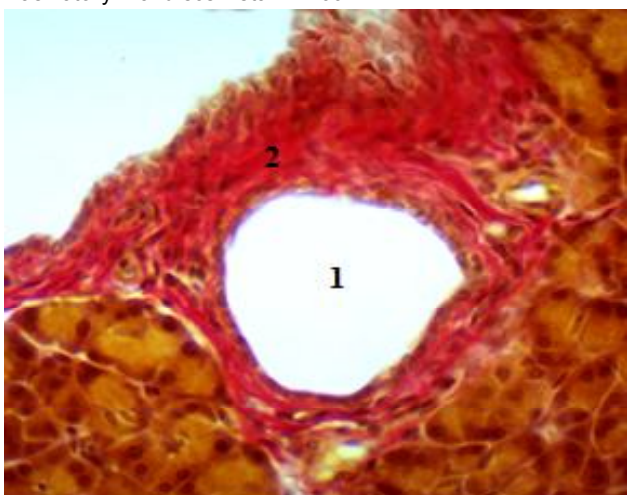


Fig. 2. Pancreas of a rat. Exocrine part. General dehydration. 7th day. Advanced fibrosis around the ducts and epithelium desquamation in the ducts. 1 - withdrawal duct, 2 - edema, wall fibrosis. Van Gieson's stain. x400.

group and by 2.2% ($p>0.05$) in comparison with the 3rd day. Acini area on the 7th day has increased by 7.2% ($p>0.05$) in comparison with the control group and by 13.2% ($p>0.05$) in comparison with the 3rd day.

The 10th day of the study demonstrated the significant structural changes in the exocrine and endocrine part of pancreas parenchyma. The gland structure when stained with haematoxylin and eosin and using Van Gieson's stain preserve lobulation, but most of the acini lose sharp contours due to swelling of the connective tissue. There is epithelium desquamation, advanced fibrosis around the excretory ducts. There are some areas of focal angiomas. There are perivascular leukocytic infiltrations and edema (Fig. 3).

Islets of Langerhans are of irregular shape, large in size due to hydropic dystrophic changes. There is lesser

quantity of β -cells located in the islet center, the nuclei of the latter are of high density, hyperchromatic, between which the connective tissue is diagnosed due to edema (Fig. 4).

The area of islets of Langerhans has increased by 55.5% ($p<0.001$) in comparison with the control group, by 56.3% ($p<0.001$) in comparison with the 3rd day; by 55.4% ($p<0.001$) in comparison with the 7th day. The acini area has increased by 25.8% ($p<0.05$) on the 10th day in comparison with the control group; by 30.6% ($p<0.01$) in comparison with the 3rd day; by 20.2% ($p>0.05$) in comparison with the 7th day.

The results analysis found the correlation between the area of islets of Langerhans and acini $r=0.407$, $p<0.05$; between the area of islets of Langerhans and dehydration degree $r=0.708$, $p<0.001$; between acini area and dehydration degree $r=0.534$, $p<0.001$.

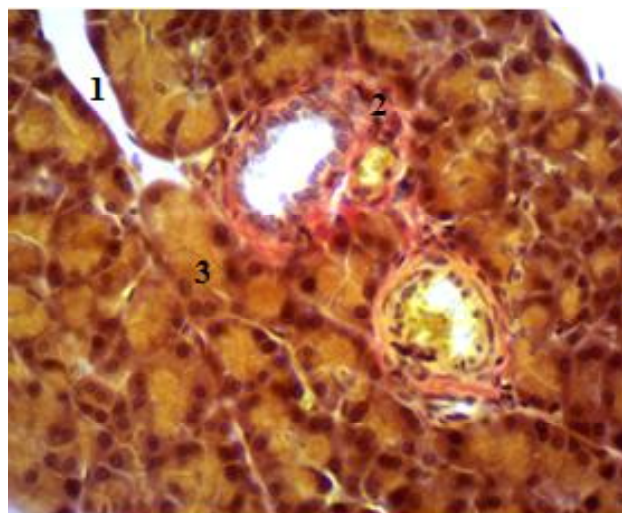


Fig. 3. Pancreas of a rat. Exocrine part. General dehydration. 10th day. Stromal edema, enlargement of the connective tissue around the vessels and heterogeneity of the secretion in the ducts. 1 - interacinic edema, 2 - lymphohistocytic infiltration, 3 - pancreatic acinus. Van Gieson's stain. x400.

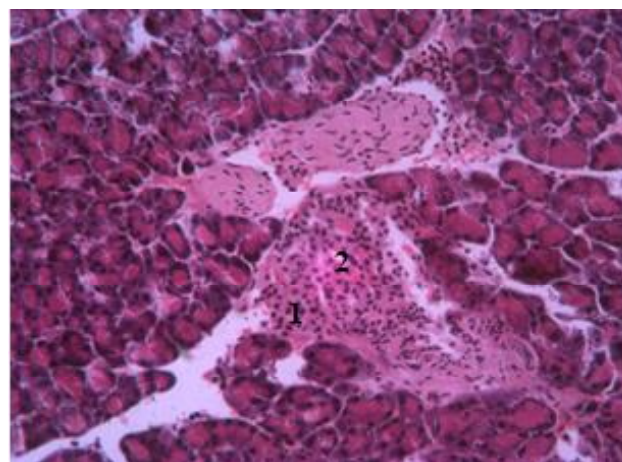


Fig. 4. Pancreas of a rat. Exocrine part. General dehydration. 10th day. Islet deformation, lesser quantity of β -cells located in the islet center. 1 - α -cells, 2 - β -cells. Hematoxylin and eosin stain. x200.

Discussion

In the conditions of general dehydration, changes in the histostructure of the parenchyma were found in all experimental series. The severity of the destructive changes in the exo- and endocrine parenchyma and microcirculatory bloodstream is progressing as the degree of severity of dehydration increases [4]. There is a large amount of data on the effect of various exogenous factors on the parenchyma of the pancreas in the literature, special attention is paid to the effects of alcohol and narcotic substances [1, 3]. The authors note the expression of degenerative changes in glandular cells and proliferation of connective tissue, obliteration and the phenomenon of endarteritis are observed in the vessels. In the excretory ducts, periductal fibrosis and epithelial proliferation are detected. Scientists have noted a decrease in the islets of Langerhans due to their wrinkling. A significant amount of work is devoted to the study of the effect of nicotine, in which the authors note the edema of cells and the compaction of nuclei with cell pycnosis. Taking into account a small number of studies in which the authors studied the effect of dehydration on the pancreatic parenchyma and their contradictory nature, we decided to conduct research

in this direction.

The prospects for further research suppose the changes study in pancreas parenchyma under the conditions of intracellular dehydration.

Conclusions

1. The analysis of pancreas morphometric data under the conditions of general dehydration showed that the histostructure of gland parenchyma undergoes changes in all experimental groups. The morphological structure proved that destructive changes in both the exocrine and endocrine parenchyma and the microcirculatory bloodstream progress with the severity degree of dehydration.

2. The significant increase in acini area and islets of Langerhans occurs under the condition of severe dehydration. The results comparison of mild and moderate dehydration in comparison with the control group has not shown significant differences. One can suppose that it was caused by adaptive-compensatory mechanisms.

3. A direct connection is established between dehydration degree and the area of acini and islets of Langerhans.

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ГІСТОМОРФОМЕТРИЧНА ОЦІНКА ЗМІН АЦИНУСІВ ТА ОСТРІВЦІВ ЛАНГЕРГАНСА ПІДШЛУНКОВОЇ ЗАЛОЗИ ЗА УМОВ ЗАГАЛЬНОГО ЗНЕВОДНЕННЯ ОРГАНІЗМУ

Ковчун В.Ю.

Зневоднення супроводжує ряд патологічних станів і викликає перебудову органів і систем організму. Мета дослідження: дослідити гістоморфометричні зміни підшлункової залози за умов загального зневоднення організму. Встановити кореляційні зв'язки між досліджуваними групами. Дослід був проведений на 35 білих щурах-самцях. Підшлункову залозу забарвлювали гематоксилін-еозином та за Ван-Гізон. Оцінювали розміри ацинусів, острівців Лангерганса та інші утворення паренхіми. Площа ацинусів на 3 добу відносно інтактної групи зменшилася на 6,5% ($p>0,05$). Площа острівців зменшилася на 2% ($p>0,05$) відносно інтактної групи. На 7 добу експерименту площа острівців збільшилася на 0,2% ($p>0,05$) відносно групи контролю; на 2,2% ($p>0,05$) відносно 3 доби. Площа ацинусів на 7 добу збільшилася на 7,2% ($p>0,05$) відносно групи контролю та на 13,2% ($p>0,05$) відносно 3 доби. Площа ацинусів на 10 добу збільшилася на 25,8% ($p<0,05$) відносно групи контролю; відносно 3 доби на 30,6% ($p<0,01$); відносно 7 доби на 20,2% ($p>0,05$). Площа острівців збільшилася на 55,5% ($p<0,001$) відносно групи контролю; відносно 3 доби на 56,3% ($p<0,001$); відносно 7 доби на 55,4% ($p<0,001$). Встановлено наявність кореляційного зв'язку між площею острівців та ацинусів $r=0,407$, $p<0,05$. Між площею острівців та ступенем зневоднення $r=0,708$, $p<0,001$; між площею ацинусів та ступенем зневоднення $r=0,534$, $p<0,001$. Морфологічно встановлено наявність деструктивних змін паренхіми підшлункової залози, що прогресують за мірою зростання ступеня тяжкості дегідратації.

Ключові слова: підшлункова залоза, острівці Лангерганса, ацинуси, зневоднення.

ГИСТОМОРФОМЕТРИЧЕСКАЯ ОЦЕНКА ИЗМЕНЕНИЙ АЦИНУСОВ И ОСТРОВКОВ ЛАНГЕРГАНСА ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ В УСЛОВИЯХ ОБЩЕГО ОБЕЗВОЖИВАНИЯ ОРГАНИЗМА

Ковчун В.Ю.

Обезвоживание сопровождается рядом патологических состояний и вызывает перестройку органов и систем организма. Цель исследования: исследовать гистоморфометрические изменения поджелудочной железы в условиях общего обезвоживания организма. Установить корреляционные связи между исследуемыми группами. Опыт был проведен на 35 белых крысах-самцах. Поджелудочную железу окрашивали гематоксилин-еозином и по Ван-Гизон. Оценивали размеры ацинусов и островков Лангерганса и другие образования паренхимы. Площадь ацинусов на 3 сутки относительно интактной группы уменьшилась на 6,5% ($p>0,05$). Площадь островков уменьшилась на 2% ($p>0,05$) относительно интактной группы. На 7 сутки эксперимента площадь островков увеличилась на 0,2% ($p>0,05$) относительно группы контроля; на 2,2% ($p>0,05$) относительно 3 суток. Площадь ацинусов на 7 сутки увеличилась на 7,2% ($p>0,05$) относительно группы контроля и на 13,2% ($p>0,05$) относительно 3 суток. Площадь ацинусов на 10 сутки увеличилась на 25,8% ($p<0,05$) относительно группы контроля; относительно 3 суток на 30,6% ($p<0,01$) в отношении 7 суток на 20,2% ($p>0,05$). Площадь островков увеличилась на 55,5% ($p<0,001$) относительно группы контроля; относительно 3 суток на 56,3% ($p<0,001$); в отношении 7 суток на 55,4% ($p<0,001$). Установлено наличие корреляционной связи между площадью островков и ацинусов $r=0,407$, $p<0,05$. Между площадью островков и степенью обезвоживания $r=0,708$, $p<0,001$; между площадью ацинусов и степенью обезвоживания $r=0,534$, $p<0,001$. Морфологически установлено наличие деструктивных изменений паренхимы поджелудочной железы, которые прогрессируют по мере роста степени тяжести дегидратации.

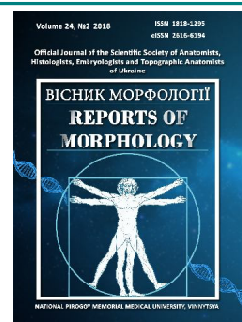
Ключевые слова: поджелудочная железа, островки Лангерганса, ацинусы, обезвоживание.



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Cephalometric studies of Ukrainian adolescents with orthognathic bite by the method of E.P. Harvold

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According to many numerical literary sources, the modern direction of provision of dental care involves an individual approach to the patient, in particular, based on his gender, age and nationality. This calls for the creation of its own regulatory bases for the population of different countries and ethnic groups, taking into account all possible criteria. The purpose of the study is to establish and analyze the cephalometric parameters by the method of E.P. Harvold in young men and women of Podillia region of Ukraine with orthognathic bite. Primary lateral teleroentgenograms of 38 young men and 55 women with normal occlusion close to orthognathic bite, obtained using the Veraviewepocs 3D device, Morita (Japan), were taken from the data bank of the research center of the National Pirogov Memorial Medical University, Vinnytsya. Cephalometric measurements were performed according to the recommendations of E.P. Harvold. Statistical processing of the obtained results was carried out in the license package "Statistica 6.0" using nonparametric methods of estimating the results. When comparing teleroentgenographic indices between boys and girls of the Podillia region of Ukraine with orthognathic occlusion, significantly higher values of the length of the upper and lower jaw, lower facial height and interjaw differences in young men were established, and Ap1uAp1l-DOP angle was significantly higher in young women. When comparing these data with the results obtained by E.P. Harvold, the boys and girls of the Podillia region of Ukraine with orthognathic bite have significantly lower values of the length of the upper and lower jaw and lower facial height. In addition, Ukrainian young women have significantly higher values of the Ap1uAp1l-DOP angle and a tendency to lower interjaw differences, while in young men, the tendency towards higher values of the cross-sectional angle than the value of these parameters obtained by E. Harvold is established. The results of the study confirm the need to create a normative basis for teleroentgenographic indices by the method of E.P. Harvold for the population of different regions of Ukraine, taking into account both sexual and age affiliation.

Keywords: lateral teleroentgenograms of the head, cephalometry, young men and women of Podillia with orthognathic bite, E.P. Harvold analysis.

Introduction

The face as the most open part of the human body plays many key roles in the modern human life, and in particular performs aesthetic function. If we talk about the lower part of the face, then the aesthetic function is ensured not only due to a smile (correct arrangement, size of teeth, etc.) and, in general, the correct arrangement of all structures of the lower face of each other (upper and lower jaw, chin, etc.).

In order to correctly assess the interrelationship of these entities, a cephalometric analysis based on the study of specific points, lines, planes and angles is used [14].

There are numerous methods of cephalometric analysis

of lateral teleroentgenograms, each of which has its own characteristics. So, at the moment, well known methods of analysis are by Steiner, Wits, Downs, Tweed, Ricketts, Burstone, McNamara [1, 4-6, 17, 18, 20, 21].

Among them, special attention should be paid to the analysis of the Norwegian scientist Egil Peter Harvold, proposed in 1974 [7]. Its peculiarity is that the position of the patient's teeth is not taken into account [15]. However, like other methods, Harvold's analysis has a significant drawback - norms laid down in this technique are specific because the study was conducted on Canadians who had European

ancestors. The modern direction of provision of dental care involves an individual approach to the patient, in particular, taking into account his gender, age and nationality.

All this pushes scientists in different parts of the planet to create their own normative bases for their own population, taking into account all possible criteria.

The purpose of the work - to establish and analyze cephalometric parameters by the method of E.P. Harvold in young men and women of the Podillia region of Ukraine with orthognathic bite.

Materials and methods

Primary lateral teleroentgenograms of 38 young men (aged 17-21 years) and 55 young women (aged 16 to 20 years) with normal occlusion close to orthognathic bite, obtained using the Veraviewepocs 3D device, Morita (Japan), taken from the data bank of the scientific-research center of National Pirogov Memorial Medical University, Vinnytsya.

According to the cephalometric method of E.P. Harvold [16] determined the following indices (Fig. 1).

Used points:

ADP (anterior Downs point, anterior point of the closing plane (**OcIPI**) by Downs) - the middle of the line connecting the cutting edges of the middle cutters upper (**Is1u**) and lower (**Is1L**) jaws;

ANS (spina nazalis anterior) - usually in the majority of cephalometric techniques is indicated as the apex of the anterior nasal ostium, forming the anterior point of the palatal plane, but in the Harvold method, this is a point on the lower contour of the anterior nasal passage where the thickness reaches three millimeters and is used for horizontal measurements. For vertical measurements a point is used on the upper contour of the anterior nasal passage where the thickness reaches three millimeters;

Ap1L (apex first inferior incisor) - the point of the top of the root of the median lower cutter;

Ap1u (apex first upper incisor) - the point of the top of the root of the median upper cutter;

Gn (gnation) - anterior point on the lower contour of the body of the mandible, in Harvold method marked as **GN**;

Is1L (incision inferior) - point located on the cutting edge of the lower middle incisor;

Is1u (incision superior) - point located on the cutting edge of the upper middle incisor;

N (nasion) - the most forward point of the fronto-nasal suture connections frontal and nasal bones in the mid-jib plane;

PDP (posterior Downs point) - rear point of closing plane (**OcIPI**) by Downs - the middle of the line, connecting the near-buccal tip of the first molars of the upper and lower jaws;

PGN (prognathion) - a point on the anterior chin contour which determines the maximum length of the lower jaw from the point (**TM**);

Pog (pogonion) - the most forward point of the bony chin performances, in the Harvold method is indicated as **PG**;

TM (temporomandibular joint) - a point on the contour of

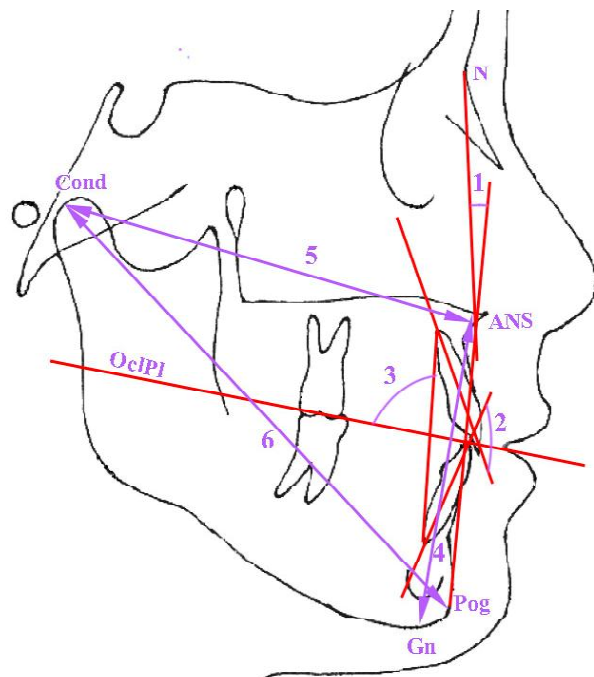


Fig. 1. Cephalometric indices determined by the cephalometric method of E.P. Harvold [16]. 1. **N-ANS-Pog (Convexity)** - angle of convexity, determines the convexity of the profile formed by the reference lines **N-ANS** and **ANS-Pog** (note - in the author's technique, the point Pog is marked as PG) ($^{\circ}$); 2. **II (Interincisal Angle)** - Angulation of axes of upper to lower incisor, formed by the reference lines **Ap1u-Is1u** and **Ap1L-Is1L** ($^{\circ}$); 3. **Ap1uAp1L-DOP** - Angle between Occlusal Plane and the connecting line between the root tips of the central upper and lower incisor ($^{\circ}$); 4. **TM-ANS** (is also found as **ANS-Cond**) (maxillary length, forward position of the maxilla) - distance from (**TM**) to spina nazalis anterior (**ANS**) determining the maxillary length (mm); 5. **TM-PGN** (is also found as **Pog-Cond**) (mandibular length) - distance from (**TM**) to (**PGN**) determining the mandibular length (mm); 6. **ANS-Gn** (Lower Face Height) - distance from Anterior nasal spine to Gnathion determining the lower face height (mm); 7. **Max-Mand** (Difference in Jaw Lengths) - difference of the lengths of the lines **ANS-Cond** and **Pog-Cond** (mm).

the mandibular fossa through which the line of the greatest length of the mandible passes, usually in most of the cephalometric techniques is indicated as a point on the vertex of the contour of the head of the mandible **Cond (condylion)**.

Statistical processing of the obtained results was carried out in the "Statistica 6.0" licensing package using nonparametric estimation methods. The reliability of the difference in values between the independent quantitative values was determined using the Mann-Whitney U test.

Results

Indicators determined by cephalometric method of E.P. Harvold [16] and A.E. Athanasiou [3] (mean with standard deviation and percentage scale) in Ukrainian young men and women with orthognathic bite are presented in Table 1.

It should be noted that part of the parameters determined

Table 1. Percentage scale of teleroentgenographic indices by E.P. Harvold method in Ukrainian young men and women with orthognathic bite.

| Indexes | Young men | | Young women | | p |
|------------------|-------------|--------------|-------------|--------------|--------|
| | M±σ | 25p-l, 75p-l | M±σ | 25p-l, 75p-l | |
| N-ANS-Pog (°) | 11.24±5.85 | 7.0-16.0 | 10.45±5.18 | 7.0-14.0 | >0.05 |
| II (°) | 130.6±7.1 | 126.0-134.9 | 130.5±8.0 | 125.4-135.5 | >0.05 |
| Ap1uAp1I-DOP (°) | 90.29±4.05 | 87.0-94.0 | 92.45±4.21 | 89.0-95.0 | <0.05 |
| ANS-Cond (mm) | 91.19±13.01 | 86.3-92.7 | 83.40±7.21 | 79.8-85.3 | <0.001 |
| Pog-Cond (mm) | 117.4±18.3 | 110.4-117.8 | 107.4±10.0 | 102.8-108.9 | <0.001 |
| ANS-Gn (mm) | 64.64±11.10 | 61.2-66.0 | 59.63± 6.40 | 56.0-61.4 | <0.001 |
| Max-Mand (mm) | 26.16±6.84 | 23.1-29.5 | 24.05± 4.51 | 21.5-26.7 | <0.05 |

Notes: M±σ - average ± standard deviation; 25p-l, 75p-l - percentage scale; p - statistical significance of differences between the sizes of young men and women.

Table 2. Comparison of teleroentgenographic indices obtained by E.P. Harvold with young men and women with orthognathic bite (M±σ).

| Indexes | Value by E.P. Harvold [16] | | Young men | Young women |
|------------------|----------------------------|----------|-----------------|----------------|
| N-ANS-Pog (°) | no data | | 11.24±5.85 | 10.45±5.18 |
| II (°) | 128±4 | | 130.6±7.1 t | 130.5±8.0 |
| Ap1uAp1I-DOP (°) | 89±5 | | 90.29±4.05 | 92.45±4.21 ** |
| | Boys | Girls | | |
| ANS-Cond (mm) | 100±4.17 | 93±3.45 | 91.19±13.01 *** | 83.40±7.21 *** |
| Pog-Cond (mm) | 127±5.25 | 119±4.44 | 117.4±18.3 ** | 107.4±10.0 *** |
| ANS-Gn (mm) | 71±5.73 | 65±4.67 | 64.64±11.10 ** | 59.63±6.40 *** |
| Max-Mand (mm) | 27 | 26 | 26.16±6.84 | 24.05±4.51 t |

Notes: *, **, *** - significant differences (p<0.05, p<0.01 and p<0.001) of corresponding indicators obtained by E.P. Harvold method with young men and women with orthognathic bite; t - trends of differences in the corresponding indicators obtained by E.P. Harvold method with Ukrainian young men and women with orthognathic bite.

by the cephalometric method of E.P. Harvold (angle II, or inter-incisive angle) are used in the analyzes proposed by C. Steiner, G. Schmuth and A.M. Schwarz and are reflected in the studies of M.O. Dmitriev [10, 11, 13].

When comparing teleroentgenographic indices obtained by the method of E.P. Harvold between young men and women of the Podillia region of Ukraine with orthognathic occlusion, established significantly higher (p<0.05-0.001) values of the length of the upper jaw (ANS-Cond), the length of the mandible (Pog-Cond), the lower face length (ANS-Gn) and maxillary difference (Max-Mand) in young men, as well as significantly higher (p<0.05) value of the Ap1uAp1I-DOP angle for young women (see Table 1).

When comparing teleroentgenographic indices of E.P. Harvold with the results obtained in young men and women of the Podillia region of Ukraine with orthognathic occlusion, established significantly lower (p<0.01-0.001) values of the length of the upper jaw (ANS-Cond), the length of the mandible (Pog-Cond) and lower face height (ANS-Gn) in Ukrainian young men and women with orthognathic bite (Table 2). In addition, young women of the Podillia region of Ukraine have significantly higher (p<0.01) values of the Ap1uAp1I-DOP angle and the trend (p=0.063) to smaller values of the maxillary difference (Max-Mand), while in young men of the Podillia region of Ukraine there is a slight trend

(p=0.078) to higher values of the inter-incisive angle (angle II) than teleroentgenographic parameters obtained by E.P. Harvold (see Table 2).

Discussion

Scientists from Bangladesh conducted a cephalometric examination of lateral teleroentgenograms using E.P. Harvold's technique of 50 young men and 50 women aged 18 to 24 years with a physiological bite. After the statistical processing of the obtained data revealed signs of sexual dimorphism - most of the studied values were greater in young men. Compared with the author's methodology, for Bangladesh girls found lower values of such indicators as: inter-incision angle (p<0.001), occlusal and radicular planes (p<0.001) and inter-jaw differences (p<0.001). Also, a significant difference was found between the position of the upper and lower jaw and the anterior lower facial height (p<0.01) [2].

A.A. Daer and A.H. Abuaffan conducted a similar study to determine the cephalometric norms by the method of E.P. Harvold for the population of Yemen. To achieve this goal, 105 young women and 89 men aged from 18 to 25 years old who had no history of trauma or illness of the tooth-jaw system and physiological bite were examined. All of them had an X-ray examination with subsequent

cephalometric analysis and statistical treatment of the results. Statistically significant differences were noted between young men and women among skeletal sagittal relationships [9].

E. Lara-Carrillo and colleagues surveyed 116 boys and 125 girls aged from 9 to 18, in the third generation of Mexican residents. The purpose of the study was to identify cephalometric norms for residents of the capital of Mexico, according to their age and sexual characteristics. After statistical data processing, manifestations of sexual dimorphism were revealed among the indicators of the position of the upper and lower jaw, the anterior lower height of the face in all age groups [19].

When comparing teleroentgenographic indices used in the E.P. Harvold analysis between young men and women of the Podillia region of Ukraine with orthognathic bite, pronounced sexual differences are established, namely, significantly higher values of the length of the upper and lower jaw, lower facial height and interjaw difference in young men, and higher angle values Ap1uAp1I-DOP for young women. In previous studies, in the analysis of cephalometric parameters by methods of C.J. Burstone and R.M. Ricketts, we also revealed pronounced sexual differences [8, 12].

Comparing teleroentgenographic indices obtained by E.P. Harvold with the magnitude of the data obtained in the young men and women of Podillia with orthognathic bite, we also established the distinct differences, namely: significantly lower values of the length of the upper jaw (allows us to estimate the position of the anterior point of the upper jaw in the arterial plane and clinically allows interpret the results as a normal position, front position or prognathism, underdevelopment or retrognathia of the upper jaw), length of the mandible (allows you to evaluate the position of the chin in the jet and the vertical planes; essentially the hypotenuse of the lower jaw takes into account not only the length of the branch and the body of the mandible but also the angle between them) and the lower height of the face (vertical absolute index characterizing the lower face height; an increase in the index testifies to the affiliation of the case to the open bite and decrease to deep bite) in Ukrainian young men and women. Also, Ukrainian young women have significantly higher values of the Ap1uAp1I-DOP angle (it allows to determine the position of the tops of the anterior group of teeth in relation to the functional plane. Individuals with second-degree pathology according to Engle have an

increased angle value, and patients with a pathology of 3rd grade by Engle have a reduced value. Consequently, clinically, this indicator helps to orient itself in the person's belonging to one or another group of sagittal anomalies) and the tendency towards lower values of the interjaw difference (which, unlike the generally accepted indicators of the angle ANB and Wits, which characterize interjaw correlation, do not depend on the vertical bite characteristics and allow the maxillofacial determination of the existence of interjaw disharmony. When taking into account the parameters of the length of the upper and lower jaws, the interjaw difference allows one to determine which of the jaws most changed as a result of anomalies of development). It should be noted that according to our research, most of the cephalometric parameters obtained by R.M. Ricketts [8] and more than half of the cephalometric parameters obtained by C.J. Burstone [12] also have significant differences with the magnitudes of these parameters obtained in young men and women of Podillia region of Ukraine with orthognathic bite.

The obtained results indicate the need to create a normative basis for teleroentgenographic indices by E.P. Harvold for the population of various ethno-territorial regions of Ukraine, taking into account both sexual and age-related affiliation.

Conclusion

1. Significant sexual differences between cephalometric parameters by E.P. Harvold between young men and women of Podillia with orthognathic bite were found: significantly greater lengths of the upper (ANS-Cond) and lower jaw (Pog-Cond), lower facial height (ANS-Gn), and maxillary difference (Max-Mand) in young men, and higher value of the angle Ap1uAp1I-DOP in young women.

2. When comparing the cephalometric parameters obtained by E.P. Harvold with the indices obtained in young men and women of Podillia with orthognathic bite, the values of the length of the upper (ANS-Cond) and the lower jaw (Pog-Cond) and lower face height (ANS-Gn) in young men and women established significantly lower values. Also, young women from Podillia have significantly higher values of the Ap1uAp1I-DOP angle and a tendency to lower values of the interjaw difference (Max-Mand), and in young men, the tendency towards higher values of the intersection angle (angle II) is established.

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ЦЕФАЛОМЕТРИЧНІ ДОСЛІДЖЕННЯ УКРАЇНСЬКИХ ЮНАКІВ ТА ДІВЧАТ З ОРТОГНАТИЧНИМ ПРИКУСОМ ЗА МЕТОДОМ Е.Р. HARVOLD

Черниш А.В.

Згідно багато чисельних літературних джерел сучасний напрямок надання стоматологічної допомоги передбачає індивідуальний підхід до пацієнта, зокрема, з урахуванням його статі, віку та національної приналежності. Це викликає необхідність створення власних нормативних баз для населення різних країн та етносів з урахуванням всіх можливих критеріїв. Мета дослідження - встановити та проаналізувати цефалометричні параметри за методом Е.Р. Harvold у юнаків і дівчат Подільського регіону України з ортогнатичним прикусом. Первинні бокові телерентгенограми 38 юнаків та 55 дівчат з нормальною оклюзією наближеною до ортогнатичного прикусу, отримані за допомогою пристрою Veraviewerpross 3D, Моріта (Японія), взяті з банку даних науково-дослідного центру Вінницького національного медичного університету ім. М.І. Пирогова. Цефалометричні вимірювання проводили згідно рекомендацій Е.Р. Harvold. Статистична обробка отриманих результатів проведена в ліцензійному пакеті "Statistica 6,0" з використанням непараметричних методів оцінки результатів. При порівнянні телерентгенографічних показників між юнаками та дівчатами Подільського регіону України з ортогнатичним прикусом встановлено достовірно більші значення довжини верхньої і нижньої щелепи, нижньої висоти обличчя та міжщелепної різниці в юнаків, а також достовірно більше значення кута $Ar1uAr1l-DOP$ у дівчат. При порівнянні даних показників з результатами отриманими Е.Р. Harvold, в юнаків і дівчат Подільського регіону України з ортогнатичним прикусом встановлені достовірно менші значення довжини верхньої і нижньої щелепи та нижньої висоти обличчя. Крім того, в українських дівчат встановлені достовірно більші значення кута $Ar1uAr1l-DOP$ та тенденція до менших значень міжщелепної різниці, а в юнаків - тенденція до більших значень міжрізцевого кута, ніж величина даних параметрів отриманих Е.Р. Harvold. Результати дослідження підтверджують необхідність створення нормативної бази телерентгенографічних показників за методикою Е.Р. Harvold для населення різних регіонів України з урахуванням як статевої, так і вікової належності.

Ключові слова: бокові телерентгенограми голови, цефалометрія, юнакі і дівчата Поділля з ортогнатичним прикусом, аналіз Е.Р. Harvold.

ЦЕФАЛОМЕТРИЧЕСКОЕ ИССЛЕДОВАНИЕ УКРАИНСКИХ ЮНОШЕЙ И ДЕВУШЕК С ОРТОГНАТИЧЕСКИМ ПРИКУСОМ ПО МЕТОДУ Е.Р. HARVOLD

Черныш А.В.

Согласно многочисленных литературных источников современное направление оказания стоматологической помощи предусматривает индивидуальный подход к пациенту, в частности, с учетом его пола, возраста и национальной принадлежности. Это вызывает необходимость создания собственных нормативных баз для населения различных стран и этносов с учетом всех возможных критериев. Цель исследования - установить и проанализировать цефалометрические параметры по методу Е.Р. Harvold у юношей и девушек Подольского региона Украины с ортогнатическим прикусом. Первичные

боковые телерентгенограммы 38 юношей и 55 девушек с нормальной окклюзией приближенной к ортогнатическому прикусу, полученные с помощью устройства Veraviewerocs 3D, Морита (Япония), взяты из банка данных научно-исследовательского центра Винницкого национального медицинского университета имени Н.И. Пирогова. Цефалометрические измерения проводили согласно рекомендациям Е.Р. Harvold. Статистическая обработка полученных результатов проведена в лицензионном пакете "Statistica 6,0" с использованием непараметрических методов оценки результатов. При сравнении телерентгенографических показателей между юношами и девушками Подольского региона Украины с ортогнатическим прикусом установлено достоверно большие значения длины верхней и нижней челюсти, нижней высоты лица и межчелюстной разницы у юношей, а также достоверно большее значение угла $Ar1uAr1l-DOP$ у девушек. При сравнении данных показателей с результатами, полученными Е.Р. Harvold, у юношей и девушек Подольского региона Украины с ортогнатическим прикусом установлены достоверно меньшие значения длины верхней и нижней челюсти и нижней высоты лица. Кроме того, в украинских девушек установлены достоверно большие значения угла $Ar1uAr1l-DOP$ и тенденция к меньшим значениям межчелюстной разницы, а у юношей - тенденция к большим значениям между-резцового угла, чем величина данных параметров, полученных Е.Р. Harvold. Результаты исследования подтверждают необходимость создания нормативной базы телерентгенографических показателей по методике Е.Р. Harvold для населения различных регионов Украины с учетом как половой, так и возрастной принадлежности.

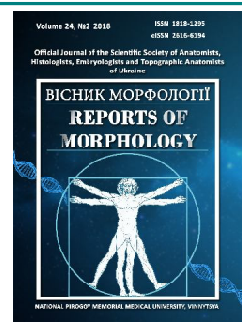
Ключевые слова: боковые телерентгенограммы головы, цефалометрия, юноши и девушки Подолья с ортогнатическим прикусом, анализ Е.Р. Harvold.



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Leading factors which influence the features of the course of the psychophysiological adaptation of students of modern medical education in the dynamics of educational year, and their complex assessment

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The assessment of the peculiarities of the processes of psychophysiological adaptation of students involves the implementation of a comprehensive analysis of a number of indicators of the functional state of the organism and of the personality traits of girls and youth on the basis of the application of methods of statistical analysis, one of the main tools of which is factor analysis. The aim of the work is to determine the leading factors influencing the peculiarities of the course of psychophysiological adaptation of students of modern institutions of higher medical education in the dynamics of the academic year. Determination of the level of development of psychophysiological functions of students was carried out with the use of the licensed computer complex "Efecton Studio", peculiarities of the person were evaluated on the basis of psychodiagnostic studies, statistical processing of the received materials was carried out using a package of programs of multidimensional statistical analysis "Statistica 6.1" on the basic procedures of factor analysis. It is determined that as the main factors in the analysis of the physiologically-determined correlates of the success of the processes of psychophysiological adaptation, the following factors should be noted: "peculiarities of dynamic efficiency" and "peculiarities of the balance of nervous processes", as well as at the beginning of the training time, the factor "peculiarities of mobility of nerve processes" and in the end it is a factor "the peculiarities of the mobility of the nervous processes and the speed of the visual-motor reactions". The main factors that were identified during the analysis of the mentally-determined correlates of the success of the processes of psycho-physiological adaptation are the following factors: "features of temperament and anxiety", "features of character properties", "features of the level of subjective control of personality", "peculiarities aggressive manifestations of personality", as well as "features of emotional burnout", which at the beginning of the training period was associated with a number of features of the mechanisms of psychological protection, at the end of it - with a number of features asthenic and depressive states.

Keywords: *students, academic year, psychophysiological adaptation, physiologically-significant correlates, mentally-significant correlates, factor analysis.*

Introduction

The study of the peculiarities of the course of psychophysiological adaptation of students who are in the institution of higher education, including in the conditions of medical institutions of higher education, involves the implementation of a comprehensive analysis of a number of indicators of the functional state of the organism and peculiarities of the personality of young men and women on

the basis of the application of methods of multidimensional statistical analysis, one of the main tools of which is the process of factor analysis [2-4, 8, 10, 12, 13, 18, 23-26, 29].

In general, factor analysis allows to quantitative assessment of the characteristics of the investigated indicators, which is directly determined on the basis of the evaluation of characteristics, the level of expression of which

is established and, therefore, makes it possible to detect a rather narrow set of properties for a significant part of the initial characteristics that characterize the relationship between the groups of these signs and certain generalized factors [1, 7, 11, 14, 20, 22, 27].

Therefore, factor analysis is attributed to statistical methods that allow us to make a completely correct statistical description of multidimensional objects, which are characterized by the presence of a plurality of quantitative attributes, and, therefore, a priori redundancy of the initial characteristics of the system under study, based on the definition of the depth indicators that actually form them and determine [1, 5, 6, 15-17, 21, 28, 30].

The purpose of the work is to determine the leading factors influencing the peculiarities of the processes of psychophysiological adaptation of students of modern institutions of higher medical education in the dynamics of the academic year, and their complex assessment based on the use of procedures of factor analysis.

Materials and methods

Scientific researchers were conducted among students of the medical faculty of the National Pirogov Memorial Medical University, Vinnytsya. 55 young women and 45 young men were assigned to the study groups.

Comprehensive assessment of the development level of the leading psychophysiological functions indicators of the students (physiologically significant correlates of the course of psychophysiological adaptation) envisaged the definition of the degree of development of characteristics that determined the features of sensorimotor reactions (the speed of simple and differentiated visual-motor response, simple audio-motor reaction), the course of the main nervous processes (mobility and balance of nerve processes), as well as the strength of the nervous system (tapping test) and the dynamics of performance in monotony conditions were carried out during academic year (at the beginning of the autumn and at the end of spring semesters) on the basis of the using computer complex "Effecton Studio", registered in the Fund of computer programs of educational purposes (registration number 1717) and has a certificate of conformity of the Institute of Information Education [9].

At the heart of determining the characteristics of the personality of students (psychologically significant correlates of psychophysiological adaptation) were psychodiagnostic techniques that provided for the establishment of individual-typological peculiarities of the person and, above all, the properties of temperament, anxiety and character, indicators of the level of subjective control, peculiarities of mental states, nature, aggressiveness, emotional burnout, mechanisms of psychological protection, etc. [19].

Applied implementation and subsequent interpretation of the results obtained using factor analysis procedures were carried out using the licensed standard application package of multidimensional statistical analysis "Statistica 6.1" (license number BXXR901E245722FA, belonging to the

National Pirogov Memorial Medical University, Vinnytsya).

Results

Considering the obtained results, it should be noted that at the beginning of the academic year the regularities of the relationships between the studied parameters and the physiologically-determined correlates of the success of the processes of psycho-physiological adaptation (y) that were established should be presented as the following relationship (1-2):

- among young women: $y = 0.408f_1 + 0.236f_2 + 0.298f_3$; (1)

where the factor f_1 was to be defined as "the features of dynamic performance" (the share of dispersion - 42,27%) and, above all, united in its structure indicators that showed the characteristics of dynamic performance in monotony conditions and data on the performance of the tapping test during all studied intervals; the factor f_2 was to be defined as "the peculiarities of the balance of nervous processes" (the proportion of dispersion - 25.10%) and combines both the actual characteristics of the equilibrium of the nervous processes and the data with respect to the amount of premature reactions and latency reactions; the factor f_3 - was to be defined as "the peculiarities of the mobility of the nerve processes" (the dispersion share was 19.22%) and included only indicators of the mobility of the nerve processes in its structure;

- among young men: $y = 0.517f_1 + 0.243f_2 + 0.192f_3$; (2)

where the factor f_1 was to be defined as "the features of dynamic performance" (the share of dispersion - 43,27%) and, above all, united in its structure indicators that depicted the characteristics of dynamic performance in monotony conditions and data on the performance of the tapping test during all the studied intervals, except for the latter; the factor f_2 was to be defined as "the peculiarities of the equilibrium of the nervous processes" (the proportion of the dispersion was 26.10%) and combining both the actual characteristics of the balance of the nervous processes and the data with respect to the number of premature reactions and delayed reactions; the factor f_3 was to be defined as "the peculiarities of the mobility of the nerve processes" (the dispersion share was 21.22%) and included only the indices of the actual mobility of the nerve processes in its structure.

At the same time, at the end of the school year, the regularities of the relationship between the studied parameters and the physiologically-determined correlates of the success of the processes of psycho-physiological adaptation (y) that have been established should be presented in the form of such relationships (3-4):

- among young women: $y = 0.430f_1 + 0.226f_2 + 0.200f_3$; (3)

where the factor f_1 was to be defined as "the features of dynamic performance" (the share of dispersion - 44.22%) and, above all, united in its structure the indicators reflecting the characteristics of dynamic performance in monotony conditions and data on the implementation of the tapping test during all studied intervals; the factor f_2 was to be defined as "the peculiarities of the balance of nervous

processes" (the proportion of dispersion was 25.96%) and combined both the actual characteristics of the balance of nervous processes and the data with regard to the amount of premature reactions and delayed reactions; the factor f3 was to be defined as "the peculiarities of the mobility of the nerve processes and the speed of the visual-motor reactions" (the proportion of the dispersion was 18.98%) and included in its structure the parameters of the mobility of the nerve processes and, unlike the initial stage of observation, characteristics of the speed of the simple and differentiated visual-motor reaction;

- among young men: $y = 0.400f_1 + 0.271f_2 + 0.203f_3$; (4)

where the factor f1 was to be defined as "the features of dynamic performance" (the share of dispersion - 40.17%) and, above all, united in its structure indicators that showed the characteristics of dynamic performance in monotony conditions and data on the implementation of the tapping test during all studied intervals; the factor f2 was to be defined as "the peculiarities of the balance of nervous processes" (the dispersion share was 25.88%) and combined both the actual characteristics of the balance of nervous processes and the data with respect to the amount of premature reactions and latency reactions; the factor f3 was to be defined as "the peculiarities of the mobility of the nerve processes and the speed of the visual-motor reactions" (the proportion of the dispersion was 17.19%) and included in its structure the parameters of the mobility of the nerve processes and, unlike the initial stage of observation, characteristics of the speed of the simple and differentiated visual-motor reaction.

At the same time, analyzing the results obtained, it should be noted that at the beginning of the school year, the patterns of relationships between the studied parameters and the psychologically determined correlates of the success of the processes of psycho-physiological adaptation (y), which were established, should be presented as the following relationship (5-6):

- among young women: $y = 0.567f_1 + 0.249f_2 + 0.216f_3 + 0.231f_4 + 0.146f_5$; (5)

where the factor f1 was to be defined as "the features of the level of subjective control of the individual" (the share of dispersion - 29.00%) and, above all, united in its structure indicators that reflect the characteristics of the general level of subjective control level and level subjective control in the field of educational relations and health and disease; the factor f2 - was to be defined as "the features of temperament and anxiety" (the share of dispersion - 19.18%) and, first of all, combines indicators of neuroticism, situational and personality anxiety; the factor f3 was to be defined as "the features of the character properties" (the fraction of the dispersion was 15.32%) and included in its structure, first of all, characteristics of the character properties of the hysteria (Hy), psychopathy (Pd) and hypomania (Ma); the factor f4 - was to be defined as "the features of aggressive manifestations of personality" (the share of dispersion - 14.38%) and united in its structure, first of all, indices of irritation, feeling of insult and index of aggressiveness; factor

f5 - was to be defined as "the features of emotional burnout and mechanisms of psychological protection" (the proportion of dispersion - 8.96%) and combines the leading characteristics of emotional burnout according to stress, resistance and exhaustion phases, as well as mechanisms of psychological protection such as mechanisms of its protection on regression, displacement and projection scales; - among young men: $y = 0.449f_1 + 0.338f_2 + 0.247f_3 + 0.167f_4 + 0.101f_5$; (6)

where the factor f1 was to be defined as "the peculiarities of the properties of nature" (the proportion of the dispersion was 24.78%) and included in its structure, first of all, characteristics of the character of the character on the scales of hypochondria (Hs), hysteria (Hy), psychopathy (Pd), paranoid (Pa), psychasthenia (Pt) and schizoid (Se); the factor f2 was to be defined as "the features of aggressive manifestations of personality" (the share of dispersion was 17.30%) and united in its structure, first of all, indicators of indirect aggression, irritation, feeling of insult and indices of aggression and hostility, the factor f3 was to be defined as "features of temperament and anxiety" (the share of dispersion - 16.30%) and combines indicators of neuroticism, situational and personality anxiety; the factor f4 was to be defined as "the peculiarities of the level of subjective control of personality" (the share of dispersion was 15.62%) and, above all, united in its structure the indicators reflecting the characteristics of the general level of subjective control and the level of sub oversight in the field of failures, learning relationships and health and disease; the factor f5 - was to be defined as "the features of emotional burnout and mechanisms of psychological protection" (the share of dispersion - 9.23%) and, first of all, combines the leading characteristics of emotional burnout according to the phases of stress, resistance and exhaustion, and also such mechanisms of psychological protection as mechanisms of its protection on regression, displacement, negation and projection scales.

At the same time, at the end of the academic year, the regularities of the relationships between the studied indicators and the mentally determined correlates of the success of the processes of the psycho-physiological adaptation (y) that have been established should be presented in the form of such interrelations (7-8):

- among young women: $y = 0.500f_1 + 0.324f_2 + 0.146f_3 + 0.215f_4 + 0.152f_5$; (7)

where the factor f1 was to be defined as "the features of emotional burnout and asthenic and depressive states" (the share of dispersion was 29.54%) and included in its structure the leading characteristics of emotional burnout according to the stresses, resistance and exhaustion phases, as well as the indicators of the degree of expression asthenic and depressive states; the factor f2 was to be defined as "the peculiarities of the level of subjective control of personality" (the share of dispersion was 17.57%) and, in the first place, united in its structure the indicators reflecting the characteristics of the general level of subjective control and the level of sub objective monitoring in the field of

achievements, educational relations and health and disease; the factor f3 was to be defined as "the peculiarities of the character properties" (the proportion of the dispersion was 16.04%) and combines, first of all, characteristics of the character of the character on the scale of the hypochondria (Hs), depression (D), hysteria (Hy), psychopathy (Pd) and hypomania (Ma); factor f5 - was to be defined as "the features of aggressive manifestations of personality" (the share of dispersion - 15.13%) and included in its structure, first of all, indicators of negativism, irritation and indices of aggressiveness and hostility; factor f5 - was to be defined as "the features of temperament and anxiety" (the share of dispersion - 9.39%) and, above all, united in its structure indicators of neuroticism, situational and personal anxiety; - among young men: $y = 0.451f1 + 0.148f2 + 0.339f3 + 0.260f4 + 0.146f5$; (8)

where the factor f1 was to be defined as "the features of emotional burnout and asthenic and depressive states" (the share of dispersion - 26.19%) and included in its structure the leading characteristics of emotional burning according to the phases of stress, resistance and exhaustion, as well as indicators of the degree of expression asthenic and depressive states; the factor f2 was to be defined as "the peculiarities of the character properties" (the proportion of the dispersion was 18.47%) and united in its structure, first of all, characteristics of the character on the scales of hypochondria (Hs), depression (D), psychopathy (Pd), psychasthenia (Pt) and hypomania (Ma); factor f3 - was to be defined as "the features of aggressive manifestations of personality" (the proportion of dispersion - 15.90%) and, above all, combined the indicators of verbal and indirect aggression, irritation and indices of aggression and hostility; the factor f4 - was to be defined as "the features of the level of subjective control of the individual" (the share of dispersion - 14.68%) and, first of all, included in its structure the indicators reflecting the characteristics of the general level of subjective control and the level of subjective control over failures, learning relationships and health and disease; factor f5 - had to be defined as "features of temperament and anxiety" (the share of dispersion - 10.17%) and, above all, united in its structure indicators of neuroticism, situational and personal anxiety.

Discussion

In the course of the conducted researches, using factor analysis procedures, the leading factors that exert a pronounced influence on the peculiarities of the processes of adaptation transformation processes and the established connections between a number of nominal indicators of the level of development of psychophysiological functions and the formation of the peculiarities of the student's personality and the level of psychophysiological adaptation are revealed. It was determined that regardless of the nature of the training load, organization of daily activities and sexual characteristics, as the main factor formations that were identified during the analysis of the physiologically-determined correlates of the progress of the processes of psycho-physiological

adaptation, it was noted the following factors: "features of dynamic performance" and "peculiarities of the balance of nervous processes", as well as at the beginning of the training period, the factor "peculiarities of the mobility of nerve processes", which included in its structure only oscillation the actual mobility of the neural processes, and in the end it is the factor of "the peculiarities of the mobility of the nerve processes and the speed of the visual-motor reactions", which included in its structure as indicators of the mobility of the nerve processes and, unlike the initial stage of observation, characteristics of the speed of a simple and differentiated visually-motor reaction.

At the same time, it should be noted that regardless of the period of study, the nature of training loads, organization of daily activities and sexual characteristics, as the main factor education, identified during the analysis of the mentally determined correlates of the success of the processes of psychophysiological adaptation, the following factors were noted: "features temperament and anxiety", "features of character properties", "peculiarities of the level of subjective control of personality", "peculiarities of aggressive manifestations of personality" and features emotional burnout" that at the beginning of training time was closely and inextricably linked with a number of features of psychological defense mechanisms, at the end of it - with those asthenic and depressive states.

It should be noted that the peculiarities of the psychophysiological adaptation of students undergoing higher education institutions during the academic year established according to the data of factor analysis are of considerable prognostic value and need to be taken into account during the creation of a preventive environment in modern higher education institutions [4, 10, 17, 21, 23, 24, 26].

Moreover, the revealed patterns must be taken into account in the application of healthcare technologies in educational institutions of different levels and profiles, the conceptual principles of effective use of which are considered: regime-adaptive, psychophysiological, personal and integrative-functional principles with relevant content which have a favorable overall impact on the processes of the formation of the health and functional state of the body of young women and men and provide high level of training preparedness of modern students [25].

The obtained results are marked by significant significance from the point of view of determining the peculiarities of the formation of the adaptive resources of the student body in higher educational establishments of the medical profile, and the formation of effective approaches to the formation of the optimal working dynamic stereotype of young women and men who are studying [10, 23]. Moreover, the presence of certain dynamic changes on the content of individual factor groups requires careful analysis during the effective training of future medical professionals [12, 13, 25, 29].

Considering the prospects for further use of the obtained data, it should be noted several of their leading aspects, namely: diagnostic, which is based on taking into account the results obtained in the development of methods for assessing

the level of expression of academic stress among students of higher education institutions, and preventive, which involves taking into account the factors that make unfavorable influence on the processes of psychophysiological adaptation of young women and men, during developing measures for their correction and improvement.

Conclusions

In the course of the conducted researches, the leading factors influencing the peculiarities of the psychophysiological adaptation of students of modern institutions of higher medical education in the dynamics of the academic year are determined, and their complex assessments are carried out on the basis of the use of factor analysis procedures. Such an approach made it possible, during the analysis of physiologically-determined correlates of the success of the processes of psycho-physiological adaptation, such as the following factors: "features of dynamic performance" and "peculiarities of the balance of nervous processes", as well as at the beginning of the training time, the factor

"peculiarities of mobility of nerve processes", which included in its structure only the indices of the actual mobility of the nervous processes, and in the end it is a factor "the peculiarities of the mobility of the nerve processes and the speed of the visual-motor reactions", which included in its structure as indicators of the mobility of nerve processes and, unlike the initial stage of observations, the characteristics of the speed of a simple and differentiated visual-motor response. However, during the analysis of the mentally determined correlates of the success of the processes of psycho-physiological adaptation, such as the following factors: "features of temperament and anxiety", "features of the nature of the character", "features of the level of subjective control of personality", "the features of aggressive manifestations of personality" as well as "features of emotional burnout", which at the beginning of the training period was closely and inextricably linked with a number of features of the mechanisms of psychological protection, at the end of it - with the indicators of asthenic and depressive states.

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ПРОВІДНІ ФАКТОРИ, ЯКІ ВПЛИВАЮТЬ НА ОСОБЛИВОСТІ ПЕРЕБІГУ ПРОЦЕСІВ ПСИХОФІЗІОЛОГІЧНОЇ АДАПТАЦІЇ СТУДЕНТІВ СУЧАСНИХ ЗАКЛАДІВ ВИЩОЇ МЕДИЧНОЇ ОСВІТИ В ДИНАМІЦІ НАВЧАЛЬНОГО РОКУ, ТА ЇХ КОМПЛЕКСНА ОЦІНКА

Мороз В. М., Макаров С. Ю.

Оцінка особливостей перебігу процесів психофізіологічної адаптації студентів передбачає здійснення комплексного аналізу цілого ряду показників функціонального стану організму та особливостей особистості дівчат і юнаків на підставі застосування методів статистичного аналізу, одним із основних інструментів якого є факторний аналіз. Метою роботи є визначення провідних факторів, які впливають на особливості перебігу процесів психофізіологічної адаптації студентів сучасних закладів вищої медичної освіти в динаміці навчального року. Визначення рівня розвитку психофізіологічних функцій студентів проводилось із застосуванням ліцензованого комп'ютерного комплексу "Effecton Studio", особливості особистості оцінювались на підставі психодіагностичних досліджень, статистична обробка отриманих матеріалів здійснювалось із використанням пакету програм багатовимірного статистичного аналізу "Statistica 6.1" на основі процедур факторного аналізу. Визначено, що як основні фактори під час аналізу фізіологічно-обумовлених корелят успішності перебігу процесів психофізіологічної адаптації, слід відзначити наступні фактори: "особливості динамічної працездатності" і "особливості рівноваженості нервових процесів", а також на початку часу навчання фактор "особливості рухливості нервових процесів" та наприкінці його - фактор "особливості рухливості нервових процесів і швидкості зорово-моторних реакцій". Як основні фактори, що були визначені під час аналізу психічно-обумовлених корелят успішності перебігу процесів психофізіологічної адаптації, слід відзначити такі фактори: "особливості темпераменту і тривожності", "особливості властивостей характеру", "особливості рівня суб'єктивного контролю особистості", "особливості агресивних проявів особистості", а також "особливості емоційного вигорання", який на початку часу навчання був пов'язаний з особливостями механізмів психологічного захисту, наприкінці його - з показниками астеничного і депресивного станів.

Ключові слова: студенти, навчальний рік, психофізіологічна адаптація, фізіологічно-значущі кореляти, психічно-значущі кореляти, факторний аналіз.

ВЕДУЩИЕ ФАКТОРЫ, ОКАЗЫВАЮЩИЕ ВОЗДЕЙСТВИЕ НА ОСОБЕННОСТИ ТЕЧЕНИЯ ПРОЦЕССОВ ПСИХОФИЗИОЛОГИЧЕСКОЙ АДАПТАЦИИ СТУДЕНТОВ СОВРЕМЕННЫХ ЗАВЕДЕНИЙ ВЫСШЕГО МЕДИЦИНСКОГО ОБРАЗОВАНИЯ В ДИНАМИКЕ УЧЕБНОГО ГОДА, И ИХ КОМПЛЕКСНАЯ ОЦЕНКА

Мороз В. М., Макаров С. Ю.

Оценка особенностей течения процессов психофизиологической адаптации студентов предусматривает осуществление комплексного анализа целого ряда показателей функционального состояния организма и личностных особенностей девушек и юношей на основе применения методов статистического анализа, одним из основных инструментов которого является факторный анализ. Целью работы является определение ведущих факторов, оказывающих воздействие на особенности течения процессов психофизиологической адаптации студентов современных учреждений высшего медицинского образования в динамике учебного года. Определение уровня развития психофизиологических функций студентов проводилось с использованием лицензированного компьютерного комплекса "Effecton Studio", личностные особенности оценивались на основании психодиагностических исследований, статистическая обработка полученных материалов осуществлялась с использованием пакета программ многомерного статистического анализа "Statistica 6.1" на основе процедур факторного анализа. Определено, что в качестве основных факторов в ходе анализа физиологически-обусловленных коррелят успешности течения процессов психофизиологической адаптации, следует отметить следующие факторы: "особенности динамической работоспособности" и "особенности уравновешенности нервных процессов", а также в начале времени обучения фактор "особенности подвижности нервных процессов" и в конце его - фактор "особенности подвижности нервных процессов и скорости зрительно-моторных реакций". В качестве основных факторов, которые были определены в ходе анализа психически-обусловленных коррелят успешности течения процессов психофизиологической адаптации, следует отметить следующие факторы: "особенности темперамента и тревожности", "особенности свойств характера", "особенности уровня субъективного контроля личности", "особенности агрессивных проявлений личности", а также "особенности эмоционального выгорания", который в начале учебного года был связан с рядом особенностей механизмов психологической защиты, в конце его - с показателями астенического и депрессивного состояний.

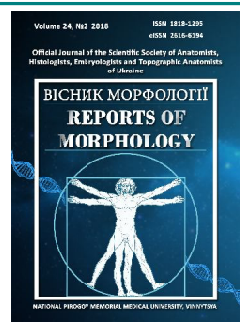
Ключевые слова: студенты, учебный год, психофизиологическая адаптация, физиологически-значимые корреляты, психически-значимые корреляты, факторный анализ.



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Histochemical picture in the skin of rats 1, 3, 7, 14, 21 and 30 days after burning of II-III degrees on the background of injection during first 7 days of 0.9% NaCl solution

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For pathogenetically grounded therapy of burn disease, aimed at reducing the severity of the pathological process, the rapid recovery of microcirculation and stimulation of reparative processes, it is necessary to study the fine mechanisms of reparative regeneration in the skin on the microscopic and biochemical levels. The purpose of the study is to investigate the state of elastic and collagen fibers, as well as glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of rats 1, 3, 7, 14, 21 and 30 days after the burning of the II-III degree, with the background of the injection during first 7 days of 0.9% NaCl solution. An experimental study of structural changes in the skin after burn injury was performed on laboratory white rats, males weighing 150-160 g. Rats were divided into 2 groups, which previously, under the conditions of propofol anesthesia 60 mg/kg internally, catheterization of the femoral vein and depilation lateral surfaces of the trunk of rats. Group 1 - animals without thermal trauma of the skin, which once a day for the first 7 days were injected intravenous infusion of 0.9% NaCl solution in a dose of 10 ml per kg. Group 2 - animals who were also injected once a day with the first 7 days of infusion of 0.9% NaCl solution in a dose of 10 ml per kg after 2-3 degree skin burns, with a total area of 21-23% of the body surface of the rat. Euthanasia of rats was performed after propofol anesthesia (60 mg/kg i/v) by decapitation. Changes in the histochemical structure of bits of skin from the edge of the wound were studied after 1, 3, 7, 14, 21, and 30 days from the beginning of the experiment. Histological sections 5-6 microns thick were stained with Weigert's elastic stain and dyed with picric acid by Van Gieson's method (for the detection of elastic and collagen fibers); the PAS + Hale reaction was performed by the Moury method (detection of glycoproteins and glycosaminoglycans in the intercellular substance of the dermis). The histochemical investigations of the area of the II-III degree of skin damaged by burns on the background of the injection of 0.9% NaCl solution found that the degree of change depends on the duration of the experiment. In the early stages of the experiment, the reorganization of the intercellular substance of the dermis manifests itself as damage to the fibrous structures of its papillary and reticular layers. Histochemically, the components of amorphous connective tissue substance are dominated by glycoproteins, the content of acidic glycosaminoglycans is small. In the late stages of the experiment (after 14, 21 and 30 days) in the connective tissue of the affected skin in the central and marginal regions poorly structured collagen and elastic fibers are observed. The pronounced "Hale"-positive coloration reflects an increase in the content of sulfated glycosaminoglycans in the amorphous substance of the connective tissue. Thus, the experimental thermal trauma of skin II-III degree on the background of the introduction during the first 7 days, 0.9% of the NaCl solution is histochemically characterized by reorganization of the intercellular substance of the connective tissue of the dermis. In the early stages of the experiment there is damage to the fibrous structures of the papillary and reticular layers, the prevalence of glycoproteins and the insignificant content of acidic glycosaminoglycans in the amorphous substance,

indicating a significant inhibition of adaptive-compensatory processes. In the long term, after the thermal defeat in the dermis, histochemically, an increase in the amount of acid glycosaminoglycans is detected and the PAS positivity of the collagen fibers of the intercellular substance is well expressed. This indicates a slow updating of the amorphous substance and fibrous structures of the connective tissue of the dermis, a violation of regenerative processes.

Keywords: thermal burns of the skin, rats, histochemical studies.

Introduction

Burns are one of the most common traumatic skin lesions. This is due to an increase in the frequency of their receipt at work, in everyday life, in conditions of regional military conflicts and disasters of peacetime [1, 32].

In the first place, thermal injury damages the skin, because it plays an important role in protecting the body from extreme environmental factors. Damage of the skin is considered to be leading in the development of burn disease, which is complicated by both pathogenesis and treatment, and most importantly - characterized by high mortality [18, 21, 33].

Burn wound is not a "stable" formation, in the process of repair it is possible to deepen it, it is associated with circulatory disorders and, first of all, with a capillary stasis, which initially causes anoxia of tissues, and then their necrosis [22]. Significant quantitative and qualitative changes in metabolic processes result in endogenous intoxication and associated with a decrease in non-specific resistance of the organism [21, 33].

Timely and complete recovery of the skin needs not only from the point of view of cosmetic and functional effectiveness, but also to save the victim's life. Deepening of the damage to the layers of the skin and deterioration of the patient's condition may occur when irrational treatment [19, 20, 34].

Morphofunctional changes in organs and tissues with thermal burns continue until the healing of burning surfaces [35]. The main issue concerns the dynamics of morphological changes in organs of indicators of homeostasis, infectious process and the regeneration scenario of burned skin. These organs include the lungs, small intestine, kidneys, spleen, etc., the study of structural changes in the modeling of burns of the skin is currently actively conducted [3, 4, 7, 8, 11, 13, 23].

For pathogenetically grounded therapy of burn disease, aimed at reducing the severity of the pathological process, the rapid recovery of microcirculation and stimulation of reparative processes, it is necessary to study the fine mechanisms of reparative regeneration in the skin on the microscopic and biochemical levels [24, 37]. Extrapolation of results obtained on the example of burns in an experiment on laboratory animals is a serious ground for the study of the effectiveness of the use of pharmaceuticals and drugs in the treatment of burn disease [6, 15, 29].

The purpose of the study is to investigate the state of elastic and collagen fibers, as well as glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of rats 1, 3, 7, 14, 21 and 30 days after the burning

of the II-III degree, on the background of the injection during first 7 days 0.9% NaCl solution.

Materials and methods

Experimental study of structural changes in the skin after burn injury was performed on laboratory white rats, males weighing 150-160 g obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Animals were kept at the Scientific and Experimental Clinic of the National Pirogov Memorial Medical University, Vinnytsya on a standard diet, with free access to water and food. The temperature in the room where the animals were kept was 24-25 °C. The research was conducted on the basis of the research laboratory of functional morphology and genetics of the development of the research center of the National Pirogov Memorial Medical University, Vinnytsya and in the laboratory of the department of pharmacology of the National Pirogov Memorial Medical University, Vinnytsya.

Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya determined that the experiments were carried out taking into account the recommendations of the European Commission regarding medical and biological research using animals, medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for the clinical evaluation of safety of pharmacological agents (GLP)".

The rats were divided into 2 groups, which previously, under the conditions of propofol anesthesia 60 mg/kg internally, catheterization of the femoral vein and depilation of the lateral surfaces of the trunk of the rats were performed.

Group 1 - animals without thermal trauma of the skin, which once a day for the first 7 days were administered intravenous infusion of 0.9% NaCl solution in a dose of 10 ml per kg.

Group 2 - animals that were also treated once a day with for first 7 days with infusion of 0.9% NaCl solution, at a dose of 10 ml per kg after burning the skin. The burnout shock was caused by applying to the shaved lateral surfaces of the trunk of the rats four copper plates (two plates on each side), which were preheated for 6 minutes in water at a constant temperature of 100 °C. [14, 28]. The surface area of each plate was 13.86 cm². The total area of defeat, calculated by the formula M. O. Lee [25], was 21-23% of the body surface of rats. Such an area at the exposure of 10 seconds is sufficient for the formation of a

2-3 degree burn (according to the classification adopted at the 20th Congress of Surgeons of Ukraine, September 2000 in Ternopil.) And causing a medium-gravity shock state [31], which has been confirmed by team of performers of the scientific work of the research center of the National Pirogov Memorial Medical University, Vinnytsya "Structural changes in the lungs in the conditions of endogenous intoxication caused by skin burn and its correction with domestic infusion drugs "lactoprotein with sorbitol" and HAES-LX-5% (experimental study)" (State Registration No. 0112U004187) [12]. Euthanasia of rats was performed after propofol anesthesia (60 mg/kg i/v) by decapitation. Changes in the histochemical structure of the skin were studied 1, 3, 7, 14, 21, and 30 days from the beginning of the experiment.

After decapitation for 5 minutes, they took pieces of skin from the edge of the wound to fix the material before the development of posthumous changes. Sections were fixed in 10% formalin solution, while the exposure time did not exceed 1-2 days. Subsequently, dehydration was carried out in spirits of increasing concentration and poured into paraffin blocks. Received on the sledge microtome MS-2 histological sections 5-6 microns thick were stained with Weigert's elastic stain and dyed with picric acid by Van Gieson's method (for the detection of elastic and collagen fibers); the PAS + Hale reaction was performed by the Moury method (detection of glycoproteins and glycosaminoglycans in the intercellular substance of the dermis) [9, 30]. Histochemical slides were investigated in a light microscope of Leitz Laborlux S.

Results

In the papillary layer of skin dermis of intact animals, thin elastic fibers are observed, which anastomoses between themselves and thicker wave-shaped undifferentiated collagen fibers (Fig. 1).

When conducting histochemical studies of the skin of white rats of the control group in the intercellular substance of its connective tissue, brightly "Hale"-positive acidic glycosaminoglycans and moderately PAS-positive glycoproteins, neutral glycosaminoglycans (Fig. 2).

In the early stages after burn injury in the intercellular substance of the connective tissue there is swelling and thickening of collagen fibers, their homogenization, destruction of elastic fibers and decay into fragments (Fig. 3). Histochemical changes in the amorphous substance of the dermis appear to be insignificant "Hale"-positive color and pronounced PAS-positive properties, indicating the disintegration of glycosaminoglycans and an increase in the number of glycoproteins (Fig. 4).

After 7 and 14 days of the experiment on preparations prepared by the Van Gieson's-Weigert's method, defective fibrous structures of the papillary and reticular skin dermis are observed at the site of the lesion. Elastic fibers are not detected (Fig. 5).

In the amorphous substance of the dermis, the predominance of glycoproteins and a small amount of

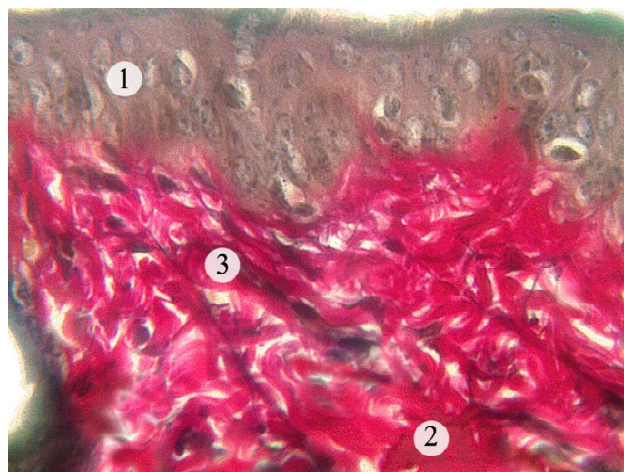


Fig. 1. Histological organization of the skin of the animal intact group. Epidermis (1), collagen (2) and elastic (3) fibers of the papillary layer of the dermis. Van Gieson's-Weigert's coloring. x 600.

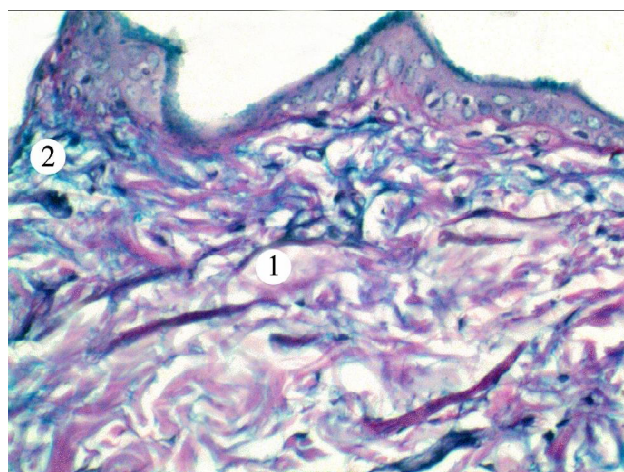


Fig. 2. Histochemical organization of skin of white rat intact group. Moderately PAS-positive collagen fibers (1) and bright "Hale"-positive sour glycosaminoglycans (2) amorphous dermis substances. Coloring by the Moury method. x 300.

acidic glycosaminoglycans was revealed histochemically, manifested by pronounced PAS-positive properties and an insignificant "Hale"-positive color (Fig. 6).

In the late stages of the experiment (21 and 30 days) in preparations painted on the Van Gieson's-Weigert's method poorly structured collagen and fragmented elastic fibers are observed in the reticular layer of the connective tissue of the skin (Fig. 7). The histochemical increase in the amount of sulfated glycosaminoglycans in the amorphous substance of the dermis, which may be due to an increase in the synthetic activity of fibroblasts. There is a well-expressed PAS-positivity of collagen fibers (Fig. 8).

Discussion

In intact skin, the fibrous structures of the dermis consist of collagen, elastic and reticular fibers. The intervals between fibers, appendages of the skin and other structural

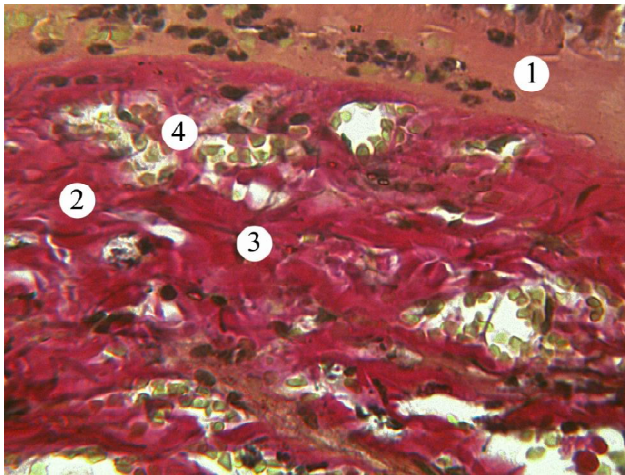


Fig. 3. Histological changes in the skin of animals after 1 day after a thermal trauma. Necrotically altered epidermis (1), thickened collagen fibers (2), fragmented elastic fibers (3), hemocapillaries (4). Van Gieson's-Weigert's coloring. x 400.

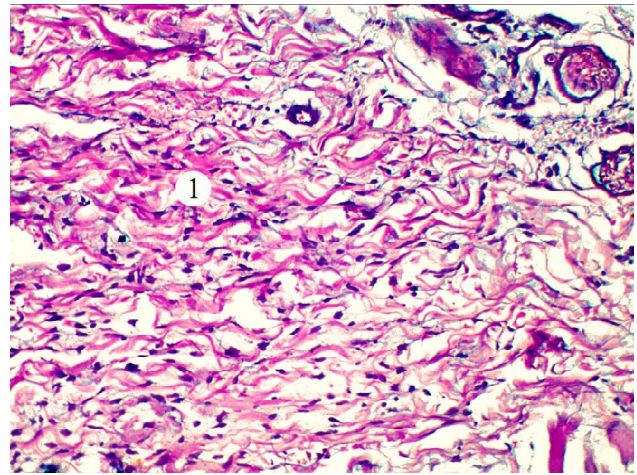


Fig. 6. Histochemical changes of the derma of the animal 7 days after a thermal trauma. PAS-positive collagen fibers (1). Coloring by the Moury method. x 300.

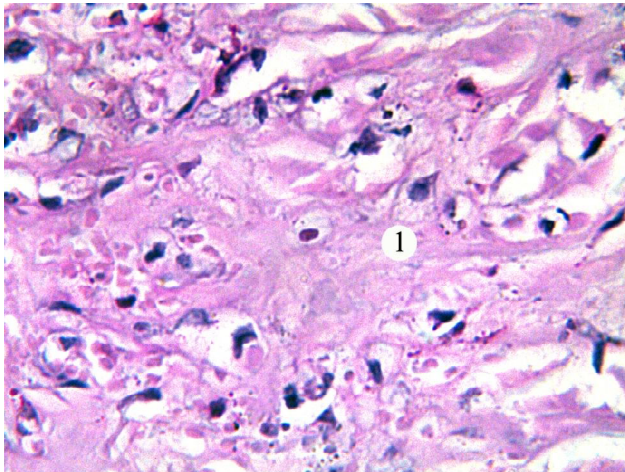


Fig. 4. Histochemical changes in the skin of the skin of the animal after 3 days after a thermal trauma. Swollen collagen fibers (1) with pronounced PAS-positive properties. Coloring by the Moury method. x 400.

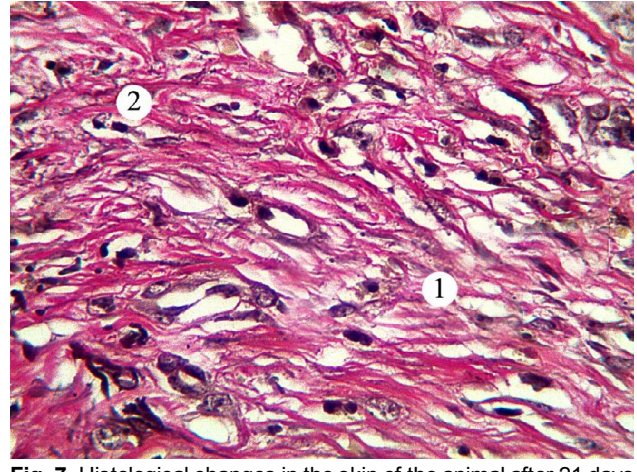


Fig. 7. Histological changes in the skin of the animal after 21 days after a thermal trauma. Collagen (1) and elastic fibers (2). Van Gieson's-Weigert's coloring. x 600.

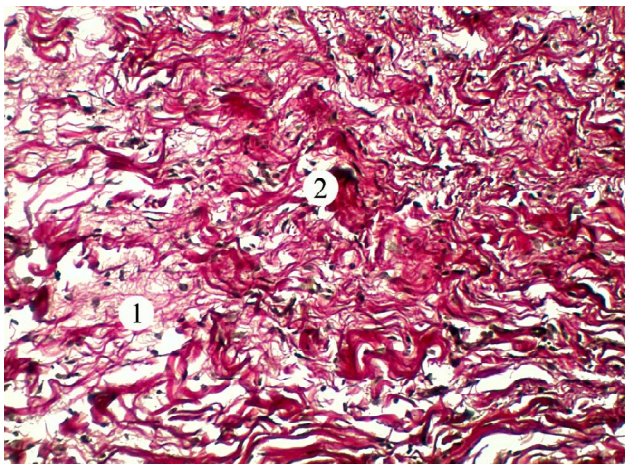


Fig. 5. Histological changes in the skin of the animal 7 days after burn injury. Damaged, thin, unstructured collagen fibers (1), altered fibroblasts (2). Van Gieson's-Weigert's coloring. x 300.

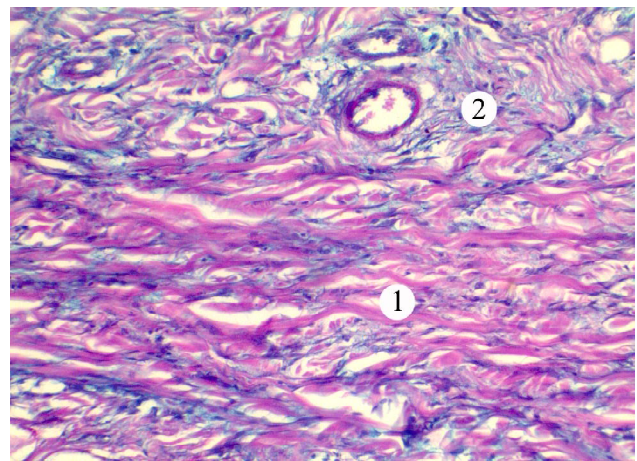


Fig. 8. Histochemical organization of skin of an animal 21 days after a thermal trauma. Components of the dermis with well-expressed PAS- (1) and "Hale"-positive (2) properties. Coloring by the Moury method. x 200.

formations are the so-called basic substance - an amorphous substance, the important components of which are glycoproteins and glycosaminoglycans [2].

At burns of II-III degree there are changes in the structure of elastic and collagen fibers, and also the balance of glycoproteins and glycosaminoglycans in the intercellular substance of the skin dermal layer [16] is disturbed. The fiber architecture, the ratio of glycosaminoglycans and glycoproteins, the fibrous component, and the cell proliferation are markers of the favorable flow of the wound process and the effectiveness of the use of drugs [17].

Having made a search cut of literary data it is established that prognostically it is beneficial:

A) when staining by Weigert's and dyeing with picric acid by Van Gieson's [30]: ordering collagen fibers and reducing their swelling, the appearance in the regenerate of elastic fibers (within a month of observation) [10, 15, 29]; predominance of the fibrous component over cell proliferate (fibroblasts), the formation of an amorphous component, which indicates the high degree of maturity of the granulation tissue (predominantly for 14 days) [5, 16, 33]; formation of the dermal plate, which is mainly represented by mature fibrous connective tissue consisting of fuchsinophilic beams of collagen fibers, infiltrated fibroblast cells (after 1 month) [26, 27, 35].

B) in the implementation of the PAS + "Hale" reaction by the Moury method [15, 17]: structuring of glycosaminoglycans [13, 36]; reduced expression of the PAS-positive properties of granulation tissue (reduction of glycoproteins) and the "Hale"-positive strengthening of the connective tissue in the area of the burn wound (increase of acidic glycosaminoglycans) by 14-21 days compared with the previous terms of the experiment [22, 37].

The histochemical investigations made by us of the area of the II-III degree of skin that was damaged by burns on the background of the introduction of a 0.9% solution of NaCl found that the degree of change depends on the duration of the experiment. In the early stages of the experiment (after 1, 3 and 7 days) the reorganization of the intercellular substance of the dermis manifests itself as damage to the fibrous structures of its papillary and reticular layers. Histochemically,

among the components of amorphous connective tissue substance is dominated by glycoproteins, the content of acid glycosaminoglycans is small, which indicates their lesion.

After experimental thermal trauma in the late stages of the experiment (after 14, 21 and 30 days), colored preparations using the Van Gieson's-Weigert's method in the connective tissue of the affected skin in the central and boundary regions of the poorly structured collagen and elastic fibers are observed. The pronounced "Hale"-positive color reflects an increase in the content of sulfated glycosaminoglycans in the amorphous substance of the connective tissue. The revealed histochemical changes are associated with non-fibrotic processes in the burn wound and suggest that the development of young granulation tissue, its maturation and transformation into connective tissue is poorly expressed.

In the future, it is promising to study the state of elastic and collagen fibers, as well as glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of rats after its burn injury on the background of preliminary administration of colloidal hyperosmolar solutions.

Conclusions

1. Experimental thermal trauma of skin of II-III degrees on the background of the injection during first 7 days of 0.9% NaCl solution is histochemically characterized by reorganization of the intercellular substance of the connective tissue of the dermis. In the early stages of the experiment there is damage to the fibrous structures of the papillary and mesenteric layers, the prevalence of glycoproteins and the insignificant content of acidic glycosaminoglycans in the amorphous substance, indicating a significant inhibition of adaptive-compensatory processes.

2. In the long term, after the thermal defeat in the dermis, histochemically, an increase in the amount of acidic glycosaminoglycans is detected and the PAS-positivity of the collagen fibers of the intercellular substance is well expressed. This indicates a slow updating of the amorphous substance and fibrous structures of the connective tissue of the dermis, a violation of regenerative processes.

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ГІСТОХІМІЧНА КАРТИНА В ШКІРІ ЩУРІВ ЧЕРЕЗ 1, 3, 7, 14, 21 ТА 30 ДІБ ПІСЛЯ ОПІКУ II-III СТУПЕННЯ НА ФОНІ ВВЕДЕННЯ ПЕРШИХ 7 ДІБ 0,9 % РОЗЧИНУ NaCl

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Для патогенетично обґрунтованої терапії опікової хвороби, спрямованої на зниження вираженості патологічного процесу,

якнайшвидшого відновлення мікроциркуляції і стимуляції репаративних процесів необхідне вивчення тонких механізмів репаративної регенерації в шкірі на мікроскопічному і біохімічному рівні. Мета дослідження - вивчити стан еластичних і колагенових волокон, а також глікопротеїнів і глікозаміногліканів у міжклітинній речовині дерми щурів через 1, 3, 7, 14, 21 та 30 днів після опіку II-III ступеня на фоні введення перших 7 днів 0,9 % розчину NaCl. Експериментальне дослідження структурних змін шкіри після її опікового ураження були виконані на лабораторних білих щурах-самцях масою 150-160 г. Щури були розподілені на 2 групи, в яких попередньо, в умовах пропофолового наркозу 60 мг/кг внутрішньо, проводили катетеризацію стегнової вени та депіляцію бічних поверхонь тулуба щурів. 1 група - тварини без термічної травми шкіри яким 1 раз на добу перші 7 днів проводили внутрішньовенну інфузію 0,9 % розчину NaCl у дозі 10 мл на кг. 2 група - тварини яким також проводили 1 раз на добу перші 7 днів інфузію 0,9 % розчину NaCl, у дозі 10 мл на кг після опіку шкіри 2-3 ступеню загальною площею ураження 21-23 % поверхні тіла щурів. Евтаназію щурів проводили після пропофолового наркозу (60 мг на кг в/в) шляхом декапітації. Зміни гістохімічної структури шматочків шкіри з крайової ділянки рани вивчали через 1, 3, 7, 14, 21, та 30 днів від початку експерименту. Гістологічні зрізи товщиною 5-6 мкм забарювали фуксиліном за Вейгертом та дофарбовували пікрофуксином за методом Ван Гізона (для виявлення еластичних та колагенових волокон); проводили ШИК+ "Хейл" реакцію за методом Моурі (виявлення глікопротеїнів та глікозаміногліканів у міжклітинній речовині дерми). Проведені гістохімічні дослідження ділянки пошкодженої опіком II-III ступеня шкіри на фоні введення 0,9 % розчину NaCl встановили, що ступінь змін залежить від терміну дослідження. У ранні терміни експерименту реорганізація міжклітинної речовини дерми проявляється пошкодженням волокнистих структур її сосочкового і сітчастого шарів. Гістохімічно серед компонентів аморфної речовини сполучної тканини переважають глікопротеїни, вміст кислих глікозаміногліканів невеликий. У пізні терміни дослідження (через 14, 21 та 30 днів) у сполучній тканині ураженої шкіри в центральних і крайових ділянках спостерігаються погано структуровані колагенові та еластичні волокна. Виражене "Хейл"-позитивне забарвлення відображає збільшення вмісту сульфатованих глікозаміногліканів у аморфній речовині сполучної тканини. Таким чином експериментальна термічна травма шкіри II-III ступеня на фоні введення перших 7 днів 0,9 % розчину NaCl гістохімічно характеризується реорганізацією міжклітинної речовини сполучної тканини дерми. У ранні терміни дослідження відбувається пошкодження волокнистих структур сосочкового і сітчастого шарів, переважає глікопротеїнів та незначний вміст кислих глікозаміногліканів у аморфній речовині, що свідчить про значне пригнічення пристосувально-компенсаторних процесів. У віддалені терміни після термічного ураження у дермі гістохімічно виявляється збільшення кількості кислих глікозаміногліканів та добре виражена ШИК-позитивність колагенових волокон міжклітинної речовини. Це свідчить про повільне оновлення аморфної речовини і волокнистих структур сполучної тканини дерми, порушення регенераторних процесів.

Ключові слова: термічний опік шкіри, щури, гістохімічні дослідження.

ГИСТОХИМИЧЕСКАЯ КАРТИНА В КОЖЕ КРЫС ЧЕРЕЗ 1, 3, 7, 14, 21 И 30 СУТОК ПОСЛЕ ОЖОГА II-III СТЕПЕНИ НА ФОНЕ ВВЕДЕНИЯ ПЕРВЫХ 7 ДНЕЙ 0,9% РАСТВОРА NaCl

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Для патогенетически обоснованной терапии ожоговой болезни, направленной на снижение выраженности патологического процесса, скорейшего восстановления микроциркуляции и стимуляции репаративных процессов необходимо изучение тонких механизмов репаративной регенерации в коже на микроскопическом и биохимическом уровне. Цель исследования - изучить состояние эластических и коллагеновых волокон, а также гликопротеинов и гликозаминогликанов в межклеточном веществе дермы крыс через 1, 3, 7, 14, 21 и 30 суток после ожога II-III степени на фоне введения первых 7 дней 0,9% раствора NaCl. Экспериментальное исследование структурных изменений кожи после ее ожогового поражения были выполнены на лабораторных белых крысах-самцах массой 150-160 г. Крысы были разделены на 2 группы, в которых предварительно, в условиях пропофолового наркоза 60 мг/кг внутрь, проводили катетеризацию бедренной вены и депиляцию боковых поверхностей туловища крыс. 1 группа - животные без термической травмы кожи которым 1 раз в сутки первые 7 суток проводили инфузию 0,9% раствора NaCl в дозе 10 мл на кг. 2 группа - животные, которым также проводили 1 раз в сутки первые 7 суток инфузию 0,9% раствора NaCl в дозе 10 мл на кг после ожога кожи 2-3 степени общей площадью поражения 21-23% поверхности тела крыс. Евтаназию крыс проводили после пропофолового наркоза (60 мг на кг в/в) путем декапитации. Изменения гистохимической структуры кусочков кожи с краевого участка раны изучали через 1, 3, 7, 14, 21 и 30 суток от начала эксперимента. Гистологические срезы толщиной 5-6 мкм окрашивали фуксиліном по Вейгерту и докрашивают пикрофуксином по методу Ван Гизона (для выявления эластических и коллагеновых волокон); проводили ШИК+ "Хейл" реакцию методом Моури (выявление гликопротеинов и гликозаминогликанов в межклеточном веществе дермы). Проведенные гистохимические исследования участка поврежденной опіком II-III степени кожи на фоне введения 0,9% раствора NaCl установили, что степень изменений зависит от срока опыта. В ранние сроки эксперимента реорганізація міжклітинного вещества дермы проявляется повреждением волокнистых структур ее сосочкового и сетчатого слоев. Гистохимически среди компонентов аморфного вещества соединительной ткани преобладают гликопротеины, содержание кислых гликозаминогликанов невелико. В поздние сроки опыта (через 14, 21 и 30 суток) в соединительной ткани пораженной кожи в центральных и краевых участках наблюдаются плохо структурированные коллагеновые и эластичные волокна. Выраженное "Хейл"-положительное окрашивание отражает увеличение содержания сульфатированных гликозаминогликанов в аморфном веществе соединительной ткани. Таким образом, экспериментальная термическая травма кожи II-III степени на фоне введения первых 7 дней 0,9% раствора NaCl гистохимически характеризуется реорганізацією міжклітинного вещества соединительной ткани дермы. В ранние сроки опыта происходит повреждение волокнистых структур сосочкового и сетчатого слоев, преобладание гликопротеинов и незначительное содержание кислых гликозаминогликанов в аморфном веществе, что свидетельствует о значительном подавлении приспособительно-компенсаторных процессов. В отдаленные сроки после термического поражения в дерме гистохимически выявляется увеличение количества кислых гликозаминогликанов и хорошо выраженная ШИК-позитивность коллагеновых волокон межклеточного вещества. Это свидетельствует о медленном обновлении аморфного вещества и волокнистых структур соединительной ткани дермы, нарушения регенераторных процессов.

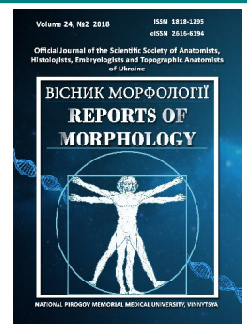
Ключевые слова: термический ожог кожи, крысы, гистохимические исследования.



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Possibility of the treatment effects on the dynamics of apoptosis processes in tissues of kidneys in acute pyelonephritis and comparative diabetes mellitus in the experiment

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Programmed cell death under conditions of an infectious-inflammatory process plays a biologically exclusively positive role in the elimination of cells. Acute inflammatory process is a phenomenon capable of excessive concentration of aggressive effectors of inflammation. The aim of the work was to assess the dynamics of ultrastructural changes and early signs of apoptosis in the kidney tissues in experimental modeling of acute pyelonephritis (AP) and concomitant diabetes mellitus (DM) of type II and I when conducting complex drug correction. The work was performed on 300 adult Wistar rats, divided into 6 groups. Fragments of the kidneys were studied and photographed in the electron microscope PEM-100-01. In the kidneys of animals of the group, where traditional medical correction was used after the friendly modeling of pyelonephritis and type I diabetes, it was found that the glomerular ultrastructure was more preserved, but part of the capillaries of the glomerulus remained deformed with a narrowed lumen, there are signs of insufficient restoration of the glomerular capillary network. In the group of animals using the proposed complex drug correction, the renal structure was preserved, the capillaries of the glomerular network with unchanged architectonics. It has been established that the traditional medical correction did not sufficiently contribute to the restoration of damaged kidney tissue ultrastructure. After carrying out the complex medical correction proposed by us after a friendly simulation of an OP of DM type II, the ultrastructure of the kidney tissue in form and structure approached that of control animals, signs of a compensatory-restorative process appeared: most of the podocytes were hyperplastic and hypertrophied. The podocytes of the outer leaflet were in the active phase of activity, as evidenced by an increase in contractile function and, possibly, the release of urine from the cavity into the lumen of the proximal tubules. The structure of the tubules and interstitial tissue is close to the structure of the group of control animals, only in the cytoplasm of the podocytes of the proximal tubules there is an increased content of lysosomes. The ultrastructure of the glomeruli was normalized; the number of hypertrophic podocytes of the inner leaflet with signs of enhanced protein synthesis increased. Changes in the structures of the cortical and medulla are similar except for the state of cytotrabeculae, where their size decreased in the medulla and signs of deformation of the plasmolemma appeared. In experimental modeling of PD and DM of types I and II, pronounced ultrastructural changes in the kidney tissues were established, and manifestations of early apoptosis processes are significantly limited. The use of the complex drug correction proposed by us stimulates the development of the reparative processes of the kidney and moderately activates apoptosis. The addition of multi-vector preparations (Armadin and Nuklex) to the complex of treatment intensifies compensatory-restorative changes in the kidneys and apoptosis, which contributes to the elimination from the renal microstructures of excess damaged cells and aggressive effectors of inflammation.

Keywords: kidneys, ultrastructure, acute inflammation, hypertrophy, medication correction, apoptosis, modeling.

Introduction

Studies of recent years have shown that programmable cell death (PCD) in conditions of a variety of pathological

conditions, which includes the infectious and inflammatory process, can probably play a biologically positive role in

the elimination of cells, the persistence of which has a pronounced negative effect on the organ and system organs [11, 19, 22, 23, 24, 26, 27, 28, 31]. With regard to the acute infectious-inflammatory process, the above-mentioned phenomenon is capable of effectively eliminating the excessive accumulation of aggressive inflammatory effector, which provides a favorable course of the disease [1, 12, 13, 15, 16, 19, 27, 28].

In studies of authors Vinnichenko L. N. (1980) and Verlander J. W. (1998) [27, 28], it has been established that kidney diseases are accompanied by a number of specific features of programmed cell death (PCD). Thus, inhibition of apoptosis is observed with most different morphological variants of glomerulopathy.

At the same time, obstructive nephropathy, reflux nephropathy, hydronephrosis, polycystitis, interstitial nephritis, and others are accompanied by its activation, reported by a number of researchers [1, 13, 14, 18]. It is known that in kidneys of a healthy adult, up to 3.0% of glomerular cells are daily exposed to apoptosis [13, 15, 20]. There are studies of a number of authors devoted to the study of the deep mechanisms of the apoptotic process and its major effectors [7, 10, 20, 24]. Separately, the so-called "mitochondrial pathway of apoptosis" is isolated, which is carried out by indirect neutralization of anti-apoptotic effects at the cellular level [1, 2, 7, 10, 25, 27]. It has been established that factors that alter oxidative-reducing homeostasis in cells, such as hypoxia and oxidative stress, can stimulate the expression of regular apoptosis modulators both *in vitro* and *in vivo* [6, 8, 9, 18, 21, 24, 29, 30]. The process of apoptosis in a different kidney disease today is not sufficiently studied. Modern drugs can influence the processes of apoptosis and, in some way, correct the ultrastructural changes of the kidneys, which require further research.

Taking into account the data obtained by us that electron microscopic studies are highly informative in the study of pathological changes and early dynamic processes of apoptosis in kidney tissues, the aim of the work was to: assess the dynamics of ultrastructural changes and early processes of apoptosis in renal tissues in the experimental modeling of acute pyelonephritis and concomitant diabetes mellitus type I and II under conditions of complex drug correction.

Materials and methods

The work was performed on 300 adult Wistar rats, divided into 6 groups: group A - control (30 intact animals); group B (35 animals, acute pyelonephritis modeling); group E (50 animals, simulation of acute pyelonephritis and type I diabetes with traditional treatment); group F (50 animals with simulation of acute pyelonephritis and type 1 diabetes mellitus in the complex treatment offered by us), group G (50 animals with simulation of acute pyelonephritis and type II diabetes with traditional treatment), group H (50 animals with simulation of acute pyelonephritis and diabetes mellitus II type with the complex treatment offered by us).

The collection of material for electron microscopic research was carried out in an experiment conducted by the authors Borysov S. O. with co-authors [4]. The ultra-thin sections obtained in the experimental work were contrasted by Reynolds E.S. [22]. The work was carried out in the laboratory of pathoanatomical and electron microscopic research.

In the traditional medication correction in a group of animals with diabetes and pyelonephritis, an intramuscular injection of antibiotic "Hepacef" injected at a dose of 30 mg per kg body weight 2 times a day for 14 days after the simulation of acute pyelonephritis. With the proposed comprehensive medical correction after the simulation of acute pyelonephritis and diabetes mellitus, in addition to the antibiotic "Hepacef" in the above scheme, received the drug "Nuklex" per os at the rate of 1 kg of weight 7 mg 3 times a day and intramuscular injection of preparation "Armadin" by 1.5 mg per 1 kg of weight 3 times a day for 14 days.

Results

In our previous study, the ultrastructure of the renal cortex and renal medulla material of the kidney of animals of the control group (A) was studied and described [4].

An electron microscopic study is performed on the kidneys of animals of group E (models of AP and DM type I with the use of traditional drug correction). While studying the renal cortex, it was found that the capillaries in the glomeruli have a focal length of the enlarged lumen, in which its content of elevated electron density is located. Endothelial cells of capillaries, mainly with unchanged ultrastructure. However, some of them differed in the clarified cytoplasm and the dilute arrangement of their organelles, clearly identified fenestri (Fig. 1).

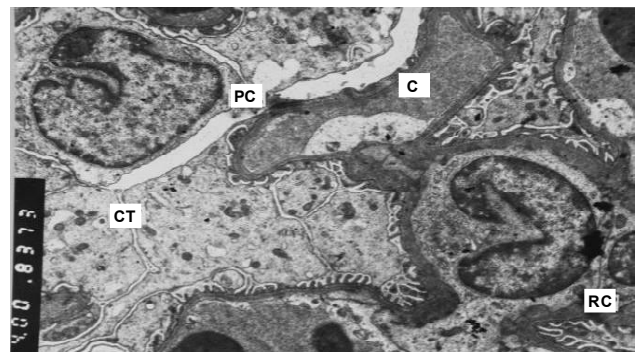


Fig. 1. Ultrastructure of rat renal cortex (group E). Traditional drug effect. Signs of edema of the cytoplasm of the endothelial cells of the capillaries and the expansion of the elements of the granular endoplasmic net in the cytotrebeculae of the glomerular capillary net. Electronic Microphotography. x4000. Here and thereafter: RC - renal cortex, C - capillary, CEC - capillary endothelial cell, CT - cytotrebecula, CP - cytopodia, CV - cavernous vesicles, CF - collagen fibrils, PC - podocyte, N - nucleus, M - mitochondria, RM - renal medulla, MC - mesangiocyte, E - erythrocyte, GER - granulated endoplasmic reticulum, IT - interstitial tissue, RPT - renal proximal tubules, BM - basement membrane, GCN - glomerular capillary network.

Basement membrane has increased tortuosity. The cavity of the capsule is significantly narrowed in places, in this section cytopedicules are single, short and thickened, cytotrabeculae are short and have a slightly electron-dense cytoplasm. In cavity we can see a substance identical to that of the capillary lumen.

In other areas, cytopodies and cytotrabeculae have a structure close to normal. Podocytes are characterized by large cell sizes, large nuclei and shallow folds of karyolemma. There are nucleolus in the karyoplasm. The cytoplasm has well expressed polysomes and mitochondria. In the podocytes of the proximal tubules, the ultrastructure is close to normal, only in some cells there is a decrease in the number of mitochondria and enlightenment of the cytoplasmic matrix. In the podocytes of the distal tubules, the devastated areas of the cytoplasm are marked centrally. Enlightenment of the main substance is manifested in interstition of the connective tissue. As for the electron microscopic picture of the renal medulla of the group E animals, we found that the capillary net of the glomeruli was well expressed. It was established that the gaps of the capillaries were extended compared to the control group material, and the area of the cavity was slightly narrowed. Enlarged electron density of lumen content. Endothelial cells of a number of capillaries with signs of hydropic changes, others have a structure close to the usual structure. The majority of podocytes of the inner layer was found to have an unmodified ultrastructure, only in some of them, expanded tanks of the granular endoplasmic grid were installed. Cytopodies are thickened in some places (Fig. 2).

In the proximal tubules, the part of the podocytes has a reduced number of mitochondria, and those in the cell are manifested with the pathology of mitochondrial and cytoplasmic basal structures, others with an increased number of mitochondria in the cells. In all cells, the number of vacuoles is reduced. In the part of the podocytes of the distal tubules there are signs of edema of the cytoplasm and the destruction of part of the organelles with a focal destruction of the basal strain. Interstitial tissue sites are determined by the clarification of its main substance. Thus, the structure of the nephron after the traditional treatment ultrastructure is more conserved than in the group with the simulation of acute pyelonephritis. To a greater extent expressed large hypertrophied, with active nuclei of podocytes, different sizes of cytotrebeculae, there was a large number of cytopedicules. Only certain cytotrabeculae are slightly deformed, noticeably compressed. In the cavity of the capsule appear freely located separate rounded forms of the cytotrabeculae. Capillaries have the shape and arrangement similar in structure of the control group of animals. They differ only in the more dense content of their lumen and the presence of part of the endothelial cells with signs of hydropic changes.

In a similar electron microscopic study, the kidneys of animals of group F (model of AP and I type of diabetes and with proposed complex of medical correction - with the use

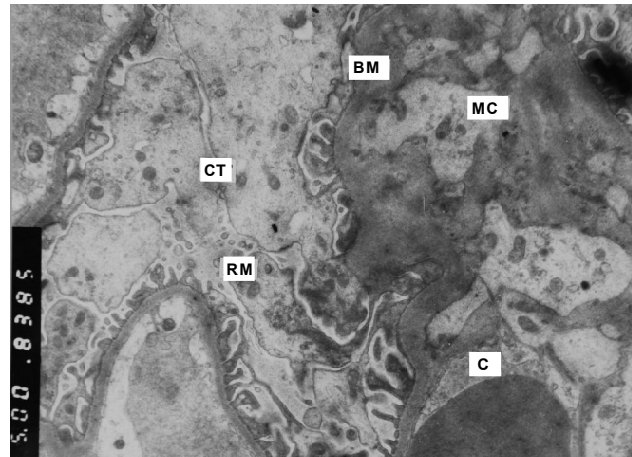


Fig. 2. Ultrastructure of rat renal medulla (group E). Traditional drug effect. Signs of edema of the cytoplasm of the endothelial cells of the capillaries, the processes of mesangial cells are well expressed. EMP. x5000.

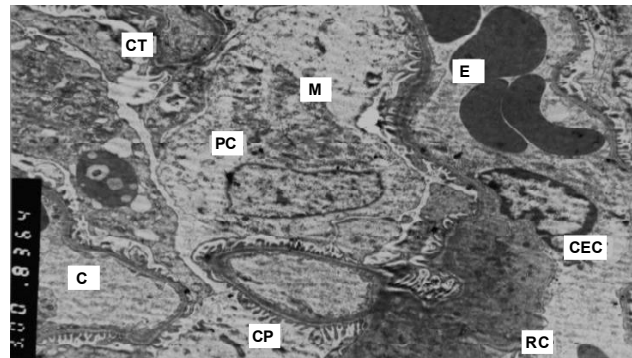


Fig. 3. Ultrastructure of rat renal cortex (group F). Proposed pharmacological effect. Hypertrophic podocytes with expanded elements of the granular endoplasmic net. EMP. x3000.

of preparations of multi-vector action of Armadin and Nuklex). In studying the renal cortex of animals, it was discovered that the ultrastructure of the glomerular capillaries is close to that of the control animals. Attention is drawn to somewhat extended areas of the contents of mesangial tissue. The capillaries in the cross section are round or close to the oval shape. Enlargement of capillaries of regular diameter with content of moderate density. There are isolated red blood cells in it. Endothelial cells in the majority are unchanged, but there are isolated cells with signs of edema (Fig. 3).

In a well-defined cavity there are large podocytes and cytotrabeculae with a large number of their intracellular organelles, as well as individual cell fragments and bubbles of various sizes. As for the electron microscopic picture of the renal medulla of animals, it was found that in the renal glomeruli the podocytes of the outer layer in the majority with the unchanged structure. Some podocytes are in a state of increased metabolic activity. The glomerular net capillaries do not differ much from such a control group, but their part has a slightly narrowed lumen. Endothelial cells with signs of edema are found on thinned areas of the cytoplasm. Fenestra also well-defined in this area. The brightness of

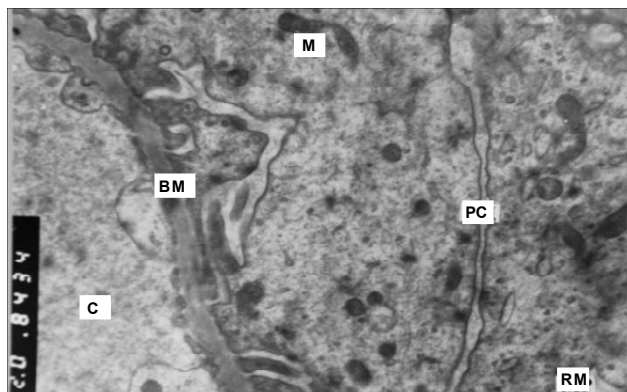


Fig. 4. Ultrastructure of rat renal cortex (group F). Proposed pharmacological correction. In the cavity of the capsule, fragments of hypertrophied cytotrabeculae are observed. EMP. x6000.

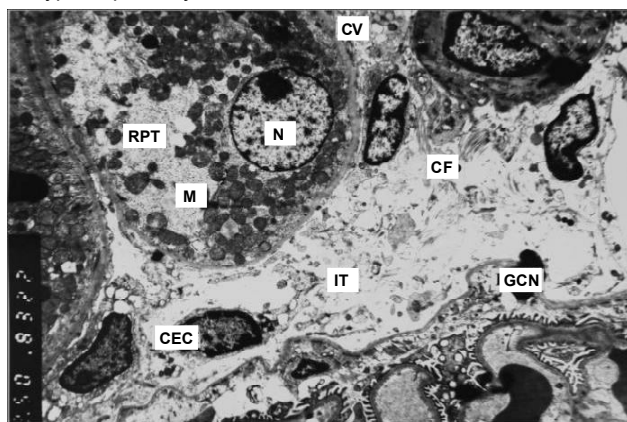


Fig. 5. Ultrastructure of rat renal cortex (group G). Traditional drug correction. Structures of interstitial tissue with signs of edema. Electronic microphotography. x2500.

some capillaries is filled with moderate density, others are more electronically dense. It contains single red blood cells or large electron-transparent vacuoles. Mesangiocytes that come in contact with capillaries are well-defined.

The cavity of the capsule is filled with a large number of large cytotrabeculae of the inner layer, which has a normal structure and is in contact with both the cytopedicules and with each other. The cytopedicules throughout the basal membrane are well expressed. In the cytoplasm of the part of cytotrabeculae, the expansion of the elements of the granular endoplasmic net and the edema of the intramitochondrial matrix with the focal destruction of the crust, which is possibly a sign of apoptosis in these cells, is noted. Locally there are podocytes of large size with a large nucleus and with an increased number of intracellular organelles, which indicates an increase in their functional activity (Fig. 4).

Ultrastructure of proximal tubules practically unchanged. Only in separate proximal tubules there are single vacuoles and lysosomes. Interstitial tissue draws attention to the increased number of cells. Ultrastructure of the distal tubules without visible structural changes.

At the same time, we carried out an electron microscopic

study of the kidneys of animals of the G group (model of AP and type II diabetes with the use of traditional drug correction). During the study of the renal cortex of animals, it was found that in the renal glomeruli the outer layer of the capsule led on the basal membrane, its podocytes had an electron-enlightened cytoplasm. The cytoplasm has well expressed polysomes and mitochondria. Detected close contacts between adjacent podocytes. In the increased in volume nuclei there is a margin of chromatin and in the central zone of the nucleus, chromatin predominates in a diffuse state. The glomerular capillaries have winding contours. Enlargement in most of the capillaries is narrowed and has an elevated electron density. It contains slightly deformed erythrocytes. Endothelial cells, in general, had signs of edema and destruction of the main part of the organelles. However, part of bodies of endothelial cells in structure is close to the same control group of animals. The space of the capsule was narrowed. It should be noted that the number of podocytes and cytotrabeculae is reduced, their area is also significantly reduced. They look wrinkled, their cytoplasm is winding (Fig. 5).

Most of the podocytes and cytotrabeculae are in a state of destruction, in which gelization of cytoplasm is observed. Cytopodies are absent somewhere. In general, they have an increased density of the cytoplasm, somewhat deformed, their structure differs indistinct, which should be considered an expression of apoptosis. The number of podocytes and cytotrabeculae in the glomerular is reduced, in the greater part of the cytoplasm has an electron-dense appearance, and its organelles are in a state of edema. Some podocytes and their processes are almost unchanged. In proximal tubules, the podocytes are in a different state: part of their usual unchanged structure, and part with signs of alteration. In such podocytes the cytoplasm is enlightened, that is, with signs of edema, especially apical part of it. In the podocytes of the distal tubules, there are also marked signs of edema of the apical area cytoplasm and a decrease in the number of organelles. It encounters a large number of vacuoles and mitochondria, which are pathologically altered (Fig. 5). In interstitial focal signs of edema of the main substance and intracellular organelles of capillaries appear.

During the study of the renal medulla of animals, it was found that the glomerular capillaries have slightly winding contours. Endothelial cells have signs of cytoplasmic edema. Other endothelial cells encountered a reduced number of organelles, expanded in the subtle parts of the cytoplasm. Some endothelial cells contained signs of activation of the cytoplasmic structures. The content of the lumen of capillaries of moderately elevated electron density. Podocytes and cytotrabeculae of large size, densely filling the cavity of the capsule. They were with a slightly enlightened cytoplasm. Cytoplasm is regularly located along the basement membrane. Somewhere they have an electron-dense cytoplasm. Some cytotrabeculae with deep degenerative changes (Fig. 6). They are located between the podocytes with unchanged ultrastructure. In proximal

and distal tubules, there are podocytes with unchanged structure and with signs of pathological changes in mitochondria and slightly enlightened cytoplasm. In the podocytes of the proximal tubules there are different sizes of lysosomes, which indicates the increased need for cells to dispose of its damaged elements. In the interstices there are manifestations of minor edema of the main substance and the cytoplasm of the endothelial cells of the microvessels that are located there.

Based on the results of the electron microscopic picture, it can be thought that the traditional treatment did not sufficiently contribute to the restoration of damaged ultrastructure of the renal tissues.

In accordance with the tasks of the study, electron microscopic study was carried out on the kidneys of animals of the group H (modeling of AP and type II diabetes in the complex treatment offered by us with the use of drugs of multi-vector action - Armadin and Nuklex).

In the study of the renal cortex of animals, it was determined that in the glomeruli the podocytes of the outer layer have an enlarged nuclei and a cytoplasm relative to the control group material. The inner layer is characterized by the fact that the cytopodies are present throughout the basement membrane, and in places they are thickened. From some cytopodies, quite large cytotrabeculae branch out. These cells have moderately electron-dense cytoplasm, in which markedly increased number of organelles. In addition, in the capsule space there are rather large podocytes in the state of hyperplasia and hypertrophy. They contain a large nucleus and organelles enriched in the cytoplasm. The nucleus has a convoluted karyolemma and its separate invaginations, which increases the surface area of the nucleus and metabolic processes with the cytoplasm. Nuclear chromatin is in a diffuse state. The condition of capillaries is practically no different from that of control animals. A smaller density of contents in the lumen of capillaries, close to such control animals was noted (Fig. 7).

Podocytes of PT and DT by their structure do not differ from such control material, there is a somewhat increased amount of lysosomes in podocytes of PT. Interstitial tissue has places slightly enlightened amorphous connective tissue substance and a large number of its large cells with large nuclei.

In electron microscopic study of the renal medulla found that the ultrastructure of the renal glomerulus is well expressed. Podocytes of the outer layer have (like in the renal cortex) increased nucleus and cytoplasm compared with the control group. In the nucleus, chromatin diffuse state predominates, and in the cytoplasm an increased number of polysomes, elements of the granular endoplasmic net, mitochondria, and Golgi complex. Most of the glomeruli capillaries in the lumbar cut have correct round or oval shape. Enlightenment of capillaries is slightly extended with moderate electron density. Endothelial cells of capillaries have an ordinary structure with clear fenestra. Cytopodies

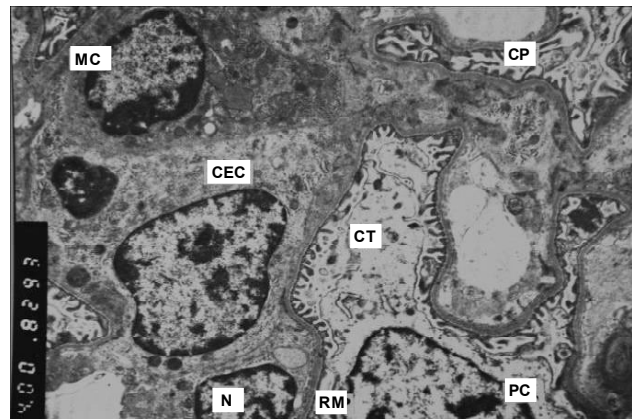


Fig. 6. Ultrastructure of rat renal medulla (group G). Traditional drug correction. The cavity of the capsule is slit-shaped. Hypertrophy of capillary endothelial cell, focal destruction of the cytopedics. Hydrophobic changes in the podocytes and cytotrabeculae of the glomerular capillary network. Electronic microphotography. x4000.

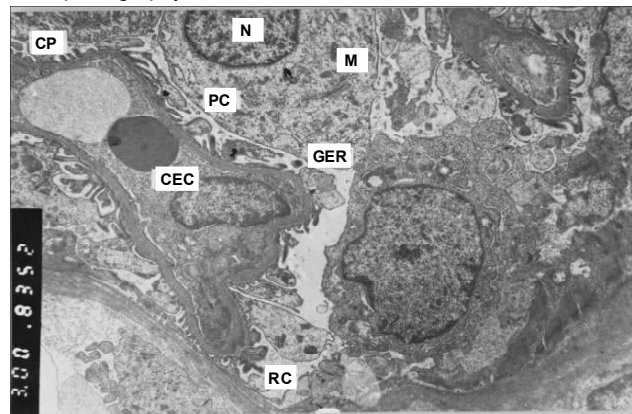


Fig. 7. Ultrastructure of rat renal cortex (group H) after the proposed drug correction. The structure of the glomerular capillary network with elements of activation of intracellular metabolic processes, both in the podocytes, and in the endothelial cells of the capillaries. Electronic microphotography. x3000.

are present throughout the length of the basement membrane. Well defined cytotrabeculae. Some of them have an increased number of intracellular structures. Part of the mitochondria is in the state of cellular degeneration, which may be due to the increased energy requirement for intracellular metabolic processes. Granular endoplasmic network tanks are expanded, which may be a manifestation of the onset of apoptosis in cells (Fig. 8).

In addition, part of the podocytes has a large size, an elevated electron density cytoplasm, in which extended elements of the granular endoplasmic net and edema of the mitochondria are observed, which is also probably due to the manifestations of apoptosis in these cells. Some of the large cytotrabeculae have a much narrower cytoplasm, almost in the form of a filiform leg. Separate cytotrabeculae are interconnected with the same narrow processes. Mesangial cells have unchanged structure, in some of them in the cytoplasm there are two nuclei, or the nuclei have

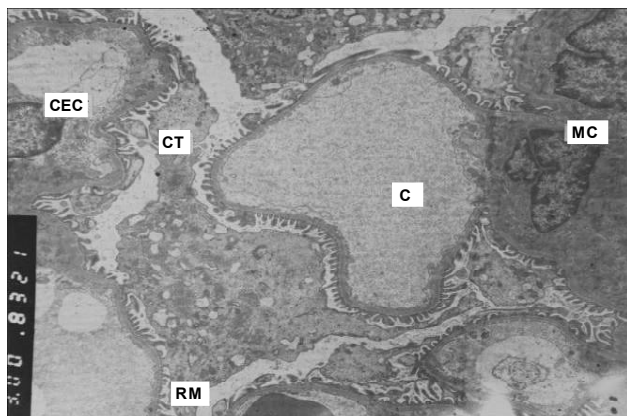


Fig. 8. Ultrastructure of the rat renal medulla (group H) after the proposed medication correction. Podocyte of the inner leaf and cytotrabeculae with the extended elements of the granular endoplasmic net and the signs of mitochondrial edema. Electronic microphotography. x3000.

deep folds of karyolemma.

The proximal and distal tubules are unchanged, they show well mitochondria, nuclei in the active state, as evidenced by the chromatin, which is in a diffuse state. Well-formed basal and apical areas of their cells. In the area of interstitial tissue local signs of minor edema of the main substance are detected. Cellular and fibrous elements of connective tissue and amorphous substance without visible structural changes.

Discussion

Taking into account the data obtained, it should be noted that in the kidney of the animal group where we used traditional drug correction after the common modeling of pyelonephritis and type I diabetes, it was found that the ultrastructure of the glomerulus is more conserved than in the group without treatment. The cavity of the capsule is more pronounced, but the part of the capillaries of the glomerulus remains deformed and with a narrowed lumen. Among the podocytes and cytotrabeculae with normal ultrastructure, there are often those with signs of degenerative changes of varying degrees of manifestation, indicating that the normal functioning of the glomerular capillary net has not been restored, which ensures the formation of primary urine and its filtration by the structures of the tubules. These data supplement and improve the researches of 2011-2014 [11, 12, 17].

The use of traditional medication effects promotes the development of reparative processes in the kidneys and moderately activates apoptosis, proved in a number of works [2, 3, 4, 5, 31].

The analysis of the ultrastructure of the nephron and tubules of rats in the group with the proposed comprehensive drug correction (with the use of preparations of multi-vector action of Armadin and Nuklex) and its comparison with the group with the traditional medication intervention showed that in the group of animals using our proposed

comprehensive drug correction, the renal structure was more preserved. The capillaries of the glomerular mesh in most cases had an ordinary architecture, endothelial cells were practically restored, fenestra differed well. The lumen of the capillaries has a high electron density. Podocytes of inner layer differs by polymorphic changes. Among the podocytes with normal structure there are cells with signs of their destruction and atrophy, as well as signs of their hypertrophy, indicating the activation of compensatory restoration processes. Separate fragments of cytotrabeculae are freely located between the podocytes, which should be considered as an expression of apoptosis. Similar fragments are observed in the material of control animals, but in smaller quantities. It should be noted that the cavity of the capsule in this material is quite extensive.

As a result of the traditional treatment of animals after the common model of pyelonephritis with type II diabetes, the structure of the nephron is partially restored. Podocytes of the outer layer in the state of strengthening the protein-synthetic and energy-forming function; in capillaries there is an edema of endothelial cells and an elevated electron density of its lumen. However, there are still signs of pathological changes in the part of capillaries and, in particular, their endothelial cells. Podocytes of inner layer in a greater number have an electron-dense cytoplasm and vacuolization of mitochondria, expanded granular endoplasmic mesh tanks. Mesangial tissue is more pronounced than that of control animals. Thus, in a significant part of the podocytes and cytotrabeculae there is a rare disposition of cytopodies. The granular cluster is concentrated in the cavity, which confirms the violation of the barrier function and the smooth penetration of substances from the lumen of the capillaries to the cavity of the capsule of high molecular weight substances. In addition, in the cavity there are isolated pinocytosis bubbles. The revealed changes in the structure of the nephrons indicate that the function of the formation of primary urine is disturbed. At the same time, in parallel with the signs of alteration of the glomerular structures, the phenomena of compensatory-restorative nature are determined, evidence of which is the restoration of the structure of some endothelial cells and their fenestra, individual podocytes. However, the gaps in capillaries have an elevated electron density. The structure of the tubules and interstitial tissue remain with the elements of hydropic changes. It should be noted that in the renal medulla, the ultrastructure of the kidney tissues is more conserved than renal cortex. It should be assumed that the traditional drug correction did not sufficiently contribute to the repair of damaged ultrastructure of the tissues of the kidney. Similar conclusions were made in the works of Bhayani S.B. with co-authors and Tirapelli L. F. with co-authors [3, 24].

After carrying out the complex medical correction proposed by us in the conditions of the common modeling of acute pyelonephritis and type II diabetes, the ultrastructure of the kidney tissues by form and structure is basically similar

to that of control animals. Significantly, there are signs of a compensatory-restorative process of podocytes. Most of these cells are in a state of hyperplasia and hypertrophy, they are significantly enlarged in body size and explode into a well-defined cavity. Cell cytoplasm is filled with a large number of organelles, which confirms the enhancement of protein-synthetic, energy-forming and other cellular functions. Cytopodiae and cytotrabeculae are also enlarged, which greatly extends the filtration zone. Capillaries are slightly expanded, their lumen content does not differ from the similar structures of control animals. Podocytes with signs of alteration, as well as signs of their apoptosis, are found singly. Somewhere in the cavity there are fragments of cytotrabeculae and signs of increased permeability of substances in the lumen of the cavity of the capsule. Podocytes of outer layer are also in the state of activating their activity, which reflects the increase of contractile function and, possibly, the release of urine from the cavity into the lumen of the PC. The structure of the tubules and interstitial tissue is close to the norm, only the cytoplasm of the PC podocytes shows an increased content of lysosomes, which reflects the active release of cells from their damaged elements.

Studies have shown that in the renal cortex ultrastructures after the proposed drug correction have the following characteristics: the ultrastructure of the glomeruli is close to normal; increased number of podocytes of the inner layer in a state of hypertrophy, in which signs of enhanced protein synthesis are manifested; EC of capillaries have a much higher restored structure compared to a group of animals receiving traditional treatment; in the cavity of the capsule there are cytotrabeculae from which the fragments of the cytoplasm are trimmed, as well as their freely located fragments are observed; in the renal proximal tubules part of the podocytes with the local destruction of structures. It should be noted that changes in the structures studied in both the renal cortex and in the renal medulla are similar with the exception of the state of cytotrabeculae having signs of deformation of plasmolemma and reduction of size in the renal medulla.

Electron microscopic studies in pathological conditions,

which were modeled in the experiment, allowed to qualitatively analyze the changes occurring in separate cells of the kidneys tissues in the dynamics of apoptosis. The method of electron microscopy provides an opportunity to obtain objective information about the degree of apoptosis in individual tissues and organs. This further increases the arsenal of methods for assessing cell membrane-pathological effects in the pathogenesis of the pathological conditions under study and their dynamics in the implementation of a comprehensive drug effect.

Conclusions

1. According to the data of electronic microscopy in the experimental modeling of acute pyelonephritis and concomitant diabetes I and II types with the use of traditional medical correction, ultrastructural changes in the tissues of the kidneys are pronounced: the renal structure is more stable, the capillaries of the glomerular net with unchanged architectonics, endothelial cells are practically restored and manifestations of early processes of apoptosis are extremely limited; between the podocytes, somewhere are freely located separate fragments of the cytotrabeculae, which should be considered as apoptotic manifestation.

2. The use of integrated therapeutic measures leads to the tendency developing of reparative processes of renal ultrastructures and promotes moderate activation of apoptosis.

3. Adding to the complex of pathogenetically induced drug correction (preparations of multi-vector action of Aradin and Nuklex) provides significant intensification of compensatory and restorative changes in the tissues of the kidney (a cytoplasm filled with a large number of organelles, which is a sign of enhancement of protein-synthetic and energy-forming cell function) and a marked increase in the processes of apoptosis, which causes elimination of the renal microstructures of the excess of damaged cells, and high content of lysosomes reflects the active release from cells their damaged elements and aggressive inflammatory effects (signs of cytoplasmic edema and destruction parts of organelles).

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МОЖЛИВОСТІ ЛІКУВАЛЬНОГО ВПЛИВУ НА ДИНАМІКУ ПРОЦЕСІВ АПОПТОЗА В ТКАНИНАХ НИРОК ПРИ ГОСТРОМУ ПІЛОНЕФРИТІ ТА СУПУТНЬОМУ ЦУКРОВОМУ ДІАБЕТІ В ЕКСПЕРИМЕНТІ
Борисов С.О., Костєв Ф.І., Борисов О.В., Молчанюк Н.І.

Запрограмована клітинна загибель в умовах інфекційно-запального процесу відіграє біологічно виключно позитивну роль в елімінації клітин. Гострий запальний процес - це явище, здатне до надмірного зосередження агресивних ефекторів запалення. Метою роботи стала оцінка динаміки ультраструктурних змін та ранніх процесів апоптоза в тканинах нірок при експериментальному моделюванні гострого пілонефриту (ГП) та супутнього цукрового діабету (ЦД) I та II типів при проведенні комплексної медикаментозної корекції. Робота виконана на 300 дорослих щурах лінії Вістар, розподілених на 6 груп. Фрагменти нірок тварин вивчали і фотографували в електронному мікроскопі ПЕМ-100-01. У нірках тварин групи, де використовували традиційну медикаментозну корекцію після співдружного моделювання пілонефриту і діабету I типу виявлено, що ультраструктура клубочка більш збережена, однак частина капілярів клубочка залишається деформованою зі звуженим просвітом, є ознаки недостатнього відновлення функціонування клубочкової капілярної сітки. У групі тварин із використанням запропонованої комплексної медикаментозної корекції ніркова структура була збереженою, капіляри клубочкової сітки із незмінною архітектонікою. Встановлено, що традиційна медикаментозна корекція недостатньо сприяла відновленню пошкоджених ультраструктур тканин нірки. Після проведення запропонованої нами комплексної медикаментозної корекції в умовах співдружного моделювання ГП і ЦД II типу ультраструктура тканин нірки за формою і структурою наближалась до такої контрольних тварин, проявились ознаки компенсаторно-відновного процесу: більшість подоцитів були гіперплазовані й гіпертрофовані. Подоцити зовнішнього листка знаходились в активній фазі діяльності, про що свідчило підвищення скорочувальної функції і, можливо, вивільнення сечі із порожнини до просвіту проксимальних каналців. Структура каналців та інтерстиційної тканини близька до структури групи контрольних тварин, лише в цитоплазмі подоцитів проксимальних каналців відмічений підвищений вміст лізосом. Ультраструктура клубочків нормалізувалась; збільшувалась кількість гіпертрофічних подоцитів внутрішнього листка з ознаками посиленого білкового синтезу. Зміни в структурах коркової та мозкової речовини аналогічні за винятком стану цитотрабекул, де їх розміри

зменшувались в мозковій речовині і з'являлись ознаки деформації плазмолемми. При експериментальному моделюванні ГП I ЦД I та II типів встановлені виражені ультраструктурні зміни в тканинах нирки, а прояви ранніх процесів апоптоза суттєво обмежені. Застосування комплексної медикаментозної корекції, запропонованої нами, стимулює розвиток репаративних процесів нирки та помірно активує апоптоз. Додавання до комплексу лікування препаратів багатовекторної дії (Армадіна та Нуклекса) інтенсифікує компенсаторно-відновлювальні зміни у нирках та апоптоз, що сприяє елімінації з ниркових мікроструктур надлишка пошкоджених клітин та агресивних ефекторів запалення.

Ключові слова: нирки, ультраструктура, гостре запалення, гіпертрофія, медикаментозна корекція, апоптоз, моделювання.

ВОЗМОЖНОСТИ ЛЕЧЕБНОГО ВОЗДЕЙСТВИЯ НА ДИНАМИКУ ПРОЦЕССОВ АПОПТОЗА В ТКАНИ ПОЧЕК ПРИ ОСТРОМ ПИЕЛОНЕФРИТЕ И СОПУТСТВУЮЩЕМ САХАРНОМ ДИАБЕТЕ В ЭКСПЕРИМЕНТЕ

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

Запрограммированная клеточная гибель в условиях инфекционно-воспалительного процесса играет биологически исключительно положительную роль в элиминации клеток. Острый воспалительный процесс - это явление, способное к надмерному сосредоточению агрессивных эффекторов воспаления. Целью работы стала оценка динамики ультраструктурных изменений и ранних признаков апоптоза в тканях почек при экспериментальном моделировании острого пиелонефрита (ОП) и сопутствующего сахарного диабета (СД) I и II типа при проведении комплексной медикаментозной коррекции. Работа выполнена на 300 взрослых крысах линии Вистар, разделенных на 6 групп. Фрагменты почек изучали и фотографировали в электронном микроскопе ПЕМ-100-01. В почках животных группы, где использовали традиционную медикаментозную коррекцию после содружественного моделирования пиелонефрита и диабета I типа установлено, что ультраструктура клубочка более сохраненная, однако часть капилляров клубочка оставалась деформированной с суженным просветом, есть признаки недостаточного восстановления функционирования клубочковой капиллярной сети. В группе животных с использованием предложенной комплексной медикаментозной коррекцией почечная структура была сохранена, капилляры клубочковой сети с неизменной архитектурой. Установлено, что традиционная медикаментозная коррекция недостаточно способствовала восстановлению поврежденных ультраструктур тканей почки. После проведения предложенной нами комплексной медикаментозной коррекции после содружественного моделирования ОП с СД II типа ультраструктура ткани почки по форме и структуре приближалась к таковой контрольных животных, проявились признаки компенсаторно-восстановительного процесса: большинство подоцитов были гиперплазированы и гипертрофированы. Подоциты наружного листка находились в активной фазе деятельности, про что свидетельствовало повышение сократительной функции и, возможно, высвобождение мочи из полости в просвет проксимальных канальцев. Структура канальцев и интерстициальной ткани близка к структуре группы контрольных животных, только в цитоплазме подоцитов проксимальных канальцев отмечено повышенное содержание лизосом. Ультраструктура клубочков нормализовывалась; увеличивалось количество гипертрофических подоцитов внутреннего листка с признаками усиленного белкового синтеза. Изменения в структурах коркового и мозгового вещества аналогичные за исключением состояния цитотрабекул, где их размеры уменьшались в мозговом веществе и появлялись признаки деформации плазмолеммы. При экспериментальном моделировании ОП и СД I и II типов установлены выраженные ультраструктурные изменения в тканях почки, а проявления ранних процессов апоптоза существенно ограничены. Применение комплексной медикаментозной коррекции, предложенной нами, стимулирует развитие репаративных процессов почки и умеренно активизирует апоптоз. Добавление в комплекс лечения препаратов многовекторного действия (Армадина и Нуклекса) интенсифицирует компенсаторно-восстановительные изменения в почках и апоптоз, что способствует элиминации из почечных микроструктур избытка поврежденных клеток и агрессивных эффекторов воспаления.

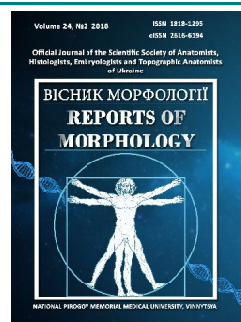
Ключевые слова: почки, ультраструктура, острое воспаление, гипертрофия, медикаментозная коррекция, апоптоз, моделирование.



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Planimetric correlations between Peyer's patches and the area of small intestine of white rats

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The largest concentration of the local immune system in the form of the nodular associations of the lymphoid tissue with the epithelium of the mucous membranes (tonsils, single lymphoid nodules and their aggregations, named Peyer's patches) is located in the gut. The paper is aimed at the study of the quantitative and planimetric correlations between the Peyer's patches and the area of the small intestine of white rats based on the visual assessment and comparative analysis of some metric values. The study was conducted on 60 white male rats, which were assigned into 2 groups of 30 animals: after the morning feeding (controls) and after a daily fasting. After the removal made in advance, the gut-associated complexes have been studied from the stomach to the cecum. The resulting experimental data have been processed on a personal computer using the EXCEL 2010 (Microsoft Excel Corp., USA) software. No significant difference in the diameter and length of the small intestine of white rats of the first and second groups was found. The total area of the small intestine wall in the control group ranged from 8666 mm² to 20724 mm², and from 8496 mm² to 20573 mm² in group II (after a daily fasting), ranking equally. Thickness parameters of the small intestine in two groups of animals were almost similar in its unchanged, within the limits of individual variability, longitudinal length. To conduct an accurate quantitative and planimetric analysis of the aggregated lymphoid nodules of the white rat's small intestine, it was advisable to distinguish 3 groups, namely: small-, medium- and large-sized. Thus, their total number varies from 12 to 28 units. Among them 8 to 17 units (on the average of 12.60±0.40) were small-sized, 2 to 11 (5.800±0.500) were medium-sized, and the large ones were not always found. For example, in the studied samples, they were absent in 6 animals, whereas 1 to 5 units were presented in the rest of animals. The area of single small Peyer's patches ranged from 1.570 to 9.800 mm², and their total area was 64.90±2.90 mm²; the area of medium samples individually ranges from 10.60 to 27.50 mm². Totally, they occupy an average area of 97.60±8.00 mm². The same value of individual large aggregated nodules is between 31.40 and 60.40 mm², which totally accounted on the average of 58.40±10.30 mm². Thus, the average statistical value of the total areas of Peyer's patches is 220.9±14.4 mm², accounting for only 2% of the total area of the small intestine wall of white rats, not including single lymphoid nodules, not exceeding 1 mm.

Keywords: Peyer's patches, small intestine, metric values.

Introduction

Currently, it has been found that the immune system of the mucous membranes of the gut is functionally consolidated with the immune mechanisms of the mucous membranes of other hollow organs (the phenomenon of the "immune solidarity of the mucous membranes") [6, 14, 16, 22, 32, 35, 36]. Apparently, the gut is characterized by the highest concentration of local immune system in the form of nodular associations of the lymphoid tissue with the

epithelium of the mucous membranes [2, 8, 10, 21, 25], involving the tonsils, single lymphoid nodules, as well as their aggregations, named Peyer's patches. Moreover, the highest concentration of the latter is found in distal part of the human ileum and appendix, which is absent in rats, as well as the presence of tonsils is unusual for this species of rodents [9, 11, 12, 19, 33, 40].

Consequently, in white rats, according to the publications,

aggregated lymphoid nodules (Peyer's patches) are located mainly in the wall of the small intestine, which is a transverse section between the stomach and cecum, accounting for about one meter in length, which is just 4-5 times shorter than in humans, being completely disproportionate relative to their body mass [3, 5, 13, 20, 24]. Taking into account that the lymphoid structures of the small intestine provide with immune control over its antigenic contents and are the initial links in the mechanisms of formation of the corresponding immune responses, it can be considered that the following planimetric parameters will serve as the most informative quantitative indices to estimate this correlation: (1) the total area of the small intestine walls; (2) the overall dimensional area of the Peyer's patches, and (3) index of the latter to the first ratio.

The paper was *aimed* at the study of the quantitative and planimetric correlation between the Peyer's patches and the area of the white rats small intestine based on the visual assessment and comparative analysis of some metric values.

Materials and methods

The study was conducted on 60 mature Wistar white male rats, weighing 200.0 ± 20.00 g, one half of which was on a daily fasting regimen before vivisection, and euthanasia of the others was carried out immediately after the morning feeding. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) of the Ukrainian Medical Stomatological Academy in compliance to the regulations on keeping experimental animals adopted by the European Parliament and Council Directive (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, №249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013) [7, 31, 34].

After euthanasia made under the overdose of thiopental sodium anesthesia (75 mg/kg animal body weight intramuscularly in the upper third of the hip of the hind paw), the anterior abdominal wall was removed from all animals. The total organs of the gut were removed from the abdominal cavity, which, with the preservation of the natural position between them, were embedded into 10% neutral buffered formalin and photographed [1, 4, 39, 41]. Within few days, after washing in running water, in the area of the pyloric sphincter, loops of the small intestine were cut off from the stomach and cecum. It was measured flat, placed on a sheet of laminated graph paper with refinement using a metal ruler (GOST 427-75) and caliper (SHTS-1 DSTU 8.113: 2009; GOST 166-89 as of 14.05.17.Cert. No.1188/0315), which was calibrated by the territorial body of the State Consumer Standard of Ukraine.

Considering that the small intestine is a tubular formation, the calculations used in mathematics for

measuring the lateral surface area of cylindrical figures are quite applicable for calculating the area of its wall. Consequently, it is necessary to know only the size of the diameter of the figure and its height or length, which is easy to measure on the selected specimens of the small intestine. With these data, the calculation of the area of its wall is initially reduced to determining its perimeter according to the formula:

$$S = 2\pi RL = \pi DL,$$

where R is the outer radius of the intestine, D is the outer diameter, L is the longitudinal length of the intestine.

The resulting value was then multiplied by the actual length of the small intestine. It would be appropriate to point at this, the only possible, simple way of calculation of the area of the small intestine, since, for some reasons, it was not mentioned in the related descriptions of the authors, cited above.

The initial digital parameters of aggregated lymphoid nodules are represented by their number in the small intestine wall and two-dimensional size of isolated ones. Given that they all have a rounded shape (orbicular or oval), their area can be calculated by conventional formulas. The area of the oval lymphoid nodules was calculated using the formula for calculating the area of an ellipse:

$$S = \pi ab,$$

where S is the area of the ellipse, π is the pi-number (3.1415), a is the length of the semimajor axis, b is the length of the minor semiaxis.

The calculation of the area of orbicular lymphoid nodules was carried out according to the formula for calculating the area of a circle:

$$S = \pi R^2,$$

where S is the area of a circle, π is the pi-number (3.1415), r is the radius of a circle [18, 38].

The resulting experimental data were processed on a personal computer using the EXCEL 2010 (Microsoft Excel Corp., USA) software.

Results

Noteworthy, the analysis of the resulting digital data has shown no significant difference in the major metric parameters of the small intestine (its diameter and length) of white rats of the first and second groups, being within the statistical accuracy, which depends entirely on the variation of quantitative indicators (Table 1). Therefore, the calculation made on the basis of these initial data of the total area of its wall in animals of the control group is from 8666 mm^2 to 20724 mm^2 ; in the group of animals undergone daily fasting, the value ranges from 8496 mm^2 to 20573 mm^2 , ranking equally. The average value was $13127 \pm 644 \text{ mm}^2$.

For better representation of the morphophysiological features of the digestive system of white rats, 2 groups of animals were formed; animals of one of the group underwent evening fasting. By this, on the one hand, the representativeness of metric studies was twice higher, and on the other hand, it has been possible to identify some

Table 1. The mean values of the area of the outer surface of small intestine of white rats of both groups.

| No. | Groups | | | | | |
|-----|--|-----------------------------|---------------------------|----------------------------|-----------------------------|---------------------------|
| | After morning feeding (control group) (n=30) | | | After daily fasting (n=30) | | |
| | D (diameter), mm | L (longitudinal length), mm | S (area), mm ² | D (diameter), mm | L (longitudinal length), mm | S (area), mm ² |
| M±m | 4.200±0.200 | 998.6±9.0 | 13127±644 | 4.200±0.200 | 1005±9 | 13313±638 |
| Min | 3 | 920 | 8666 | 3 | 902 | 8497 |
| Max | 6 | 1100 | 20724 | 6 | 1092 | 20573 |

Notes: S - area, D - diameter, L - longitudinal length, M - mean value, m - error of mean, Min - minimal value, Max - maximum value.

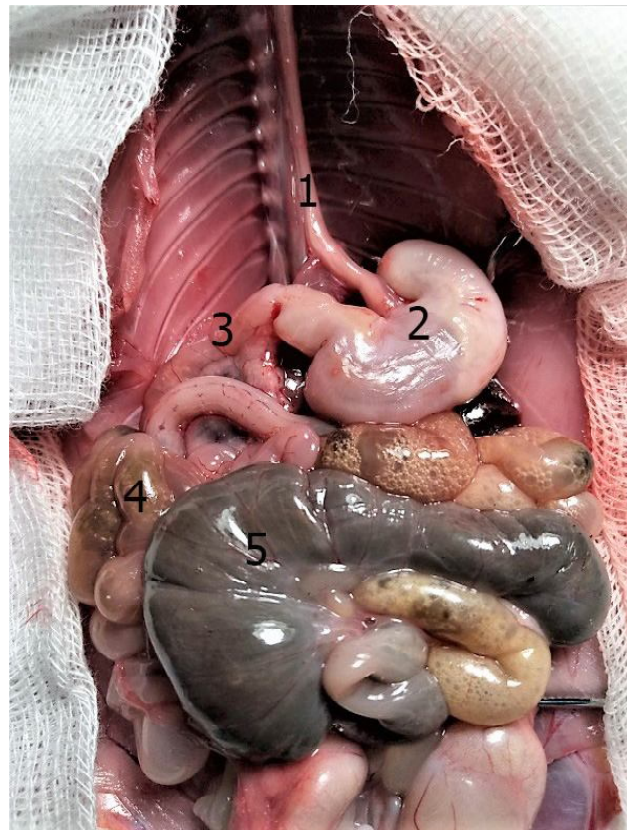


Fig. 1. Location, configuration and general dimensions of the stomach and cecum of white rats after daily fasting. 1 - esophagus; 2 - stomach; 3 - duodenum; 4 - loops of the small intestine; 5 - cecum.

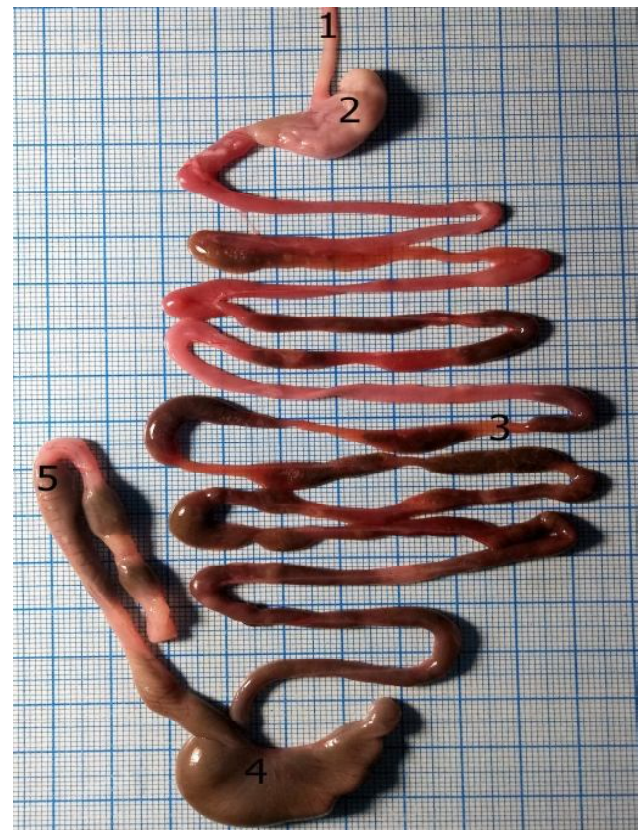


Fig. 2. Total specimen of the white rats' gut after daily fasting. 1 - esophagus; 2 - stomach; 3 - loops of the small intestine; 4 - cecum; 5 - colon.

morphological peculiarities of changes in the gut that occur during their daily feeding cycle. Here it is reasonable to give them a general assessment. Notably, in animals after a daily fasting, the stomach is collapsed, while the cecum looks more dilated (Fig. 1). At the same time, the small intestine, which is the transitive part between them, has heterogeneous lengthwise alternation of distensions, containing portions of chyme of different size, which are separated from each other by constrictions of different expressions. And the dimensional frequency of these distensions increases in the distal direction, i.e., towards the cecum (Fig. 2). A completely opposite picture occurs in animals, killed immediately after the morning feeding. The difference was mainly in their small intestine which was of homogenous thickness that significantly simplified

measuring of its thickness, whereas in the first group of animals, calculation of its average thickness had to be made from the measurements in the region of several thickenings and narrowings. Finally, the thickness parameters of the small intestine in two groups of animals turned out to be similar, as mentioned above, with an unchanged, within the limits of individual variation, its longitudinal length. Consequently, in the process of transitive movement of the chyme from the stomach into the cecum the white rats' small intestine undergoes only homogenous, isometric deformation while preserving its basic dimensional parameters.

Peyer's patches are clearly visualized without optical instruments in the form of somewhat whitish orbicular or oval protrusions of various sizes that are faintly visible on

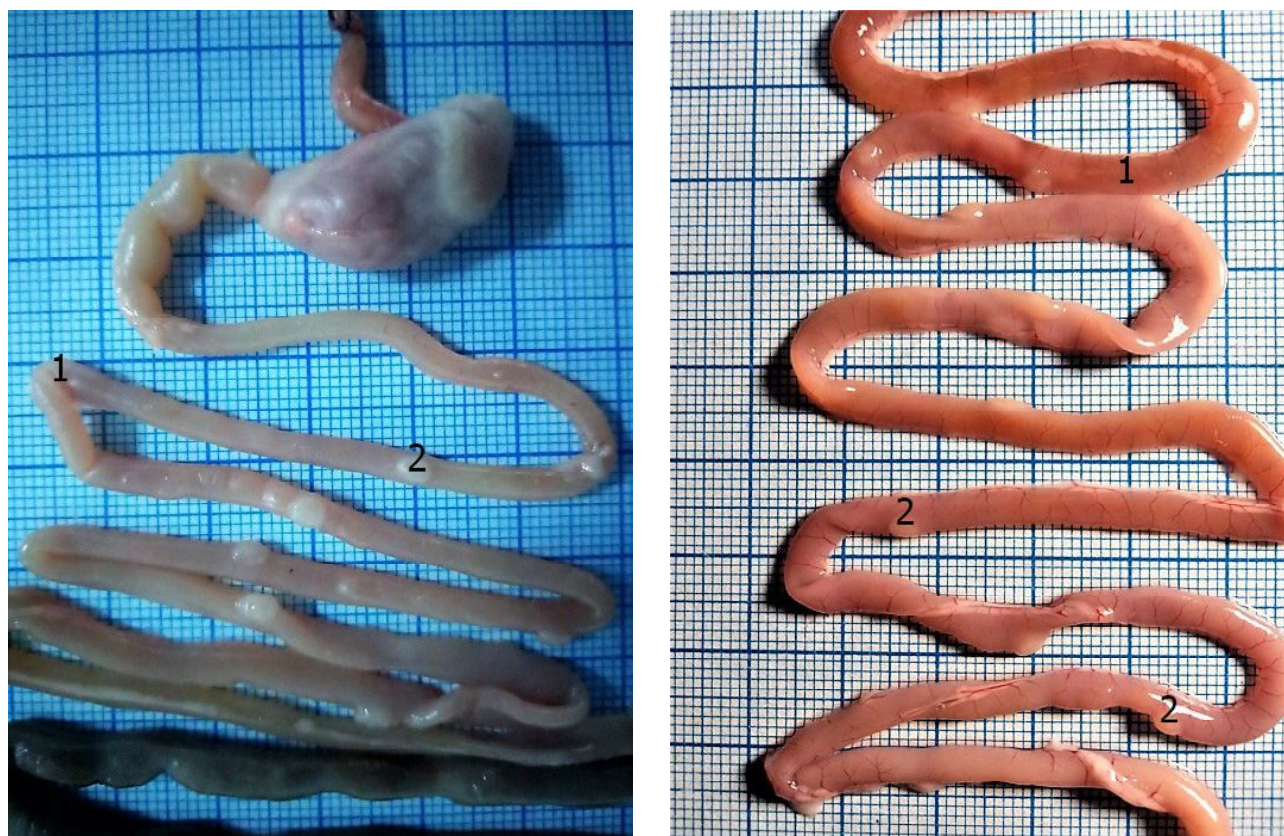


Fig. 3. Outer surface of the small intestine loops. 1 - small intestine, 2 - Peyer's patches.

Table 2. The resulting data of the quantitative and planimetric analysis of the aggregated lymphoid nodules (Peyer's patches) of the white rats small intestine.

| No. | Total number of PPs | Number and area (S) of PPs size | | | | | | Total area of PPs (mm ²) |
|-----|---------------------|---------------------------------|----------------------|-------------|----------------------|-------------|----------------------|--------------------------------------|
| | | Small | | Medium | | Large | | |
| | | Number | S (mm ²) | Number | S (mm ²) | Number | S (mm ²) | |
| M±m | 19.90±0.70 | 12.60±0.40 | 64.90±2.90 | 5.800±0.500 | 97.60±8.00 | 1.500±0.300 | 58.40±10.30 | 220.9±14.4 |
| Min | 12 | 8 | 1.570 | 2 | 10.60 | 0 | 31.40 | 87.30 |
| Max | 28 | 17 | 9.800 | 11 | 27.50 | 5 | 60.40 | 406.7 |

Notes: PPs - Peyer's patches, S - area, M - mean value, m - error of mean, Min - minimal value, Max - maximum value.

the outer surface of the white rats' small intestine along its entire length, starting from the duodenal part and reaching almost the cecum (Fig. 3). Moreover, their alternation in shape and size in such longitudinal direction is rather voluntary and changeable. However, in the total big combinational variability of their distribution along the length of the small intestine, certain regularity is observed in the form of smoothly increasing concentration of lymphoid tissue towards the cecum, which specifically results in enlargement of Peyer's patches and the last of them is the largest. If the shape of the aggregated lymphoid nodules is not an essential morphological criterion in their evaluation, then their dimensions should be taken into account, since they directly depend on the number of single lymphoid nodules associated in them. In this regard, for more clear quantitative and planimetric analysis of

aggregated lymphoid nodules of the white rats' small intestine, it is reasonable to distinguish 3 groups among them, namely: small-, medium- and large-sized, which were separately subjected to mathematical analysis (Table 2). The findings show, first of all, the great variability of the total quantitative composition of lymphoid formations and their metric parameters. Thus, their total number varies from 12 to 28 units (the average value was 19.90±0.70). Among them 8 to 17 units (on the average of 12.60±0.40) were small-sized, 2 to 11 (5.800±0.500) were medium-sized, and the large ones were not always found. For example, in the studied samples, they were absent in 6 animals, whereas 1 to 5 units were presented in the rest of the animals.

It has been subsequently established that the area of single small-sized Peyer's patches ranged from 1.570 to

9.800 mm², and their total area was 64.90±2.90 mm²; the area of medium-sized samples individually ranges from 10.60 to 27.50 mm². In aggregate, they occupy an average area of 97.60±8.00 mm². The same value of individual large aggregated nodules is between 31.40 and 60.40 mm², which totally accounted on the average of 58.40±10.30 mm².

Discussion

The anatomy of the gut of laboratory rats has not been fully elucidated in publications, and the available scarce data are not systematized, while it is known that the shape, structure and topography of the organs of the gastrointestinal tract in vertebrates are quite variably [23, 30, 37, 40].

The similarity of the structural organization of the organs and tissues of humans and some animals determines the use of the latter for the experimental modeling of various diseases occurred in clinical practice [2, 17, 23]. However, for a deeper understanding and adequate interpretation of the findings of the experiment and the development of methods for their correction, certain species characteristics and differences that are typical for specific animals should be taken into account as well as the quantitative parameters of their tissues, organs and systems for comparison.

Accurate digital data on metric values of the albino rats' small intestine are presented in the "Results" section. However, such parameter as the mean statistical value of the area of the small intestine (13127±644 mm²) is noteworthy since it does not coincide with similar values obtained by S.A. Kashchenko et al. [15, 26, 27, 28, 29], stating that the surface area of the small intestine is at the average of 9121±35 mm² in albino male rats weighing 250-280 g, that is approximately 300.0 mm² less than the calculated value. Apparently, it can be explained differently. For example, when measuring dimensions of small intestine, which is, morphologically, rather variable gut-associated organ, the errors in the results are always inevitable; this entirely depends on its functional state on the eve of the vivisection of animals, as well as their individual status and weight. The latter factor can be minimized by selecting animals of approximately equal

weight (200.0±20.0 g).

Notably, the similar algorithm have been used by other researchers in their investigations [2, 10, 13, 14, 22], and it would be appropriate to refer to their findings during the study. Specifically, the dependence of the above metric values on the functional state of the digestive system of the experimental animals is crucial and should be taken into account during the study.

Regarding the quantitative and topographical indices of Peyer's patches, they are mainly concentrated in the small intestine on the wall opposite to the site of the mesentery attachment [5, 9, 27, 30, 35]. Many morphologists have been conducting such studies, though no detailed analysis of Peyer's patches has been carried out, with the distribution of the latter into groups (small-, medium- and large-sized). The resulting quantitative data on the group lymphoid formations (Peyer's patches) of the small intestine, as well as the values of their size and area, contribute to enhancement of expertise, which is beneficial both for theoretical and practical medicine.

Prospects for further investigations are the study of the lymphoepithelial structures of the small intestine of white rats on the immunohistochemical level, as well as in the simulation of various pathological states.

Conclusions

The simple addition of the mean values of the total areas of small-, medium- and large-sized Peyer's patches gave a value of 220.9±14.40 mm², which is only 2% of the total area of the small intestine of albino rats, the metric value of which, according to above data, is equal to the average of 13.127±644 mm². However, the superficial contact of the lymphoid tissue with the contents of the small intestine is limited by this value; single lymphoid nodes are not taken into account, since only some of them can be visible on its outer surface in the form of single whitish spots of not more than 1 mm. In fact, their amount is much bigger; they are distributed in large quantities in the mucous membrane of the small intestine, occupying an intermediate position between the Peyer's patches.

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ПЛАНІМЕТРИЧНІ СПІВВІДНОШЕННЯ МІЖ ПЕЙЕРОВИМИ БЛЯШКАМИ І ПЛОЩЕЮ ТОНКОЇ КИШКИ БІЛИХ ЩУРІВ

Гринь В.Г.

Травний тракт відрізняється найбільшою концентрацією місцевого представництва імунної системи у вигляді вузликів асоціацій лімфоїдної тканини з епітелієм слизових оболонок (мигдалики, поодинокі лімфоїдні вузлики і їх групові скопчення - пейєрові бляшки). Мета - вивчити кількісне і планіметричне співвідношення між пейєровими бляшками і площею тонкої кишки білих щурів на основі візуальної оцінки і порівняльного аналізу деяких метричних показників. Дослідження проведено на 60 білих щурах-самцях, яких розділили на 2 групи по 30 тварин: після ранкового годування (контрольна група) і після добового голодування. Після попереднього видалення, досліджували комплекси шлунково-кишкового тракту від шлунку до сліпої кишки. Отримані експериментальні дані оброблені на персональному комп'ютері пакетом прикладної та статистичної програми EXCEL 2010 (Microsoft Excel Corp., США). Діаметр і довжина тонкої кишки білих щурів першої і другої групи між собою достовірно не відрізнялися. Загальна площа стінки тонкої кишки в контрольній групі склала від 8666 мм² до 20724 мм², а у 2 групі (після добового голодування) від 8496 мм² до 20573 мм², що виявилось практично рівнозначним. Параметри товщини тонкої кишки у двох груп тварин виявилися близькими при незмінній, в межах індивідуальної варіативності, її поздовжньої довжини. З метою проведення коректного кількісного і планіметричного аналізу групових лімфоїдних вузликів тонкої кишки білих щурів доцільно було виділити 3 групи, а саме: малого, середнього і великого розміру. Так, загальна їх кількість варіює в межах від 12 до 28 одиниць. Серед них малих форм налічується від 8 до 17 одиниць (в середньому - 12,60±0,40), середніх - від 2 до 11 (5,800±0,500), а великі зустрічаються не завжди. Наприклад, у вивчених вибірках вони були відсутні у 6 тварин, тоді як у всіх інших їх налічувалося від 1 до 5 одиниць. Площа окремих малих пейєрових бляшок перебувала в діапазоні від 1,570 до 9,800 мм², а загальна їх площа дорівнює 64,90±2,90 мм²; площа середніх зразків окремо коливається від 10,60 до 27,50 мм². В сукупності вони займають площу в середньому 97,60±8,00 мм². Те ж значення окремих великих групових вузликів знаходиться між 31,40 і 60,40 мм², що в їх сукупному значенні дорівнює в середньому 58,40±10,30 мм². Таким чином, встановлено середньостатистичне значення загальної площі пейєрових бляшок, яке дорівнює 220,9±14,4 мм², що становить лише 2% від загальної площі стінки тонкої кишки білих щурів, не враховуючи поодинокі лімфоїдні вузлики, величиною не більше 1 мм.

Ключові слова: пейєрові бляшки, тонка кишка, метричні показники.

ПЛАНІМЕТРИЧЕСКИЕ ОТНОШЕНИЯ МЕЖДУ ПЕЙЕРОВЫМИ БЛЯШКАМИ И ПЛОЩАДЬЮ ТОНКОЙ КИШКИ БЕЛЫХ КРЫС

Гринь В.Г.

Пищеварительный тракт отличается самой большой концентрацией местного представительства иммунной системы в виде узелковых ассоциаций лимфоидной ткани с эпителием слизистых оболочек (миндалины, одиночные лимфоидные узелки и их групповые скопления - пейєровы бляшки). Цель - изучить количественное и планиметрическое отношение между пейєровыми бляшками и площадью тонкой кишки белых крыс на основе визуальной оценки и сравнительного анализа некоторых метрических показателей. Исследование проведено на 60 белых крысах-самцах, которых разделили на 2 группы по 30 животных: после утреннего кормления (контрольная группа) и после суточного голодания. После предварительного удаления, исследовали комплексы желудочно-кишечного тракта от желудка до слепой кишки. Полученные экспериментальные данные обработаны на персональном компьютере пакетом прикладной и статистической программы EXCEL 2010 (Microsoft Excel Corp., США). Диаметр и длина тонкой кишки белых крыс первой и второй группы между собой достоверно не отличались. Общая площадь стенки тонкой кишки в контрольной группе составила от 8666 мм² до 20724 мм², а во 2 группе (после суточного голодания) от 8496 мм² до 20573 мм², что оказалось практически равнозначным. Толстые параметры тонкой кишки у двух групп животных оказались близкими при неизменной, в пределах индивидуальной вариабельности, ее продольной длины. С целью проведения корректного количественного и планиметрического анализа групповых лимфоидных узелков тонкой кишки белых крыс целесообразно было выделить 3 группы, а именно: малого, среднего и большого размера. Так, общее их количество варьирует в пределах от 12 до 28 единиц. Среди них малых форм насчитывается от 8 до 17 единиц (в среднем - 12,60±0,40), средних - от 2 до 11 (5,800±0,500), а большие встречаются не всегда. Например, в изученных выборках они отсутствовали у 6 животных, тогда как у всех остальных их насчитывалось от 1 до 5 единиц. Площадь отдельных малых пейєровых бляшек находилась в диапазоне от 1,570 до 9,800 мм², а общая их площадь равна 64,90±2,90 мм²; площадь средних образцов в отдельности колеблется от 10,60 до 27,50 мм². В совокупности они занимают площадь в среднем 97,60±8,00 мм². То же значение отдельных больших групповых узелков находится между 31,40 и 60,40 мм², что в их совокупном значении равно в среднем 58,40±10,30 мм². Таким образом, установлено среднестатистическое значение общих площадей пейєровых бляшек равно 220,9±14,4 мм², что составляет всего лишь 2% от общей площади стенки тонкой кишки белых крыс, не учитывая одиночные лимфоидные узелки, величиной не превышающей 1 мм.

Ключевые слова: пейєровы бляшки, тонкая кишка, метрические показатели.

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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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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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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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