МОР в лютеиновой фазе ОМЦ. Взаимосвязи между степенью механической болевой чувствительности, а также концентрацией МОР и уровнями лютеинизирующего гормона и пролактина не обнаружено.

რეზიუმე

მექანიკური ტკივილის მგრძნობელობის და μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციის კორელაცია ოვარიულ-მენსტრუალური ციკლის სხვადასხვა ფაზაში

მ. აფხაზავა, ი. კვაჭაძე, მ. ცაგარელი, დ. მჟავანაძე, გ. ჭიჭინაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ფიზიოლოგიის დეპარტამენტი

ბოლო ათწლეულის პერიოდის მრავალი კვლევის შედეგები მიუთითებს სასქესო ჰორმონების გავლენაზე ტკივილის მგრძნობელობაზე და რეაქციაზე ანალგეზიური საშუალებების მიმართ. წინამდებარე კვლევის მიზანს წარმოადგენდა კორელაციის შეფასება მექანიკური ტკივილის მგრძნობელობას და μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციას, სასქესო ჰორმონების დონესა და მექანიკური ზეწოლის ზღურბლის ხარისხს, მექანიკური ტკივილის ზღურბლს, მექანიკური ტკივილისადმი გამძლეობასა და μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციას შორის.

დადგენილია მექნიკური დაწოლისადმი ზღურბლის, მექნიკური ტკივილის ზღურბლის და მექნიკური ტკივილისადმი გამძლეობის შემცირება ოვარიულ-მენსტრუალური ციკლის (ომც) ლუთეინურ ფაზაში. გამოვლენილია ლუთეინურ ფაზაში მექნიკური ტკივილის ზღურბლის შემცირების ხარისხის დამოკიდებულება μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციის ცვლილებაზე (შემცირება/მომატება). დადგენილია კორელაცია მექანიკური ტკივილის მგრძნობელობის მაჩვენებელსა და ფოლიკულმასტიმულირებელი ჰორმონის კონცენტრაციას შორის ომც-ის ფოლიკულურ ფაზაში, ასევე, მექანიკური ტკივილის მგრძნობელობის მაჩვენებელს, პროგესტერონის კონცენტრაციასა და μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციას შორის ლუთეინურ ფაზაში. დამოკიდებულება მექანიკური ტკივილის მგრძნობელობის მაჩვენებელს, μ-ოპიოიდური რეცეპტორის ცილის კონცენტრაციასა და მალუთეინიზებელი ჰორმონისა და პროლაქტინის დონეს შორის დადგენილი არ არის.

MORPHOLOGICAL ASSESSMENT OF NO-SYNTHASE DISTRIBUTION IN OVERACTIVE BLADDER AND STRESS URINE INCONTINENCE IN ANIMAL MODELS ADMINISTERED WITH EXPERIMENTAL PHARMACOCORRECTION REGIMENS

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The manifestations of overactive bladder (OAB) and stress urinary incontinence (SUI) are now referred to as lower urinary tract symptoms, caused by not only local lesions of the genitourinary tract in both sexes, but also by a number of common systemic influences involved in the regulation of all functions of the lower urinary tract, regardless of gender differentiation [5,25]. Available data suggest the widespread prevalence of OAB and SUI all over the world and, at the same time, poor scope and quality of medical care provided to patients. Despite modern diagnostic techniques applied, there is still no consensus on the etiology and pathogenesis of these diseases. Along with the neurogenic, myogeneic, and urothelial models of OAB and SUI genesis, the effects of hormones and ischemia on OAB and SUI are actively studied. The role of oxidative stress and influence of nitric oxide (NO) on the functional activity of smooth myocytes is considered the most important, perhaps, the involvement of NO and its fractions in the pathogenesis of OAB and SUI remains unclear [5,11-14].

This is the very reason why the immunohistochemical evaluation of distribution of various NO-synthase fractions in the structural elements of the bladder wall in overactivity and stress urinary incontinence prior and following the administration of Mirabegron, Spasmex, Quercetin and their combination with Testosterone and Estradiol became the objective of this work.

Material and methods. Experiments on reproduction of OAB model were performed on 300-g sexually mature white laboratory female rats. For this purpose, the animals were divided into two groups. The first group was a control one, where the rats were daily intraperitoneally administered 0.3 ml of sterile physiological saline for 14 days. Animals of the second, experimental group,

intraperitoneally received 0.3 ml of Homviotensin solution, containing 0.45 mg of Reserpinum once daily for 14 days for development of OAB model. The solution was obtained by grinding tablets in sterile conditions, followed by dissolving the powder in a physiological solution. We used n. puddle transection for SUI development. The reproduction of models was proven by histological studies. In the OAB group, starting from Day 14, we daily administered Mirabegron (Astelas Pharma Europe B.V.) solution, 1 ml, rough a gastric probe for 14 and 28 days (8 mg, 1/6 tablet dissolved in 1 ml of distilled water); Quertin (PAT NVTs Borshchahivskyi KhFZ) - daily through the gastric probe for 14 and 28 days, 1 ml of solution containing Quercetin, 10 mg (1/4 tablet dissolved in 1 ml of distilled water); Spasmex (Dr.R.Pfleger GmbH) - intraperitoneally daily 1 ml of the solution containing 0.4 mg of Trospium Chloride (1/4 tablet per 10 ml of physiological saline); Testosterone Propionate (PAT Farmak) – intramuscularly daily 0.05 ml of finished solution containing 1 mg Testosterone for 14 and 28 days, and Divigel (Orion Corporation, Finland) – daily application of 0.2 g of the gel on the shaved area of the back, containing 0.2 mg Estradiol for 14 and 28 days; the doses did not change in combinations of medicines. Quercetin and its combination with hormones in the aforementioned doses were administered in the SUI group. In total, 460 rats were used, 20 experimental animals in each group.

When working with laboratory animals, we adhered to the requirements of the "Scientific and Practical Recommendations for Management of Laboratory Animals" of the State Pharmacological Center of the Ministry of Health of Ukraine (Minutes No. 8 dated June 22, 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 the material in alcohol of growing concentration, clearing in chloroform, and sealing in paraffin. 5-7 μm-thick sections were stained with hematoxylin-eosin and picrofuchsin under van Gieson, and with resorcin-fuchsin under Weighert methods [1]. Immunohistochemical studies were conducted by arranging an indirect immunoperoxidase reaction with monoclonal antibodies (MCA) in endothelial, inducible and factions neuronal NO-synthase (eNOS, iNOS and nNOS, respectively) produced by Thermo Fisher scientific. The reaction was visualized using UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen kit (Thermo scientific). The immunohistochemical staining was assessed by semiquantitative method, under which 4 categories were isolated: 0 (-) - negative reaction (<5% of cells were stained), 1 (+) - weak staining (10-30% of positively stained cells), 2 (+ +) - a moderately pronounced reaction (31-60% of positively stained cell), and 3 (+++) - intense staining (> 60% of cells or almost all cells of the epithelium were positively stained). [21,26] The expression coefficient (EC) was calculated after each observation using the formula: $EC = \Sigma (i \times v)/100$, where i - the intensity of staining in points (from 0 to 3), v - the percentage of stained cells (from 0 to 100% with the most pronounced reaction in 10 fields of view at x400) for each value of i. [9, 22]. The data was statistically processed using the Microsoft Office Excel 2007 program. The differences were considered statistically significant at $p < 0.05$. Bladder parts obtained from animals before treatment served as controls. The content of cellular elements was determined proceeding from a unit of conditional space ($1mm^2$). The histological preparations were microscoped and photographed using an optical microscope OLIMPUS BX 41 with magnifications of 40, 100, 200 and 400 times.

Results and their discussions. *Overactive bladder.* Following a 14-day administration of Homviotensin, an immunohistochemical assay revealed the emergence of an inducible fraction (iNOS) in interstitial cells located between the muscle fibers and in the subepithelial layer of the bladder wall, and in the vascular endothelial cells and perinuclear cytoplasm segments of the transitional epithelium of the mucous membrane (Fig. 1). No expression of iNOS in the control group was determined. Along with this, there was a statistically significant eNOS expression drop in the endothelial cells of the bladder wall compared to the control group ($p < 0.001$). The level of expression of the neuronal fraction of NO-synthase was also low in the nerve cells compared to the control group and was associated with degenerative changes in the nerve fibers.

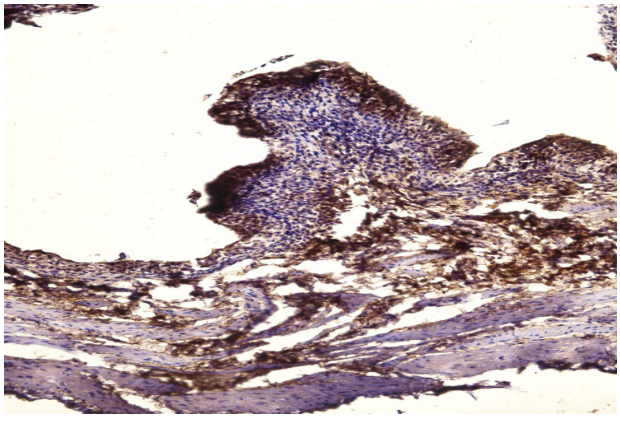

Fig. 1. Strong expression of inducible fraction of NO-synthase (iNOs) in interstitial cells of bladder muscle vessels and the transient epithelium of the mucous membrane. Day 14, OAB. Immunohistochemical marking - iNOs, x 100

Compared to OAB, an increase in expression of endothelial fraction (eNOS) was observed in the Mirabegron pharmaco-correction group, with the highest expression indexes obtained in its combination with hormonal Testosterone - 0.87 ± 0.03, and Testosterone with Estradiol - 0.89 ± 0.04, respectively (Table 1).

Table 1. Intensity of expression endothelial (eNOS), inducible (iNOS) and neuronal (nNOS) NO-synthase fractions in the bladder wall at disorders of urodynamics of the lower urinary tract on Day 14 of the experiment

Animal groups\pharmacorrection regimens		eNOS	iNOS			nNOS
		EN	EP	EN	IC	N
Control group		0.93±0.11	-	-	-	1.03±0.33
OAB		0.12±0.01†	0.95±0.52	1.06±0.03	2.55±0.95†	0.13±0.01†
SUI		0.38±0.07†	0.24±0.01	-	1.04±0.11	0.18±0.09
Pharmacorrection of OAB	Mirabegron	0.75±0.07*	-	-	0.81±0.02*	0.46±0.07*
	Spasmex	0.14±0.006†	0.79±0.02	0.91±0.03	1.99±0.01	0.15±0.006†
	Quercetin	0.69±0.11*	-	-	0.84±0.02*	0.55±0.04*
	Mirabegron+Testosterone	0.83±0.03*	-	-	0.31±0.03*	0.71±0.02*
	Mirabegron+Estradiol	0.45±0.01*	-	-	0.48±0.03*	0.53±0.02*
	Mirabegron+Testosterone+Estradiol	0.86±0.01*	-	-	0.3±0.02*	0.82±0.03*
	Spasmex+Testosterone	0.24±0.01√	0.35±0.04	0.59±0.01*	1.29±0.09*	0.19±0.009√
	Spasmex+Estradiol	0.18±0.008√	0.62±0.02	0.75±0.02	2.12±0.05√	0.16±0.006√
	Spasmex+ Testosterone+Estradiol	0.26±0.01*	0.32±0.03	0.5±0.022	1.25±0.1	0.22±0.01*
	Quercetin+Testosterone	0.85±0.01*	-	-	0.37±0.04*	0.74±0.01*
	Quercetin+ Estradiol	0.7±0.09*	-	-	0.76±0.0*	0.6±0.01*
	Quercetin+ Testosterone+Estradiol	0.8±0.02*	-	-	0.39±0.03*	0.74±0.02*
	Testosterone	0.5±0.01*	-	-	0.4±0.03*	0.59±0.08*
	Estradiol	0.34±0.01*	-	-	0.65±0.03*	0.25±0.01*
	Testosterone+Estradiol	0.61±0.01*	-	-	0.39±0.03*	0.39±0.02*
Pharmacorrection of SUI	Quercetin	0.58±0.01^	-	-	0.56±0.02	0.37±0.04
	Testosterone	0.51±0.02^	-	-	0.54±0.02^	0.35±0.03^
	Estradiol	0.43±0.02^	-	-	0.68±0.02^	0.29±0.01^
	Testosterone+Estradiol	0.5±0.03^	-	-	0.59±0.02^	0.36±0.01^
	Quercetin+Testosterone+ Estradiol	0.65±0.02^	-	-	0.45±0.02^	0.58±0.01^

*note: OAB - overactive bladder, SUI - stress urinary incontinence, eNOs - endothelial fraction of NO-synthase, iNOs - inducible fraction of NO-synthase, NO - nitrous oxide, EP – epithelium, EN – endothelium, IC – interstitial cells, N – neurocytes. - no expression; * - p<0.001 compared to OAB; † - p<0.001 compared to control; ^ - p<0.001 compared to SUI; √ - p>0.05 compared to OAB*

Following the OAB Spasmex treatment, on Day 14 of the experiment we did not find any significant difference in expression of eNOS, iNOS, and nNOS in the structural elements of the bladder wall compared to the experimental animal group receiving Homviotensin as a sole therapy (p>0.05, Table 1), while its combination with Testosterone (p <0.001, Tables 1, 2) and Testosterone + Estradiol presented a slight decrease in iNOS expression compared to OAB.

In the groups Mirabegron+OAB, Quercetin+OAB and their combination with hormonal medicines, we observed a significant grow of expression of endothelial and neuronal NO-synthase fractions (Table 1) already at this observation phase. It should be noted that we observed the highest indicators in the group of animals receiving the combination of Quercetin+Testosterone. Along with this, there was a statistically significant reduction of iNO expression noted both in a group of Quercetin and its combination with hormones (p <0.001), and in a group of Mirabegron in combination with hormonal medicines (Table 1).

In the event of sole administration of hormonal medicines, at this research phase, the best indicators characterized by a decrease in the marking of iNOS and an increase in the expression of eNOS and iNOS were obtained in Testosterone and Testosterone+Estradiol groups compared to Estradiol-alone group (Table 1).

On Day 28 of the experiment, we observed weak labeling of both inducible and neuronal and endothelial NO-synthase fractions (Figure 2) in the OAB group without pharmacological correction, which was accompanied by sclerotic changes in the hypertrophied bladder wall and mucosal atrophy. At the same time, iNOS labeling in endothelial cells and epithelium was weak in intensity, yet a fairly high level of expression remained in interstitial cells (Table 2).

Table 2. Intensity of expression of endothelial (eNOS), inducible (iNOS) and neuronal (nNOS) NO-synthase fractions in the bladder wall at disorders of urodynamics of the lower urinary tract on Day 28 of the experiment

Animal groups/ pharmacorrection regimens		eNOS	iNOS			nNOS
		EN	EP	EN	IC	N
Control group		0.99±0.03	-	-	-	1.04±0.03
OAB		0.13±0.01†	0.9±0.04	0.89±0.03	1.9±0.02	0.15±0.01†
SUI		0.25±0.01†	-	-	0.88±0.02	0.11±0.006†
Pharmacorrection of OAB	Mirabegron	0.79±0.03*	-	-	0.71±0.02	0.66±0.03*
	Spasmex	0.13±0.01	-	-	1.5±0.15	0.12±0.008
	Quercetin	0.72±0.03*	-	-	-	0.72±0.03*
	Mirabegron+Testosterone	0.87±0.03*	-	-	-	0.83±0.02*
	Mirabegron+Estradiol	0.59±0.02*	-	-	-	0.65±0.03*
	Mirabegron+Testosterone+Estradiol	0.89±0.04*	-	-	-	0.89±0.008*
	Spasmex+Testosterone	0.26±0.01*	-	-	0.95 ±0.04*	0.16±0.009
	Spasmex+Estradiol	0.15±0.01√	-	-	1.18±0.08	0.17±0.006√
	Spasmex+ Testosterone+Estradiol	0.29±0.02*	-	-	1.21±0.1	0.2±0.01√
	Quercetin+Testosterone	0.88±0.03*	-	-	-	0.9±0.02*
	Quercetin+ Estradiol	0.71±0.01*	-	-	-	0.69±0.02*
	Quercetin+ Testosterone+Estradiol	0.96±0.03*	-	-	-	0.98±0.05*
	Testosterone	0.65±0.02*	-	-	-	0.63±0.03*
	Estradiol	0.43±0.03*	-	-	0.11±0.08	0.27±0.006*
	Testosterone+Estradiol	0.63±0.02*	-	-	-	0.57±0.02*
Pharmacorrection of SUI	Quercetin	0.62±0.02^	-	-	-	0.59±0.02^
	Testosterone	0.59±0.02	-	-	-	0.4±0.01^
	Estradiol	0.49±0.02^	-	-	0.15±0.008	0.3±0.01
	Testosterone+Estradiol	0.57±0.03^	-	-	-	0.52±0.01^
	Quercetin+Testosterone+ Estradiol	0.8±0.02^	-	-	-	0.7±0.02^

*note: OAB - overactive bladder, SUI - stress urinary incontinence, eNOs - endothelial fraction of NO-synthase, iNOs - inducible fraction of NO-synthase, NO - nitrous oxide, EP – epithelium, EN – endothelium, IC – interstitial cells, N – neurocytes. - no expression; * - p<0.001 compared to OAB; † - p<0.001 compared to control; ^ - p<0.001 compared to SUI;√ - p>0.05 compared to OAB*

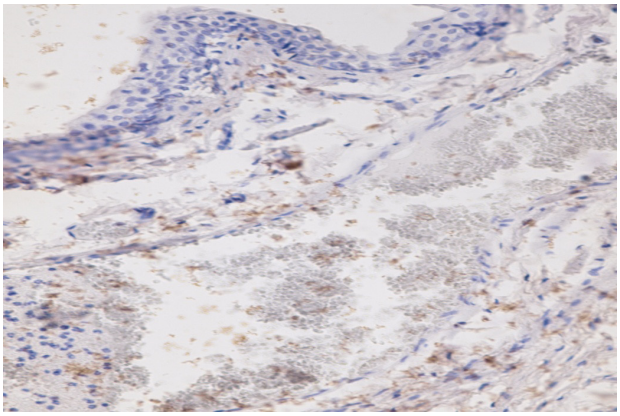

Fig. 2. Low expression of endothelial fraction of NO-synthase (eNOs) in endothelial cells of the subepithelial vessels and bladder muscle vessels. Day 28, OAB. Immunohistochemical marking - eNOs, x 200

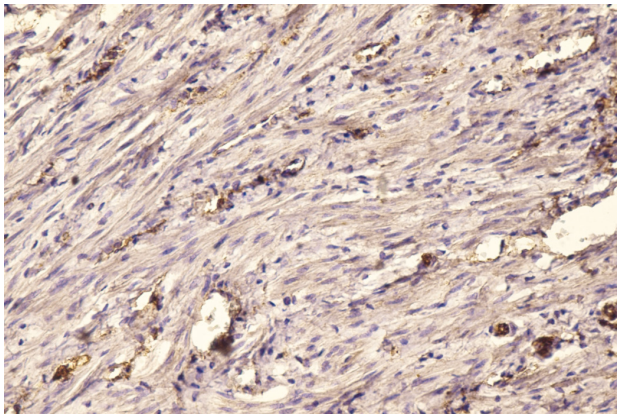

Fig. 3. Moderate expression of endothelial fraction of NO-synthase (eNOs) in endothelial cells of bladder muscle vessels. Day 28, Quercetin+Testosterone+Estradiol. Immunohistochemical marking - eNOs, x 400

It`s worthy to emphasize that compared to Mirabegron and Quercetin in the experimental animals receiving Spasmex, dystrophic changes in muscle and nerve fibers were progressing and accompanied by muscle sclerosis.

The eNOS expression coefficient in the event of administration of Mirabegron (0.87±0.03, 0.59±0.02, 0.89±0.04), Quercetin (0.88±0.03, 0.71±0.01, 0.96±0.03) and their combination with Testosterone and Estradiol was significantly increasing ($p < 0.001$) at this study period compared to OAB (0.13±0.01, $p < 0.001$); the same trend was observed also in the analysis of expression of NO-synthase neuronal isoform (Table 2). The NO inducible fraction was not determined in the above groups in the given period of the experiment.

Stress urinary incontinence following n. pudendus ligature. The immunohistochemical evaluation of iNOS expression of the bladder wall in the animal models subjected to n. pudendus ligature presented with the similar results compared to OAB group in terms of expression of the inducible NO-synthase fractions in interstitial cells of the bladder muscle wall, but in contrast to OAB, no expression of iNOS was registered in the endothelial cells and the transitional epithelium of the mucosa. The main morphological manifestation was atrophic changes in the bladder wall and a decrease in eNOs and nNOS expression. As we can see from Table 2, the expression coefficient of eNOs and nNOS was statistically significantly lower than 0.12±0.01 and 0.13±0.01 ($p<0.001$) compared to the control group (0.93±0.11 and 1.03±0.33, respectively).

On Day 14 of Quercetin and Mirabegron administration in combination with hormones in SUI+Quercetin+Testosterone+Estradiol and SUI+Mirabegron+Testosterone+Estradiol groups, when expression of iNOS was low and determined only in isolated interstitial cells of the muscle layer, sometimes absolutely absent, the quantitative indicators of eNOS and nNOS expression in endothelial cells and nerve cells of the bladder wall were improving (Table 1).

On Day 28 of the experiment, the atrophic changes in the bladder wall were accompanied with sclerotic changes with a predominantly diffuse growth of collagen fibers between smooth myocytes in the group of experimental animals with n.pudendus ligation. The inducible fraction of NO-synthase was virtually absent in the group of Mirabegron, Quercetin and their combination with hormones. The expression of eNOS and nNOS was approaching the control group by the intensity (Table 2).

When using hormones at this period of the study, Testosterone and Testosterone in combination with Estradiol demonstrated better efficacy in reducing the expression of iNOs in interstitial muscle cells and subepithelial units of the own plate of the bladder wall, compared with the group receiving Estradiol alone (Table 2). Expression of eNO and nNOs was growing predominantly in Testosterone and Testosterone+Estradiol groups compared to n.pudendus-ligated animals not subjected to pharmacological correction.

The data on reduction of endothelial and neuronal isoforms and increase of the inducible fraction of nitric oxide in OAB suggest an intense formation of toxic NO metabolites. According to literature [2,4,8], both excessive and lacking nitric oxide may have a toxic effect on cells, associated with both direct action on iron-containing enzymes and the formation of a strong oxidant, a highly reactive and toxic free radical peroxynitrite compound. In turn, inhibition of mitochondrial enzymes leads to a decrease in ATP production, while peroxynitrite can directly damage DNA that activates protective mechanisms with subsequent stimulation of Poly-(ADP-Ribose) synthetase, reduces ATP level, and may lead to a cell death due to DNA damage. [8]. Continuous imbalance in the NO-ergic system leads to a disturbance of adaptive-compensatory mechanisms, which manifests in changes in NO-synthase activity, disturbance of the functional state and loss of integrity of the morphological structure of the bladder wall. In our study, we established a reduction in expression of NO-synthase endothelial isoform and amplified expression of the inducible isoform of the enzyme in interstitial cells surrounding smooth myocytes in OAB and SUI models, which indirectly suggested the intense formation of toxic products of nitric oxide metabolism. Therefore, according to our research and other authors' data, interstitial cells are the main target of NO in detrusor at OAB and SUI [8,23].

Endogenous formation of NO, as a vasodilator, is very important for maintaining vascular microcirculation and the integrity of structural components of the bladder wall. The mechanism for increasing the level of NO-synthase endothelial isoform in the endothelium of blood vessels under Quercetin administration may be associated with a decrease in its inactivation by free radicals, which proves antioxidant and anti-ischemic properties of Quercetin. Under normal conditions, Quercetin activates HIF-1α in various cell cultures, increases expression of the vascular endothelial growth factor in them and GLUT-1 glucose conveyor. Quercetin blocks asparaginyl hydroxylase, the factor that inhibits HIF, which inactivates HIF-1α under normal conditions [24]. In addition, the antioxidant activity of flavonoids is preconditioned by inhibition of tyrosine phosphorylation and activation of phospholipase D in activated neutrophils, myeloperoxidase, and binding transitional metals involved in degradation of H_2O_2 into a hydroxyl radical. Formation of flavonoid complexes with metal ions is also possible, thus limiting their access to fatty acids of cell membrane phospholipids. In our study, the administration of Quercetin led to a reduction of oxidative stress both in OAB and SUI, that was quite in agreement with the conclusions of other researchers [3,7].

In addition, in our previous study, we established a significant suppression of potassium channels in OAB models and their recovery following Quercetin administration [10]. Comparing these findings with the present results, we can predict that one of the mechanisms for restoring potassium channels under Quercetin administration may be growing expression of eNOS followed by activation of protein kinase G, which leads to a decrease of Ca^{2+} by inhibiting phospholipase C and formation of inositol 1,4,5-trisphosphate. These transmitters also stimulate relaxation by activating potassium

channels (K+channels), which is in line with the data of other authors, for example, hydrogen sulfide activates protein kinase G, which leads to relaxation of muscle fibers by activating ATP-dependent K + channels [18,19]. The relaxing effect of NO-synthase is closely related to activation of soluble guanylate-cyclase and accumulation of cyclic guanosinemonophosphate (cGMP). The excessive concentration of GMP activates the cGMP-dependent protein kinase and ATPase, involved in dephosphorylation of light myosin chains, thus leading to the release of calcium from muscle cells and, as a result, to vasodilation [6].

It should be noted that along with the sole administration of Quercetin and Mirabegron, a positive effect was also achieved by their combination with hormones, especially with Testosterone. Moreover, a statistically significant increase in eNOS was achieved in Testosterone group both in OAB and in SUI compared to sole administration of Estradiol. As we known, the deficiency of Testosterone and nitric oxide is manifested by endothelial dysfunction (endothelial-dependent vasodilatation distortion resulting from poor bioactivity of nitric oxide, its excessive degradation, disorders in the system of antioxidant protection, or disturbance of endothelial NO-synthase expression) [15, 16]. Reduced Testosterone levels or Testosterone aromatization in Estradiol may result in a disturbance of endothelial NO-synthase function and NO deficiency [16]. Sex hormones are directly involved in the regulation of NO-synthase activity (blood circulation, innervation, oxygen supply of tissues, metabolism and energy metabolism; endothelium-dependent vasodilatation; endothelium-independent neuronal-mediated vasodilation) [5]. One of the common mechanisms of such universal effect of androgens on the lower urinary tract may be involvement of Testosterone in activation of NO-synthases and L-arginases, which are proactive in the synthesis of nitric oxide in both sexes [20]. According to our study, animal groups of combination of Quercetin with Testosterone and Quercetin with Testosterone and Estradiol demonstrated an increase in the synthesis of NO-synthase endothelial fraction throughout the experiment, thus confirming the involvement of hormones in activation of eNOS.

Conclusions. 1. The immunohistochemical analysis of expression of inducible, endothelial and neuronal NO-synthase fractions under experimental overactive urinary bladder and stress urinary incontinence has confirmed its involvement in the pathogenetic mechanisms of urodynamic disorders of the lower urinary tract. According to our study, the main cells that produce NO-synthase and are involved in the contractile activity of smooth myocytes are interstitial cells of the bladder muscle wall.

2. The group of experimental animal models receiving Spasmex did not present any reliable evidence of its effect on expression of NO-synthase isoforms in OAB, but a combination of the medicine with Testosterone presented with a statistically significant ($p < 0.001$) decrease of iNOS expression in the bladder interstitial cells.

3. Sole Mirabegron therapy and especially its combination with Testosterone and Estradiol demonstrated a positive trend of eNOS and nNOS immunohistochemical expression indices and a statistically significant decrease of iNOS expression ($p < 0.001$) in interstitial bladder wall cells in OAB and SUI.

4. The results of the study proved a positive effect of Quercetin for treatment of experimental OAB and SUI, its high efficacy in combination with Testosterone and Estradiol confirmed by a significant increase and stabilization of eNOS and nNOS expression and disappearance of iNOS expression in interstitial cells of the bladder wall.

Taking into account the leading role of nitric oxide in the pathogenesis of OAB and SUI, further study of distribution of endothelial, neuronal and inducible NO-synthase fractions in the structural elements of the bladder wall opens the prospects for changing existing perceptions of the mechanism of urodynamic disorders and the development of new medicines for correction and optimization of the treatment policy.

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SUMMARY

MORPHOLOGICAL ASSESSMENT OF NO-SYNTHASE DISTRIBUTION IN OVERACTIVE BLADDER AND STRESS URINE INCONTINENCE IN ANIMAL MODELS ADMINISTERED WITH EXPERIMENTAL PHARMACOCORRECTION REGIMENS

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The objective of the study was immunohistochemical evaluation of distribution of various NO synthase fractions in the structural elements of the bladder wall under stress urinary incontinence and its overactivity prior and post Mirabegron, Spasmex, Quercetin therapies and their combinations with Testosterone and Estradiol.

Using the immunohistochemical method, we studied the expression of the main fractions of NO synthase in experimental models of hyperactive bladder (OAB) and stress urinary incontinence (SUI). We found that OAB and SUI were characterized by emergence of expression of the inducible fraction (iNOS) predominantly in the interstitial cells of the muscular layer of the bladder and reduced expression of endothelial (eNOS) and neuronal (nNOs) NO synthase fractions. In contrast to Spasmex, Mirabegron and Quercetin in combination with Testosterone and Estradiol contributed to stabilization of eNOS and nNOs expression already at early observation phases, and reduced the level of iNOS expression with its further disappearance in the later observation period.

Keywords: overactive bladder, stress urinary inconsistency, immunohistochemical evaluation, NO synthase, pharmacocorrection.

РЕЗЮМЕ

МОРФОЛОГИЧЕСКАЯ ОЦЕНКА РАСПРЕДЕЛЕНИЯ NO-СИНТАЗЫ ПРИ ГИПЕРАКТИВНОМ МОЧЕВОМ ПУЗЫРЕ И СТРЕССОВОМ НЕДЕРЖАНИИ МОЧИ В ЭКСПЕРИМЕНТЕ ПОД ВЛИЯНИЕМ ФАРМАКОКОРЕКЦИИ

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Цель исследования – иммуногистохимическая оценка распределения различных фракций NO-синтазы в структурных элементах стенки мочевого пузыря при стрессовом недержании мочи и его гиперактивности до и после лечения мирабегроном, спазмексом, кверцетином и их комбинацией с тестостероном и эстрадиолом.

На экспериментальных моделях гиперактивного мочевого пузыря (ГАМП) и стрессового недержания мочи (СНМ) с помощью иммуногистохимического метода изучена экспрессия основных фракций NO-синтазы. Установлено, что при ГАМП и СНМ появляется экспрессия индуцибельной фракции (iNOS) преимущественно в интерстициальных клетках мышечного слоя мочевого пузыря, снижается экспрессия эндотелиальной (eNOS) нейрональной (nNOs) фракций NO-синтазы. Мирабегрон и кверцетин в сочетании с тестостероном и эстрадиолом в отличие от спазмекса способствуют стабилизации экспрессии eNOS

и nNOs уже на ранних сроках наблюдения, а также снижают уровень экспрессии iNOS с ее исчезновением на поздних сроках наблюдения.

Принимая во внимание ведущую роль оксида азота в патогенезе ГАМП и СНМ, дальнейшее изучение распределения эндотелиальных, нейронных и индуцибельных фракций NO-синтазы в структурных элементах стенки мочевого пузыря открывает перспективы для изменения существующих представлений о механизме уродинамических расстройств и разработки новых лекарств для коррекции и оптимизации лечебной тактики.

რეზიუმე

NO-სინთაზას განაწილების მორფოლოგიური შეფასება ჰიპერაქტიური შარდის ბუშტის და შარდის სტრესული შეუკავებლობის დროს ფარმაკოკორექციის ზემოქმედებისას ექსპერიმენტში

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კვლევის მიზანს წარმოადგენდა NO-სინთაზას სხვადასხვა ფრაქციის განაწილების იმუნოჰისტოქიმიური შეფასება შარდის ბუშტის კედლის სტრუქტურულ ელემენტებში შარდის სტრესული შეუკავებლობის და მისი ჰიპერაქტიურობის დროს მირაბეგრონით, სპაზმექსით, კვერცეტინით და ტესტოსტერონთან და ესტრადიოლთან მათი კომბინაციით მკურნალობამდე და მკურნალობის შემდეგ.

ჰიპერაქტიური შარდის ბუშტის (ჰაშბ) და შარდის სტრესული შეუკავებლობის (შსშ) ექსპერიმენტულ მოდელებზე იმუნოჰისტოქიმიური მეთოდით შესწავლილია NO-სინთაზას ძირითადი ფრაქციების ექსპრესია. დადგენილია, რომ ჰაშბ-ის და შსშ-ის დროს ვლინდება ინდუცებელური ფრაქციის (iNOS) ექსპრესია უპირატესად შარდის ბუშტის კუნთოვანი შრის ინტერსტიციულ უჯრედებში, მცირდება NO-სინთაზას ენდოთელური (eNOS) და ნეირონული (nNOs) ფრაქციების ექსპრესია. მირაბეგრონი და კვერცეტინი ტესტოსტერონთან და ესტრადიოლთან შერწყმით, სპაზმექსისგან განსხვავებით, დაკვირვების ადრეულ ეტაპზევე ხელს უწყობს eNOS-ის და nNOs-ის ექსპრესიის სტაბილიზებას, ასევე, ამცირებს iNOS-ის ექსპრესიის ხარისხს, სრული გაქრობით დაკვირვების გვიან ეტაპებზე.

აზოტის ოქსიდის წამყვანი როლის გათვალისწინებით ჰაშბ-ის და შსშ-ის პათოგენეზში, შარდის ბუშტის კედლის სტრუქტურულ ელემენტებში NO-სინთაზას ენდოთელური, ნეირონული და ინდუცებელური ფრაქციების განაწილების შემდგომი კვლევა ხსნის ახალ პერსპექტივებს არსებული შეხედულებების შეცვლისათვის უროდინამიკური დარღვევების მექანიზმების შესახებ, ასევე, ახალი პრეპარატების შემუშავებისათვის კორექციისა და მკურნალობის პოლიტიკის ოპტიმიზებისათვის.

THE STATE OF THE CYSTATHIONINE GAMMA-LYASE / H_2S SYSTEM IN THE LIVER AND SKELETAL MUSCLES OF RATS WITH HYPERCHOLESTEROLEMIA UNDER SIMVASTATIN ADMINISTRATION

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Nowadays among the drugs for treatment, primary and secondary prevention of atherosclerotic cardiovascular events statins represent one of the most powerful agents because in addition to beneficial lipid-lowering action, statins seem to have wide spectrum of non-lipid-mediated pleiotropic effects such as anti-inflammatory, anti-thrombogenic, anti-proliferative, immunomodulatory properties [8,10,12].

Statins have demonstrated the beneficial effects of treatment cardiovascular disorders, diabetes mellitus, peripheral arteries diseases, produced a significant reduction in incident myocardial infarction, stroke and death from cardiovascular disease in all patients [2, 4]. The mechanism of statins action is associated with inhibition of the key reaction of cholesterol synthesis pathway - the formation of mevalonic acid, which is the source of isoprenoid equivalents. Thus, the first stage in the cholesterol synthesis chain thereby is interrupted: acetyl coenzyme A → mevalonate → 5 melovalonate pyrophosphate → isopentenyl pyrophosphate → 3.3 dimethyl pyrophosphate → geranyl pyrophosphate → farnesyl pyrophosphate → squalene → lanosterol → cholesterol. However, the effects of statins extend beyond their cholesterol-lowering action, inhibition of HMG-CoA reductase, the regulatory enzyme of the pathway, results in disturbances in practically all vital cellular processes, such as the formation of a component of the mitochondrial electron-transport chain of ubiquinone and the posttranslational protein glycosylation and prenylation, which are necessary for the regulation of numerous cellular functions [5,12]. Thus, the most common side effects have been associated with