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[1]IATSYNA O.I., [2]VERNYGORODSKYI S.V, [1]KOSTYEV F.I.

THE IMPACT OF PHARMACOCORRECTION ON MORPHOGENESIS OF OVERACTIVE DETRUSOR AND URODYNAMIC STRESS INCONTINENCE

[1]Odessa National Medical University, Ministry of Public Health;
[2]National Pirogov Memorial Medical University, Vinnitsa, Ukraine

SUMMARY

Purpose of the study was to provide histo- and immunohistochemical evaluation of the quantitative composition of bladder wall connective tissue under stress urinary incontinence and its overactivity prior- and post-treatment with Mirabegnon, Spasmex, Quercetin and their combinations with testosterone and estradiol.

We studied main components of the connective tissue bladder wall frame on the experimental models of overactive bladder (OAB) and stress urinary incontinence (SUI). Mirabegonon in combination with testosterone and estradiol proved to stabilize the production of Type 3 and 4 collagens already at early observation stages in the OAB group, while Quercetin in combination with testosterone and estradiol proved to be more effective when administered at the late observation stage both in OAB and SUI models. Along with the stabilizing effect on formation of collagens and elastic fibers, Quercetin demonstrated the most pronounced antisclerotic effect ($p = <0.05$) compared to other medicines. In terms of efficacy as OAB treatment, the study medicines can be placed in the following sequence starting from the lowest: Spasmex '! Mirabegon '! Quercetin, keeping in mind that the best results were obtained by combinations thereof with testosterone and estradiol.

One of the pressing and still unresolved questions in urology is a problem of stress urinary incontinence (SUI) and overactive bladder (OAB). Almost 22 million individuals in European countries suffer from this serious syndrome, but only 27% of them receive treatment, which is indicative of inadequate attention paid

to this problem by both patients and medical specialists. The periodicity of symptoms in aging subjects grows up to 30% in people over 65 and up to 40% in individuals over 70 [10]. The available data suggest the multifactorial development of OAB, but the question of OAB pathogenesis remains unclear. In the recent time, the neurogenic theory of OAB emergence has been challenging by a theory of morphological changes that occur in smooth myocytes of detrusor and their interaction with the extracellular matrix (ECM). The mere fact of disturbance of intercellular junctions acting as conducting paths is one of the leading mechanisms of OAB development [7].

The main components of a bladder wall are smooth muscle fibers with extracellular matrix (ECM) consisting predominantly of collagen and elastin between them. Collagen is responsible for resistance to rupture and integrity, while elastin provides elasticity and flexibility of tissues. In turn, structural and functional changes of collagen and elastin influence on contractile activity of smooth myocytes [8]. The quantitative and qualitative data from the literature sources on different types of collagen [1,3,4] in patients with SUI and OAB are quite contradictory, as some authors insist on a decrease of Type 1 collagen content and 1:3 ratio [9], while others indicate an increase in the amount of Type 1 and 3 collagen [6, 11]. That is why the purpose of this work was to provide histo- and immunohistochemical evaluation of the quantitative composition of bladder wall connective tissue under stress urinary incontinence and its overactivity prior and post-treatment with Mirabegnon, Spasmex, Quercetin and their combinations with testosterone and estradiol.

MATERIALS AND METHODS. The experiments on reproduction of OAB model were conducted on 300 g sexually mature white laboratory female rats. For this purpose, 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 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, Mirabegnon 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 Borshchahivskyi 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 0.2 g (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 22.06.2012).

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 [1,2].

The immunohistochemistry assay was performed using DAKO and ThermoFisher Scientific paraffin blocks and reagents with monoclonal antibodies to Collagen IV (Clone CIV 22) - a marker of basal

membrane connective tissue, Collagen 1 (Clone COL-1) and Collagen 3 (Clone FH-7A) with EnVision™FLEX 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. Bladder areas obtained from animals before treatment served as controls. The content of cellular elements was evaluated as per unit of conventional space (1 mm^2). The histological preparations were microscoped and photographed using OLIMPUS BX 41 optical microscope at 40, 100, 200 and 400x magnification.

RESULTS AND DISCUSSIONS. ***Overactive bladder.*** After 14-day administration of Homviotensin, the morphological analysis revealed significant hypertrophy of the bladder wall compared to the control group ($p<0.001$). At the same time, hypertrophy of muscle fibers was accompanied with degenerative changes associated with vacuolation of the cytoplasm. The thickness of the muscular layer was 0.97±0.05 mm (Table 1).

Table 1. The thickness of bladder wall muscle layer (mm) in models with lower urinary tract urodynamics disturbance during the study

Animal group/ Pharmacocorrection type		14 days	28 days
Control group		0.41±0.013	0.42±0.009
OAB		0.97±0.05†	0.89±0.017†
SUI		0.14±0.009†	0.16±0.008†
OAB pharmacocorrection	Mirabegnon	0.65±0.029*	0.66±0.026*
	Spasmex	0.87±0.031$^{\vee}$	0.73±0.026$^{\vee}$
	Quercetin	0.67±0.029*	0.61±0.023
	Mirabegnon+Testosterone	0.75±0.026*	0.67±0.032*
	Mirabegnon+Estradiol	0.62±0.028*	0.61±0.019*
	Mirabegnon+Testosterone+Estradiol	0.56±0.022*	0.57±0.021*
	Spasmex+Testosterone	0.85±0.027$^{\vee}$	0.79±0.031$^{\vee}$
	Spasmex+Estradiol	0.81±0.03$^{\vee}$	0.75±0.024$^{\vee}$
	Spasmex+ Testosterone+Estradiol	0.79±0.026	0.68±0.015*
	Quercetin+Testosterone	0.66±0.026*	0.62±0.022*
	Quercetin+ Estradiol	0.63±0.031*	0.57±0.017*
	Quercetin+ Testosterone+Estradiol	0.57±0.034*	0.48±0.008*
	Testosterone	0.76±0.027*	0.69±0.025*
	Estradiol	0.74±0.029*	0.68±0.027*
	Testosterone+Estradiol	0.71±0.03*	0.63±0.022*
SUI pharmaco-correction	Quercetin	0.2±0.013$^{\&}$	0.27±0.009$^{\&}$
	Testosterone	0.17±0.006$^{\&}$	0.21±0.012$^{\&}$
	Estradiol	0.18±0.008$^{\&}$	0.19±0.009$^{\&}$
	Testosterone+Estradiol	0.21±0.011$^{\&}$	0.3±0.014$^{\&}$
	Quercetin+Testosterone+ Estradiol	0.26±0.015$^{\&\&}$	0.33±0.012$^{\&\&}$

Note: * - $p<0.001$ compared to OAB; $^{@}$ - $p<0.05$ compared to OAB, † - $p<0.001$ compared to control; $^{\&}$ - $p<0.05$ compared to SUI; $^{\&\&}$ - $p<0.05$ compared to SUI+Quercetin,$^{\vee}$ - $p>0.05$ compared to OAB.

The connective tissue surrounding vessels was swollen with uneven edema and stained with picrofuchsin in pale pink color. The area of collagen fibers was 0.35 ± 0.025 mm^2, elastic fibers - 0.16 ± 0.03 mm^2 (Table 2). Collagen fibers gradually displaced smooth myocytes, and took up to 1/3 of the muscle layer. Elastic fibers spread along the muscular beams and vascular walls and, in most cases, presented degenerative changes associated with fragmentation and lysis (Fig. 1).

Table 2. Area of collagen and elastic fibers (mm) 2 per 1 mm^2 of bladder wall in models with lower urinary tract urodynamics disturbance during the study

Animal group / Pharmacocorrection type		Collagen fibers		Elastic fibers	
		14 days	28 days	14 days	28 days
Control group		0.14±0.013	0.15±0.011	0.05±0.007	0.05±0.04
OAB		0.35±0.025†	0.88±0.021†	0.18±0.026†	0.13±0.014†
SUI		0.24±0.037†	0.47±0.046†	0.03±0.004†	0.02±0.004†
OAB Pharmacocorrection	Mirabegnon	0.23±0.022*	0.7±0.035*	0.11±0.011$^{@}$	0.09±0.005*
	Spasmex	0.31±0.016	0.81±0.017	0.15±0.02	0.13±0.009
	Quercetin	0.21±0.02*	0.65±0.019*	0.12±0.019$^{@}$	0.08±0.005
	Mirabegnon+Testosterone	0.25±0.022*	0.64±0.022*	0.11±0.009$^{@}$	0.1±0.008
	Mirabegnon+Estradiol	0.23±0.02*	0.62±0.027*	0.12±0.011$^{@}$	0.11±0.009
	Mirabegnon+Testosterone+Estradiol	0.19±0.02*	0.58±0.019*	0.11±0.017$^{@}$	0.08±0.006$^{@}$
	Spasmex+Testosterone	0.3±0.015	0.72±0.023	0.13±0.015	0.12±0.008
	Spasmex+Estradiol	0.29±0.016	0.69±0.023	0.12±0.016	0.11±0.009
	Spasmex+ Testosterone+Estradiol	0.29±0.018	0.66±0.023*	0.1±0.013*	0.1±0.005
	Quercetin+Testosterone	0.26±0.024$^{@}$	0.61±0.026*	0.11±0.012$^{@}$	0.11±0.007
	Quercetin+ Estradiol	0.25±0.017$^{@}$	0.57±0.015*	0.11±0.015$^{@}$	0.12±0.008
	Quercetin+ Testosterone+Estradiol	0.18±0.019*	0.52±0.013*	0.1±0.011$^{@}$	0.07±0.005*
	Testosterone	0.28±0.018$^{@}$	0.64±0.016*	0.13±0.011	0.12±0.007
	Estradiol	0.25±0.017$^{@}$	0.61±0.018*	0.11±0.008$^{@}$	0.1±0.007
	Testosterone+Estradiol	0.23±0.021*	0.58±0.013*	0.09±0.007$^{@}$	0.08±0.007$^{@}$
SUI Pharmaco-correction	Quercetin	0.21±0.013	0.33±0.021	0.04±0.003	0.05±0.004
	Testosterone	0.23±0.012	0.4±0.01	0.05±0.005	0.06±0.004
	Estradiol	0.21±0.013	0.38±0.017	0.04±0.005	0.06±0.005
	Testosterone+Estradiol	0.2±0.013$^{\&}$	0.37±0.016$^{\&\&}$	0.06±0.006$^{\&}$	0.07±0.004$^{\&}$
	Quercetin+Testosterone+Estradiol	0.19±0.012$^{\&}$	0.27±0.009$^{\&}$	0.08±0.006$^{\&}$	0.07±0.005$^{\&}$

Note: * - p<0.001 compared to OAB; $^{@}$ - p<0.05 compared to OAB, † - p<0.001 compared to control; $^{\&}$ - p<0.05 compared to SUI; $^{\&\&}$ - p<0.05 compared to SUI+Quercetin.

Fig. 1. Fragmentation and focal lysis of elastic fibers in the own bladder mucosa plate. OAB, Day 14. Weigert's Resorcin-Fuchsin X 200.

The expression of Type 1 and 3 collagens was found predominantly between muscle fibers, perinuclearly in the cytoplasm, and sometimes in the nuclei of smooth myocytes and interstitial cells, suggesting a change in their differentiation towards the fibroblast variety. At the same time, the number of poorly differentiated and young fibroblasts increased. Fibers, formed by Type I collagen, were not observed continuously; usually only limited fragments appeared to be fuchsinophilic, which were characterized by an average degree of affinity with the stain.

Compared to Type 1 collagen, Type 3 collagen is presented in OAB wall structural elements much more frequently, and the content of fibrous structures was highest in the subepithelial parts of the muscle layer and interstitial tissue (Table 3). Type 4 collagen expressed mostly along the basal membranes of submucosal vessels, muscle and serous membrane in the form of a brown strip with more intense presentation of the microcirculatory bed vessels of submucous layer. The surrounding collagen fibers periodically interchanged with incompletely formed vascular walls, suggesting an incomplete angiogenesis (Fig. 2). In this case, the share of Type 1 collagen was 20.9 ± 0.82%, Type 3 - 13.2 ± 0.38% and Type 4 - 19.9 ± 0.72% (Table 3).

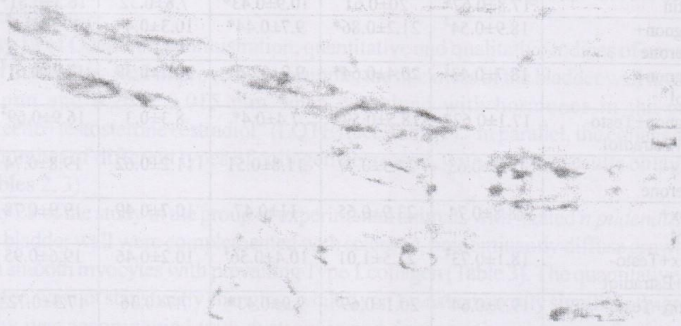

Fig. 2. Development of new blood vessels in connective tissue of submucosal wall of the bladder wall with moderate Type 4 collagen expression in the walls. OAB, Day 14. Type 4 collagen IHC reaction x 400.

After Spasmex therapy, we observed the progression of dystrophic and degenerative changes in collagen and elastic fibers, with no significant changes in the quantitative composition of different types of collagen compared to the Homviotensin group revealed ($p<0.05$, Table 3). However, in the Mirabegnon group, Quercetin and its combinations with hormonal medicines, well at current observation period, presented a significant reduction in both dystrophic changes in smooth myocytes and hypertrophy of the muscle layer, as compared with a non-treatment group (see Table 1). In parallel, the area of collagen ($p<0.001$), elastic fibers ($p<0.05$) and the quantitative percentage of Type 1, 3 and 4 collagens (Table 3) decreased.

Fig. 3. The growth of collagen fibers (red), partially interlaying the muscle layer of the bladder wall. OAB, Day 28. Van Gieson's picrofuchsin staining x 400.

Table 3. The constituent part of various types of collagen in the connective tissue of the bladder wall, as a percentage per 1 mm^2, in models with disorders of urodynamics of the lower urinary tract during the study

Animal group/Pharmaco-correction type (Ph.C.t.)		Type 1 collagen		Type 3 collagen		Type 4 collagen	
		14 days	28 days	14 days	28 days	14 days	28 days
Control group		12.4±0.77	13±0.74	4.08±0.41	4.2±0.41	6.85±0.59	5.78±0.51
OAB		20.9±0.82†	27.4±1.88†	13.2±0.38†	12.1±0.5†	19.9±0.72†	19.1±0.78†
SUI		19.4±0.88†	21.3±0.74†	12.1±0.48†	10.8±0.62†	13.3±0.83†	11.72±0.6†
OAB – Ph.C.t.	Mirabegnon	17.7±1.02	22±0.71	10.5±0.61*	9±1.04	17.3±0.86‡	16±0.66
	Spasmex	20.5±0.68	25±1.13	12.5±0.5	11.1±0.38	19.2±0.62	18.5±0.52
	Quercetin	17.8±0.67*	20±0.61	10.9±0.43*	7.8±0.32	16.3±0.51*	14.5±0.73*
	Mirabegnon+ Testosterone	18.9±0.54‡	21.2±0.86*	9.7±0.44*	10.3±0.44	17.6±0.6‡	16.5±0.71‡
	Mirabegnon+ Estradiol	18.7±0.43‡	20.4±0.64*	9.9±0.56*	10.7±0.39	17.3±0.61‡	16.1±0.91‡
	Mirabegnon+Testo-sterone+Estradiol	17.1±0.62*	18.5±0.52*	7.4±0.4*	8.3±0.3	16.9±0.69‡	15.9±0.54*
	Spasmex+ Testosterone	19.2±0.63	24.3±0.77	11.8±0.51	11.2±0.62	19.8±0.74	18.2±0.84
	Spasmex+ Estradiol	18.8±0.74	23.9±0.65	11±0.47	10.7±0.49	19.9±0.78	18.9±0.64
	Spasmex+Testo-sterone+Estradiol	18.1±0.73‡	21.5±1.01	10.4±0.56‡	10.2±0.46	19.6±0.95	17.9±0.61
	Quercetin+Testo-sterone	19.9±0.64‡	20.1±0.69‡	9.9±0.43*	7.7±0.36	17.3±0.72‡	13.6±0.47*
	Quercetin+ Estradiol	18.6±0.61‡	19.2±0.74*	10.1±0.6*	7.9±0.38	17±0.64‡	12.7±0.49*
	Quercetin+Testo-sterone+Estradiol	17.4±0.6*	16.8±0.46*	9.6±0.56*	6.4±0.3	16.1±0.5*	8.3±0.73*
	Testosterone	20.5±0.84	22.1±0.83*	10.9±0.58‡	10.7±0.59	19.1±0.79	17.3±0.81‡
	Estradiol	19.6±0.58	21.5±0.85*	10.3±0.59‡	10.2±0.53	18.2±0.67	16.9±1.05*
	Testosterone+Estradiol	17.9±0.64‡	19.2±0.74*	9.1±0.31*	9.1±0.48	17.8±0.64‡	16.3±0.57*
SUI – Ph.C.t.	Quercetin	15.1±0.5	16.8±0.77	9.7±0.61	7.7±0.59	10.1±0.64	7.7±0.59
	Testosterone	18.5±0.45	17.6±0.79$^{\wedge}$	10.4±0.63	8.8±0.35$^{\wedge}$	11.4±0.6	9.3±0.55
	Estradiol	18.7±0.51	18.3±0.78	9.9±0.48$^{\wedge}$	8.3±0.42$^{\wedge}$	11.3±0.67	9±0.52
	Testosterone+Estradiol	16.3±0.59	16.3±0.61$^{\wedge}$	8.3±0.53$^{\wedge}$	8±0.42$^{\wedge}$	10.5±0.71$^{\wedge}$	8.7±0.77$^{\wedge}$
	Quercetin+Testo-sterone+ Estradiol	14.8±0.35$^{\wedge}$	15.2±0.33$^{\wedge}$	7.6±0.6$^{\wedge}$	6.9±0.43$^{\wedge}$	8.1±0.54$^{\wedge\wedge}$	7.1±0.37$^{\wedge}$

Note: * - p<0.001 compared to OAB; $^{\#}$ - p<0.05 compared to OAB, † - p<0.001 compared to control; ‡ - p<0.05 compared to SUI; $^{\&\&}$ - p<0.05 compared to SUI nerve +Quercetin.

On Day 28 of the experiment, after administration of Homviotensin without pharmaceutical correction, an intensive formation of mature collagen fibrils occurred in the intercellular matrix due to the growth in number of mature fibroblasts, followed by development of fibrillar fiber bundles. We observed the progress in degenerative changes in the form of focal homogenization, fragmentation of collagen and elastic fibers. Collagen fibers grew predominantly in their own layer, splitting the muscle layer, and, in most cases, associated with a growth of fuchsinophilia, which could be easily visualized by Van Gieson's picrofuchsin staining (Fig. 3).

87% smooth muscle fibers were replaced with collagen ones, while the latter occupied 2/3 of the muscle layer. Qualitative changes in connective tissue, such as fragmentation, weakening of fuchsinophilia (yellow staining by the course of fibers), and fiber thickening associated with development of sclerous fields were observed along with quantitative changes.

It should be noted that sclerotic changes in the animal models receiving Spasmex progressed, unlike the Mirabegon group, where the animals presented with statistically significantly better results (see

Table 2). Administration of Quercetin and its combinations with testosterone and estradiol resulted in statistically significant decrease ($p < 0.001$) of the collagen area (0.65 ± 0.019 mm^2 and 0.58 ± 0.019 mm^2), and elastic fibers (0.08 ± 0.005 mm^2 and 0.07 ± 0.005 mm^2) at this study phase compared to Homviotensin (0.88 ± 0.021 mm^2 and 0.13 ± 0.014 mm^2, respectively), and the quantitative composition of collagens (Tables 2, 3), suggesting the antisclerotic properties of Quercetin.

Stress urinary incontinence following n. pudendus ligation. The morphological study of the bladder wall of experimental animals subjected to *n. pudendus* ligation presented opposite results compared to the OAB group. After 14 days of treatment, atrophic changes in the bladder wall were observed, which were manifested by a significant thinning of the muscular layer. As one can see from Table 2, its thickness on this stage of the study was 0.14 ± 0.009 mm compared to the control group - 0.41 ± 0.013 mm, and OAB - 0.97 ± 0.05 mm.

On Day 14 of Quercetin administration, quantitative and qualitative indices of connective tissue components of the bladder wall improved; for example, the thickness of the bladder wall muscle layer was 0.2 ± 0.013 mm and 0.26 ± 0.015 mm when combined with hormones in the group "nerve ligation+Quercetin+testosterone+estradiol" (LQTE) (see Table 1). In parallel, the collagen, elastic fiber area, and the number of different types of collagen decreased, with the best results obtained in LQTE group (see Tables 2, 3).

On Day 28 of the study in the group of experimental animals with ligated *n.pudendus*, the atrophic changes of the bladder wall were complemented with sclerotic, predominantly diffuse growth of collagen fibers between smooth myocytes with prevailing Type 1 collagen (Table 3). The quantitative composition of Type 3 collagen was not statistically changing, while Type 1 was statistically significantly growing (Table 3). The atrophy was accompanied with dystrophic and degenerative changes of smooth myocytes. Resorcinol fuchsine Weigert staining of elastic fibers in the muscular layer revealed that the elastic fibers were unevenly distributed and their number sharply reduced compared to the control group. In addition, changes to elastic fibers in the form of swelling and formation of lumps were found. Many fibers were presented in the form of grains ("granular" disintegration of elastic fibers), rods or stripes, well stained with resorcin-fuchsin in a dark blue color.

Quercetin in combination with testosterone and estradiol contributed to stabilization of smooth muscle components of the bladder wall, which was manifested by their focal hypertrophy compared to the control group, and statistically significantly inhibited the synthesis of various types of collagen (see Table 1.3), which resulted in a reduction of the area of sclerotic fields (Table 2).

Combinations of hormonal medicines presented the best therapeutic effect in both groups compared with their single administration. In this case, the efficacy may be presented in the following sequence starting from the lowest: testosterone → Estradiol → TE → Quercetin → QTE (Table 1,2,3).

Signs of connective tissue lesion (thinning of fibers, tissue swelling, loss of spatial orientation), and dilatation of blood vessel lumen were found in 81% of the studies, when the volume of fibrous tissue reached 1/3 of smooth muscle fibers in 67% and 62% of OAB and SUI cases at early stage, and 2/3 in 83 and 75%, respectively, after 28 days of the experiment.

The revealed increase in Type 3 collagen content and its superiority over Type 1 after 14 days of survey correlated with the increase in number of elastic fibers at this stage and suggested an incomplete angio- and fibrilogenesis, which could be considered as adaptive reaction to changing the functional state of the bladder as a result of Homviotensin administration and cutting *n. pudendus*. At the same time, the increased disorganization of elastic component in the stroma and blood vessels contributed to development of dyscirculatory processes in the organ. In addition, hyperlastosis might have been preconditioned with compensatory mechanisms in the event of weakening the biomechanical functions of the collagen frame. Changes in the elastic frame cause excessive stretching ability of the bladder muscular wall, which leads to deterioration of microcirculation, development of connective tissue ischemia, and increased fibrillogenesis.

The myogenic concept of OAB development suggests that the cause of bladder overactivity is a change in the detrusor myocytes associated with disruption of intercellular junctions that act as conducting pathways. Spontaneous or induced contractions of individual myocytes may result in synchronous reduction

of a significant amount of muscle cells, which leads to involuntary contractions of the detrusor [4,12]. The quantity and quality of collagen and elastin in the connective tissue is maintained by the exact balance between synthesis, posttranslational transformation and degradation [5]. In our study, we observed a decrease in the number of Type 3 collagen and elastic fibers in the muscular wall with simultaneous increase in Type 1 collagen in both groups after 28 days of the experiment, which was accompanied by activation of fibrillogenesis and led to sclerosis of the muscular wall. Replacement of Type 1 and 3 collagens with more elastic Type 4 collagen could also affect the mechanical properties of tissues [3]. Growing expression of Type 3 collagen on the background of high expression of metalloproteinases is a reflection of the processes of remodeling (synthesis and degradation) of damaged tissues, or tissues subjected to continuous mechanical load [13]. It should be noted that the completeness of tissue restoration is determined by the ratio of activity of biosynthesis processes and ECM components' catabolism. Along with the structural rearrangement of collagen fibers in our study, degenerative changes in elastic fibers (granular decomposition, fragmentation, decrease in quantity) were found. All this can lead to a significant loss of elastic properties of the tissues studied, disturbance of their tensile strength and damaging the musculoskeletal frame. In our opinion, the damage of connective tissue matrix (collagen and elastic fibers) may be a very cause of recurrent urinary incontinence. Consequently, at later stages of the disease, fibrillogenesis with sclerotic changes leads to degradation of tissue elasticity and microcirculation disturbances, which stimulates the synthesis of Type 1 collagen even more.

Therefore, the main pathomorphological changes in the bladder wall of the proposed models were manifested by structural rebuilding (remodeling) with dissociation, disorganization, and disintegration of muscle bundles, weakening or strengthening of fuchsinophilia, and fraying with deformation of surrounding structures of the muscle layer connective tissue frame, increasing immature Type 3 and 4 collagens at the early stages, and Type 1 at the late observation stages. Flavonoid Quercetin in combination with testosterone and estradiol showed a pronounces anti-sclerotic effect compared with other medicines, confirmed by a decrease in the synthesis of Type 1, 3, and 4 collagens and, respectively, the area of sclerous lesions.

CONCLUSIONS. 1. Morphological changes that occurred in models of overactive bladder and stress urinary incontinence in animals with ligation of *n.pudendus* proposed by us confirm the staging changes starting from compensatory hypertrophy of the wall with degenerative changes of smooth myocytes, violation of intercellular junctions due to growing volume of immature Type 3 and 4 collagens in the early observation period (14 days), which eventually result in depletion of adaptive properties with subsequent decompensation and sclerosis of urinary bladder wall at the late stage of the experiment (28 days).

2. The resulted models meet the requirements for evaluation of qualitative indicators in the study of morphological changes in OAB and CNC, and can be used as a baseline at preclinical stage of the studies in the future.

3. No statistically reliable evidence of Spasmex efficacy in OAB models was found in a group of experimental animals administered the medicine in combination with hormones.

4. We established a positive trend of morphological parameters at early stages (14 days) with a sole administration of Mirabegnon and especially its combination with testosterone and estradiol, while Quercetin and its combination with hormonal medicines proved to be more effective at later stages of the study (28 days).

Further study of the morphological aspects of the OAB and SUI problems will make possible to make a differentiated choice of pharmacocorrection management and will improve the effectiveness of restoring the detrusor condition, taking into account the molecular and cellular disorders.

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[1]А.И.ЯЦИНА, [2]С.В.ВЕРНИГОРОДСКИЙ, [1]Ф.И. КОСТЕВ

МОРФОГЕНЕЗ ДЕТРУЗОРА ПРИ ЕГО ГИПЕРАКТИВНОСТИ И СТРЕССОВОМ НЕДЕРЖАНИИ МОЧИ ПОД ВЛИЯНИЕМ ФАРМАКОКОРРЕКЦИИ

[1]Одесский Национальный медицинский университет Министерства здравоохранения Украины; [2]Винницкий национальный медицинский университет им.Н.И. Пирогова

РЕЗЮМЕ

Цель исследования – гисто- и иммуногистохимическая оценка количественного состава соединительнотканного каркаса стенки мочевого пузыря при стрессовом недержании и его гиперактивности до и после лечения мирабегроном, спазмексом, кверцетином и их комбинации с тестостероном и эстрадиолом.

На экспериментальных моделях гиперактивного мочевого пузыря (ГАМП) и стрессового недержания мочи (СНМ) изучены основные составляющие соединительнотканного каркаса стенки мочевого пузыря. Установлено, что мирабегрон в сочетании с тестостероном и эстрадиолом способствует стабилизации продукции коллагенов 3 и 4 типа уже на ранних сроках наблюдения в группе ГАМП, в то время как кверцетин в комбинации с тестостероном и эстрадиолом проявил себя эффективнее при более длительном применении на поздних сроках наблюдения, как при ГАМП так и при СНМ. Наряду со стабилизирующим влиянием на образование коллагеновых и эластичных волокон кверцетин имел наиболее выраженный антисклеротический эффект ($p<0,05$) по сравнению с другими препаратами. По своей эффективности при ГАМП исследованные препараты можно разместить в следующей последовательности спазмекс → мирабегрон → кверцетин, при этом следует отметить, что наилучшие результаты были получены при комбинации их с тестостероном и эстрадиолом.

ა.იაცინა, ს.ვერნიგოროდსკი, ფ.კოსტევი

ფარმა-კორექციის ზემოქმედება ზეაქტიურ დეტრუზორის მორფოგენეზისა და შარდის სტრესული შეუკავებლობის დროს

ოდესის ეროვნული სამედიცინო უნივერსიტეტი; ნ.პიროგოვის სახელობის ვინიცის ეროვნული სამედიცინო უნივერსიტეტი; უკრაინა