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THE EXPERIMENTAL MODELLING OF STRESS URINARY INCONTINENCE

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Abstract

The stress urinary incontinence (SUI), especially in women, constitutes most frequent complication of urogynecological diseases. Adequate and a stable reproducible model of SUI in white laboratory rats was created, using bilateral ligature of pudendal nerves. This guarantees a stable signs of SUI, which had progressed while the experiment duration enhanced. In accordance to the urodynamical investigations data, the leak pressure of the urine first drop in operated laboratory animals was registered; results of histological and morphometric investigations had witnessed about the ureter's diameter enhancement and the muscles tone lowering. In accordance to the investigation data, the ureter's and its sphincter denervation was stable, and the model application had permitted to obtain stable reproducible results.

The received model meets the requirements for qualitative indicators in the study of morphological changes in hyperactive urinary bladder model and can be used as a basis in the preclinical stage of research. The results of pathomorphological studies with revealed peculiarities of rearrangement of collagen fibers, degenerative changes of the elastic in the

target organ, taking into account the anatomic-functional orientation of action, form the basis for further clarification of the mechanisms of occurrence and determination of the SUI recurrence.

Key words: stress urinary incontinence, experimental modeling, rats, urodynamics.

Urinary incontinence, especially in women, is an urgent problem all over the world. The most common type of urinary incontinence is stress urinary incontinence (SUI), or urinary incontinence during the exercise, its frequency is about 50%, the frequency of urgent and mixed urinary incontinence - 14 and 32%, respectively. The SNS is a multifactorial disease, its etiology and pathogenesis is not well understood, so it is very difficult to reproduce its adequate experimental model [1].

Most authors point to the prevalence of stress urinary incontinence (SNA) among women under 50 years of age, after 60 - dominated by mixed. Its frequency in menopause is 8-27% [2-4]. The situation is complicated by the fact that various types of urinary disorders are combined with a complex of adverse vaginal symptoms, which causes the "urogenital syndrome". It can be traced firstly both atrophic and dystrophic processes interaction in the estrogen-dependent structures of the structures: the bladder, urethra, vagina, the small pelvis and pelvic ground muscles [5, 6].

The experimental trials should be provided according to the following items. The first aspect is the sensory activity of the lower urinary tract, which includes the concept of afferent signaling, including signal transmission and afferent traffic, signal transmission, sensitization and conscious perception. Nerve endings of the lower urinary tract are densely concentrated under the urothelium. In this place, they undergo the action of mediators released from the urothelium [7], cellular effects and the action of cytokines [8]. The second aspect is the motor control due to the hyperactivity of the muscle – urinary bladder emptying (MUBE) in many patients with overactive bladder syndrome. The motor function includes the processes that cause the MUBE reduction. Many changes in the properties of smooth muscle of the bladder in overactive bladder syndrome and MUBE activity are described, on the basis of which the "myogenic hypothesis of MUBE activity" is proposed. This confirms the thesis that hyperactive contractions of MUBE arise due to increased excitability and the spread of contraction in the muscle [9].

This process can be further modified by efferent nerve fibers, interstitial cells, and local mediators [10]. This condition is normal when urinating, however, it should not be manifested in the placement of urine, as the CNS inhibitory effects suppress it.

The third aspect is the reflexes of the lower urinary tract; since urinary retention in the urinary bladder requires inhibition of MUBE [11], it can be assumed that the activation of the urinary bladder motility or its emptying results in hyperactivity in the MUBE or the involuntary allocation of urine with an imperative urge. The motor activity of the reflexes of the lower urinary tract is based on the ascetic providers of sensory information, integrated at different levels of the central nervous system [12], and is sensitive to stress factors.

SUI incidence and statistical data justifies the need for research using appropriate animal models. One of these models has been developed and used by us in our research.

Materials and methods

The experimental white rats during the trials were divided into two groups. In the control group, only the cut of the skin on both sides of the large skeletal muscle was carried out on the rats, after which the skin wound was sewn.

N.n. pudendi were bandaged on both sides under anesthesia with the aim of SUI modeling. Anesthesia during surgery was performed by 5% thiopental (0.2 ml) and 1% propofol (0.4 ml) i.p. administration. After skin dissection over the large sciatic nerve along the perimysium the muscle was extracted until the sciatic nerve visualization. Its branches (of the n. pudendus) were bandaged.

The wound was seamed layer by layer. All animals have undergone operative intervention satisfactorily.

We carried out the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (2006), the Guidelines for the Core and Use of Laboratory Animals (National Academy Press, Revised, 1996) and the American Heart Association's Guidelines for the Use of Animals in Research" during the experiment.

Before the SUI modeling and 14 and 30 days after the model reproduction in rats, the urinary bladder pressure required for the first urine droplet (LPP - Leak Point Pressure) was recorded. Before the study, animals were given anesthesia, as with surgical intervention. In the bladder, a catheter RE 50 was injected through the urethra, filled with water. The volume of the bladder was considered to be the one in which urination occurred after the introduction of the maximum volume of water. This indicator is needed to further determine the volume of fluid in measuring LPP.

The leakage pressure of the first drop of urine was determined by administering a fluid equal to half the volume of the bladder. Gently pressed on the anterior abdominal wall, recorded pressure, which was allocated the first drop of urine.

Six LPP measurements were sequentially performed. Urodynamic studies were conducted according to a specially developed system "Uro-Pro", which included such devices.

1. Pressure meter of the multi-channel device "Rhythm" (NVP Saturn).
2. Information processing unit.
3. Pump peristaltic SR15 firm Thomas.
4. The catheter draining module for urethra and sphincter profilometry (Patent of the Ukraine N72059, 10.08.2012).
5. Mobile rack with data logger.
6. A set of sensors collected in the measuring system.

Special software is developed for registration, processing and printing of the received data. The installation menu uses the standard terminology, ICS (International Continente Society), which meets international requirements. The installation was made in accordance with the safety standards for medical electrical equipment IEC 601-1 type BF, which provided a special degree of protection against electric shock.

Urodynamic studies confirmed the creation of a SUI model after 14 days, in 2 weeks and 1 month the animals of both groups were withdrawn from the experiment by overdose of 5% solution of thiopental-sodium. Allocated the bladder together with the urethra, macroscopic preparations were fixed in 10% formaldehyde solution for 24 hours, the preparations were prepared according to the generally accepted method. Preparations of transverse sections of the urethra thickness of 5 - 7 microns were stained with hematoxylin and eosin, picrofuxin for van Gizon, using the Schiff-iodine acid (SIA) method for McManus.

Microphotographs were obtained using a light-optical microscope Leica ICC50 HD, morphometric studies were performed using the Paradis Image Analysis Software.

For SUI model confirmation according to the morphometric study, the area of the outer perimeter of the circular layer of the urethral muscle was determined. The index of the urethral lumen area (IS_{int}) as ratio $S_{int}/S_{urethr} \times 100$ was calculated, and it was evaluated as a morphometric indicator relevant to the SUI model.

The area of rats urethra was measured within the outer perimeter of the circular layer of the muscle, the boundaries of the adventitious membrane were difficult to determine, so it was not taken into account. The estimation of the ratio of these two indicators according to the morphometric study allowed to level out the measurement errors that arose in obtaining

cross sections of the urethra as a result of deformation during fixation and consolidation. The use of such an indicator as the area of the urethral lumen (IS_{int}) made it possible to obtain more reliable data.

Results and their discussion

LPP indexes in the control group rats did not differ significantly from those in intact animals. In animals of the main group, after 14 days after the creation of the model, they decreased by 28.6%, after 30 days - by 39.7%. According to histological studies, in the rats of the control group, the lumen of the urethra is predominantly slit-shaped (Fig. 1), its area, with increasing duration of observation, probably did not change.

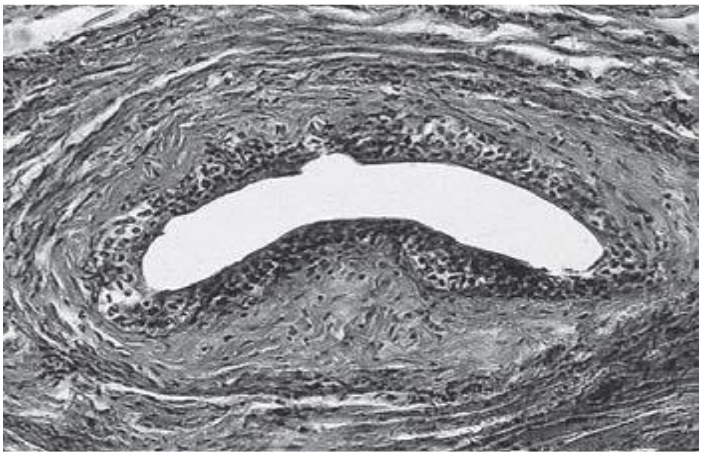

Fig. 1. Microfoto. Rats urethra (control group) after 14 days. Hematoxylin and eosin colour. Increase x120

Animals of the main group, after 14 days of reproduction of the model, were taking out of the trials, the histological and morphometric studies were performed. According to histological studies, pathological changes in both groups of animals were not detected, however, in rats of the main group, there was a slightly increased desquamation of the epithelium from the surface of the epithelial plate of the mucous membrane, a moderately expressed atony of the muscle of the bladder and urethra without its pronounced thinning.

In the urethra, the formation of deep folds of the mucous membrane (Fig. 2), an increase in the area of the lumen compared with that in the control was revealed, as evidenced by an increase in the diameter of the equivalent circle of the urethra lumen by 1.4 times.

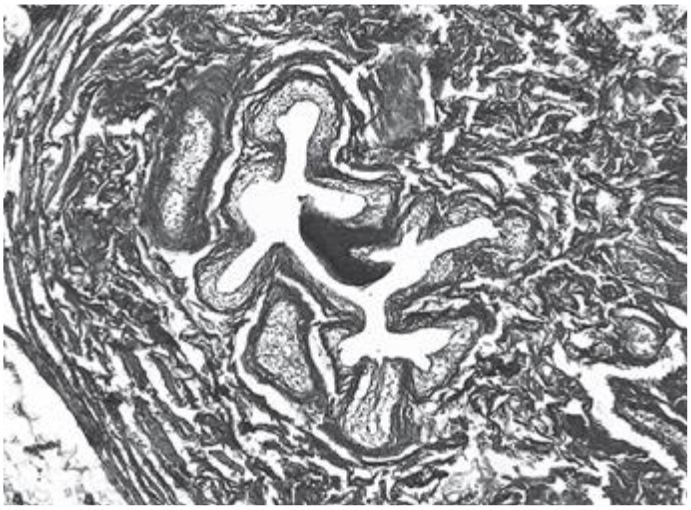

Fig. 2. Microfoto. Rats urethra (the main group), 14 days after the model induction. Picrofuscin for van-Gizon colour. Increase x 100.

After 30 days in the animals of the main group, an increase in the area of the urethral lumen was observed, the muscle atony progressed, as evidenced by an increase in the urethra (Fig. 3), the epithelial plate of the mucous membrane on some sites was grown both due to loss of tone and increased cell desquamation.

Urethral IS_{int} in animals of the main group, after 14 days of reproduction, the model was 19.73, in the control group - 7.93; after 30 days - 26,17, that is, its increase by the 14th day and preservation of the tendency to increase in the future testified to the receipt of reproducible results.

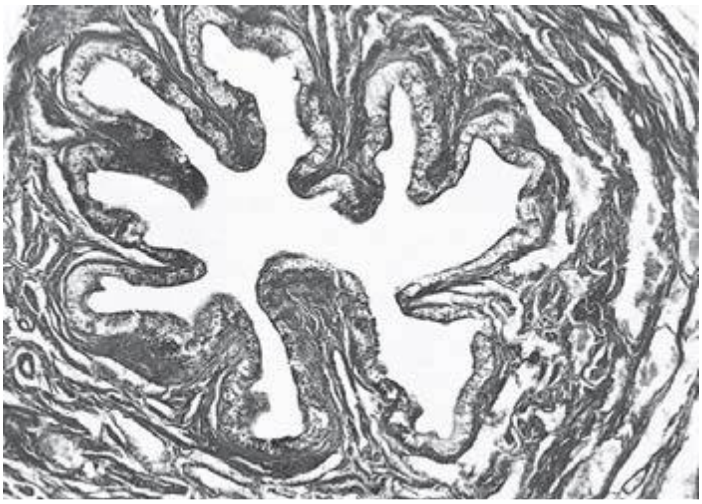

Fig. 3. Microfoto. The rats urethra (the main group), 28 days after the model induction. SIA-reaction. Increase x100.

To summarize, it should be noted that signs of disorganization of connective tissue (thinning of fibers, swelling of the tissue, loss of spatial orientation), dilatation of the lumen of the blood vessels is established in 81% of cases. At the same time, the volume of fibrous tissue reached 1/3 of smooth muscle fibers in 67% and 62% of cases with overactive bladder syndrome and SUI at an early stage and 2/3 in 83% and 75% in 28 days of the experiment.

The more intense increase in the content of collagen type 3 than type 1 after 14 days of observation correlated with the growth of elastic fibers. This indicated an unfinished angiopathy and fibrilogenesis, which could be considered as an adaptive reaction to a change in the functional state of the bladder as a result of the experiment. At the same time, the strengthening of the disorganization of the elastic component in the stroma and vessels contributed to the development of circulatory processes in the body. The hyperelastosis additionally may be due to compensatory mechanisms with the relaxation of the biomechanical functions of the collagen framework. Changes in the elastic framework cause excessive stretching of the muscular wall of the bladder, which leads to deterioration of the microcirculation, the development of connective tissue ischemia, and increased fibrilogenesis.

It should be noted that the value of tissue restoration is determined by the ratio of the activity of the extracellular matrix components both biosynthetic and catabolic processes. Along with the structural rearrangement of collagen fibers in our study, degenerative changes of elastic fibers (their granular decomposition, fragmentation, decrease in quantity) were detected. All this can lead to a significant loss of elastic properties of the tissues studied, breaking their tensile strength and damaging the musculoskeletal framework. In our opinion, damage to the connective tissue matrix (collagen and elastic fibers) may be the cause of the development and occurrence of relapses of urinary incontinence.

Thus, the objective data obtained confirming the achievement of experimentally stress urinary incontinence in rats provides evidence of further research as the basis of which this model will be used.

The results of pathomorphological studies with revealed peculiarities of rearrangement of collagen fibers, degenerative changes of the elastic in the target organ, taking into account the anatomic-functional orientation of action, form the basis for further clarification of the mechanisms of occurrence and determination of the recurrence of the SNA.

Conclusions

1. The SUI model reproduction through n.n. pudendi bandaging one should register the urodynamic indexes consistent decrease and corresponding urethral morphological and morphometric changes.

2. The SUI model is adequate, reproducible,cheap and can be used during preclinical studies to develop the SUI modern methods of treatment And prophylaxis.

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