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THE EXPERIMENTAL OVERACTIVE BLADDER MODELLING

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Abstract

An experimental study was conducted, which resulted in the development of a hyperactive urinary bladder model. Animals of the main group administered once daily for 14 days i.p. 0.3 ml of the hombiotensin, containing 0.45 mg of reserpine. This model development is confirmed by histological studies results. Morphological changes that arose in reproduction of the model of hyperactive bladder are determined by the following stages. Namely: compensatory hypertrophy of the wall with degenerative changes in smooth myocytes, disturbances of intercellular bonds due to the increase of immature collagen type 3 and type 4 in the early stages of observation (14 days), eventually leads to the depletion of adaptive capabilities with subsequent decompensation and sclerosis of the bladder wall at a late end of the experiment (28 days).

The obtained 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.

Key words: hyperactive urinary bladder, experimental modeling, rats, hombiotensin, degeneration, collagen.

According to the International Society for Urinary Disorders [1], overactive bladder syndrome (OBS) is an imperative urge to urinate with involuntary urinary excretion or without such an increase, usually with an increase in the frequency of urination during the day and night, in the absence of a diagnosed infection or explicit pathological changes.

The incidence of OBS is 12-23% [2]. It is believed that urinary incontinence may be due to the relative weakness of the bladder neck and the outer muscle - the urethra closure in women. Also, a significant increase in the incidence of OBS in women with an age is noted: from 2% - in patients aged 18-24 years, to 19.1% - from 65 to 74 years.

OBS significantly impairs the quality of life of patients; with this symptom of imperative urges to urinate has the strongest effect. According to a large study conducted in the United States, the United Kingdom and Sweden, the symptoms associated with a violation of the urine deposit have a greater impact than other symptoms of lower urinary tract damage [3]. In addition, the treatment of OBS requires significant financial costs. The estimate of population expenditure depends on the accuracy of the data on the distribution of OBS . The average annual cost per patient with OBS in the United States in 2000 was US $ 1,925, on a national scale - tens of billions of dollars [4].

The main symptom of OBS is the imperative urge to urinate, in which the patient suddenly has an irresistible urge to empty the bladder, which can not be postponed [1]. The imperative urge to urinate should not be confused with the strong desire to empty the bladder, which is, in essence, a normal sensation that occurs when the functional volume of the bladder reaches. Patients often express anxiety about the "leakage" of urine with compelling appetites for urination. This "fear of leakage" and "fear of pain" distinguishes patients who have imperative urge to urinate with OBS and with pain syndrome of the bladder [5].

In the OBS , the imperative urge for urination, patients usually feel in the perineum, at the base of the penis or vagina / urethra. The pain of the bladder syndrome is characterized by pain over the pubic, although the patient also experiences some discomfort in the region of the perineum (urethra/vagina/penis) [6]. Increasing the frequency of daytime urination is a complaint of patients who think that urination is occurring too often during the day [1]. This standardized definition does not include the minimum amount of urination, since most observations have no significant differences in the functioning of normal and hyperactive bladder in terms of the objective frequency of urination. At present there is no evidence base

that would help establish a threshold for determining the high frequency of urination during the day.

OBS is a symptomatic diagnosis. In contrast, the hyperactivity of the bladder emptying muscle is determined from the data of urodynamic observation; it is characterized by spontaneous contraction of the muscle during the filling phase, which can be spontaneous or provoked [1]. These terms can not be interchangeable, since in patients with OBS, according to the urodynamic study, no hyperactivity of bladder emptying muscle is detected.

To understand the pathophysiology of OBS, it is necessary to create experimental models for animals, but, given the subjectivity of the symptom complex, the interpretation of results in animals needs special attention. Since imperative urges for urination in animals can not be determined, mediated factors were analyzed, in particular, changes in the site of urination, the allocation of urine without reducing the bladder. No experimental model can fully reflect the subjective, objective and related factors inherent in a particular clinical situation. The models were reproduced in rats with partial obstruction of the bladder outlet, with spontaneous hypertension, hyperlipidemia, various neurological lesions and knockout of some genes [7]. The search for the most effective models continues.

We conducted our own study, which resulted in the development of a hyperactive bladder model.

Materials and methods

Experiments on OBS model reproduction were performed on sexually mature white laboratory female rats, body weight 300 g. Animals were divided into two groups. Rats of the control group were i.p. injected of 0.3 ml of sterile isotonic sodium chloride solution for 14 days.

Animals of the main group received hombiotensin, once daily for 14 days, i.p., 0.3 ml, containing reserpine (0.45 mg). The tablet was triturated under sterile conditions and dissolved in a isotonic solution of sodium chloride. The components of the drug normalize intracardiac hemodynamics, myocardial contractility, arterial tone, renal function, cardioprotective effect, which provides a better course of the experiment and reduces the mortality of animals. The reproduction of the model is confirmed by the results of histological studies.

During work with laboratory animals, they complied with the requirements of "Scientific and practical recommendations for the maintenance of laboratory animals and work with them", State Pharmacological Center Health Care Ministrty of Ukraine (Protocol No. 8 from 22.06.2012).

7, 14, 21, 28 days of animals were withdrawn from the experiment by overdose of 10% solution of sodium thiopental, bladder removed, fixed in 10% formaldehyde solution for 24 hours, dehydrated in alcohols of increasing concentration, clarified in chloroform and sealed in paraffin.

Sections with thickness of 5 - 7 microns were stained with hematoxylin and eosin, picrofusin for van Gizon. To evaluate the functional activity of the tissues, a Shiff-iodine acid (SIA) method was used as for McManus. The preparations were photographed and studied using a light microscope Leica ICC50 HD.

The data obtained were verified statistically.

Results and their discussion

In order to study the functional state of the detrusor in experimental animals prior to the modeling of GASM, as well as its features after, the cystotonometric indices were evaluated in the comparative aspect (Table 1).

Table 1

Indicators of cystometometry in experimental animals (M±m)

Data / Groups	Basal pressure (cm H_2O)	Amplitude (cm H_2O)	Frequency of contractions per 10 min	Duration of contractions, min
Control, n=10	2,15±0,22	28,2±0,17	4,96±0,36	2,25±0,28
OBS , 14 day	5,19±0,37*	50,66±2,1*	10,35±0,65*	4,15±0,17*
OBS , 28 day	4,95±0,15	48,79±0,8	8,1±0,17	3,9±0,27

Note: * - p<0,001, the significant difference compared with the same indexes in the control group.

Table 1 shows that baseline pressure increased by 2.4 times (p<0.001) to 5.19±0.3 cm H_2O relative to the control group at 14 days after the OBS reproduction. amplitude of reductions in 1,8 times to 50,66±2,1 cm H_2O (p<0,001), their frequency in 10 minutes. twice as much as 10.35 ± 0.65, and their duration per minute (from 2.25±0.28 to 4.15±0.17, p<0.001) increased in practically the same number (1.8 times). It should be noted that the corresponding indices of cystotonometry in the group of experimental animals on the 28th day of the simulated OBS did not have a statistically significant difference with the previous values. Thus it became possible to prove the effectiveness and stability of the result of the experiment.

In animals in the control group, the condition of the bladder did not change throughout the experiment period, the model was reproduced already at the 14th day, although at the beginning of experimental studies, the drug was administered within 30 days.

According to histological studies, the epithelial plate of the mucous membrane of rats is represented by a transitional epithelium consisting of 6 to 8 rows of epithelial cells, and its own plate of the mucous membrane is moderately dense. The mucous membrane and submucosal layer formed numerous shallow folds of moderate width. Changes in the muscle showed its moderate tone. The adventitious membrane is formed by a loose connecting cloth, without any features.

The transition epithelium was located on its own plate of the mucous membrane, which separated it and the muscle. The latter dominated collagen fibers and encountered single elastic fibers (Fig. 1).

At immunohistochemical examination of the walls of the urinary bladder animals, the control group of elastin and collagen type I and type III was found predominantly in their own plate and, to a lesser extent, in the detrusor itself (Fig. 2). specific values of collagen type I for 14 days were 12.4 ± 0.77, type III 4.08 ± 0.41, and no probable changes remained.

After 7 days, in the rats of the main group, marked dystrophic changes in the epithelial plate, its local hypertrophy, and the consolidation of its own plate of the mucous membrane were observed (Fig. 3). Sealing the stroma of the muscle and significant increase in the tonus of the smooth muscle cells were noted.

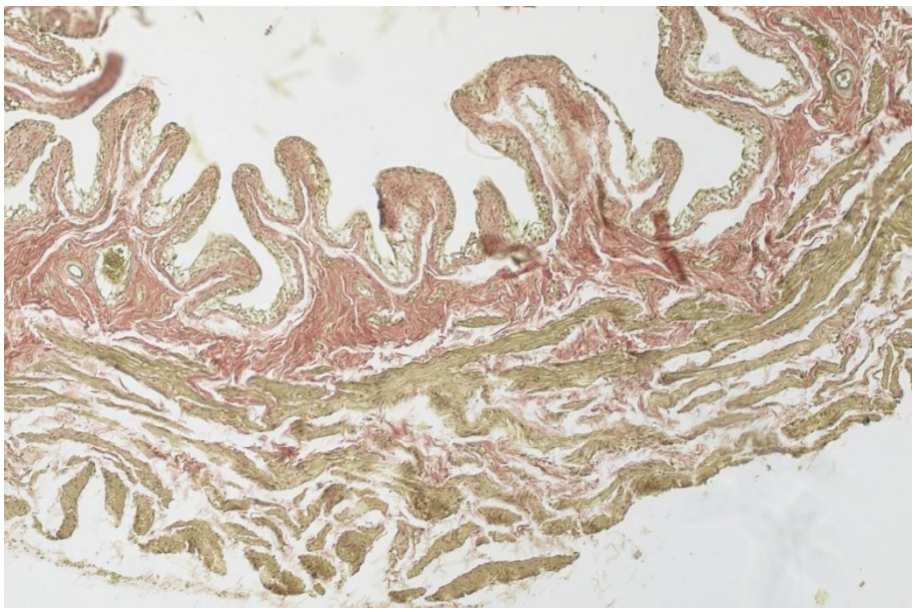

Fig. 1. The advantage of collagen fibers in the submucosal mucous membrane of the urinary bladder. Control group. van Gizon coloring. Increase x100.

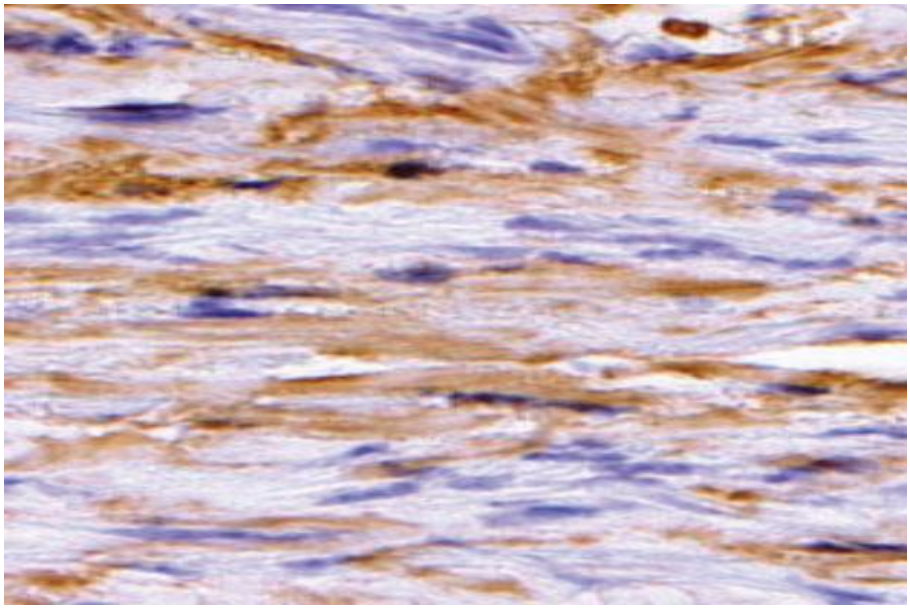

Fig. 2. Type I collagen in the muscle wall of the bladder. Control group. Immune-histochemical marking by the first type anticollagen antibody. Increase x 400.

At the 14th day in animals, atrophic changes in the epithelial plate of the mucous membrane were observed, and the desquamation of its cells increased (Fig. 4). In some small areas, the actual plate of the mucous membrane is almost naked, the density of blood vessels in it is reduced.

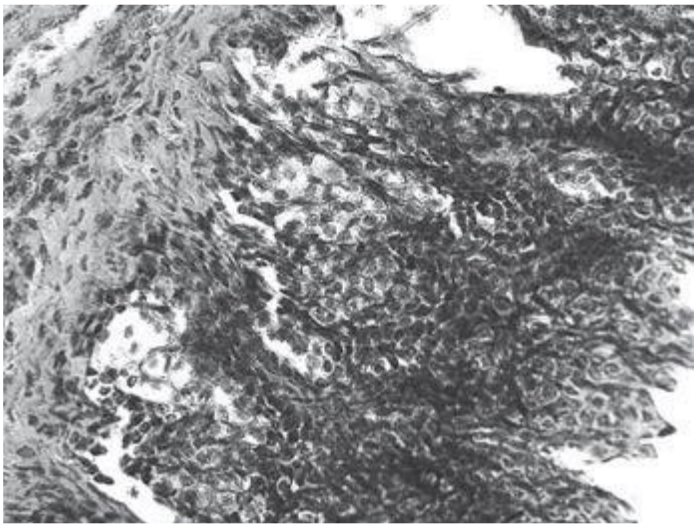

Fig. 3. Microfoto. The wall of the urinary bladder of the rat. Mucous membrane epithelial plate hypertrophy, the 7th day. Hematoxylin and eosin colour. Increase x400.

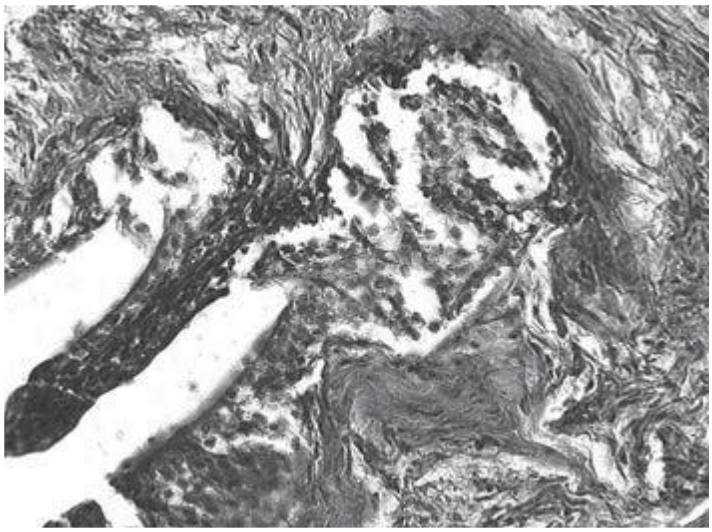

Fig. 4. Microfoto. Dystrophic changes in the epithelial plate of the mucous membrane of the bladder, the area of desquamation. The 14th day. SIA-reaction. Increase x 400.

The mucous membrane and submucosal layer formed long narrow folds as a result of pronounced contraction of muscle smooth muscle cells.

After 21 days in the bladder, dystrophic changes in the epithelial cells, sealing of the own plate of the mucous membrane, edema of collagen fibers were observed. Ischemic changes and edema were detected in the smooth muscle cells of the muscle.

At the 28th day, dystrophic changes in the epithelial plate of the mucous membrane were noted. An increase in the desquamation of epithelial cells in some areas caused a significant thinning of the plate, sometimes up to the complete removal of cells. The mucous membrane and submucosal layer formed wide short folds, the seals of the own plate of the mucous membrane were observed; in it and in the submucosal layer, venous plethora, edema was noted. In the muscle there were signs of increased tone of myocytes. Progressed degenerative changes in the form of focal homogenization, fragmentation of collagen and elastic fibers.

At this time, the area of collagen fibers in conditions of OBS model significantly increased to 0,88±0,021 against 0,35±0,025 per 14 days and 0,15±0,011 in the control group. At the same time, the area of elastic fibers significantly decreased (0.13±0.014 vs. 0.18±0.026, respectively). Collagen fibers developed predominantly from the side of their own plate, penetrating and straightening the muscular layer, with their fuscinophilia increasing in most cases, which was clearly noticeable when stained with picrofuxin for van Gyzon (Fig. 5).

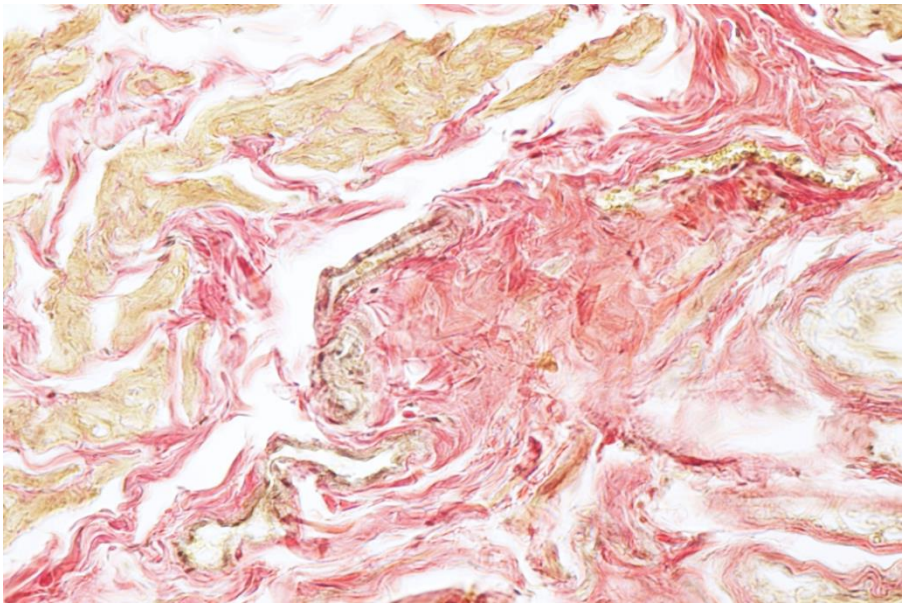

Fig. 5. Growth of collagen fibers (red color), which sometimes splits the muscular layer of the wall of the bladder. OBS , 28 days. van Gizon colour. Increase x400.

The hombiotensin injection to rats starting with the 7th day of the trial caused dystrophic changes in the epithelial plate of the mucous membrane. Since the 14th day, the desquamation of epithelial cells has increased. In the early stages of observation, signs of hypertrophy predominated, in the future (from the 14th day), mostly atrophic changes were noted. The proper plate of the mucous membrane is compact, edematous. The increase in tone of the muscle was stable and maintained until the end of the experiment, in addition, in the muscular area, areas of ischemic changes were observed.

Thus, an excessive tonus caused a violation of trophics, which most manifested itself in pathological changes in the mucous membrane and muscle.

Morphological changes that arose in reproduction of the model of hyperactive bladder are determined by the stage. Namely: compensatory hypertrophy of the wall with degenerative changes in smooth myocytes, violation of intercellular bonds due to the increase of immature collagen type 3 and type 4 in the early stages of observation (14 days), eventually leads to the depletion of adaptive capabilities with subsequent decompensation and sclerosis of the bladder wall at a late end of the experiment (28 days).

The obtained model meets the requirements for qualitative indicators in the study of morphological changes in OBS and can be used as a basis in the preclinical stage of research.

Conclusions

1. OBS is a common condition that significantly affects the quality of life of patients. Differential diagnosis of OBS requires the elimination of severe pathological conditions and other important factors, each treatment option has its own limitations that need to be kept in mind to avoid serious problems.

2. The development and use of an appropriate experimental model will allow solving significant practical as well as theoretical issues.

3. The proposed hyperactive bladder model proved to be effective, reproduced in all animals of the main group.

4. The model for qualitative estimations of structural changes in the future can be used as a basis for preclinical studies of the effectiveness of treatment of patients with disorders of urination

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