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Research paper

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Immunohistochemical evaluation of the effect of neurotrophic factors on the bladder of rats with infravesical obstruction after administration of biologically active compositions

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Abstract. *The present study aimed to investigate the immunohistochemical characteristics of the bladder (UB) of rats with infravesical obstruction (IVO) after intraperitoneal administration of biologically active compositions (BACs) (conditioned media (CM) of native and cryopreserved cultures of mantle gliocytes (MG) obtained from the spinal ganglia (SG)).*
Methods. IVO was reproduced by surgical ligation. BACs were intraperitoneally injected for 10 days. The relative area of expression of S100 protein and actin, as markers of bladder nerve and smooth muscle cells, was calculated. The experimental animals were divided into groups as follows: 1 - intact control (n=15); 2 - CM from the culture of native MG (n=7); 3 - CM from the culture of cryopreserved MG (n=6); 4 - animals with IVO without treatment (n=15).
Results. BACs obtained from the culture of native and cryopreserved MG were involved in the remodeling of the bladder structure, which changed during IVO. Visual assessment of the UB tissue specimens subjected to immunohistochemical labelling with antibodies to S100 protein and actin revealed an increase in the relative area of positive labelling in animals of groups 2 and 3 compared to group 4 (untreated). Statistical analysis demonstrated an increase in the relative area of expression of the studied markers of nervous and muscle structures by both indicators in animals of group 2 by 91.6% and 78.9% (p = 0.004; p = 0.002, respectively) compared to group 4 (without treatment). A somewhat different trend was observed when comparing the results of group 3. A statistically insignificant increase in the relative area of S100 protein expression in the UB tissues (p>0.05) and a significantly higher expression of actin by 78.8% (p=0.001) were found compared to the untreated group.
Conclusions. The positive effect of neurotrophic factors contained in the secretions of native/cryopreserved SG cell cultures on the expression of immunohistochemical markers of nerve and smooth muscle cells of the bladder in the course of IVO in rats has been found. It has been determined that cryopreservation affects the nature of the biological activity of the secretions of SG cell culture. At the same time, the administration of CM of cryopreserved culture has been proven to be effective in correcting the effects of IVO and similar to the effect of CM of native culture.

Key words: *immunohistochemical markers, S100, actin, protein expression, infravesical obstruction, spinal ganglion, mantle gliocytes, conditioned medium.*

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Імуногістохімічна оцінка впливу нейротрофічних факторів на сечовий міхур щурів з інфравезикальною обструкцією після введення біологічно активних композицій

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Резюме. *Мета роботи — вивчення імуногістохімічних характеристик сечового міхура (СМ) самок щурів із інфравезікальною обструкцією (ІВО) після внутрішньочеревного введення біологічно активних композицій (БАК) (кондиційованих середовищ (КС) нативної і криоконсервованої культур мантійних гліоцитів (МГ), отриманих зі спінальних гангліїв (СГ)).*

Методи. ІВО моделювали лігатурним методом. БАК вводили тваринам внутрішньочеревно протягом 10 діб. Розраховували відносну площу відносної площі експресії білку S 100 та актину, як маркерів нервових і гладеньком'язових клітин сечового міхура. Тварин поділили на групи: 1 — інтактний контроль (n=15); 2 — КС від культури нативних МГ (n=7); 3 — КС від культури кріоконсервованих МГ (n=6); 4 — тварини з ІВО без лікування (n=15).

Результати. БАК, отримані від культури нативних і криоконсервованих МГ, брали участь у ремоделюванні структури сечового міхура, яка змінювалась під час ІВО. За візуальною оцінкою препаратів тканини СМ, які було піддано імуногістохімічному міченню антитілами до білку S100 та актину, встановлено збільшення відносної площі позитивного мічення у тварин груп 2 і 3 порівняно з групою 4 (без лікування). Шляхом статистичного аналізу було встановлено збільшення відносної площі експресії досліджуваних маркерів нервових і м'язових структур за обома показниками у тварин групи 2 на 91,6% та на 78,9% (p = 0,004; p = 0,002 відповідно), порівняно з групою 4 (без лікування). Дещо інша тенденція була при порівнянні результатів групи 3. Встановлено статистично не значуще збільшення відносної площі експресії у тканинах СМ білку S100 (p>0,05) та значно більші показники експресії актину на 78,8% (p = 0,001), порівняно з групою без лікування.

Висновки. Встановлено позитивний вплив нейротрофічних факторів, які містяться у секретомах нативної/кріоконсервованої культур клітин СГ на експресію імуногістохімічних маркерів нервових і гладеньком язових клітин сечового міхура при ІВО у щурів. Визначено, що кріоконсервування впливає на характер біологічної активності секретомів культури клітин СГ. При цьому введення КС кріоконсервованої культури було ефективним для корегування наслідків ІВО і схожим на вплив КС нативної культури.

Ключові слова: *імуногістохімічні маркери, S100, актин, експресія білків, інфравезікальна обструкція, спінальний ганглій, мантійні гліоцити, кондиційоване середовище.*

Introduction. Urodynamic disorders due to infravesical obstruction (IVO) are an urgent problem in modern urology. More than 50% of elderly men are predisposed to IVO due to benign prostatic hyperplasia (BPH) [1]. IVO occurs in 8-10% of women as a complication of surgical treatment of urinary incontinence [2].

In conditions of prolonged chronic bladder (UB) obstruction and increased urethral resistance, blood circulation is impaired, and cell ischaemia, detrusor hypertrophy, and inflammatory processes may occur. One of the consequences of the structural and functional restructuring of cell layers is the loss of the receptor apparatus and denervation of the bladder wall, which can be more than 50% in this pathological condition [3].

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IVO causes multiple physiological and structural changes in smooth muscle (SM), transitional cell epithelium, nerves, and vasculature. Ultimately, this can lead to a loss of smooth muscle function and contractility, or cause progressive severe hypertrophy and increased contractility of the UB [4, 5].

It has also been morphologically proven that outflow obstruction causes neuronal degeneration (partial denervation) in the smooth muscle of the UB [6, 7, 8]. The loss of functional synapses can reduce the release of contractile neurotransmitters and thus reduce the magnitude of the bladder contractile response [9]. Studies using experimental animals have also shown that the presence of an obstruction to urine outflow affects the nervous control of the UB activity [3, 7, 10, 11]. IVO is usually accompanied by a decrease in the innervation density of the hypertrophied bladder [12]. IVO changes neural networks in the central nervous system, which can cause organ dysfunction [6, 10, 13, 14].

At present, reinnervation of the urethra is an urgent task in urology, reconstructive surgery, and tissue engineering of the urinary tract. According to current data, cultures of cells of neural origin, in particular Schwann cells and nerve cells obtained from various sources, are used to solve such problems [15, 16].

It is known that glia derivatives are able to produce a number of neurotrophic factors (NFs) that promote neuronal regeneration and stimulate the directed growth of nerve cell axons [17, 18]. This is valuable for the reinnervation of the UB and restoration of the pool of nerve endings in the bladder wall.

Currently, research is being conducted on the cultivation and cryopreservation of spinal ganglion (SG) cell culture and the generation of cell-free media containing NFs [19, 20]. However, there has been no attempt so far to use SG cell culture, secretions, and other biological products to restore the UB receptor pool and improve its contractility in IVO.

The research aims to investigate the effect of biologically active compositions (conditioned media of native and cryopreserved cell cultures) obtained from spinal ganglia on the expression of S100 proteins and actin in the bladder of rats with infravesical obstruction.

Materials and Methods. Investigations were carried out on 6-month-old white outbred rats (250–320g, n=43). Manipulations in animals were performed in accordance with the Law of Ukraine 'On Protection of Animals Against Cruelty' (No. 3447-IV dated 21.02.2006) in compliance with the requirements of the Committee in Bioethics of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Kharkiv), in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Study design. The culture of mantle gliocytes (MG) was obtained from the SG of neonatal piglets by an enzymatic method [19]. The initial seeding concentration of cells in both cases was 6×10^4 cells/cm^2. On the 21st day of cultivation, the medium was collected from all Petri dishes, and combined; an aliquot was taken and used in further experiments. Part of the SG cell cultures after cultivation for 6 days was detached from the substrate with 0.25% trypsin-EDTA solution with Hanks salts (Biowest, France) and cryopreserved as a suspension in cryoprotective medium with 10% dimethyl sulfoxide (DMSO) with a cooling rate of 1 °C/min to –40 °C and further immersion in liquid nitrogen. The cells were kept at –196 °C for 1 month, after which they were thawed, washed from DMSO, and cultured for 20 days. On day 21, the culture medium was collected from all samples, combined; an aliquot was taken and used in further experiments. IVO was modeled by the ligature method [21]. After 1.5 months, the ligature was removed and the next day the animals were started to become injected with the BACs intraperitoneally for 10 days. Conditioned medium obtained from native and cryopreserved MG cultures were injected with 0.6 mL/kg of body weight. The animals were taken out of the experiment by decapitation under anesthesia on the 56th day from the start of IVO modeling. The animals were divided into groups: 1-control (C); 2-the introduction of conditioned medium from the culture of native MG (CMCNMG); 3-introduction of conditioned medium from the culture of cryopreserved MG (CMCCMG); 4-without treatment (UT).

Detection of S100 and Actin expressions in the urinary bladder. A Rady-to-Use test kit with monoclonal antibodies (MAbs) against Smooth Muscle Actin 1A4 (DAKO, Denmark) was used as a marker of bladder muscle structures. As a marker of peripheral glia cells, MAbs against S-100A protein (Thermo Scientific, Germany) at 1:100 dilution was used. To visualise the immunohistochemical staining with the first antibodies, the UltraVision Quanto Detection Systems HRP Polymer (Thermo Scientific) was used. DAB (diaminobenzidine) was used as a chromogen.

Stained sections were examined using a Primo Star microscope (Carl Zeiss) with AxioCam software (ERc 5s).

The qualitative assessment of immunohistochemical staining was determined by the presence or absence of brown staining of cellular structures [22].

For quantitative evaluation, the relative area of positive labelling was calculated using AxioVision Rel 4.7 image processing software as the ratio of the total area of stained structures (in μm2) to the standard section area (in μm2) multiplied by 100% according to the method [23].

When calculating quantitative characteristics of structural components in the urinary bladder, in each case, a micro-photograph was measured 30 times [23].

Statistical analysis. The results were statistically processed using Excel (Microsoft, USA) and Statistica 10 (StatSoft, USA). Quantitative data were presented as median (Me) and quartiles (Q1; Q3) and were assessed using nonparametric Kruskal-Wallis and Mann-Whitney tests with the Dunnett's test for post-hoc comparisons. Differences were considered significant at $p<0.05$.

Results. _Immunohistochemical studies._ Visual assessment of the SM tissue specimens subjected to immunohistochemical labelling with antibodies to protein S100 and actin revealed an increase in the relative area of positive labelling in animals of group 2 compared to group 4 (untreated) (Fig. 1).

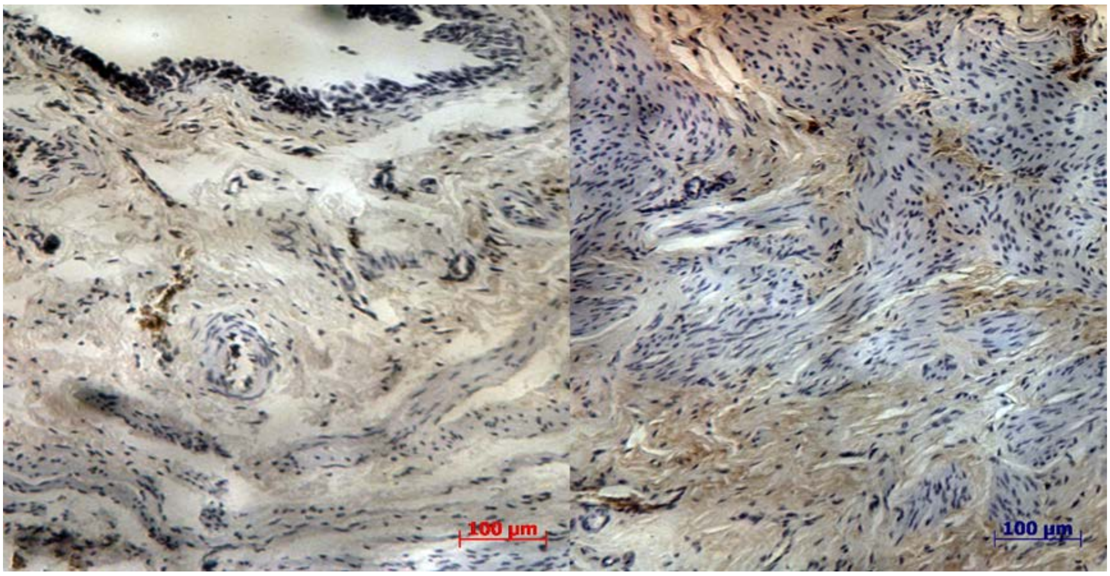

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Fig. 1. UB tissue of rats of group 2 (injection of CM from native cell culture). Immunohistochemical study of S 100 (a) and actin (b) expression.

Also, visual assessment revealed an increase in the relative area of positive labelling of nervous and muscle structures with antibodies to proteins S100 and actin in group 3 compared to group 4 (untreated) (Fig. 2).

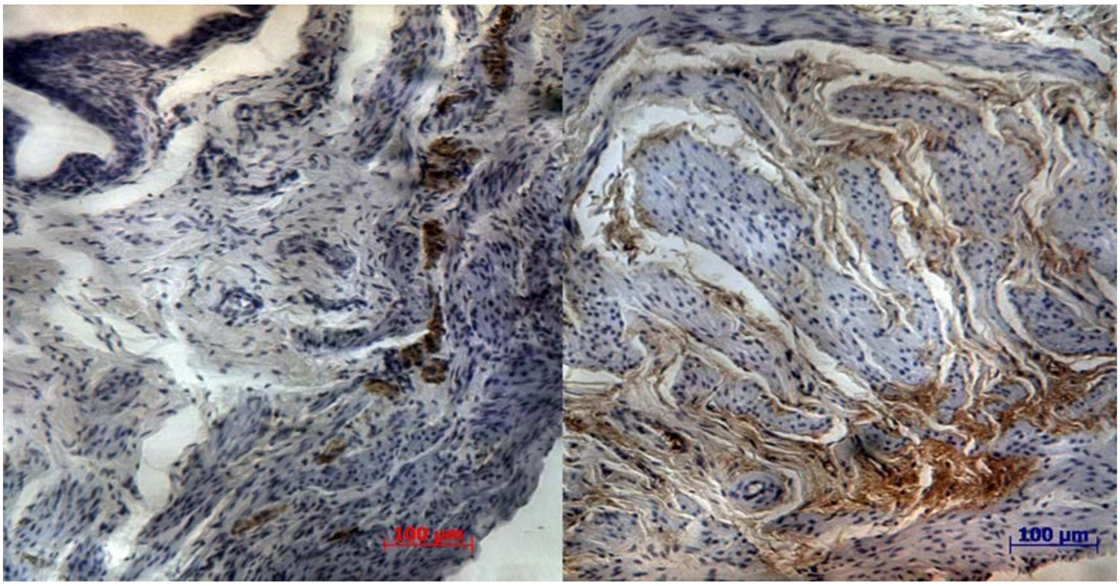

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Fig. 2. UB tissue of group 3 rats (injection of CM from cryopreserved cell culture). Immunohistochemical study of S 100 (a) and actin (b) expression.

Morphometric characteristic of the urinary bladder. Statistical analysis revealed an increase in the relative area of expression of the studied markers of nervous and muscle structures by both indicators in animals of group 2 by 91.6% and 78.9% (Fig. 3, $p = 0.004$; $p = 0.002$, respectively) compared to group 4 (without treatment).

A somewhat different trend was observed when comparing the results of group 3. A statistically insignificant increase in the relative area of expression of S100 protein in the UB tissues (Fig. 3, $p>0.05$) and significantly higher actin expression by 78.8% (Fig. 3, $p=0.001$) were found compared to the untreated group.

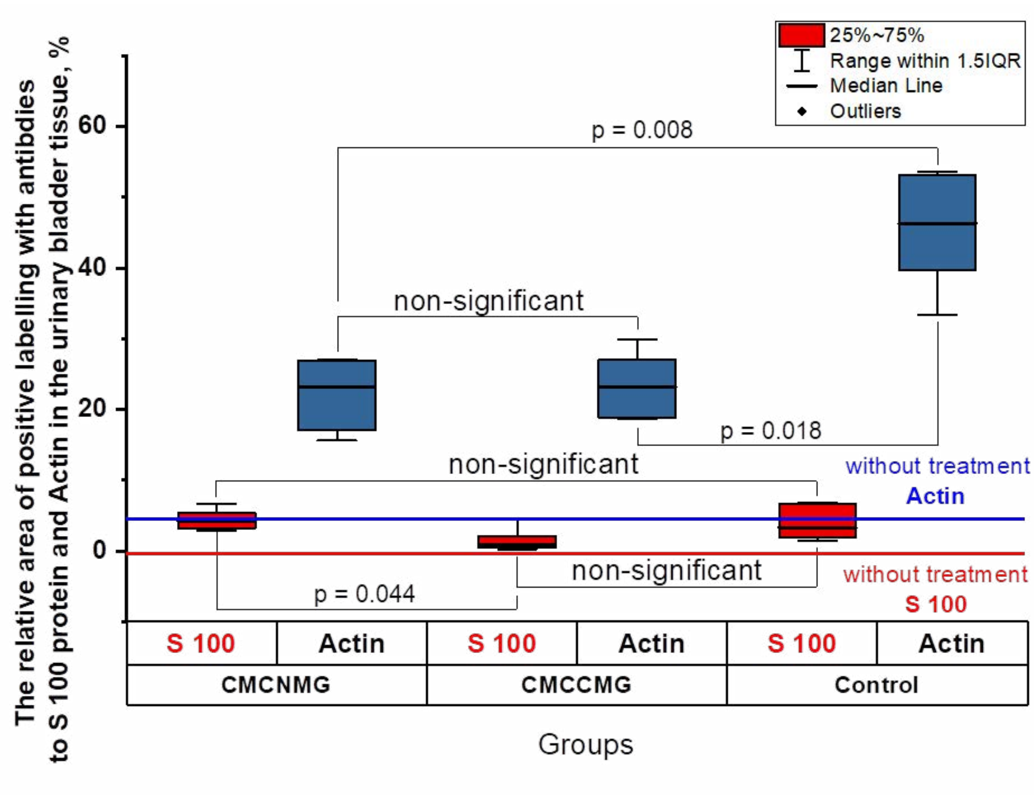

Fig. 3. Changes in the relative area (%) of positive labelling with antibodies to S100 protein and actin in the UB tissue of animals of groups 2, 3 and 4. Blue columns - positive labelling to actin, red columns - positive labelling to S100 protein.

Discussion. The analysis of the works of previous authors, which studied the effect of BACs obtained from SG on the morphological and functional parameters of the UB of experimental animals and humans with IVO, showed the existence of a small number of studies on this subject [24, 25]. Most of the scientific works used stem cells for tissue engineering [26, 27], studied the effect of increased contractility of the UB and endogenous NFs, as well as growth factors on the morphology and function of the urinary tract [28, 29], the effect of high hydrostatic pressure on various cell cultures [30], and the role of NFs at neurodegenerative diseases [31].

We have suggested that it is possible to improve the contractile function of the UB by restoring the innervation of the wall using secretions from cultures of neural cells. However, up to now, no attempts have been made to use glial cell culture, secretions, and other biological products to restore the UB receptor pool and improve its contractility in the case of IVO.

Infravesical obstruction causes denervation, which leads to an increase in the mass of the UB, and characteristic changes in contractile activity and morphology. There is an increase in the number and volume of bladder smooth muscle along with a decrease in the density of nerve endings and contractile proteins in the organ tissue [32]. These changes demonstrate the occurrence of the process of remodelling of the UB with the participation of several cellular compartments, namely transitional cell epithelium, mucosal lamina propria, detrusor smooth muscle, connective tissue, and neurons [8].

S100 protein actively affects the functions of the nervous system, tissue repair and regeneration, inflammation, infections, and cell growth and differentiation [33]. S100-positive neural structures and nerves are present in the UB tissues [34].

UB smooth muscle cells contain contractile proteins (myosin and actin) and proteins that regulate their interaction in response to neuroendocrine stimulation. These cells also synthesise most of the extracellular matrix surrounding them in the UB tissue. In addition, smooth muscle cells are involved in remodelling of the UB in many conditions that affect organ function [35].

Hypertrophy of UB smooth muscle, which occurs as a result of IVO, may be associated with fluctuations in the number of different actin isoforms, both in the direction of increase/decrease and in the absence of any changes at all. The contraction of the smooth muscle of the UB is initiated by an increase in the concentration of free Ca^{2+} in the cytoplasm of smooth muscle cells [36].

Actin is a component of the contractile apparatus and cytoskeleton of smooth muscle cells. Functionally, it is involved in muscle contraction and other types of cell motility. Actin in the smooth muscle of the UB accumulates in conditions when there is a stressful increase in mechanical work, as a result of which the muscle mass of the organ increases. Thus, hypertrophy of the smooth muscle of the UB may be associated with changes in the composition of contractile proteins [37].

Actin is used as a marker for myoblasts and smooth muscle cells. Hypertrophic UB muscles with IVO express increased levels of actin compared to normal tissues. A sufficient level of actin appears to be a prerequisite for the generation of normal SM contraction force [38, 39].

Neurotrophic factors or similar biologically active substances prevent nerve cell death at an early stage of IVO and increase nerve density in the UB tissue, as well as limit the severity of detrusor hypertrophy. This slows down the development of functional decompensation of the organ in the case of long-term obstruction [40].

Low values of the median relative area of S100 protein and actin expression were found in group 4 (without treatment) - 0.4 (0.07; 1.04) and 4.9 (2.3; 9.3) compared to intact animals - 3.3 (1.7; 6.7) and 46.3 (39.7; 53.1), respectively (Fig. 3, $p < 0.05$). In contrast, an increase in the median relative area of S 100 protein expression was detected in groups 2 and 3 compared to the control. The aforementioned index in group 2 (CM of the native culture of SG cells) - 4.2 (3.2; 5.5) was significantly higher than in rats with IVO without treatment (Fig. 3, $p < 0.05$). Higher values of the median relative area of actin protein expression were found in groups 2 (CM of native SG cell culture) - 23.2 (17.1; 26.9) and 3 (CM of cryopreserved SG cell culture) - 23.1 (18.9; 27.1) compared to rats with IVO without treatment (Fig. 3, $p < 0.05$). However, this indicator in the above groups was lower than in intact animals. Thus, it was found that BACs obtained from SG affect the expression of S100 proteins and actin in the tissue of the UB of rats with IVO, which results in changes in the morphological and functional parameters of the bladder.

NFs-like activity was previously established in CM obtained from cultures of native and cryopreserved cells from the SG of newborn piglets [41].

NFs functions include regulation of axonal and dendritic growth, formation and functioning of synapses, cell migration, cell proliferation, and survival of adult neurons. It has been established that NFs affect the restoration of organ innervation of taking into account these facts [42].

Cryopreservation is an integral part of the technology for the production of CM, as cells are stored in a cryobank before cultivation. In this regard, a natural question arises about the effect of freezing on cells. In previous studies, it has been established that cryopreservation of MG in a cryoprotective medium with 7.5-10% DMSO allows maintaining cell viability at the level of 83-87% [19]. At the same time, the expression of neurotrophic factors remains at a level close to control values [41].

In our research, we did not investigate which types of NFs affect the reinnervation and remodeling of the UB. The data obtained indicate the prospect of discovering new drugs based on NFs contained in the secretions of native/cryopreserved SG cell cultures.

Conclusions. Thus, the use of biologically active compositions obtained from spinal ganglia contributed to an increase in the relative area of S100 protein and actin expression in the bladder tissue of animals with infravesical obstruction, which indicated their stimulating effect on the processes of reinnervation and remodelling of the organ.

However, the precision mechanisms by which NFs-containing BACs affect the expression of the above proteins in the bladder tissue of mammals with infravesical obstruction are not yet clear and require further study.

Conflict of interest statement. The authors have no competing interests to declare.

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Author Contributions

Vyacheslav Globa: experiment and methodology, analyzed and interpreted the data, and was a major contributor to writing the manuscript;

Galyna Bozhok: conceived the presented concept, designed the study, experiment, and methodology;

Evgeniy Legach: final manuscript editing and research management;

Yana Samburg: analyzed and interpreted the data, edited the final manuscript;

Olga Godlevska: histopathological evaluation and data interpretation;

Olena Vlasenko: data collection and analysis

All authors discussed the results and commented on the manuscript.

References:

1. *Reddy SVK, Shaik AB.* Non-invasive evaluation of bladder outlet obstruction in benign prostatic hyperplasia: a clinical correlation study. Arab Journal of Urology. 2019; 17 (4): 259–64. doi: 10.1080/2090598X.2019.1660071.
2. *Malde S, Solomon E, Spilotros M, Mukhtar B, Pakzad M, Hamid R, et al.* Female bladder outlet obstruction: Common symptoms masking an uncommon cause. LUTS: Lower Urinary Tract Symptoms. 2017; 11 (1): 72–7. doi: 10.1111/luts.12196.
3. *Gosling JA, Gilpin SA, Dixon JS, Gilpin CJ.* Decrease in the autonomic innervation of human detrusor muscle in outflow obstruction. Journal of Urology. Ovid Technologies (Wolters Kluwer Health).1986;136(2):501–4. doi: 10.1016/s0022-5347(17)44930-5.
4. *Juan YS, Chuang SM, Jang MY, Chun-Hsiung Huang, Chou Y, Wen-Jeng Wu, Longet C.* Basic research in bladder outlet obstruction. Incon Pelvic Floor Dysfunct. [Internet].2011;5(1):1–6. Available from: http://www.tcs.org.tw/issue/folder/5_1/1-6.pdf.

5. *Levin RM, Longhurst PA, Monson FC, Kato K, Wein AJ.* Effect of bladder outlet obstruction on the morphology, physiology, and pharmacology of the bladder. The Prostate. Wiley.1990;17 (S3):9–26. doi: 10.1002/pros.2990170503.
6. *Speakman MJ, Brading AF, Gilpin CJ, Dixon JS, Gilpin SA, Gosling JA.* Bladder outflow obstruction—a cause of denervation supersensitivity. Journal of Urology. Ovid Technologies (Wolters Kluwer Health).1987;138(6):1461–6. doi: 10.1016/s0022-5347(17)43675-5.
7. *Milicic I, Buckner SA, Daza A, Coghlan M, Fey TA, Brune ME, et al.* Pharmacological characterization of urinary bladder smooth muscle contractility following partial bladder outlet obstruction in pigs. Eur J Pharmacol.2006; 532(1-2):107–14. doi: 10.1016/j.ejphar.2005.12.076.
8. *Fusco F, Creta M, De Nunzio C, Iacovelli V, Mangiapia F, Li Marzi V, et al.* Progressive bladder remodeling due to bladder outlet obstruction: a systematic review of morphological and molecular evidences in humans. BMC Urol. 2018; 18 (1):15. doi: 10.1186/s12894-018-0329-4.
9. *Lin ATL, Juan Y-S.* Ischemia, hypoxia and oxidative stress in bladder outlet obstruction and bladder overdistention injury. Low Urin Tract Symptoms.2012;4 (1):27-31. doi: 10.1111/j.1757-5672.2011.00134.x
10. *Steers WD, Kolbeck S, Creedon D, Tuttle JB.* Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest. 1991;88(5):1709-15. doi: 10.1172/JCI115488.
11. *Steers WD, Tuttle JB.* Mechanisms of disease: the role of nerve growth factor in the pathophysiology of bladder disorders. Nat Clin Pract Urol. 2006;3(2):101-10. doi: 10.1038/ncpuro0408.
12. *Zeng J, Xie K, Jiang C, Mo J, Lindstrom S.* Bladder mechanoreceptor changes after artificial bladder outlet obstruction in the anesthetized rat. Neurourol Urodyn. 2011;31(1):178–84. doi: 10.1002/nau.21219.
13. *Yoshimura N, Ogawa T, Miyazato M, Kitta T, Furuta A, Chancellor MB, et al.* Neural mechanisms underlying lower urinary tract dysfunction. Korean J Urol. 2014;55(2):81-90. doi: 10.4111/kju.2014.55.2.81.
14. *Ekman M, Zhu B, Swärd K, Uvelius B.* Neurite outgrowth in cultured mouse pelvic ganglia - Effects of neurotrophins and bladder tissue. Auton Neurosci. 2017;205:41-49. doi: 10.1016/j.autneu.2017.03.004.
15. *Adamowicz J, Juszczak K, Bajek A, Tworkiewicz J, Nowacki M, Marszałek A, et al.* Morphological and urodynamic evaluation of urinary bladder wall regeneration: muscles guarantee contraction but not proper function-a rat model research study. Transplant Proc.2012;44(5):1429-34. doi: 10.1016/j.transproceed.2012.01.144.
16. *Kim JH, Lee SR, Song YS, Lee HJ.* Stem cell therapy in bladder dysfunction: where are we? And where do we have to go? Biomed Res Int. 2013; 2013: 930713. doi: 10.1155/2013/930713.
17. *Hansebout CR, Su C, Reddy K, Zhang D, Jiang C, Rathbone MP, et al.* Enteric glia mediate neuronal outgrowth through release of neurotrophic factors. Neural Regen Res.2012;7(28):2165–75. doi: 10.3969/j.issn.1673-5374.2012.028.001.
18. *Collazos-Castro JE, García-Rama C, Alves-Sampaio A.* Glial progenitor cell migration promotes CNS axon growth on functionalized electroconducting microfibers. Acta Biomater. 2016; 35: 42–56. doi: 10.1016/j.actbio.2016.02.023.
19. *Ali S, Moiseieva N, Bozhok G.* Cryopreservation of cell culture derived from dorsal root ganglia of neonatal pigs. Problems of Cryobiology and Cryomedicine. 2020; 30(2): 158-68. doi: 10.15407/cryo30.02.158.
20. *Ali SG, Kovalenko IF, Bozhok GA.* Some phenotypic characteristics of the dorsal root galglia cell culture of neonatal piglets. Bulletin of Problems Biology and Medicine.2019; 3(152): 46-50. doi: 10.29254/2077-4214-2019-3-152-46-50.
21. *Zhang N, Ma L, Zhang J, Chen J.* Improved model for the establishment and evaluation of detrusor overactivity in female Wistar rats. Int Braz J Urol. 2014;40(3):414-21. doi: 10.1590/S1677-5538.IBJU.2014.03.17.
22. *Buchwalow IB, Böcker W.* Immunohistochemistry: Basics and Methods. Berlin Heidelberg.2010:151 p. doi: 10.1007/978-3-642-04609-4.
23. *Avtandilov GG.* Medical morphometry. Management. M.:Medicine;1990:384 p.
24. *Arms L, Vizzard MA.* Neuropeptides in lower urinary tract function. Handb Exp Pharmacol.2011;(202):395-423. doi: 10.1007/978-3-642-16499-6_19.
25. *Mundy AR.* Neuropeptides in lower urinary tract function. World Journal of Urology.1984;2 (3):211–5. doi: 10.1007/bf00327003.
26. *Beckel JM, de Groat WC.* Neural control of lower urinary tract function. Oxford Res Encycl Neurosci.2019. doi: 10.1093/acrefore/9780190264086.013.270.
27. *Imamura T, Ishizuka O, Nishizaw O.* Bone marrow–derived cells regenerate structural and functional lower urinary tracts. Regenerative Medicine and Tissue Engineering.2013. doi: 10.5772/55558.
28. *Sellers D, Chess-Williams R, Michel MC.* Modulation of lower urinary tract smooth muscle contraction and relaxation by the urothelium. Naunyn-Schmiedeberg's Archives of Pharmacology.2018;391(7):675–94. doi: 10.1007/s00210-018-1510-8.

29. *Sidler M, Aitken K, Jiang J, Bijos D, Belik J, Bägli DJ.* Finding NeMO-nerve-sparing mid-urethral obstruction: a pathophysiologically accurate model of rodent partial bladder outlet obstruction. Urology.2017;105:208.e1-208.e9. doi: 10.1016/j.urology.2017.03.032.
30. *Liu S, Tao R, Wang M, Tian J, Genin GM, Lu TJ, et al.* Regulation of cell behavior by hydrostatic pressure. Applied Mechanics Reviews. 2019;71 (4): 0408031—4080313. doi: 10.1115/1.4043947.
31. *Skaper SD.* The neurotrophin family of neurotrophic factors: an overview. Methods Mol Biol. 2012;846:1-12. doi: 10.1007/978-1-61779-536-7_1.
32. *Zhu B, Ekman M, Svensson D, Lindvall JM, Nilsson B-O, Uvelius B, et al.* Array profiling reveals contribution of Cthrc1 to growth of the denervated rat urinary bladder. Am J Physiol Renal Physiol. 2018;314(5):F893-F905. doi: 10.1152/ajprenal.00499.2017.
33. *Donato R, Geczy CL, Weber DJ.* S100 Proteins. Encyclopedia of Metalloproteins. 2013;1863—74. doi: 10.1007/978-1-4614-1533-6_48.
34. *Daub B, Schroeter M, Pfitzer G, Ganitkevich V.* Expression of members of the S100 Ca2-binding protein family in guinea-pig smooth muscle. Cell Calcium. 2003;33(1):1-10. doi: 10.1016/s0143-4160(02)00167-7.
35. *Zheng Y, Chang S, Boopathi E, Burkett S, John M, Malkowicz SB, et al.* Generation of a human urinary bladder smooth muscle cell line. In Vitro Cell Dev Biol Anim. 2012;48(2):84-96. doi: 10.1007/s11626-011-9473-9.
36. *Hashimoto N, Kiyono T, Saitow F, Asada M, Yoshida M.* Reversible differentiation of immortalized human bladder smooth muscle cells accompanied by actin bundle reorganization. PLoS One. 2017;12(10):e0186584. doi: 10.1371/journal.pone.0186584.
37. *Zhang EY, Stein R, Chang S, Zheng Y, Zderic SA, Wein AJ, et al.* Smooth muscle hypertrophy following partial bladder outlet obstruction is associated with overexpression of non-muscle caldesmon. Am J Pathol.2004;164(2):601-12. doi: 10.1016/S0002-9440(10)63149-5.
38. *Zimmerman RA, Tomasek JJ, McRae J, Haaksma CJ, Schwartz RJ, Lin HK, et al.* Decreased expression of smooth muscle α-actin results in decreased contractile function of the mouse bladder. J Urol. 2004;172(4 Pt 2):1667-72. doi: 10.1097/01.ju.0000139874.48574.1b.
39. *Duan LJ, Qi J, Huang T, Gu X, Xu D, Kong XJ, et al.* Pirfenidone attenuates bladder fibrosis and mitigates deterioration of bladder function in a rat model of partial bladder outlet obstruction. Mol Med Rep. 2015;12(3):3639-3647. doi: 10.3892/mmr.2015.3814.
40. *Hughes FM, Sexton SJ, Ledig PD, Yun CE, Jin H, Purves JT.* Bladder decompensation and reduction in nerve density in a rat model of chronic bladder outlet obstruction are attenuated with the NLRP3 inhibitor glyburide. Am J Physiol Renal Physiol. 2019;316(1):F113-F120. doi: 10.1152/ajprenal.00400.2018.
41. *Nesteruk HV, Alabedalkarim NM, Kolot NV, Komaromi NA, Protsenko OS, Legach EI.* Effect of conditioned media from glial cell cultures on the reproductive system of female rats of different ages. Problems of Endocrine Pathology. 2022;79(2):88—96. doi: 10.21856/j-PEP.2022.2.13.
42. *Gill BC, Balog BM, Dissaranan C, Jiang H-H, Steward JB, Lin DL, et al.* Neurotrophin therapy improves recovery of the neuromuscular continence mechanism following simulated birth injury in rats. Neurourol Urodyn. 2013;32(1):82-7. doi: 10.1002/nau.22264.