

Morphological Parameters of Bladder Rats with Infravesical Obstruction with the Introduction of Biologically Active Compositions

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Abstract: The morphological features of the urinary bladder (UB) after administration of biologically active compositions (BACs) containing neurotrophic factors (NFs) in female rats with infravesical obstruction (IVO) were studied. IVO was modeled by the ligation method. A cryo extract of the spinal ganglia (SG), conditioned media from cultures of native and cryopreserved mantle gliocytes (MG), and the drug Cortexin were used as the BACs. The BACs were injected intraperitoneally to the animals for 10 days. The morphometric parameters of the UB were determined by studying all its shells and their structure. The BACs obtained from the SG participated in the remodeling of the UB wall, which was changed during the IVO. Exogenous NFs insignificantly affected the hyperplasia of the transitional epithelium, stimulated regression and progression of the enlarged muscle layer of the detrusor, increased the proliferation of connective tissue, and increased the wall thickness of the UB. Proceeding from the above, NFs reduced the indices of the ratio of the muscle layer/wall of the UB, mediated by IVO.

Key words: Bladder morphology, infravesical obstruction, spinal ganglion, cryo extract, cryopreservation.

1. Introduction

Currently, urination disorders caused by infravesical obstruction (IVO) are observed in 21.5% of the world's population. IVO causes multiple changes in the smooth muscles of the UB, transitional epithelium, nerves and vasculature. This leads to progressive hypertrophy and impaired UB contractility [1].

Today, methods of regenerative medicine are relevant, restoring disturbed physiological processes and affecting the survival, differentiation and growth of cells of various tissues of the urinary system [2]. For these purposes, neurotrophic drugs are used, one of which is cortexin, which have a predominant effect on the nervous system. Correction of the main pathogenetic mechanisms of IVO, such as hypertrophy and impaired UB contractility, requires a combination of therapeutic and surgical approaches,

often with unsatisfactory results [3].

There are works showing the participation of NFs in the remodeling of the UB wall after IVO [4]. This allows the use of BACs (extracts, conditioned media, lysates) to influence the morphological and functional properties of UB in health and disease.

NFs are localized in tissues with high proliferative activity, especially in glial cells of the peripheral and central nervous systems [5]. Spinal ganglia (SG) and cell cultures from them are a potential source of NFs [6].

Currently, there are few studies that study the possibility of using BACs obtained from SG on the morphological indicators of UB in IVO.

2. Materials and Methods

2.1 Study Design

White outbred rats were used for research: females weighing 250-320 g and 6 months of age. All manipulations with animals were carried out in

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accordance with the Law of Ukraine “On the Protection of Animals from Cruelty” (No. 3447-IV dated 02.21.2006) in compliance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes” (Strasbourg, 1986).

The cryo extract was obtained from the SG of sexually mature rats using an original method [7]. After that, the cryo extract was passed through a filter with a pore diameter of 0.22 μm (TPP, Switzerland) for sterilization and packed in 1.5 mL in microtubes Eppendorf (Greiner Bio-One GmbH, Germany), followed by freezing in a refrigerator at $-18\text{ }^{\circ}\text{C}$. The protein concentration in the SG cryo extract was determined by the Bradford method and amounted to 0.1 mg/mL [8]. Before use, the cryo extract was warmed in a water bath ($42\text{ }^{\circ}\text{C}$) with continuous shaking for 1-2 min and centrifuged in a mini-centrifuge (2,000 g) for 10 min.

The culture of mantle gliocytes (MG) was obtained from the SG of neonatal piglets by an enzymatic method [9]. The initial seeding concentration of cells in both cases was 6×10^4 cells/cm². 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 (PanEco, Russia) and cryopreserved as a suspension in cryoprotective medium with 10% dimethyl sulfoxide (DMSO) with a cooling rate of $1\text{ }^{\circ}\text{C}/\text{min}$ to $-40\text{ }^{\circ}\text{C}$ and further immersion in liquid nitrogen. The cells were kept at $-196\text{ }^{\circ}\text{C}$ 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 [10].

After 1.5 months, the ligature was removed and the next day the animals were started to become injected

the BACs intraperitoneally for 10 days. Cryo extract of SG, conditioned medium obtained from native and cryopreserved MG cultures were injected with 0.6 mL/kg of body weight. Cortixin (Geropharm, Russia) was administered at a dose of 1.0 mL/kg of body weight [11]. 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 neurotrophic drug Cortixin (NDC); 3-introduction of conditioned medium from the culture of native MG (CNMG); 4-introduction of conditioned medium from the culture of cryopreserved MG (CCMG); 5-introduction of SG cryo extract (SGC); 6-without treatment (U).

2.2 Morphologic Analysis of the Urinary Bladder

For morphological assessment, UB fragments were subjected to histological tracing, stained with hematoxylin and eosin according to the standard technique [12]. Microphotography was carried out using an AmScope light-optical microscope, model XYL-403 (China) with a digital camera. The morphometric analysis of micrographs of serial sections of the UB was performed using the AxioVision Rel 4.7 image processing software (Carl Zeiss, Germany). The medium height of the transitional epithelium, the thickness of the muscle layer, the total wall thickness of the UB, the index of the ratio of the muscle layer/wall of the UB on histological preparations were measured on cross sections. Ten (10) measurements were performed on 1 slice, 5-6 slices were counted for each sample.

2.3 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. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 General Histological Characteristic of the Urinary Bladder

In the morphological study of the UB of control rats, the mucous membrane was covered with transitional epithelium, which was located on a loose lamina of its own. The muscular layer had an ordered structure in the form of several layers of smooth muscles with a thin layer of connective tissue between them. The serous membrane was represented by a thin layer of connective tissue outside the organ in Fig. 1A.

In the UB of animals treated with Cortexin (NDC), the transitional epithelium was moderately hypoplastic, there was a slight proliferation of the connective tissue of the lamina propria. The muscle layers of the lamina propria and detrusor retained a normal structure, but in places the muscles were hypertrophied. The spaces of connective tissue between the muscle layers expanded. The serous membrane was moderately thickened in Fig. 1B.

In animals of the 3rd group (CNMG), the surface of the mucous membrane of the UB was covered with hyperplastic epithelium. The lamina propria looked sharply thickened; its muscle layer was atrophied in places. The detrusor muscles lost their structure, consisted of groups of thickened muscle fibers with a small amount of connective tissue between them. The serous membrane was sharply thickened in Fig. 1C.

In the 4th group of rats (CCMG), the transitional epithelium of the UB looked sharply hyperplastic, but in some places there were areas of atrophy. The muscle layer of the lamina propria and detrusor muscles were sharply hypertrophied, with a moderate content of connective tissue between the layers. The serous membrane was sharply thickened in Fig. 1D.

In rats treated with a cryo extract of SG (SGC), foci of normal and hypoplastic transitional epithelium alternated in the mucous membrane of the UB. The lamina propria was almost replaced by connective tissue, and its muscle elements were partially

atrophied. The muscular layer was moderately hypertrophied and consisted of regularly spaced smooth muscle bundles with a small amount of connective tissue. The serous membrane was slightly thickened in Fig. 1E.

Microscopic assessment of UB in rats of the 6th group (U) showed that the transitional epithelium was sharply hypoplastic and atrophied in places, and there were areas of its complete absence. The lamina propria was thinned, completely replaced by connective tissue, with the presence of hypertrophied muscles in the form of separate foci. The muscle layers lost their structural orientation, were represented by sharply hypertrophied bundles of muscle fibers, between which the connective tissue layer was reduced. The serous membrane looked sharply thickened in Fig. 1F.

3.2 Morphometric Characteristic of the Urinary Bladder

Measurement of the average height of the transitional epithelium of the UB revealed a decrease in the 5th and 6th groups by 24.3% and 20.5% (Fig. 2A), respectively, compared with the control. But the difference in this indicator between the 6th group (U) and the animals that received the BACs was statistically insignificant (Fig. 2A, $p > 0.05$), except for the 3rd group. The thickness of the muscle layer of the UB increased in animals of the 4th, 5th and 6th groups by 42.07%, 18.5% and 37.26% (Fig. 2B), respectively, compared with the control. At the same time, the degree of muscle hypertrophy in the 2nd and 3rd groups was minimal and approached the control values, but statistically significantly differed from the indicators of the 6th group.

Morphometric analysis revealed an increase in the total wall thickness of the UB in all groups, except for the 3rd (CNMG), by 21.95%, 39.99%, 32.5% and 32.74% (Fig. 2C), respectively, compared with control. It is interesting that the indicators of the 1st (C) and 3rd groups were similar, but different from the values

of the 6th group (U).

When examining the index of the ratio of the muscle layer/wall of the UB, a decrease of 1.2 times

was found in the 2nd (NDC) and 3rd (CNMG) groups, compared with the rest of the groups (Fig. 2D). The highest index values were in the 6th group (U).

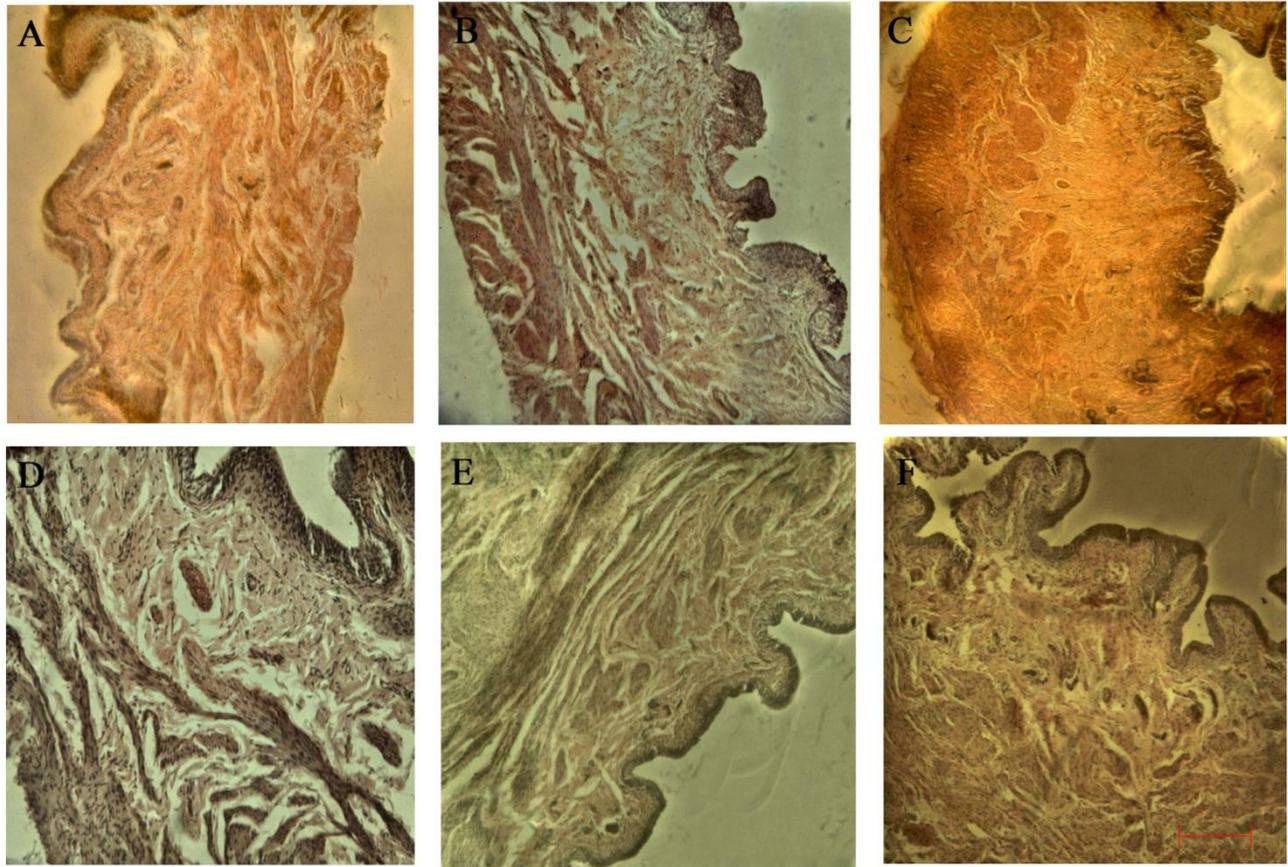


Fig. 1 Histopathology examination of different studied groups (H and E stain, observed at 10× magnification; scale bar, 50 μm). (A): control; (B): neurotrophic drag cortexin; (C): conditioned culture medium of native MG; (D): conditioned culture medium of cryopreserved MG; (E): cryo extract of SG; (F): without treatment.

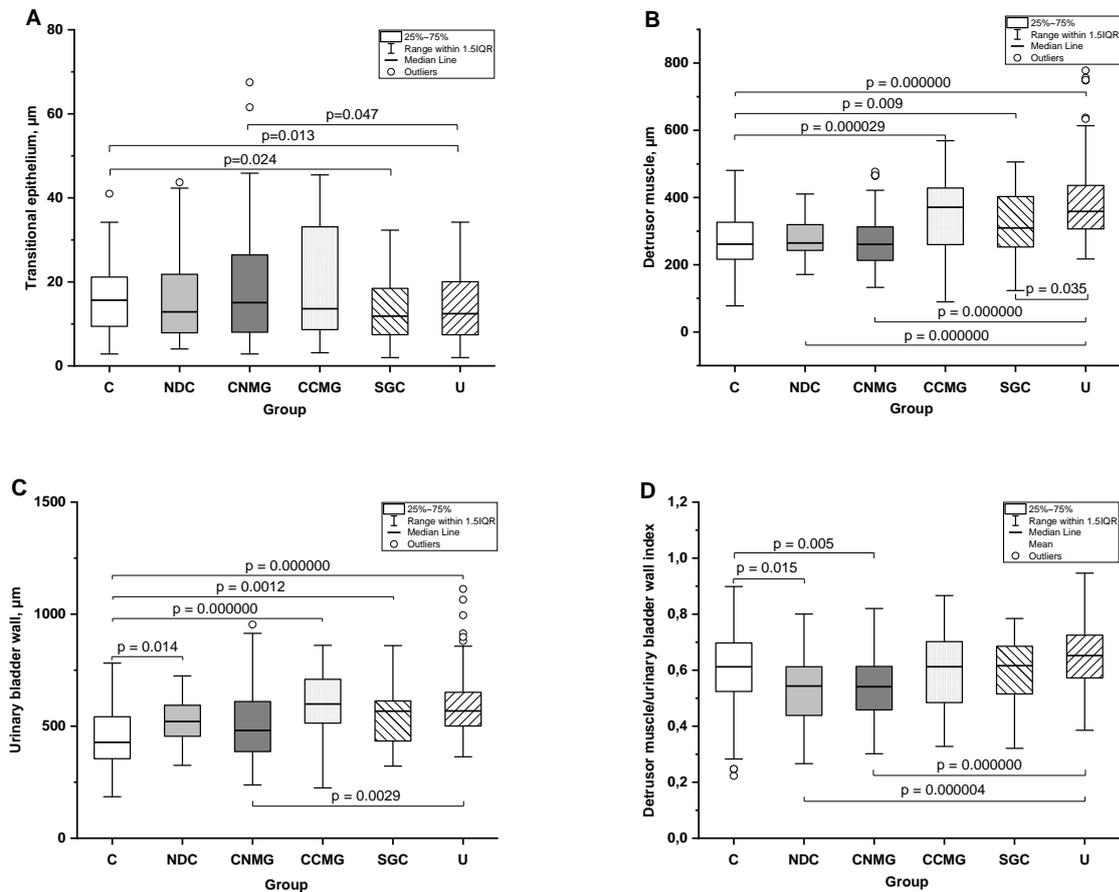


Fig. 2 The average height of the transitional epithelium (A), the thickness of the muscle layer (B), the total wall thickness of the UB, (C) and index of the ratio of the muscle layer/UB wall, (D) of different studied groups.

4. Discussion

We searched for works that studied the effect of BACs obtained from SG on the morphofunctional indicators of UB in experimental animals and humans after IVO. The obtained results indicate a small number of studies on this topic [4, 13]. Basically, in the majority of scientific works, stem cells were used for the purpose of tissue engineering [2, 14], the effect of increased contractility of UB and endogenous NFs, growth factors on the morphology and function of the urinary tract [12, 15-18], the effect of high hydrostatic pressure on different cell cultures [19], the role of NFs in neurodegenerative diseases [5, 20]. The increased interest in this topic was caused by the results we obtained in previous works. We investigated the contractility of the UB and the biochemical

parameters of the blood of rats after IVO due to the use of BACs obtained from SG. In animals that were injected with a cryo extract of SG and conditioned medium obtained from a culture of cryopreserved MG, the most pronounced increase in body weight, the smallest indicators of UB weight and mass coefficient were found, and an increase in contractile activity and maximum amplitude of bladder contractions in response to various stimuli was noted [21, 22]. The results of the current study were probably obtained by us due to the fact that NFs mediate hypertrophy of smooth muscle cells, hyperplasia of transitional cell epithelium and connective tissue [4, 23]. At the same time, different types of growth factors and NFs can be both mitogens and growth inhibitors, depending on the type of cells and the state of cell differentiation, and can also express each other in UB tissues [12, 13,

18]. Also indicative are the obtained indices of the muscle layer/wall of the UB, which have proven effective in detecting hypertrophy of the bladder wall due to IVO [24]. Based on the foregoing, it can be assumed that, in the future, it is advisable to use NFs obtained from SG and from the MG culture after freezing-warming, due to their wide spectrum of action on the morphological and functional properties of UB. This is possible due to the fact that after cryopreservation the ratio of morphological cell types changes, that is, their production of NFs [25]. At the same time, the use of neurotrophic drugs such as cortexin is less justified due to their predominant effect mainly on the nervous system. Our studies have confirmed the versatile effects of NFs along with increased hydrostatic pressure due to IVO on the growth, differentiation and apoptosis of smooth muscle cells of transitional cell epithelium and UB detrusor [14, 18, 19].

5. Conclusions

A pronounced effect of exogenous NFs on transitional epithelial hyperplasia was not observed in experimental IVO (reduction in the height of the transitional epithelium only in the SGC group by 24.3%). The introduction of native BACs stimulated regression of the enlarged UB detrusor to the control values, the thickness of which usually increases with IVO. At the same time, the use of NFs obtained by freezing-warming showed a positive effect on detrusor hypertrophy, presumably due to additional growth factors, increasing it by 18.5% (SGC) and 42.1% (CCMG). The use of BACs increased the proliferation of connective tissue and increased the wall thickness of the UB by 22%, 40%, 32.5% and 33%. BACs obtained from SG reduce the indices of the ratio of the muscle layer/wall of the UB, mediated by IVO, by an average of 11.5%.

All of the above conclusions allow us to speak about the positive participation of exogenous NFs in the remodeling of the UB wall changed during IVO.

This will make it possible not only to use BACs obtained from SG for therapeutic purposes, but also to develop methods for the prevention and prediction of many pathologies.

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