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Morphological pattern of kidneys in rats with infravesical urinary obstruction following administration of biologically active compositions containing neurotrophic factors

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Abstract. *The research aimed to study the morphological characteristics of kidneys after intraperitoneal administration of biologically active compositions (BACs) in rats with infravesical obstruction (IVO).*
Methods. IVO was reproduced by surgical ligation. BACs were injected intraperitoneally for 10 days. The morphometric parameters of the kidneys were determined by examining their structure (the areas of renal corpuscle, glomerulus, the cavity of the glomerular capsule, outer and inner diameters of the tubules, the tubular index, the outer diameter and area of the vascular lumen). The experimental animals were divided into the following groups: Group 1 was saved as an intact control group, Group 2 received "Cortexin", Group 3 was given CMCNMG, Group 4 received BCM, and Group 5 was not treated (U).
Results. BACs obtained from a culture of native mantle gliocytes (MG) and BCM were involved in the remodeling of the kidneys' structure being changed during IVO. The renal corpuscle area was found to be increased in groups 2, 4, and 5 by 42.3%, 193.9%, and 72.3%, respectively, compared with the control group. At the same time, the extent of renal corpuscle hypertrophy in group 3 was minimal and approached the control values. The glomerulus area expanded by 35.4%, 181.1%, and 34% in animals of groups 3, 4, and 5, respectively, compared with the control group. However, the difference in the index between group 5 and BAC-treated animals was not significant, except for group 4. The Bowman's capsule area increased by 117.6%, 235.8%, and 186%, in groups 2, 4 and 5, respectively, compared with the control group. Interestingly, the values in groups 1 and 3 were in agreement but differed from group 5. The lowest values of outer and inner diameters of renal tubules were determined in group 3, which were close to those in the control group but significantly different from those of other groups. An increased tubular index (by 1.35 and 1.4 times) was revealed in groups 3 and 4, compared with other groups. The lowest values were found in groups 2 and 5. The maximum values of the average diameter and area of the vessel lumen were determined in groups 3 and 4, and the minimum values were obtained in group 5. It is noteworthy that the indices of these groups were significantly different from those of group 5.
Conclusions. The research results suggest that the phenomena of glomerulosclerosis, tubular atrophy, and renal interstitial fibrosis in rats can be alleviated, as well as the morphological structure of rat kidneys can be partially restored against the background of IVO after administration of CMCNMG and, to a lesser extent, BCM due to the multidirectional action of neurotrophic factors.

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Морфологічний патерн нирок щурів з інфравезикальною обструкцією після введення біологічно активних композицій, що містять нейротрофічні фактори

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Резюме. *Метою дослідження було вивчення морфологічних особливостей нирок після внутрішньоочеревинного введення біологічно активних композицій (БАК) у щурів з інфравезикальною обструкцією (ІВО).*

Методи. ІВО була відтворена шляхом лігування. БАК вводили внутрішньочеревно протягом 10 діб. Визначали морфометричні показники нирок шляхом вивчення їх структури (площу ниркового тільця, площу клубочка, площу порожнини капсули клубочка, зовнішній та внутрішній діаметри канальців, канальцьовий індекс, зовнішній діаметр та площу просвіту судин). Піддослідні тварини були розподілені на групи: 1 – інтактний контроль (К); 2 – введення «кортексіну»; 3 – кондиційоване середовище культури нативних мантійних гліоцитів (КСКНМГ); 4 – базове середовище культивування (БСК); 5 – без лікування.

Результати. БАК, отримані від культури нативних мантійних гліоцитів (МГ) та БСК, брали участь у ремоделюванні структури нирок, яка змінюєтьсяь під час ІВО. Виявлено збільшення площі ниркових тілець у 2-й, 4-й та 5-й групах на 42,3%, 193,9% та 72,3% відповідно, порівняно з контрольною групою. У той же час ступінь гіпертрофії ниркового тільця у 3-й групі була мінімальною та наближалася до контрольних значень. У тварин 3-ї, 4-ї та 5-ї груп площа клубочків збільшилася відповідно на 35,4 %, 181,1 % та 34 % порівняно з контрольною групою. Однак різниця в індексі між 5-ю групою та тваринами, які отримували БАК, була несуттєвою, за винятком 4-ї групи. Площа простору капсули Боумена збільшилася у 2-й, 4-й та 5-й групах на 117,6%, 235,8% та 186%, відповідно, порівняно з контролем. Цікаво, що значення 1-ї та 3-ї груп збігалися, але відрізнялися від 5-ї групи. Найнижчі показники зовнішнього та внутрішнього діаметрів ниркових канальців були визначені у 3-й групі, які були близькі до показників контрольної групи, але вірогідно відрізнялися від показників інших груп. У 3-й та 4-й групах виявлено підвищення тубулярного індексу (у 1,35 та 1,4 рази) порівняно з іншими групами. Найнижчі показники виявлені у 2-й та 5-й групах. Максимальні значення середнього діаметра та площі просвіту судини визначені у 3-й та 4-й групах, мінімальні у 5-й. Слід зазначити, що показники цих груп значно відрізнялися від 5-ї групи.

Висновки. Результати експерименту свідчать про можливість зменшення явищ гломерулосклерозу, канальцевої атрофії та ниркового інтерстиціального фіброзу (НІФ), а також часткового відновлення морфологічної структури нирки щурів на тлі ІВО після введення КСКНМГ та меншою мірою БСК за рахунок різноспрямованої дії нейротрофічних факторів (НФ).

Ключові слова: *морфологічні показники, інфравезікальна обструкція, обструктивна уропатія, нирковий фіброз, спінальний ганглій, мантійні гліоцити, кондиційоване середовище.*

Introduction. The term 'obstructive uropathy' (OU) refers to a complex of changes in the structure and functions of renal parenchyma, predominantly of the tubulointerstitial type, which develops as a result of the disordered urine passage of functional or organic origins at the level of the pyelocaliceal, ureteropelvical, vesicoureteral segments or is the consequence of infravesical obstruction (IVO) [**1-3**].

Chronic tubulointerstitial nephritis develops as a result of the progression of epithelial to mesenchymal transition (EMT) and it is characterized by tubular atrophy, RIF, and inflammation, which is also a constant component of chronic kidney disease (CKD) in general and OU, in particular [**4-6**].

CKD is one of the most prevalent diseases that pose a threat to human health and life, affecting more than 10% of the world's population. By 2030, 14 out of every 100,000 people could die from CKD [**7, 8**]. CKD is a global pandemic that is growing rapidly [**1, 9, 10**], and in some cases, the progression of the disease cannot be prevented with modern treatments [**2, 7, 11**].

Among the tools of regenerative medicine, cell therapy is primarily considered a therapeutic intervention that improves the outcomes of kidney diseases. This approach has shown promise in terms of improving renal function, but some safety issues remain to be resolved before the clinical application. These include immune rejection, pulmonary embolism and teratoma formation due to uncontrolled proliferation of pluripotent stem cells (SC) [**7, 11-14**].

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Stem cells can also act in a paracrine manner by secreting the growth factors and cytokines that can promote regeneration [2, 15, 16].

Renal interstitial fibrosis is an important pathological sign of renal aging and CKD, which can be regulated by SC through their secretion [3, 7, 16].

Neurotrophic factors (NFs) play several key roles in renal physiology, including immune modulation, tissue proliferation/regeneration, microbiota and electrolyte balance, which contribute to the maintenance of renal homeostasis. However, under pathological conditions, the NFs can stimulate inflammation, fibrosis, coagulation, and oncogenesis. Recently, both in vivo and in vitro studies have shown explosive advances regarding the protective role of NFs in various renal diseases [8, 15-18].

Evidence suggests that the NFs derived from the glial cell line are necessary for the development of parasympathetic, intestinal, and motor neurons during spermatogenesis and kidney morphogenesis, and may also be the factors in the survival of damaged kidneys cells [6, 15, 19, 20].

For this purpose, neurotrophic drugs are used including Cortexin, which acts mainly on the nervous system. The idea of using the mantle gliocytes (MG) and their secretome may be promising in the rehabilitation therapy of kidneys in OU due to IVO.

The present study aimed to evaluate the morphological characteristics of the kidneys after intraperitoneal administration of biologically active compositions in rats with IVO.

Materials and Methods. The study was carried out on 6-month-old white outbred rats (250–320g, n=48). Manipulations of 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) and 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 MG was obtained from the spinal ganglia (SG) of neonatal piglets by an enzymatic method [21]. The BCM contained – MEM with the addition of 10% fetal bovine serum (FBTS, BioSera, France). Cells were cultured in plastic Petri dishes with an area of 9 cm^2, which were treated with poly-D-lysine (Orange Scientific, Belgium) at 37°C in an atmosphere with 5% CO2. The initial seeding concentration of cells in both cases was 6 104 cells/cm^2. On day 21 of cultivation, the medium was collected from all Petri dishes and pooled; an aliquot was taken and used in further experiments. IVO was reproduced by surgical ligation [22]. After 6 weeks, the ligature was removed and the next day, the animals began to be injected intraperitoneally with the BACs for 10 days. Conditioned medium obtained from the culture of native mantle gliocytes (CMCNMG) and BCM were injected at 0.6 ml/kg of body weight. Cortexin (Geropharm, Russia) was administered at a dose of 1.0 ml/kg of body weight [23]. The animals were removed from the experiment by decapitation under anesthesia on day 56 after the start of IVO modeling. The experimental animals were divided into the following groups: Group 1 was saved as an intact control group, Group 2 received "Cortexin", Group 3 was given CMCNMG, Group 4 received BCM, and Group 5 was not treated.

Morphologic analysis of kidneys. For morphological assessment, the kidney fragments were histologically processed and stained with hematoxylin and eosin according to the standard method [24]. Microphotography was carried out using an AmScope light-optical microscope, model XYL-403 (China), and a digital camera. Morphometric analysis of micrographs of serial sections of kidneys was performed using the AxioVision Rel 4.7 image processing software (Carl Zeiss, Germany). The areas of the renal corpuscle, glomerulus, glomerular capsule cavity, outer and inner diameters of tubules, the tubular index (the ratio of the tubule wall thickness to its lumen diameter), the outer diameter, and the area of the vessel lumen on histological specimens were measured in cross-sections. When calculating the quantitative characteristics of structural components in the kidney, one micro-photograph was measured 30 times in each case [25].

Statistical analysis. The results were statistically processed using Excel (Microsoft, USA) and Statistica 10 (Statsoft, USA). Quantitative data were expressed as median (Me) and quartiles (Q1; Q3) and 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. General histological characteristic of kidneys. Morphological examination of kidneys of intact control rats revealed the renal corpuscles with elliptical and spherical glomeruli in the cortex. The glomeruli were of normal size, with thin unfolded loops, no evidence of the proliferation of glomerular cells, and an increased mesangial matrix. The vessels were consistent without signs of pathological changes. The kidney stroma consisted of loose, unaltered connective (interstitial) tissue. Intact distal and proximal renal tubules were noted in the renal parenchyma, forming nephrons together with blood capillaries (Fig. 1A, 1B).

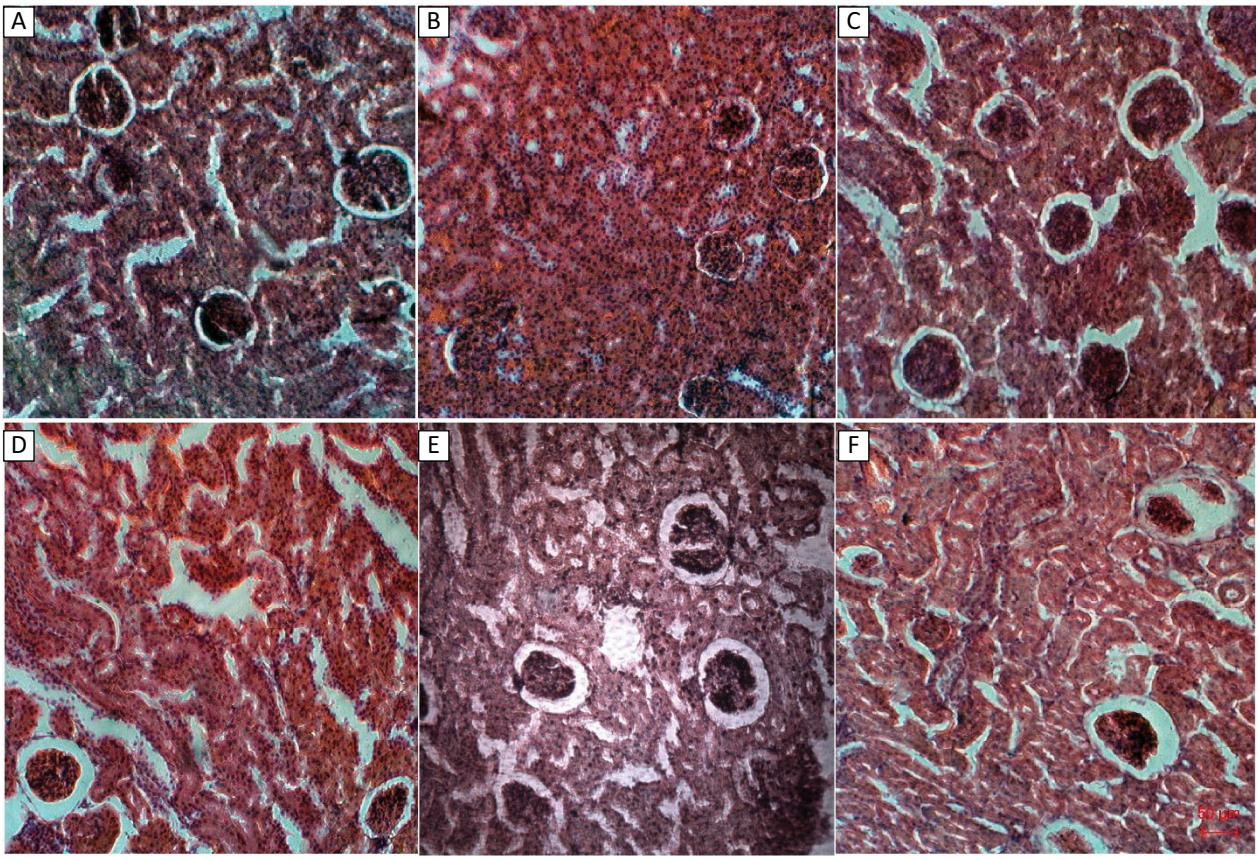

Fig. 1. Histopathological examination of the different groups examined (H and E stain; scale bar, 50 μm).
(A), (B): intact control; (C): Cortexin drug; (D): conditioned medium obtained from the culture of native mantle gliocytes; (E): basic culture medium; (F): no treatment.

In group 2 kidneys (Cortexin), the renal corpuscles with glomeruli of various shapes were found in the cortical layer. The glomeruli were moderately enlarged, with evidence of segmental cell proliferation of the glomerulus. GS and partial atrophy of the glomeruli with the expansion of Bowman's space were often detected. Defragmentation of the glomerular capillary network with single areas of mesangiolysis was multifocal. Altered renal tubules were determined (partially with tubular degeneration and atrophy, signs of regeneration of collecting ducts and tubules, everywhere expanded in diameter with desquamated epithelial cells in the lumen, sometimes with tubular hyperplasia, also single cysts). Tubular necrosis areas were identified. The vessels were changed throughout: enlarged in diameter with a narrow lumen. Multifocal interstitial fibrosis, segmental inflammatory infiltrates, single infarcts, and hemorrhages were detected (Fig. 1C).

In group 3 animals (CMCNMG), the renal cortex was found to have nonenlarged renal corpuscles with spherical and elliptical glomeruli, the latter predominating. The glomerular capillary network largely retained its normal structure. The glomerular capillary network largely retained its normal structure. The glomeruli were enlarged, with evidence of cell proliferation and a moderate increase in the mesangium. The phenomenon was multifocal in nature. In most cases, the Bowman's space was unaltered, not expanded. Most tubules were not enlarged, not dilated. The expressed hyperplasia of the tubule epithelium was found. Everywhere there was evidence of regeneration of the nephron structural elements (tubular basophilia and cell hyperplasia in the cortex and medulla without hypertrophy accompanying them, cell hyperplasia of the glomerulus basement membrane, absence or minimal diffuse peritubular and interstitial cell infiltrate, the vascular network restructuring). The diameter of the vessels was practically unchanged, their lumen was sufficient, sometimes expanded. Focal interstitial fibrosis and isolated inflammatory infiltrate were detected (Fig. 1D).

In group 4 rats (BCM), the enlarged renal corpuscles with elliptical, focally rounded, and polygonal glomeruli were determined in the cortical substance. The glomeruli were sharply enlarged with evidence of cell hyperplasia/hypertrophy. Defragmentation of the glomerular capillary network with the mesangiolysis areas was segmental. GS and partial glomerular atrophy were detected focally. Severe enlargement and expansion of the Bowman's space, which was frequently filled with cylinders, desquamated cells, and erythrocytes, was noted. Multifocally hyperplastic renal tubules are sharply enlarged and dilated in diameter, and the lumen is filled with cellular content. Areas with signs of degeneration and regeneration, partial atrophy, and single necrosis were identified. The diameter of the vessels was

increased, and the lumen was moderately sclerotic and narrowed. Focal interstitial fibrosis, single infarctions and inflammation occurred (Fig. 1E).

Microscopic assessment of the kidneys of group 5 rats (U) revealed enlarged renal corpuscles with glomeruli of various shapes in the cortical zone: often rounded or flattened, rarely ellipsoidal. Moderate enlargement of glomeruli with defragmentation of the glomerular capillary network and mesangiolysis was multifocal. Total and segmental destruction of renal corpuscles occurred. GS and atrophy with enlargement of the Bowman's space were frequently identified. Mostly it was filled with cell masses and cylinders. Focal glomerular and interstitial amyloidosis was detected. In the kidney parenchyma, multiple dilated tubules with tubular atrophy, degeneration (basophilia, nuclei accumulation, loss of the lining epithelium, single cell necrosis, pigment accumulation), and vacuolization of the epithelium were noted. Desquamated epithelial cells and cylinders were frequently noted in the lumen of the tubules. Regeneration of the tubular epithelium after degeneration in the form of hyperplasia and cell hypertrophy was focal in nature. Multiple cysts were found. The vessels were altered throughout sclerosed, greatly reduced in diameter with a narrow lumen. Multifocal interstitial fibrosis and inflammatory infiltrate, segmental infarctions, and hemorrhages (Fig. 1F).

The vessels were altered throughout sclerosed, greatly reduced in diameter with the narrow lumen. Multifocal interstitial fibrosis and inflammatory infiltrate, segmental infarcts, and hemorrhages were found (Fig. 1, F).

Morphometric characteristics of the kidneys. Measurement of the renal corpuscle area revealed an increase in groups 2, 4, and 5 by 42.3%, 193.9%, and 72.3%, respectively, compared with the intact control group (Fig. 2A). At the same time, the extent of renal corpuscle hypertrophy in group 3 was minimal and approached the control values, and it was maximal in group 4. The indices of both groups were significantly different from those of group 5 (U) (Fig. 2A, $p>0.05$), except for group 2.

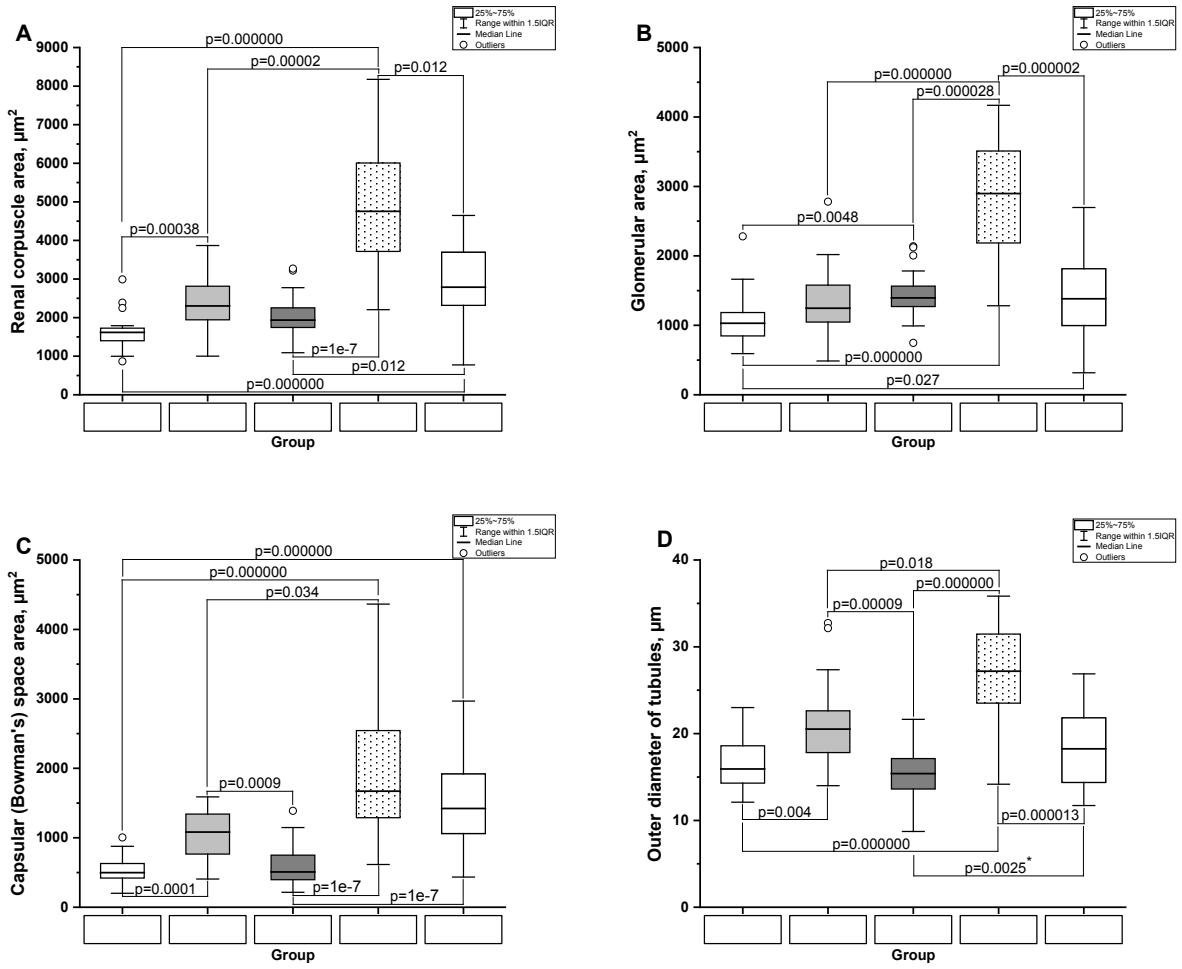

Fig. 2. Renal corpuscle area (A), glomerular area (B), capsular (Bowman's) space area (C), and outer diameter of tubules (D) of the examined groups.

The glomerulus area was increased in the animals of groups 3, 4 by 35.4%, 181.1%, and 34%, respectively, compared with the intact control group (Fig. 2B). However, the difference in this index between group 5 and BACs-treated animals was not significant except for group 4 ($p>0.05$).

Bowman's capsule area was enhanced by 117.6%, 235.8%, and 186% in groups 2, 4, and 5, respectively, compared with the intact control group (Fig. 2, C). Interestingly, the values of groups 1 (C) and 3 were in agreement but differed from those of group 5 (U).

Measurement of the outer diameter of tubules revealed an increase of 28.9% and 71% in groups 2 and 4, respectively, compared with the intact control group (Fig. 2D). At the same time, the extent of tubular enlargement in group 3 was minimal and approached the control values, and in group 4 it was maximal, which distinguished them from the corresponding indices of group 5 (U).

An increased inner diameter of tubules by 72.8%, 37.5%, and 31.2% was detected in groups 2, 4, and 5, respectively, compared with the intact control group (Fig. 3A). But the difference in this index between group 5 and BACs-treated animals was not significant, except for group 3 (Fig. 3A, $p>0.05$).

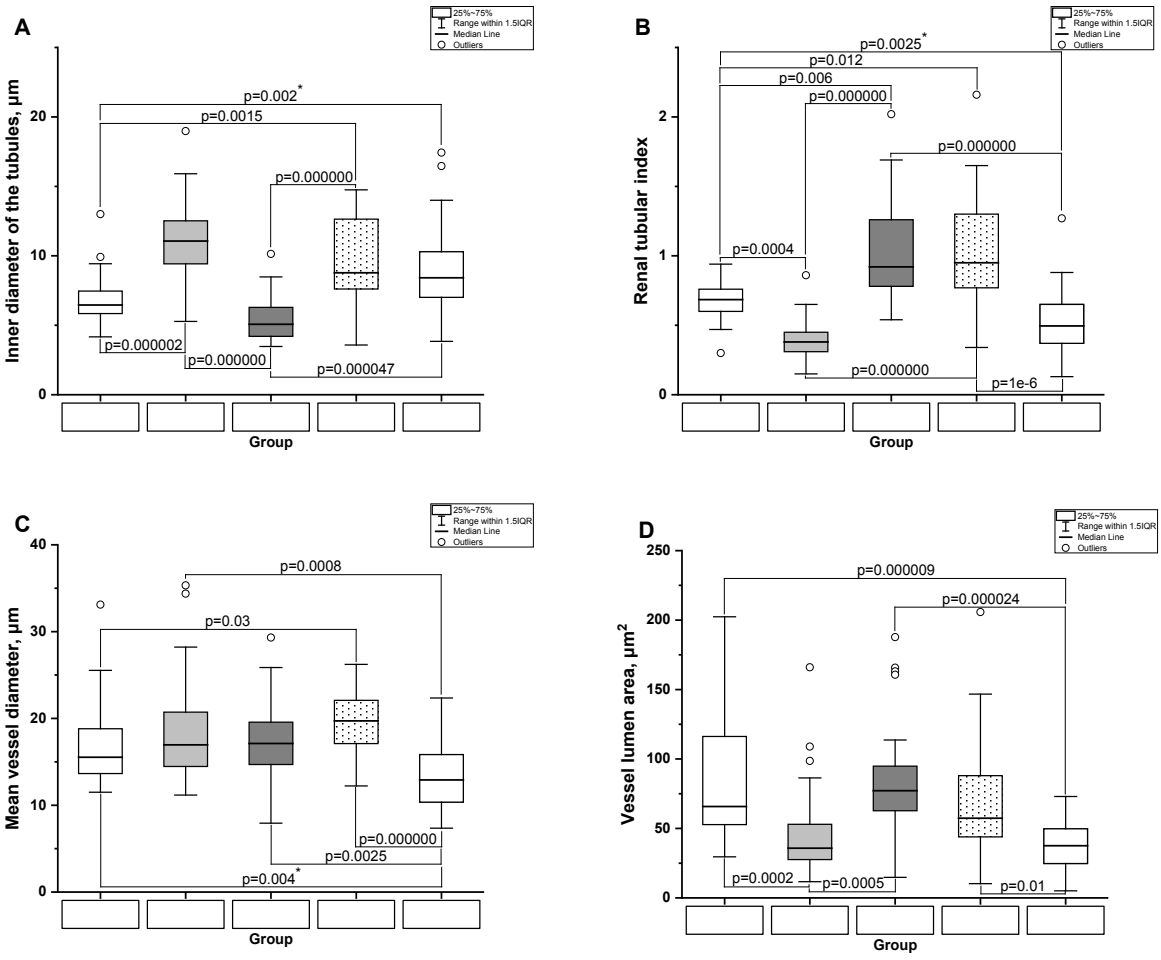

Fig. 3. Inner diameter of the tubules (A), renal tubular index (B), mean vessel diameter (C), and vessel lumen area (D) in the examined groups.

Examination of the renal tubular index revealed an increase of 1.35- and 1.4-fold in groups 3 (CMCNMG) and 4 (BCM), respectively, compared with the other groups (Fig. 3B). Groups 2 (Cortexin) and 5 (U) had the lowest index values.

Morphometric analysis revealed an increase in the mean vessel diameter in all groups except group 5 (U) by 9%, 10.3%, and 27.1%, respectively, compared with the intact control group (Fig. 3C). Besides, the size of the vessels in groups 2, 3 and 4 was significantly higher than in group 5 (U).

The vessel lumen area decreased by 45.7% and 43% in groups 2 and 5, respectively, compared with the intact control group (Fig. 3D). The vessel lumen area in group 3 was maximum and greater than the control values, and in group 4 it did not differ significantly from the latter. Interestingly, the scores of both groups were significantly different from those of group 5 (U), except for group 2 (Cortexin) (Fig. 3D, $p>0.05$).

Discussion. We searched for the papers that studied the effect of BACs obtained from SG on the morpho-functional parameters of kidneys of experimental

animals and humans after IVO. The results of such a search were meager [6, 14, 19, 26]. Basically, in most scientific reports the SCs and conditioned medium (CM) isolated by them were used for cell therapy and tissue engineering [7, 8, 11, 12, 15, 18]; and investigated the effect of high hydrostatic pressure on the renal tissue [1, 2, 5] or examined the role of endogenous NFs and growth factors in the morphology and kidney function [9, 10, 16, 17, 19, 27]. Moreover, the issue was evaluated in neurodegenerative diseases [6, 20, 23, 28], embryonic development of various organs [14, 19, 29], and the pathogenesis of various pathological conditions [3, 26]. The greater interest in this subject was triggered by our own previous flings. Previously, we have studied the morphology and functions of the urinary bladder (UB) as well as biochemical parameters of the rats' blood after IVO, the histological features of the uterus and its contractile activity in rats of all ages after the administration of BACs derived from SG. Animals injected with cryoextract of SG and CM obtained from the culture of cryopreserved MG were found to have the lowest UB weight and mass coefficient, normal values of the average thickness of the myometrium and endometrium, as well as increased contractile activity and the maximum amplitude of contractions of the UB and uterus in response to different stimuli [30-34].

Our findings in the current study were likely due to the fact that NFs mediate a decrease in the phenomena of GS and the extent of renal tubules expansion; increase the tubular index due to the protective and regenerative effects on the tubular epithelium and angiogenesis; reduce the severity of interstitial inflammation and RIF, including due to the modulation of immunological processes [6, 14, 16]. Also, NFs improve urodynamic parameters of the UB and regulate the water-salt balance while maintaining renal homeostasis, which reduces the severity and negative consequences of OU [5, 8, 31]. The effect of BACs on the conversion of macrophages into renal glomeruli or interstitium cells was revealed. At the same time, macrophages can enhance kidney damage under the action of NFs, playing a dual role in kidney pathology [1, 14, 26].

NFs play a crucial role in the survival and differentiation of visceral neurons during the development of an organism and can regulate the cell plasticity of adult neurons and non-neuronal visceral systems [11, 20, 29].

Interestingly, different types of growth factors and NFs can be both mitogens and growth inhibitors depending on the cell type and the state of cell differentiation, and can also express each other in the kidney and UB tissues [10, 28].

Established tubular indices are also indicative, which have been shown to demonstrate a reduction in the progression of the EMT in kidneys due to IVO [3, 6, 12]. NFs are responsible for the transmission of signals that can alter the behavior and physiology of target cells [19, 28].

Our previous report has confirmed the versatile effects of NFs, together with increased hydrostatic pressure due to IVO, on the growth, differentiation, and apoptosis of kidney and UB cells [33]. At the same time, the use of neurotrophic drugs such as Cortexin is less justified because they act mainly on the nervous system.

This study was experimental. Sufficient data regarding the effects of BACs administration is still required to translate this therapy into effective clinical treatment.

Conclusions. IVO leads to an increase in the area of the renal corpuscle and the Bowman's capsule space, an increase in the size of the renal tubules, and a decrease in the tubule index and diameter of the renal vessels. The administration of CMCNMG against the background of IVO normalizes the area of the renal corpuscle and the Bowman's capsule space, the outer and inner diameter of the renal tubules, and increases the tubular index, diameter, and vessel lumen of the kidney. The use of "Cortexin" against the background of IVO slightly decreases the area of renal corpuscle and glomerulus, does not affect the area of the Bowman's capsule space, increases the outer and inner diameter of the renal tubules, decreases the tubule index, and moderately enhances the diameter and lumen of the renal vessels.

Based on the above, the results of the experiment indicated the possibility of reducing the phenomena GS, tubular atrophy and RIF, as well as partially restoring the morphological structure of the rat kidneys against the background of IVO by introducing CMCNMG and, to a lesser extent, BCM due to the multidirectional effect of NFs. However, the mechanisms by which BACs containing NFs regulate physiological and pathological responses in kidneys 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;

Mykola Chyzh: histopathological evaluation and data interpretation;

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

Olga Godlevska: histopathological evaluation and data interpretation;

All authors discussed the results and commented on the manuscript.

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