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MACROSCOPIC AND MICROSCOPIC CONDITIONS OF THE SINGLE KIDNEY AND URETEROPELVIC JUNCTION AFTER THE CONTRALATERAL NEPHRECTOMY

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To date, there is no single concept on the patterns of morphogenesis of a single kidney compensatory hypertrophy. The purpose of the work is to give macroscopic and microscopic characteristics of the single kidney and the ureteropelvic junction structure after nephrectomy in the experiment. The experimental study was performed on 84 sexually matured white male rats weighing 180-200 g. All animals of the experimental group underwent surgery: nephrectomy of the left kidney. Statistical processing of the obtained results was carried out by means of the "Statistica 5.5" license package using non-parametric methods of assessing the results. It is established that in a single kidney there are processes of gradual developing compensatory hypertrophy of the organ. The sequential periods of postoperative changes were identified.

Key words: single kidney, ureteropelvic junction, nephrectomy, morphology.

The work is a fragment of the research project "Features of compensatory-adaptive processes with various diseases and damages of the human and animal body and clinical-experimental substantiation of new surgical treatment methods", state registration No. 0118U007342.

Today it is proved that in patients with a single kidney the rates of filtration-excretory function [1] and glomerular hyperfiltration [2] are significantly lower, which prognostically indicates a high risk of rapid progression of chronic kidney disease. Recent data indicate that after unilateral nephrectomy, the risk of chronic renal failure is by 3-5 times higher than in people with two kidneys [9]. Patients with a single kidney are most vulnerable to end-stage renal failure [8]. A potential risk of developing renal failure has also been established in donors, especially those who lost the kidney at a young age (18-24 years) [4]. A single kidney after contralateral removal may be more vulnerable to additional stress than congenital single kidney forms. Researchers [10] have shown that patients with a congenital single kidney with a kidney length of less than 120 mm or with proteinuria had a higher risk of renal failure.

We have found that the deterioration of the nitrogen excretion function in the single kidney and the increase in blood pressure correlated with the stage of renal ptosis [3], which is naturally due to changes in the topography of the single kidney after contralateral removal.

The pathogenesis of renal failure in patients with a single kidney may be significantly different from the pathogenesis of renal failure in the absence of nephrectomy. During the compensatory renal reaction remaining after nephrectomy, a greater tendency to develop chronic kidney disease and progression of terminal renal failure was detected [9].

However, to date, there is no single concept on the patterns of morphogenesis of a single kidney compensatory hypertrophy.

The purpose of the study was to establish the macroscopic and microscopic characteristics of the single kidney and the ureteropelvic junction structure after nephrectomy in the experiment.

Materials and methods. The experimental study was performed on 84 adult white male rats weighing 180-200 g, which were kept on a standard diet at the vivarium of Vinnytsya Pirogov Memorial National Medical University. The keeping and manipulation of animals was carried out in compliance with the "General Ethical Principles of Animal Experimentation", approved by the First National Congress on Bioethics (Kyiv, 2001), and was also guided by the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and the provisions of the "Preclinical Pharmacological Safety Assessment (GLP) Rules".

All animals were divided into two groups (42 in each): the first was the control, the second was the experimental group. In the control group under the ketamine analgesia opening of the abdominal cavity was performed, after which the abdominal wall was sutured in layers.

All animals of the experimental group were performed nephrectomy of the left kidney. Rats under general intramuscular analgesia (aminazine 10 mg/kg and ketamine 20 mg/kg) underwent left nephrectomy by cutting the renal hilum between the two ligatures with subsequent removal of the organ. Animals were sacrificed by intra-pleural administration of sodium thiopental at the dose of 50 mg/kg in 7, 14, 21 and 30, 60 and 90 days after the nephrectomy. Macroscopic assessment and description of the animals' kidneys were performed after their removal. Their weight was determined with VLR-200 laboratory scales to the accuracy of 0.1 mg, the length, width and thickness of the organ were measured using a caliper to the

accuracy of 0.05 mm. The kidney volume was calculated by the formula: V = 0.523 × a × b × s, where a is the length, b - the width, c - the thickness of the kidney.

A single kidney was removed for histological examination together with the ureteropelvic junction to detect compensatory and pathological changes in these structures that occurred within the specified time intervals. Pieces of 1 x 1 cm were cut from the renal parenchyma, and pieces of the same size were cut from the ureteropelvic junction so that 1/3 of the kidney pelvis and the ureteral section up to 1 cm in length got into the histological section. The material was fixed in 10% neutral formalin solution (pH 7.2-7.4) for 24-48 hours, then it was treated with alcohols of increasing concentration and embedded in paraffin. The resulting sections were stained with hematoxylin-eosin. Semifine sections were stained with toluidine blue.

Study of the ureteropelvic junctions and renal parenchyma microscopic structure was performed using an OLIMPUS BX41 light microscope at 100, 200, 400 and 800 magnifications. The morphometric parameters of structural changes were determined using a microscope graticule and Image Tools 3.6 software.

In the course of histometric studies, a series of images were obtained, which were used for linear measurements of each layer in the walls of ureteropelvic junctions. The thickness of the walls, perimeters and diameters of different sections of the ureteropelvic junctions' walls were determined. The percentage of structural elements in the muscular layer was also determined.

Statistical analysis of the obtained results was performed by means of "STATISTICA 5.5" software using non-parametric methods of the obtained results assessment.

Results of the study and their discussion. In the morphological study it was found that in the control group rats at the optic level in the renal cortex renal corpuscles were observed, which were round or oval structures. They have a vascular glomerulus and a Shumlyansky-Bowman capsule. The capsule consists of two epithelial leaflets, with a small space between them. Vascular glomerulus is formed of capillaries between the afferent and the efferent arterioles. The organ's stroma is represented by thin layers of loose connective tissue, which is located between the tubules and surrounds the vessels.

The outer leaflet of the renal corpuscle's capsule is formed by flat cells that had elongated nuclei and narrow cytoplasmic areas. The inner capsule leaflet tightly covered the glomerular capillaries on all sides. It is formed by irregular shaped epitheliocytes - podocytes. From their bodies several long broad processes proceeded - cytotrabecules. The latter were in contact with the basement membrane, and on the opposite side of this membrane there were endothelial cells of the glomerular hemocapillaries, which had narrow cytoplasm (fig. 1).

Morphometrically, the mean values of renal corpuscles' areas in the control group of white rats on the seventh day of the experiment were 4813±109 μm², the mean values of vascular glomeruli - 4308±94 μm², and the mean values of the capsule's lumen - 505±13 μm². After experimental nephrectomy, changes in all the nephron's components are present in the kidneys of immature animals against the background of vascular reorganization. In the cortex, most of the renal corpuscles are hypertrophied, blood filling of vascular glomeruli hemocapillaries was observed in them. The capsules' lumens are also increased compared to renal corpuscles of the control group animals.

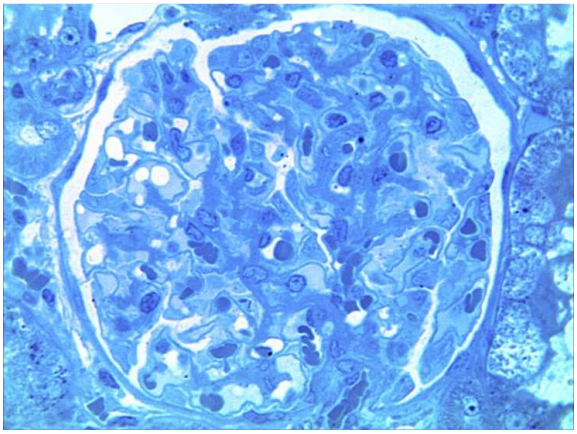

Fig. 1. Microscopic organization of nephron components in the renal cortex of a mature animal in the control group. Semifine section, staining with toluidine blue. x 800.

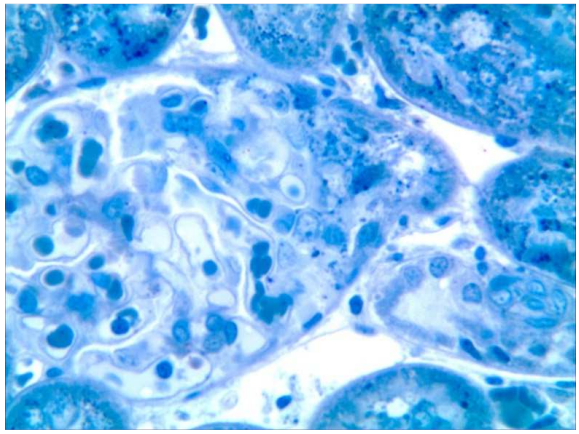

Fig. 2. Microscopic changes of the renal nephron corpuscle's cortex on the 7th day after contralateral nephrectomy. Semifine section, staining with toluidine blue. x 800.

Morphological examination of the ureteropelvic junction revealed that in the control group rats, the wall of the ureter was formed by the mucous, muscular, and serous (adventitial) layers. The structural features of each layer and their ratio were established in microscopic examination. The mucous membrane

of the ureter is formed by two layers - the epithelial and proper mucous plate of the mucous membrane. No peculiar features were noted.

Macroscopically, in the experimental group animals on the 7th day after removal of the contralateral kidney, the single right kidney was enlarged, the normal anatomical shape and surface of the kidney was preserved. The kidney weight was by 4.9% larger than the kidney weight of the control group in the same period and amounted to 0.98±0.01g, the kidney cubic capacity enlarged by 19.9% compared to the data at the beginning of the study and amounted 646.04±15.05 mm^3. Renal parenchyma retained differentiation into cortical and medullar zones. The topographic structure of the ureteropelvic junction was similar to that in the control group. It was found that the mean value of renal corpuscles area were by 15.8% higher than in the control group animals. The growth rate was an average of 2.3% per day.

The mean values of vascular glomeruli and capsule lumens' areas also increased significantly by 14.9% and 25.3%, respectively, compared to the control group animals with the mean growth rate of 2.1% and 3.6% per day, respectively. The mean area of the proximal renal nephron tubules increased to 1075±31 μm², and the mean lumen area was 428±12 μm². The mean values of the areas of the distal renal nephron tubules in this period of the experiment were 834±20 μm², and the mean values of their lumen areas were 395±11 μm². These values were by 52.5% and 45.8%, respectively, higher than in the control group animals. Microscopically, hypertrophied renal corpuscles were observed in semifiine sections of the renal cortex. In their vascular glomeruli, some of the blood capillaries had wide lumens (fig. 2).

The convoluted tubules of the nephrons are enlarged in size, both proximal and distal. The nuclei of the epitheliocytes had a circular, light karyoplasm with small basophilic blobs of heterochromatin. Protein inclusions in the form of rounded, dark, different size structures were observed in the cytoplasm of proximal cells. They are located mainly in the basal parts of the epitheliocytes cytoplasm. Closer to the basement membrane, there is striation, and the apical areas are optically lighter and have a brush-like border.

Two weeks after removal of the contralateral kidney, macroscopically, the single kidney was even larger than 7 days after, its capsule was shiny, tense. The kidney's weight was by 13.6% greater than that of the control group in the same period and amounted to 1.08±0.02 g, the kidneys cubic capacity increased by 47.7 % compared to the data at the beginning of the study and made 989.61±18.24mm^3.

Histological studies showed that on the 14th day after the experimental nephrectomy in the animals' kidneys there were greater changes of the vascular bed and parenchyma than in the previous term of the experiment. Hypertrophied renal corpuscles dominated the cortex. Lumens of the capsules was increased compared to the renal corpuscles of the control group animals. The area of the vascular glomerulus increased by 15.7% in comparison with that in the control group, and by 4.1% in comparison with the seven-days term, which made 0.6% per day. The lumen area of the Shumlyansky-Bowman capsule increased by 23.4% compared to the control and by 1.8% compared to the seven-days term. The growth rate during this period averaged 0.3% per day.

In the course of the compensatory response in the kidney remaining after unilateral nephrectomy, the area of the proximal convoluted tubules also increased. After 14 days it was larger by 11.67% compared to the animals of the seven-days experiment. The rate of the proximal convoluted tubules' area increase was 1.7%.

Three weeks after the surgery, the kidney weight was by 25.5% larger than that of the control kidney in the same period and amounted to 1.17±0.04 g, the kidney's cubic capacity was greater by 55.5 % compared to the data at the beginning of the study and was 1162.73±27.80 mm3.

Histologically, in addition to hypertrophied renal corpuscles in the cortex, the number of atrophied, diminished renal corpuscles increased. The capsule lumens were enlarged and uneven (fig. 3).

Destructive changes in the proximal and distal convoluted tubules of nephrons increased. Tubules with significant lumens were detected. Morphometrically, it was found that the mean value of the renal corpuscles area during this period of study was by 16.14% larger than in the control group animals. Compared to the previous term of the study, the area of the renal corpuscles was only by 2.9% larger. The mean daily growth was 0.4%. Indices of the proximal convoluted tubules area in the single kidney increased by 7.5% compared to the animals on the 14th day of the study.

The performed histological studies showed that on the 30th day after the experimental nephrectomy, the destructive changes in the kidneys are similar to the previous term of the experiment. Edema of the stroma connective tissue was accompanied by focal leukocyte infiltration. In the cortex there are both hypertrophied and atrophied, reduced in size renal corpuscles. The lumens of the capsules are enlarged and uneven (fig. 4).

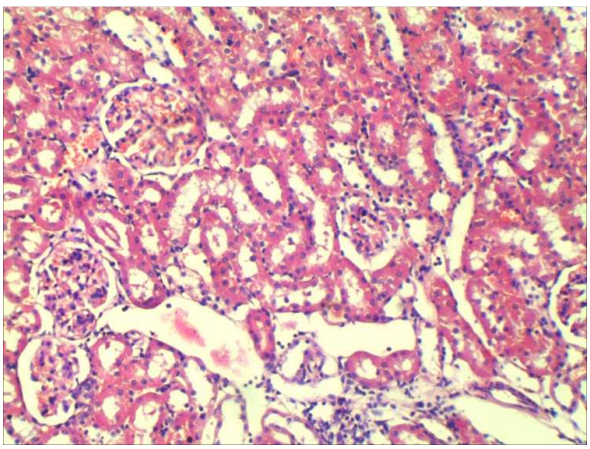

Fig. 3. Microscopic condition of the kidney cortex on the 21st day after the contralateral nephrectomy. Renal corpuscles and nephron tubules are altered. Staining with hematoxylin and eosin. x 200.

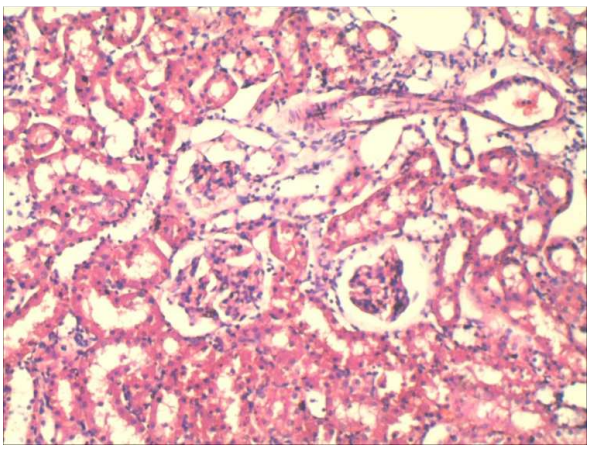

Fig. 4. Microscopic condition of the renal cortex on the 30th day after the contralateral nephrectomy. Renal corpuscles and nephron tubules are altered. Staining with hematoxylin and eosin. x 200.

On the 30th day postoperatively, the mean renal corpuscles area increased by 15.2% compared to the control group animals. Compared to the previous term of the experiment, the area of the renal corpuscles was only by 2.1% larger. The mean daily growth was 0.3%. The mean values of vascular glomeruli are by 13.5% higher than in the animals of the control group and by 1.6% higher than in the previous period. The mean daily growth was 0.2%. The lumen area of the capsule was reliably greater by 5.5% than in the animals of the previous experimental period. Compared to the animals in the control group it was greater by 26.4%. The mean daily growth in this period was 0.8%. The proximal convoluted tubules area was larger by 3.1% compared to the animals on the 21st day of study, but the rate of increase was smaller.

On the 60th day of the postoperative period after nephrectomy, besides hypertrophied, atrophied, reduced renal corpuscles are observed. In their vascular glomeruli, the blood capillaries had mostly small lumens, made by erythrocytes. In the Shumlyansky-Bowman capsules small lumens were noted. The mean value of the renal corpuscles area during this period of study is by 17.1% larger than in the control group animals. Compared to the previous term of the experiment, the renal corpuscles area was by 3.9% larger. The mean daily growth was 0.1%.

The mean values of vascular glomeruli area were by 16.8% higher than in the control group animals and by 6.4% higher than in the previous period. The mean daily growth was 0.2%. The lumen area of the capsule was reliably larger by 8.0% than in the animals of the previous experimental period. Compared to the animals of the control group it was by 28.5% higher. The mean daily growth during this period was 0.3%.

On the 90th day after the experimental nephrectomy, it was found morphometrically that the mean value of renal corpuscles area was by 16.6% higher than in animals of the control group. Compared to the previous term of the experiment, the area of the renal corpuscles was only by 1.8% larger. The mean daily growth was 0.06%. The mean values of vascular glomeruli area were by 15.7% higher than in the control group animals and by 2.8% higher than in the previous period. The mean daily growth was 0.09%. The lumen area of the capsule was reliably larger by 6.2% than in the animals of the previous experimental period. The mean daily growth in this period was 0.2%.

Thus, the morphometric analysis showed that the highest growth rate of the structural components of the renal corpuscles in the single kidney after the contralateral nephrectomy was observed in the near postoperative period until the seventh day, and subsequently it decreased. The growth rates of the proximal and distal convoluted tubules were greater than those of the renal corpuscles components.

In the later period after the kidney removal (60 and 90 days), more pronounced destructive and sclerotic changes occurred. Morphological changes in the wall of the ureteropelvic junction and ureters lied in the increased amount and expansion of intermuscular collagen fibers.

Atrophic processes were followed by further fibrotic degeneration. Against the background of progressing sclerosis, phenomena of deep destruction and dissociation were observed in the elastic frame, particularly in the submucous membrane. The performed microscopic studies of the renal cortex in semifine sections showed that on the 60th and 90th days (long terms after nephrectomy) there were signs of compensatory hypertrophy and destructive changes were revealed in the components of nephrons. Many renal corpuscles are hypotrophic, some podocytes have basophilic, pycnotically altered, irregular nucleus. In their vascular glomeruli, blood capillaries have mainly small lumens that are filled with red blood cells.

According to the literature, intraglomerular hypertension caused damage to podocytes and loss of selective permeability of the slit diaphragm filtration function between the processes of podocytes, causing proteinuria [5], which led to renal failure progression [6]. Studies on the animals kidneys have shown that glomerular hyperfiltration was a key risk factor for chronic kidney disease in nephrectomy-exposed rats [7].

Conclusion

In the single kidney there were processes of the organ's compensatory hypertrophy gradual development. Sequential periods were established: the period of early postoperative changes (in the experiment - 7-14 days after the contralateral kidney removal); the recovery period (from 14 days to 1 month in the experiment). In these terms, there took place the initial compensatory changes, the initial phenomena of hypertrophy, which testified to the strengthening of all functions in the single kidney. In later periods of the study (60-90 days postoperatively), some nephrons developed destructive changes.

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Реферати

МАКРОСКОПІЧНИЙ І МІКРОСКОПІЧНИЙ СТАН ЄДИНОЇ НИРКИ І МИСКОВО-СЕЧОВІДНОГО СЕГМЕНТУ ПІСЛЯ НЕФРЕКТОМІЇ КОНТРАЛАТЕРАЛЬНОЇ

Півторак В.І., Монастирський В.М., Окаєвич О.А., Булько І.В., Сміюха О.А.

До теперішнього часу немає єдиної концепції про закономірності морфогенезу компенсаторної гіпертрофії єдиної нирки. Метою роботи було дати макроскопічну і мікроскопічну характеристику структури єдиної нирки і мисково-сечовідного сегмента після нефректомії в експерименті. Експериментальне дослідження виконано на 84 статевозрілих білих щурах-самцях масою 180-200 грамів. Всім тваринам дослідної групи виконували оперативне втручання - нефректомію лівої нирки. Статистична обробка отриманих результатів проведена в ліцензійному пакеті "Statistica 5.5" з використанням непараметричних методів оцінки результатів. Встановлено, що в єдиній нирці відбуваються процеси поетапного розвитку компенсаторної гіпертрофії органу. Виділили послідовні періоди післяопераційних змін нирки.

Ключові слова: єдина нирка, мисково-сечовідний сегмент, нефректомія, морфологія.

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МАКРОСКОПИЧЕСКОЕ И МИКРОСКОПИЧЕСКОЕ СОСТОЯНИЕ ЕДИНСТВЕННОЙ ПОЧКИ И ЛОХАНОЧНО-МОЧЕТОЧНИКОВОГО СЕГМЕНТА ПОСЛЕ НЕФРЭКТОМИИ КОНТРАЛАТЕРАЛЬНОЙ

Пивторак В.И., Монастырский В.Н., Окаевич А.А., Булько И.В., Смиюха А.А.

К настоящему времени нет единой концепции о закономерностях морфогенеза компенсаторной гипертрофии единственной почки. Целью работы было дать макроскопическую и микроскопическую характеристику структуры единственной почки и лоханочно-мочеточникового сегмента после нефрэктомии в эксперименте. Экспериментальное исследование выполнено на 84 половозрелых белых крысах-самцах массой 180-200 граммов. Всем животным опытной группы выполняли оперативное вмешательство - нефрэктомию левой почки. Статистическая обработка полученных результатов проведена в лицензионном пакете "Statistica 5.5" с использованием непараметрических методов оценки результатов. Установлено, что в единственной почке происходят процессы поэтапного развития компенсаторной гипертрофии органа. Выделили последовательные периоды послеоперационных изменений почки.

Ключевые слова: единственная почка, лоханочно-мочеточниковый сегмент, нефрэктомия, морфология.

Рецензент Єрошенко Г.А.