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ANNOTATION

Dissertation work for the PhD degree
specialty 6D110100 "Medicine"

Topic: «the morphological substantiation of the use of the decellularized bovine-derived peritoneum in nephropexy (experimental study)»

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Relevance.

Nephroptosis is a disease that manifests itself by omission of the kidney. The cause of the disease is a weakening (congenital or acquired) of the ligamentous apparatus of the kidney. Omission is accompanied by tension of the neurovascular bundle. This pathology it is one of the most common diseases of the retroperitoneal space with a frequency of identifications in 1.54% of women and 0.12% men. Among patients suffering from nephroptosis, about 70 % are women in the between the ages of 20 and 50[1].

Complications of nephroptosis may include in the form of vascular pathology or urodynamic disorders, with a more pronounced stage of nephroptosis, it leads to chronic renal failure [2,3,4,5]. It is known that the level of markers of oxidative stress increases with the progression of chronic kidney disease and significantly correlates with the level of renal function, which is accompanied by increased oxidative stress, which manifests itself in the following ways: secondary degradation products of lipid peroxidation - malondialdehyde and a product of antioxidant protection-glutathione peroxidase [6].

By expressed an effective treatment method for nephroptosis is surgical correction using modern technologies and materials that do not have side effects in the implantation zone [7].

It is known that the use of well-known synthetic implants in surgery can be accompanied by long-term complications in the form of chronization of the process with the formation of gross scarring changes in the implantation zone, leading to inactivity and morphofunctional disorders of the organ. Among the severe complications in the post-implantation period, fistula formation and pressure sores should be noted, which affects the general somatic condition and quality of life of patients due to hypertension and the development of severe pain syndrome [8-13].

The search for alternative materials began more intensively in the mid-19th century. In recent scientific studies, it has been noted that biological implants are an alternative material in reconstructive surgery. One of the first and active proponents of this hypothesis was N. I. Krause [14].

So currently, biological implants that represent an extracellular matrix (ECM)are promising[15] are implants obtained from animal and/or human donor material by tissue engineering. The extracellular matrix is a complex of functional and structural proteins, These include glycoproteins, proteoglycans, chondroitin, hyaluronic acid, and collagens. The leading property is the maintenance and preservation of the tissue framework, which is involved in the processes of signal transmission, regulation of cell growth and differentiation, as well as in apoptosis of cellular elements, what makes it possible to use extracellular matrix as implants in fabric engineering design and regenerative medicine [16-20].

The mechanisms by which these bio-frames promote constructive remodeling and favorable clinical outcomes include the release or creation of effector molecules that attract endogenous stem/progenitor cells to the scaffold site and modulate the immune response, in particular the activation of anti-inflammatory

macrophage [21]. Also, nanovesicles embedded in extracellular matrices (matrix nanovesicles) provide a mechanistic understanding of the inductive properties of the ECM biomaterial and regulation of tissue homeostasis [22].

Despite the progress in the study of biocompatible materials in urology and medicine in general, the assessment of morphological and biochemical processes remains insufficiently studied issues of post-implantation complications such as fibro-sclerotic process in the contact zone, as well as the state of the degree of activity of malondialdehyde and glutathione peroxidase in the body on the implant. In this regard, scientific research is of practical interest, dedicated to the comparative analysis of the state of histostructure and biochemical processes in the contact zone decellularized matrix of the xenoperitoneum with kidney tissue and paranephral tissue, since we have not found any works covering this problem in the literature available to us.

Research purpose: to carry out morphological and biochemical substantiation of the use of the decellularized bovine-derived peritoneum (extracellular matrix) in comparison with non-decellularized xenoperitoneum and UltraPro mesh during nephropexy in an animal experiment.

Research objectives:

1. To study the comparative analysis of malondialdehyde level and glutathione peroxidase activity in kidney tissues and blood plasma of laboratory animals decellularized bovine-derived peritoneum (extracellular matrix) in comparison with non-decellularized xenoperitoneum and UltraPro mesh.
2. To evaluate the macroscopic characteristics of implantation zones in dynamics during nephropexy with different types of implants.
3. To perform a comparative histological analysis of the cell infiltrate in the area of contact of kidney tissue with the extracellular matrix of xenoperitoneum, non-decellularized xenoperitoneum and UltraPro mesh.
4. To perform a comparative morphometric analysis of the connective tissue maturation process in representative areas of contact of various types of implants with kidney tissue and peri-renal adipose tissue.

Scientific novelty.

1. For the first time, a method of nephropexy was developed and implemented using various types in an experiment on rats.
2. For the first time, a morphological description is given with the following lines: morphometric analysis of the reparative process of connective tissue neoplasms during implantation of non-decellularized xenoperitoneum and extracellular matrix of xenoperitoneum.
3. For the first time, it was established that the use of the extracellular matrix of xenoperitoneum during nephropexy in rats in the long-term period shows a less pronounced collagenization process with fibrosis, while the collagen structure is organized, ordered, without signs of chronization of the process compared to the implantation of the UltraPro mesh and non-decellularized xenoperitoneum .

4. For the first time, a comparative analysis of malondialdehyde and glutathione peroxidases in the kidney tissue and blood of rats when using the extracellular matrix of xenoperitoneum for nephropexy.

Relationship of the dissertation to other research papers.

The dissertation work was carried out within the framework of the grant-funded research project of the Ministry of Education and Science of the Republic of Kazakhstan on the topic 3242/GF4 "Development and implementation of new types of implants for laparoscopic nephropexy" with state registration No. 0115RK00306.The research was conducted at the Department of Pathological Anatomy and Molecular Biochemistry of KSMU.

Practical significance:

1. The practical significance of the dissertation work is that the results of the study can be used as a basis for a clinical study of the use of the extracellular matrix of xenoperitoneum in nephropexy.
2. Papplication form the extracellular matrix of xenobitoneum during nephropexy is characterized by low activity in the biochemical results of malondialdehyde indicators in kidney tissue and blood plasma of rats relative to groups using non-decellularized xenobitoneum and UltraPro mesh.
3. Comparative morphological analysis using extracellular matrix in nephropexy was accompanied by a step-by-step reparative process in the area of contact of kidney tissue with the matrix with the formation of mature connective tissue, without involvement of the surrounding tissue in the adhesive process and without a chronic reparative-regenerative process.

Implementation in practice.

Received certificate of state registration of rights to the copyright object No.1672 from 10.08.2016 "Methods for modeling nephropexy in an experiment" [23] and Patent of the Republic of Kazakhstan for invention No. 43378 dated 09.10.2017 "Method of surgical correction of nephroptosis"[24] were introduced during scientific and experimental studies in nephropexy.

Based on the results of the experimental study, a scientific monograph was co-authored: "Results of modeling nephropexy by various methods in an experiment "[25], approved at the meeting of the Academic Council of Karaganda State Medical University, Protocol No. 7 of 24.01.2018. This publication can be recommended to specialists dealing with issues of nephropexy in a mobile kidney and for students of higher educational medical institutions. institutions during the course of operative urology and clinical pathomorphology.

Materials and methods of research.

Research design.

An experimental comparative study was conducted to assess the morphological and biochemical features of the interaction of the extracellular matrix of xenoperitoneum , non-decellularized xenoperitoneum and UltraPro mesh with kidney and perinephral adipose tissue of short-haired mature rats. This study

was approved by the Bioethics Committee of Karaganda State Medical University Protocol No. 69 of 26.11.2015. The experiment was conducted with compliance with the recommendations European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes (Strasbourg, 18.03.1986). Experimental work on the maintenance and care of laboratory animals, met the standards given in the manuals Guide for care and use of laboratory animals. Eight edition. ILAR publication [26] and American medical associations veterinarians American Veterinary Medical Association Guidelines for the Euthanasia of Animals: 2013 Edition.

The study was conducted on 144 short-haired white mongrel mature rats of both sexes, of the same age, weighing 200-220 g. In a biochemical study of malondialdehyde and glutathione peroxidase activity in tissue and blood plasma, additional data were obtained 6 short-haired white mongrel sexually mature rats that formed the control group for biochemical research.

The animals were randomly assigned to 3 groups and 6 subgroups of 8 individuals. 3 study groups were formed in accordance with the implant material used: in the first group, the following method was used: decellularized (extracellular) matrix of xenoperitoneum ; in the second group - notdecellularized xenoperitoneum ; in the third group - macroporous partially absorbable light mesh UltraPro. Each subgroup corresponded to the standard period of withdrawal of the animal from the experiment: 7 days; 14 days; 21 days; 30 days; 90 days and 180 days. Identification of animals was carried out by assigning an individual number to each experimental animal with a dye label on the dorsal surface of the body.

The experimental study was carried out on the basis of the pathomorphological laboratory of the Department of Pathological Anatomy and on the basis of on the basis of the vivarium of Karaganda State Medical University. Rat blood plasma and kidney cryohomogenizate were used as the material of the biochemical study. MDA and GPO indicators were evaluated at the Department of Molecular Biology of KSMU. For microscopic examination, a tissue fragment was resected in the implant area. A representative site consisted of a fragment of the contact of kidney tissue, paranephral tissue with the implant outside the suture material. Material collection for histological examination, it was performed according to the generally accepted method. Owriting of macroscopic examination and glass preparations was carried out on the basis of the Department of Pathological Anatomy of Karaganda State Medical University.

Method of manufacturing a decellularized (extracellular) matrix of xenoperitoneum .

Scientific research under the under the leadership of Abugaliev K. R. and Ogai V. B. Processing of the decellularized (extracellular) matrix was carried out on the basis of the scientific laboratory of "General Genetics" LLP. Decellularization principle peritoneal samples were performed using the detergent-enzymatic method. Then a decellularization solution was added

containing 0,25% sodium dodecyl sulfate and 0.5% Triton X. The decellularization procedure was repeated 2 times. Sterilization xenogenic material was produced using gamma radiation [27].

Methods of statistical processing of research results.

SPSS Statistics 22 was used for statistical analysis. Data analysis was performed at the significance level α=0.05. The normality of the distribution of quantitative data was checked using the Kolmogorov-Smirnov test. Quantitative data were presented using the median and quartiles. For qualitative data, the proportion and 95% confidence interval of the proportion were calculated. The Mann-Whitney and Kruskell-Wallace U test was used to compare independent samples. The Pearson-Spearman correlation coefficient was used to estimate the relationship.

Main provisions submitted for defense

1. The implantation of non-decellularized xenoperitoneum and UltraPro mesh revealed the formation of fibrosclerotic tissue in the area of contact between implants and kidney tissue.
2. When using the extracellular matrix of xenoperitoneum biomaterial as an implant for nephropexy no morphological signs of chronic persistence inflammatory reaction and fibrosis were detected and no increase in the level of malondialdehyde in the long term period was detected;
3. For the first time, it was established on the basis of morphological, morphometric and biochemical data that the extracellular matrix of xenoperitoneum is the most optimal for performing nephropexy in comparison with non-decellularized xenoperitoneum and UltraPro mesh.

Conclusions:

1. In all study groups *blood plasma* there were no statistically significant differences in these MDA parameters on day 30 relative to the control, and no significantly significant differences were found on day 21 when evaluating the glutathione peroxidase index in all experimental groups. In the group with the use of the extracellular matrix of xenoperitoneum , MDA values in *kidney tissue* they corresponded to the control group for 90 days (p=0,382 in contrast to the comparison groups, where MDA activity was maintained for a longer period of time, implantation of non-decellularized xenoperitoneum was performed on day 180 (p=0,161), and in the group with UltraPro, there was a significantly significant difference relative to the control group (p=0.001). The activity of glutathione peroxidase in animal tissue in the group of extracellular matrix of xenoperitoneum did not show statistically significant differences relative to the control, which accounted for 30 days of exposure, and in the comparison groups, the activity of GPO was in accordance with the control group on day 180 (p=0.446; p=0,442).
2. A comparative analysis of the macroscopic picture of the kidney contact zone when using different types of implants showed that the formation of delicate thin strands of fibrous connective tissue occurs by 21-30

days (p=0.118). By 90-180 the strength of adhesions and the degree of involvement of surrounding tissues were statistically significantly higher in animals in the group with the use of UltraPro mesh and undecellularized xenobitoneum (p=0.016).

3. The comparative histological analysis of cell infiltrate in the contact zone of kidney tissue during nephropexy with the extracellular matrix of the xenoperitoneum , it showed an active decrease in the cell pool and a dynamic increase in the population of stromal cells. and the presence of collagen fibers, which means the formation of a mature connective tissue contact in the implantation zone by 21-30 days of exposure. In the group with the use of non-decellularized xenoperitoneum and with the UltraPro mesh, an active effect was detected collagen formation, sharp increase in the stromal cell population by 14-21 days of exposure (p=0,045) with weak lymphocytic infiltration (p=0.004) in nephropexy, which indicates the formation of connective tissue in these experimental groups.

4. The morthometric analysis of the histostructure in the contact zone in groups using the extracellular matrix of xenoperitoneum and non-decellularized xenoperitoneum showed the formation of a mature scar on the 21st - 30th day of exposure during nephropexy. When using the UltraPro mesh as an implant, the formation of a connective contact was noted on days 14-21, followed by the process of fibrosis (p=0.007). In the remote days of the experiment with mature and well-organized fibrous and fibrovascular tissue was determined using the extracellular matrix of xenobitoneum in all cases: collagen fibers were ordered compositionally (p=0.016) in contrast to the groups with UltraPro mesh and non-decellularized xenoperitoneum , where it was revealed hoatically located collagon colocons (p=0.0016), significantly greater collagen deposition (p=0,000).

Based on the results of the dissertation, the following can be formulated: ***practical recommendations*:**

Macroscopic, histomorphometric, and biochemical analyses have shown that xenoperitoneum used for nephropexy has a positive dynamics of the reparative process with the formation of connective tissue contact in a representative area, there are no signs of a chronic process, there is a higher rate of reduction of malondialdehyde and early activation of glutathione peroxidase in the post-implantation period. Based on morphological and biochemical results, it can be considered as a more preferable material for clinical research in nephropexy.

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