

зв'язки та альвеолярного відростку щелеп, що підтверджується суттєвим збільшенням концентрації вільного оксипроліну, глікозаміногліканів та N-ацетилнейрамінової кислоти. Застосування APDTC за умов СЗВ істотно зменшує у м'яких і кістковій тканинах пародонта деполімеризацію колагену, протеогліканів та сіалоглікопротеїнів, обмежує ступінь резорбції альвеолярного відростка щелеп. Зроблено висновок, що застосування цієї сполуки при СЗВ є ефективним засобом корекції дезорганізації сполучної тканини пародонта шурів.

Ключові слова: ядерний фактор каппа В, системна запальна відповідь, біополімери сполучної тканини, колаген, протеоглікани, глікопротеїни, пародонт.

Стаття надійшла 20.04.2019 р.

связки и альвеолярного отростка челюстей, что подтверждается существенным увеличением концентрации свободного оксипролина, гликозаминогликанов и N-ацетилнейраминової кислоти. Применение APDTC при СВО существенно уменьшает в мягких и костной тканях пародонта деполімеризацию коллагена, протеогликанов и сиалогликопротеинов, ограничивает степень резорбции альвеолярного отростка челюстей. Сделан вывод, что применение этого соединения при СЗВ является эффективным средством коррекции дезорганизации соединительной ткани пародонта крыс.

Ключевые слова: ядерный фактор каппа В, системный воспалительный ответ, биополимеры соединительной ткани, коллаген, протеогликаны, гликопротеины, пародонт.

Рецензент Срошенко Г.А.

DOI 10.26724/2079-8334-2020-1-71-183-187

UDC 616.711-003.8-007.17-047.58:599.323.4]-085.382.036.8

V.M. Zaporozhan, O.L. Appelhans, O.V. Tsyurupa
Odesa National Medical University, Odesa

EFFECTIVENESS OF THE PLATELET-RICH PLASMA APPLICATION AT DIFFERENT SIMULATION PERIODS OF DEGENERATIVE DISC DISEASE IN RATS

e-mail: aleksakusher@gmail.com

The article describes the study of the platelet-rich plasma application effectiveness for tissue regeneration of intervertebral discs in rats with simulated degenerative disk disease of the caudal vertebrae for 60 and 90 days. The experiment involved 80 rats, which were divided into four groups: Group I – rats with a simulated pathology without correction for 60 days, Group II – rats with a simulated pathology without correction for 90 days, Group III – rats with a pathology and its correction for 60 days, Group IV – rats with a pathology and its correction for 90 days. The described morphological changes in the intervertebral disk tissue suggest that the application of platelet-rich plasma in the pathology simulation for 60 days leads to the degenerative process inhibition and restores the intervertebral disk structure. The application of platelet-rich plasma for 90-day pathology simulation is less effective.

Key words: degenerative disk disease, intervertebral disc, fibrous ring, nucleus pulposus, platelet-rich plasma.

The work is a fragment of the research project "To develop and substantiate methods of correction of liver fibrous changes at chronic hepatitis and cirrhosis", state registration No. 0116U008927.

Spinal diseases take the second place among the causes of temporary disability, and, eventually, often lead to permanent disability [2]. In 90% of cases, spinal diseases are based on degenerative disk disease (DDD) [3]. At the age of 30 years, signs of DDD are detected in 57% of cases, and at the age of 60 years and older – in 100% [3]. In Ukraine, annually about 1 million patients with DDD seek medical help, more than 16 thousand of them subsequently become disabled. Thus, this pathology is of great importance not only for the medical, but also for the socio-economic sphere.

Currently, the study of the effectiveness of tissue therapy application to regenerate the intervertebral disc (IVD) structure after DDD is a promising area. The literature data indicate the effectiveness of using some growth factors for the intervertebral disk morphology regeneration after DDD in the experiment [9]. The use of platelet-rich plasma (PRP) on IVD tissues in laboratory rats after the acute IVD injury in the early stages of the DDD formation has a positive effect [7, 8]. However, often DDD-associated visits to medical institutions for qualified care occur after the first clinical manifestations, when the pathological process is already expressed.

The purpose of work was to study the efficiency of using PRP in the DDD simulation for 60 and 90 days.

Materials and methods. The study was carried out on Whistar rats of both sexes aged 4-6 months (80 animals), which were divided into four groups: Group I – animals with DDD for 60 days, without correction; Group II – animals with DDD for 90 days, without correction; Group III – animals with DDD for 60 days, which were injected with PRP; Group IV – animals with DDD for 90 days, which were injected with PRP. A separate group (10 animals) studied as intact animals. Animal preparation, anesthesia, surgery, postoperative care and terminal sacrifice were carried out in accordance with the Law of Ukraine "On protection animals from brutal treatment" No. 27, Art. 230 of 2006, and the general principles of ethics of experiments on animals and the Code of Ethics for Ukrainian Scientists.

The experiment was performed in the research laboratory of the Department of Normal and Pathological Clinical Anatomy of Odessa National Medical University (ONMedU) by forming static compression-distension of the caudal vertebrae of spinal column [5]. The surgical procedure took place under general ether anesthesia. 2% lidocaine solution was used for pain relief. The procedure was carried out in two stages. First, a stump was formed by the tail resection at the level of CcXIV-CcXV. Then the stump was sutured to the muscles and ligaments of the lumbosacral spine. After treating the surgical wound with an antiseptic and applying the postoperative dressing, the animals were placed in a warm container until awakened, and then transferred to the cells. Pathology was simulated within 60 and 90 days after surgery. Then, animals of Groups III and IV were injected subcutaneously in the base of the tail with 0.1 ml of PRP twice with an interval of seven days. PRP was obtained immediately before administration, by isolating it from whole blood on a SmartPrep preparation (manufactured by Harvester Corp, USA). The first day of the experiment was considered the day after the last PRP injection. Animals were sacrificed on the 14th and 28th day of the experiment via ether overdose inhalation.

After the experiment, a section with two adjacent vertebrae in the area of the greatest tail flexure was excised for the pathomorphological study. The obtained objects were formalin-fixed, decalcified, and further prepared and stained with hematoxylin-eosin according to standard methods [1]. Histologic specimens were studied using a Leica DM750 light microscope using standard microscopy and morphometric methods [1]. The significance of differences between the two samples was determined using Student's parametric criterion. The Student's coefficient value was determined with the number of degrees of freedom equal to (n_1+n_2-2) . The significance of the differences between the two samples (p) was calculated using the distribution table. The difference was considered significant if the probability of a random difference did not exceed 0.05 ($p \leq 0.05$).

Results of the study and their discussion. The intervertebral discs of intact animals consisted of a centrally located nucleus pulposus, which was surrounded on the periphery by a fibrous ring with an almost symmetrical shape. The nucleus pulposus (NP) had a regular oval shape, consisted of large notochordal cells and small chondrocytes. Notochordal cells were located mainly in the central parts of the nucleus in clusters of 4-6 cells, and chondrocytes were located on the periphery. The fibrous ring (FR) was represented by plates of collagen fibers of the longitudinal direction with fibroblasts and chondrocytes located between them. At the border of the IVD with the epiphysial cartilage of neighboring vertebrae, end plates consisting of round and spindle-shaped basophilic cells located in 1-2 layers were located parallel to each other. The IVD thickness of intact animals on both sides was almost the same and averaged 1.75 ± 0.09 mm. The NP cross-section area in the central part of the disk was equal to 4.21 ± 0.16 mm² (table 1).

Table 1

Morphometric indicators of intervertebral discs in rats

Parameter	Control	Group I	Group II	Group III		Group IV	
				in 14 days	in 28 days	in 14 days	in 28 days
FR on compression side (mm)	1.75 ± 0.11	$1.22 \pm 0.12^*$	$0.62 \pm 0.05^*$	1.61 ± 0.13	1.59 ± 0.12	$0.78 \pm 0.05^*$	$0.76 \pm 0.05^*$
FR on distension side (mm)	1.75 ± 0.11	2.14 ± 0.15	1.48 ± 0.05	2.16 ± 0.22	2.02 ± 0.19	1.76 ± 0.08	1.70 ± 0.08
NP area (mm ²)	4.21 ± 0.16	$2.37 \pm 0.21^*$	$0.91 \pm 0.12^*$	$2.97 \pm 0.19^*$	$2.96 \pm 0.19^*$	$1.18 \pm 0.05^*$	$1.17 \pm 0.05^*$

* - $p \leq 0.05$ – the difference is significant relative to the control

Histological examination of IVD in Group I (simulation of DDD for 60 days) revealed the asymmetry of its structures. On the compression side, a decrease in the thickness of the fibrous ring was observed. Its plates were flattened and stratified. In the central areas of compression, ruptures of collagen fibers with the presence of polymorphic fissures and foci of fibrinoid necrosis were noted. Between the fibers were single fibrocytes with signs of dystrophy. The thickness on the compression side was 1.22 ± 0.12 mm, and on the distension side – 2.14 ± 0.15 mm. The nucleus pulposus was reduced in size and displaced towards distension. Its area in the central part of the disk was 2.37 ± 0.21 mm². The nuclear matrix was heterogeneous, consisting of complexes of 3-4 notochordal cells of preserved structure and areas without cells. There were signs of vacuole dystrophy in the nucleus cells. The end plate consisted of predominantly preserved cells located in 1-2 rows, and contained areas of necrosis (fig. 1).

On histological preparations of IVD in animals of Group II, a sharp asymmetry of their structures was noted. The IVD thickness on the compression side was 0.62 ± 0.05 mm. The FR collagen fibers on the compression side were sharply compressed, and in some places they were broken. Among them are single fibrocytes and chondrocytes with signs of dystrophy. On the distension side, the FR collagen fibers were stretched, stratified, and contained polymorphic fissures and multiple ruptures. The IVD thickness on the

distension side was 1.48 ± 0.05 mm. There were signs of vacuole dystrophy in the FR cells. The nucleus pulposus was irregular in shape, sharply reduced in size and displaced towards distension. Its matrix contained significant cell-free foci. Notochordal cells were located singly and in groups of 2-3 cells, forming clusters. Most of the cells were in the stage of hydropic degeneration. The end plate contained numerous foci of necrosis and ossification (fig. 2).

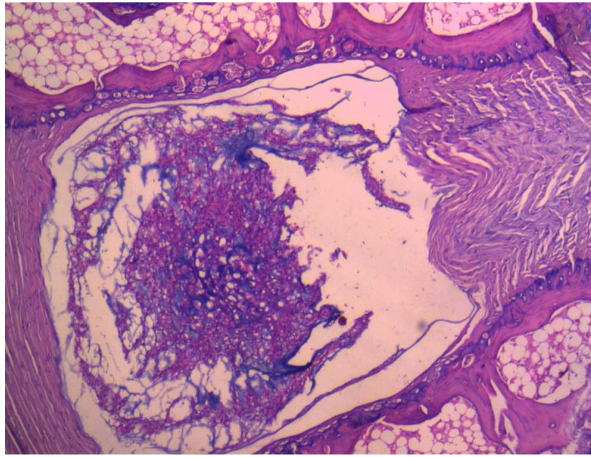


Fig. 1. Morphology of the intervertebral disc of a rat with simulated DDD for 60 days. Staining: hematoxylin, eosin, x 40

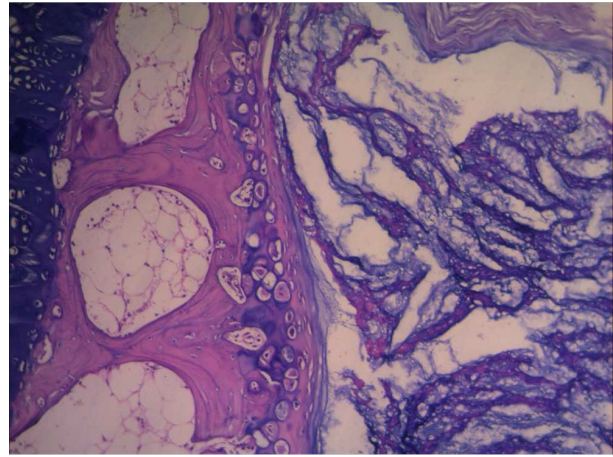


Fig. 2. Morphology of the intervertebral disc of a rat with simulated DDD for 90 days. Staining: hematoxylin, eosin, x 100

The study of preparations of the Group III showed the restoration of the intervertebral disk structure, mainly due to the fibrous ring tissues. 14 days after the PRP administration, the restoration of the fibrous ring thickness (1.61 ± 0.13 mm) was observed on the compression side. The fibers were less flattened and stratified than in a group without correction. Chondrocytes of 2-4 cells with signs of proliferation were located between the fibers. There were no foci of fibrinoid necrosis. The FR thickness on the distension side was 2.16 ± 0.22 mm. The nucleus pulposus was displaced towards distension and reduced in volume. Its cross-sectional area was 2.97 ± 0.19 mm². The nuclear matrix contained clusters of 3-4 notochordal cells with moderate polymorphism and vacuolated cytoplasm. Signs of synthetic activity were observed in the cells. An increase in the chondrocytes number is noted near the EP, and in the plate itself the number of necrosis foci is reduced and there are signs of synthetic cell activity. 28 days after the PRP administration, signs of synthetic activity of the disk cells persisted. The fibrous plates of the FR outer layer were stratified with breaks. The inner layers of the FR were without breaks. Pockets of fibrinoid necrosis were absent or were very isolated. Between the fibers were located chondrocytes with signs of proliferation. The thickness of the fibrous ring on the compression side was 1.59 ± 0.12 mm, on the distension side – 2.02 ± 0.19 mm. The nucleus pulposus retained a displaced position in the disk; its area in the central part of the disk remained almost unchanged (2.96 ± 0.19 mm²). In the nuclear matrix, against the background of large cells with signs of dystrophy collected in groups, the number of small single cells visually increased.

In the IVD tissues of animals of Group IV, the nucleus pulposus asymmetry and a decrease in the disk thickness on the compression side were preserved. On the 14th day after the PRP administration, in the outer layers of the FR there are signs of structure restoration against the background of a long degenerative process. The plates of the FR outer layer were stratified, contained longitudinal fissures and single breaks. Foci of fibrinoid necrosis of 10-30 μm, reaching up to 1/2 of the fibrous ring thickness, were noted in the compression area. The plates of the FR inner layer were more preserved and organized. Hypertrophic fibrocytes with signs of synthetic activity were found between the fibers and near the foci of necrosis. The disk thickness on the compression side was 0.78 ± 0.05 mm, and on the distension side – 1.76 ± 0.08 mm. The nucleus pulposus cut area in the central part of the disc was 1.18 ± 0.05 mm². Its matrix was heterogeneous and contained cell-free areas and cavities. Notochordal cells were located singly or clustered in 2-3 cells. Some cells were in a state of vacuole dystrophy, while others formed a heterogeneous matrix around themselves. The end plates were thinned and contained single cells with signs of synthetic activity. Numerous cells with a preserved structure of various sizes were observed near the EP.

On the 28th day of the experiment, the positive effect of PRP on the disc tissue was observed. The plates of the FR outer layer were disorganized and stratified, contained longitudinal fissures 20–40 μm in size. The fiber ruptures in the inner layers of the FR were single. Foci of fibrinoid necrosis were noted on the compression side. Fibroblasts with signs of synthetic activity were located between the FR plates. The

disk thickness on the compression side was 0.76 ± 0.05 mm, and on the distension side – 1.70 ± 0.08 mm. The nucleus pulposus cut area in the central part of the disc was 1.17 ± 0.05 mm². The matrix of the nucleus pulposus was compacted and contained cell-free areas. Notochordal cells of the nucleus pulposus were represented by solid clusters and elongated complexes. Against the background of notochordal cells in the stage of vacuole dystrophy and necrobiosis, smaller cells were determined. The end plates contained chondrocytes with signs of synthetic activity.

Studying the histological preparations of animals of Group I and II, and comparing them with the control, revealed the occurrence and progression of degenerative changes in the tissues of FR and NP. This is evidenced by increasing the FR fibrinoid necrosis foci and polymorphic fissures, clusters destruction and an increase in the NP cell-free areas, an increase in the EP necrotic sections, as well as signs of hydropic degeneration of the disk cells. Morphometric analysis revealed a decrease in the fibrous ring thickness on the compression side to 69.7% (Group I) and 35.4% (Group II) of the intact disk thickness. The nucleus pulposus was displaced toward distension, its area in the central part of the disk decreased to 56.3% (Group I) and 21.6% (Group II) of the intact disk.

A study of the intervertebral discs of animals of the Groups III and IV revealed the effectiveness of the PRP administration for the regeneration of degenerative discs. This is evidenced by an improvement in the structure of the fibrous ring plates, a decrease in the number and size of fibrinoid necrosis foci, as well as signs of fibrocyte proliferation. Matrix restoration, the appearance of synthetic activity of notochordal cells and an increase in the number of chondrocytes are noted in the NP tissues. Near the EP, there is an increase in the chondrocytes number, and in the plates themselves – a decrease in the number of necrosis foci and a high synthetic activity of the cells.

After 14 days, the PRP administration in Group III contributed to the restoration of the 92% FR thickness of the intact disc on the compression side. On the distension side, the thickness of the fibrous ring was 23% greater than the intact discs. The nucleus pulposus area after correction amounted to 70.5% of intact discs. A comparison of disk preparations after 14 and 28 days from the PRP administration did not reveal statistically significant differences, which can indicate a prolonged effect of growth factors.

14 days after the PRP administration to the Group IV of animals, the fibrous ring thickness on the compression side was 44.6%, and the NP area was 28% of intact discs. This indicates a lower effect with the PRP administration in the late terms of DDD simulation.

PRP is a small fraction of plasma with a high concentration of platelets. The regenerative effect of PRP is based on the effect of a cocktail of growth factors [11, 12]. With the PRP administration into degenerative discs at the early stages of DDD simulation (60 days), a lot of biologically active growth factors are released that contribute to tissue repair by activating the regenerative potential of disk cells [11]. The effect of the PRP administration is observed after two weeks and persists for at least another four weeks. Against the background of a longer DDD simulation (up to 90 days), when the number of disc cells and their regenerative potential are reduced, the PRP administration has a lower effect.

Of course, we cannot say that our experimental DDD simulation in rats fully reflects the natural course of spinal diseases, if only because of the presence of ongoing compression on the discs in case of illness. However, the literature data indicate that this is one of the closest morphological manifestations of the options for DDD simulation [4].

Obviously, the PRP administration has an active regenerative effect on the IVD tissues for 28 days, and possibly longer, which can be revealed in the course of further experiments. It should be remembered that the quality of PRP, prepared using commonly used laboratory centrifugation procedures may vary [13].

Conclusions

1. The formation of static compression-distension of the caudal vertebrae of spinal column in rats for 60 days leads to a pronounced degenerative disk disease, which is manifested by mechanical displacement and damage to the tissues of the intervertebral disc, resizing of the disc components, as well as the occurrence of degenerative processes in the cells themselves.

2. The formation of static compression-distension of the caudal vertebrae of spinal column in rats for 90 days leads to the progression of degenerative disk disease in the intervertebral discs and the development of irreversible tissue changes.

3. The administration of platelet-rich plasma during the DDD simulation for 60 days is effective in tissue regeneration and helps to restore the disk structure.

4. The use of platelet-rich plasma during the DDD simulation for 90 days has a positive effect on the inhibition of the pathological process in the disks, but, however, a significant restoration of morphology is not observed.

References

1. Bahriy MM, Dibrova VA, Popadynets OH, Hryshchuk MI. Metodyky morfolohichnykh doslidzhen: Monohrafiya. Vynnytsya. Nova knyha. 2016. 328 s. [in Ukrainian]
2. Bobryk YuV. Kompleksna reabilitatsiya khvorykh na osteokhondroz khrehta iz nevrolohichnymy proyavamy z vykorystanniam novykh dyferentsiynykh pidkhodiv. Krym. derzh. med. un-t im. S.I. Heorhiyevskoho. Yalta. 2013. 26–36. [in Ukrainian]
3. Kradinov AI, Chernorotov VA, Chernorotova YeV. Diagnosticheskiye i prognosticheskiye kriterii osteokhondroza sheynogo otdela pozvonochnika pri sanatorno-kurortnoy meditsinskoj reabilitatsii. Promeneva diahnozyka, promeneva terapiya. 2012; 4: 49–53. [in Russian]
4. Malyshkina SV, Dedukh NV, Levshin AA, Kosterin SB. Modelirovaniye degeneratsii mezhpozvonkovogo diska v eksperimente na zhivotnykh. Ortopediya, travmatologiya i protezirovaniye. 2015; 1: 114–124. [in Russian]
5. Kholodkova OL, Tsyurupa OV, Badyin IYu, vynakhidnyky; Sposib modelyuvannya deheneryatyno-dystrofichnoho urazhennya khvostovoho viddilu khrehta u shchuriv. Patent Ukrayiny No.106085. 2015 lystop.18. [in Ukrainian]
6. Abrams GD, Frank RM, Fortier LA, Cole BJ. Platelet-rich plasma for articular cartilage repair. Sports Med Arthrosc. 2013; 21:213–219.
7. Anitua E, Padilla S. Biologic therapies to enhance intervertebral disc repair. Regen Med. 2018 Jan; 13(1):55–72.
8. Cho H, Holt DC 3rd, Smith R, Kim SJ, Gardocki RJ, Hasty KA. The Effects of Platelet-Rich Plasma on Halting the Progression in Porcine Intervertebral Disc Degeneration. Artif Organs. 2016; 40(2):190-5.
9. Feng C, Liu H, Yang Y, Huang B, Zhou Y. Growth and differentiation factor-5 contributes to the structural and functional maintenance of the intervertebral disc. Cell Physiol Biochem. 2015; 35(1):1–16.
10. Gullung GB, Woodall JW, Tucci M A, James J, Black DA, McGuire RA. Platelet-rich plasma effects on degenerative disc disease: analysis of histology and imaging in an animal model. Evid Based Spine Care J. 2011; 2(4):13–18.
11. Hu X, Wang C. An experimental study on the effect of autologous platelet-rich plasma on treatment of early intervertebral disc degeneration. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2012; 15:977–983.
12. Li P, Zhang R, Zhou Q. Efficacy of platelet-rich plasma in retarding intervertebral disc degeneration: a meta-analysis of animal studies. Biomed Res Int. 2017; 2017(2):1–10.
13. Menchisheva Y, Mirzakulova U. Preparation of platelet-rich plasma: methods, problems of standardization. Vestnik KazNMU. 2018; 3: 236–240.

Реферати

**ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ
ЗБАГАЧЕНОЇ ТРОМБОЦИТАМИ ПЛАЗМИ
ПРИ РІЗНИХ ТЕРМІНАХ МОДЕЛЮВАННЯ
ДЕГЕНЕРАТИВНО-ДИСТРОФІЧНОГО
УРАЗЕННЯ ХРЕБТА У ЩУРІВ**

Запорожан В.М., Аппельханс О.Л., Цюрупа О.В.

У статті описано дослідження ефективності застосування збагаченої тромбоцитами плазми для відновлення тканин міжхребцевих дисків щурів з модельованим дегенеративно-дистрофічним ураженням хвостового відділу хребта протягом 60 та 90 днів. В експерименті брали участь 80 щурів, які були розподілені на чотири групи: I група – щури з модельованою патологією протягом 60 днів без корекції, II група – щури з модельованою патологією протягом 90 днів без корекції, III група – щури з патологією 60 днів та корекцією, IV – щури з патологією 90 днів та корекцією. Описанні морфологічні зміни тканин дисків дають змогу стверджувати, що застосування збагаченої тромбоцитами плазми при моделюванні патології 60 днів призводить до гальмування дегенеративного процесу та відновлює структуру диску. Застосування збагаченої тромбоцитами плазми при моделюванні патології 90 днів має менший позитивний ефект.

Ключові слова: дегенеративно-дистрофічне ураження хребта, міжхребцевий диск, фіброзне кільце, драглисте ядро, збагачена тромбоцитами плазма.

Стаття надійшла 28.04.2019 р.

**ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ
ОБОГАЩЕННОЙ ТРОМБОЦИТАМИ ПЛАЗМЫ
ПРИ РАЗЛИЧНЫХ СРОКАХ МОДЕЛИРОВАНИЯ
ДЕГЕНЕРАТИВНО-ДИСТРОФИЧЕСКОГО
ПОРАЖЕНИЯ ПОЗВОНОЧНИКА У КРЫС**

Запорожан В.Н., Аппельханс Е.Л., Цюрупа А.В.

В статье описано исследование эффективности применения обогащенной тромбоцитами плазмы для восстановления тканей межпозвоночных дисков крыс с моделируемым дегенеративно-дистрофическим поражением хвостового отдела позвоночника в течение 60 и 90 дней. В эксперименте участвовали 80 крыс, которые были разделены на четыре группы: I группа – крысы с моделируемой патологией в течение 60 дней без коррекции, II группа – крысы с моделируемой патологией в течение 90 дней без коррекции, III группа – крысы с патологией 60 дней и коррекцией, IV – крысы с патологией 90 дней и коррекцией. Описанные морфологические изменения тканей дисков позволяют утверждать, что применение обогащенной тромбоцитами плазмы при моделировании патологии 60 дней приводит к торможению дегенеративного процесса и восстанавливает структуру диска. Применение обогащенной тромбоцитами плазмы при моделировании патологии 90 дней менее эффективно.

Ключевые слова: дегенеративно-дистрофическое поражение позвоночника, межпозвоночный диск, фиброзное кольцо, студенистое ядро, обогащенная тромбоцитами плазма.

Рецензент Єрошенко Г.А.