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Effect of potentiated cryopreserved mesenchymal stem cells in intervertebral disc repair

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The aim of the study was to investigate the effect of cryopreserved mesenchymal stem cells (CrMSCs) from different tissue (bone marrow, adipose tissue, cartilage) potentiated with some growth and differentiation factors (TGFβ and bFGF) on intervertebral disc (IVD) cartilage repair using compression model of degenerative damage. In the group with self-healing histological changes in IVD cartilage tissue were pronounced. Administration of untreated CrMSCs (regardless of their origin) regenerated IVD structure up to a moderate degree on the 45th day. After therapy with TGFβ-potentiated cells, the histological structure of the disc remained similar to untreated CrMSC influence, which according to the integrated semi-quantitative scale corresponded to moderate degenerative changes. Whereas, the administration of bFGF-potentiated cells contributed to the restoration of the disc structure up to a mild degree of histological damages. In the latter case, there was an increase in cellularity of the fibrous ring, regeneration of cracks and gaps, and restoration of collagen febrile structure in annulus fibrosus on the 45th day after therapy. At the same time, the average cell density of fibrous rings increased by 1.35, 1.50, and 1.39 times and IVD height grew by 1.33, 1.36, and 1.37 times for bone marrow-, adipose- and cartilage-derived CrMSC of potentiated by bFGF respectively compared to untreated analog. Thus, administration of CrMSCs from all studied sources activates regenerative processes in degenerated intervertebral discs of rats, the rate of which increases using of bFGF-potentiated cells.

Key words: cryopreservation; cultivation; mesenchymal stem cells; b-fibroblast growth factors; transforming growth factor β; intervertebral disc; histological structure.

INTRODUCTION

Low back pain is a significant problem in low- and middle-income countries, which is growing every year, mainly due to population growth and aging [1]. Most authors are of the opinion that the pathogenesis of chronic low back pain is based on degenerative changes in the intervertebral discs (IVDs). In this case, disc degeneration is a multifactorial process, the main causes of which include excessive mechanical action, incorrect motor stereotype, genetic predisposition, violation of adequate nutrition of cellular elements, etc. [2]. The influence of all these factors leads to a decrease in the cellularity of the disc structural components, which causes a very low density of cells in the IVD of adults compared to other tissues (cells occupy approximately 0.5-1% of the volume of IVD tissues) [3]. As a result, the disc loses the ability to regenerate (in its tissues there is an imbalance between the synthesis and degradation of components of the intercellular substance in favor of the latter), and degeneration processes are started [4].

Given that one of the key elements of the chain of IVD degeneration is a decrease in the number of cells and their metabolic activity, it can be assumed that cell therapy of this pathology should show a fairly high clinical efficacy [5]. In fact, the administration of mesenchymal stem cells (MSCs), which have high regenerative potential and the ability to differentiate in several directions, including chondrogenic, has already given promising results in this pathology [6]. In our previous

studies, we have also obtained good outcomes: MSCs contributed to IVD restoration providing the most pronounced stimulatory effect on the fibrochondrocyte density in annulus fibrosus [7]. But, isolation of stem cells, their cultivation and, if necessary, differentiation is only the first stage of their application in regenerative medicine. In order to use obtained stock of stem or specialized cells anywhere and at any time they must be frozen and stored at low temperatures. And although the application of cryopreserved and native MSCs had similar results cryopreserved MSCs showed a "lag" therapeutic effect that reduces the effectiveness of treatment [7].

One of the advantages of MSCs is that they can be isolated from various tissue sources of the human body. The main of them and still the most common is bone marrow [8], where MSCs are represented by a small population of fibroblast-like cells (approximately 0.01% of bone marrow cells), which helps maintain an undifferentiated state of hematopoietic stem cells [9]. But currently, methods for obtaining them from adipose, cartilage, tendon, and other tissues have been developed [10]. Depending on the source, biological properties of MSCs are not exactly the same [11]. It has been proved that foetal MSCs showed a significantly higher expansion capacity and different paracrine secretion pattern than maternal MSCs did [12]. MSCs derived from bone marrow, adipose tissue and chorion demonstrate strong capacities for differentiation towards adipogenic, chondrogenic, and osteogenic lineages. MSCs from liver have a lower adipogenic potential, and MSCs derived from umbilical cord or amnion are weakly differentiated in osteogenic direction. [11]. At the same time, synovial-derived cells show higher chondrogenic differentiation ability than other common MSC sources [13]. This variability of MSCs is probably associated with different content of precommitted cells at their source. However, the microenvironment into which MSCs are cultivated/transplanted plays a key role in determining both MSC biology and clinical improvement [14].

It is known that MSCs can promote regeneration not only by direct cell differentiation but also by secretion of growth factors [15, 16]. The latter are one of the most important molecular families involved in regeneration. With the help of protein receptors, growth factors bind to target cells and cause intracellular responses. It should be noted that the regulators of direct chondrogenic differentiation of cells are a number of intracellular signaling molecules and growth factors including b-fibroblast growth factors (bFGF), transforming growth factor β (TGFβ), cartilage-derived morphogenetic proteins (CDMPs), bone morphogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1) and Wingless factors (Wnt) [17].

The aim of the study was to investigate the effect of cryopreserved mesenchymal stem cells from different sources (bone marrow, adipose tissue, cartilage) potentiated with some growth and differentiation factors (TGFβ and bFGF) on IVD cartilage regeneration in vivo.

METHODS

Animals and ethics statement. The work was carried out on 50 male outbred white rats (5-month-old) weighing 350 ± 50 g (mean ± s.d.) and 10 male outbred white rats (2.5-month-old) weighing 150 ± 25 g. All manipulations with animals were performed in accordance with international bioethical norms of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the protocol of the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (No. 2014-02).

Isolation and culture of MSCs. Rats weighing 150 ± 25 g were tissue donors. Primary cell suspension of bone marrow was obtained from resected fragments of the femur (3-4 mm^3). Cells were isolated by washing with Hanks' solution ("PAA", Austria), followed by passing through a needle with decreasing diameter, followed

by centrifugation at 1500 rpm for 5 min. The resulting cell suspension was seeded on plastic culture flasks with an area of 25 cm^2 ("PAA", Austria). The seed concentration of the cells was 10^3 per cm^2 of the flask. The total number of cells was counted by the traditional method in the Goryaev chamber. Cell viability was determined by supravital trypan blue staining ("Fluka", India), and the relative number of unstained cells was counted.

The primary suspensions of cells from fragments of adipose (75 ± 3 mg) and cartilage (25 ± 3 mg) tissues were obtained from the corresponding biopsies by enzymatic disaggregation. To do this, tissue samples were washed with Hanks' solution with gentamicin (150 μg/ml) ("Farmak", Ukraine) and incubated in a type II collagenase solution (1.5 mg/ml) ("PanEco", Russia) at 4°C for 18 h. After that cells were isolated from biopsies by resuspension followed by centrifugation at 1500 rpm for 3 min. A culture medium was added to the precipitate and resulted from cell suspensions were sown with an initial cell density of 10^4 per cm^2 of culture flask. The culture medium in all cases contained: Iscove's Modified Dulbecco's Medium (IMDM, "PAA", Austria), 10% fetal bovine serum ("HyClone", USA), kanamycin (150 μg/ml) ("Farmak", Ukraine) and amphotericin B (5 μg/ml) ("PAA", Austria). The culture medium (5 ml) was changed every three days.

Cryopreservation of MSCs. Cryopreservation of MSC cultures was carried out on the 2-3 passage under the protection of 10% dimethylsulphoxide ("PanEco", Russia) with the addition of 20% fetal bovine serum. Cryopreservation solution was prepared on the culture medium. A cooling rate was 1°C/min to −80°C followed by immersion in liquid nitrogen [18]. Thawing was carried out in a water bath at 40°C until the appearance of a liquid phase. Removal of cryoprotectant was carried out by adding Hanks' solution 1:9 followed by centrifugation at 1500 rpm for 5 min. All resulting cultures after cryopreservation had a phenotype $CD45^-$(<1%) $CD90^+CD73^+CD105^+$ (>90%) and were able to differentiate in the osteogenic, adipogenic, and chondrogenic directions.

Potentiation of cryopreserved MSCs by growth factors. Potentiation of cryopreserved MSCs (CrMSCs) from all studied sources using 5 ng/ml TGFβ ("Sigma-Aldrich", USA) and 10 ng/ml bFGF ("Sigma-Aldrich", USA) was carried out under culturing conditions during 12 days. The culture medium consisted of IMDM, 10% fetal bovine serum, kanamycin (150 μg/ml) and amphotericin B (5 μg/ml) with the addition of appropriate growth factor at the indicated final concentrations.

Animal compressive model of IVD degenerative damage. Modeling of degenerative damage of IVD tissues was performed by compression method on rats weighing 350 ± 25 g. To do this, the animal was anesthetized by an intraperitoneal injection of ketamine (10 mg/kg, "Biolik", Ukraine) combined with xylazine (1 mg/kg, "Bioveta", Czech Republic). The skin of the tail and back was depilated and disinfected with 5% iodine alcohol solution. A skin incision was made along the midline 1 cm cranial to the lumbar-sacral joint where a subcutaneous pocket was formed, and haemostasis was provided. Then a resection of 2/5 of length of a caudal spine at the level of IVD Co14-Co15 was performed providing haemostasis from the vessels of the tail. The stump formed during resection was inserted into the formed subcutaneous pocket and fixed with a continuous mattress suture to the skin of the back. In order to form a stronger fixation of the tail and a bend of a smaller diameter, an anastomosis between the skin of the tail and back length of 1.5 cm was additionally imposed by three successive interrupted sutures caudal to the stitched stump. During 5 days of the postoperative period animals received Brovaseptol ("Brovapharma", Ukraine) 4 g/kg of feed to prevent septic complications and ketofen ("Merial S.A.S.", France) at a dose of 2 mg/kg for analgesia.

Treatment of IVD degenerative damage. On

the 60th day after the formation of lordosis in the caudal spine, animals were anesthetized as described above and the action of compression was stopped by tail and back separating. The dorsal surface of IVD Co6-Co7 was bluntly skeletonized and a bed was formed in this area. After thorough hemostasis, a gelatin sponge ("Ankerpharm", Germany) measuring 0.8×0.8×0.5 cm was placed into bed, onto which 0.1 ml of cell suspension in Hanks' solution containing 0.5×10^6 CrMSCs was applied. Depending on the type of administrated cells, the animals were divided into ten groups (n = 5): comparison group – self-healing; experimental group 1 – bone marrow-derived CrMSCs; experimental group 2 – bone marrow-derived CrMSCs + TGFβ; experimental group 3 – bone marrow-derived CrMSCs + bFGF; experimental group 4 – adipose-derived CrMSCs; experimental group 5 – adipose-derived CrMSCs + TGFβ; experimental group 6 – adipose-derived CrMSCs + bFGF; experimental group 7 – cartilage-derived CrMSCs; experimental group 8 – cartilage-derived CrMSCs + TGFβ; experimental group 9 – cartilage-derived CrMSCs + bFGF. After the application of cell suspension, the skin of the tail was sutured with knotted sutures with haemostasis monitoring and disinfection. Then animals were given antibiotic prophylaxis and analgesia for 5 days.

Histomorphometric analysis. For histological studies on day 45 of the therapy, areas of the spine at the level of Co5-Co8 were resected, labeled and placed in 10% formalin solution for 10 days for fixation. Celloidin sections with a thickness of 7 μm were made on a microtome ("PFM", Germany) in the sagittal direction. Staining was performed with hematoxylin-eosin and picrofuchsin (van Gieson method). Microscopy was carried out on ZEISS Primo Star light microscope ("Carl Zeiss Microscopy GmbH", Germany). Microphotographs of sagittal spine sections from the central part of the blocks were used for morphometry and were processed with ZEISS ZEN 2 (blue edition) ("Carl Zeiss Microscopy GmbH", Germany). The height of IVD Co6-Co7 was measured, and average cell density was evaluated in dorsal part of its fibrous ring.

For a complex assessment of observed histological changes in cartilage tissue of IVD Co6-Co7, an integrated semi-quantitative scale was used. It took into account the course of the collagen fiber bundles, the degree of collagen fiber fragmentation, the size of cracks, the area of hypo-/acellularity, the state of fibrous ring/nucleus pulposus boundary, the severity of fuchsinophil staining, and the state of the adjacent vertebrae apophyses. Each criterion in this scale was scored from 0 to 4 points, and a minimum total number of points (7-8 points) corresponded to normal intact rats of the same weight, the number of points from 9 to 16 was defined as mild, from 17 to 22 points as moderately expressed and from 23 points as pronounced degenerative changes of IVD cartilaginous tissue [7].

Statistical analysis. Statistical analysis of the obtained results was performed using the program "Statistica 8" ("StatSoft Inc.", USA) using non-parametric criteria (Kruskal-Wallis one-way analysis of variance on ranks and Student-Newman-Keuls multiple comparison test). The critical value of the significance level was assumed to be 0.05.

RESULTS

IVD histological structure evaluation. In IVD Co6-Co7 of animals from the comparison group signs of degenerative damage without visual reparative changes were found (Fig. 1). A course of collagen bundles in the fibrous ring was disrupted; they had a serpentine shape, underwent fibril disintegration, and were fragmented, forming cracks and gaps. Around the latter, in addition to areas with low fibrochondrocyte density, there were places with their complete absence, which had the appearance of unstructured swollen sharply eosinophilic tissue. This indicated pronounced degenerative changes in collagen fibers.

Preserved fibrochondrocytes were isolated or formed isogenic groups and were heterogeneous in structure: along with cells with ovoid nuclei which are typical for this type of cartilage tissue large chondrocytes with spherical nuclei were found in territorial proximity to the fibrous ring cracks. At the edges of the disc, the formation of single infiltrates of fibroblasts was observed, which were replaced by foci of fibrous tissue.

Partial restoration of cartilage structure was determined at the histological sections of IVD Co6-Co7 of animals on the 45th day after administration of suspension of bone marrow-, adipose- or cartilage-derived CrMSCs (Fig. 2A-C).

In all cases, the fibrous ring had a lamellar organization with a radial course of bundles of collagen fibers. However, in the areas of the dorsal part of the fibrous ring adjacent to the nucleus pulposus, in the groups of adipose- and cartilage-derived CrMSCs, the collagen fibers usually had a serpentine shape. In addition, the degree of defibrillation of collagen fibers and the size of cracks and gaps of the fibrous ring reduced significantly in all groups with the use of unstimulated CrMSCs compared to the self-healing group. Fibroblast-like elongated cells with dense ovoid nuclei were located along and inside the bundles of collagen fibers. The cell density from the site of CrMSC administration was significantly higher compared to the opposite part of the fibrous ring, although its central areas on the dorsal side of the disc remained uncellular. The highest cell density was observed in the outer areas of the dorsal part of fibrous ring (i.e. in the area directly adjacent to the location of the carrier with cells).

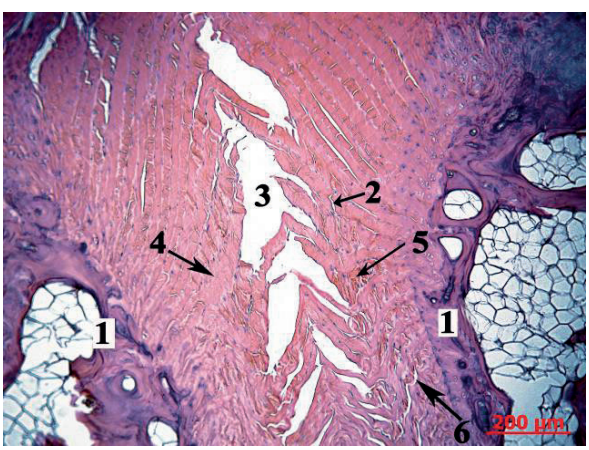

Fig. 1 The dorsal part of IVD Co6-Co7 of rat from comparison group with self-healing (day 45). 1 – Adjacent apophyses; 2 – cracks of collagen fibers; 3 – gaps in annulus fibrosus; 4 – acellular areas; 5 – eosinophilic areas; 6 – serpentine-shaped collagen bundles. Microphotograph, staining with hematoxylin and eosin

On day 45 after local administration of bone marrow-, adipose- or cartilage-derived CrMSC suspensions potentiated with TGFβ, regenerative processes were represented by reducing the size of cracks and gaps of collagen fibers and increasing cellularity in the outer parts of the fibrous ring as well as in adjacent apophyses (Fig. 2D-G). The fibrous ring cells were dominated by fibroblast-like elongated cells with dense ovoid nuclei, which were located in chains or groups along and inside the bundles of collagen fibers. However, central acellular areas were noted in the case of cartilage-derived CrMSCs + TGFβ (Fig. 2G), and after the application of bone marrow-derived CrMSCs + TGFβ large chondrocytes with spherical nuclei were presented in the center of the dorsal part of fibrous ring at the site of cracks and gaps indicating a tenseness of reparative processes in these areas (Fig. 2D). Such cells were sometimes placed in lacunae that indicated their high synthetic activity. In addition, in all cases of CrMSCs potentiated with TGFβ, the degree of defibrillation of collagen fibers was significantly reduced compared to the self-healing group.

The use of CrMSCs potentiated with bFGF had a more pronounced regenerative effect compared to cells stimulated with TGFβ (Fig. 2H, I). In groups with bone marrow- and adipose-derived CrMSC administration there was an increase in cellularity, regeneration of cracks and gaps, and restoration of collagen fibrillar structure. As a result, only small central areas of the dorsal part of fibrous ring remained cell-free. However, among all the studied

groups, the maximum regenerative effect was observed in the case of cartilage-derived CrMSCs + bFGF, after administration of which there was the almost complete restoration of the cartilaginous tissue structure of the dorsal part of IVD Co6-Co7, except slight defibrillation of collagen fibers and fibrous ring/nucleus pulposus border discontinuity (Fig. 2J).

Integrated semi-quantitative assessment of histological changes. An integrated assessment of histological changes in the fibrous ring (Table 1) revealed that in animals of the comparison group the total number of points corresponded to pronounced degenerative changes on the 45th day of therapy.

There was an improvement in hystological structure to moderate degenerative damage in animals with CrMSC therapy without potentiation (regardless of cell source). A similar trend was observed when using TGFβ-potentiated cells. The administration of bFGF-stimulated CrMSCs helped to improve the

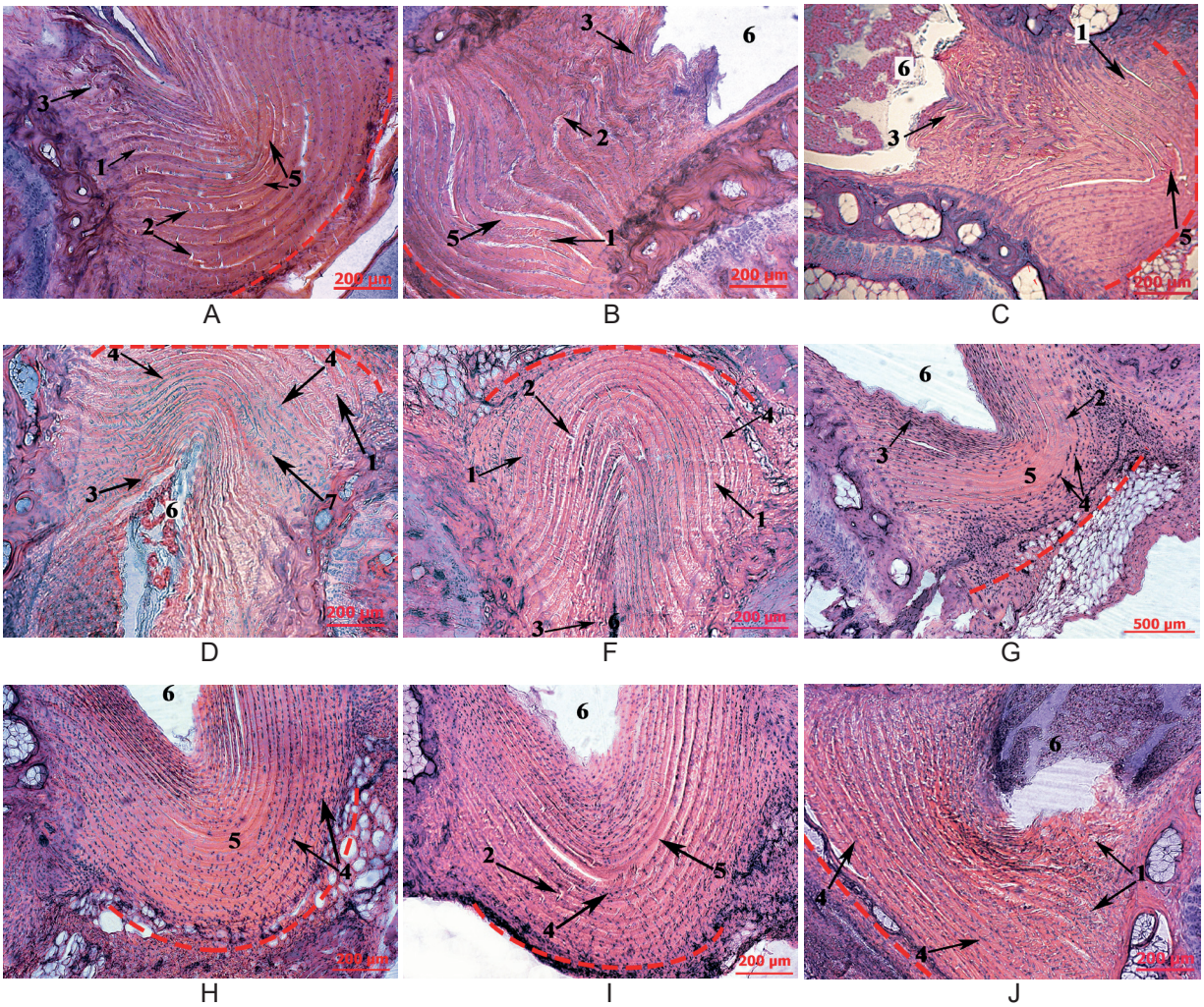

Fig. 2. Dorsal part of IVD Co6-Co7 of rat after local administration (day 45): A – bone marrow-derived CrMSCs; B – adipose-derived CrMSCs; C – cartilage-derived CrMSCs; D – bone marrow-derived CrMSCs + TGFβ; F – adipose-derived CrMSCs + TGFβ; G – cartilage-derived CrMSCs + TGFβ; H – bone marrow-derived CrMSCs + bFGF; I – adipose-derived CrMSCs + bFGF; J – cartilage-derived CrMSCs + bFGF. 1 – Defibrillation of collagen fibers; 2 – cracks in annulus fibrosus; 3 – serpentine shaped collagen bundles; 4 – chains of fibroblast-like cells; 5 – acellular areas; 6 – nucleus pulposus; 7 – large chondrocytes in lacunae; red dotted line is the side of CrMSC administration. Microphotograph, staining with hematoxylin and eosin

Table 1. Integrated semi-quantitative assessment of histological changes in a fibrous ring of IVD Co6-Co7 after administration of CrMSCs (day 45), the total number of points

Animal group	Source of CrMSCs		
	bone marrow	adipose tissue	cartilage tissue
CrMSCs	18.3 (15-22)	19.0 (16-22)	17.1 (13-19)
CrMSCs + TGFβ	17.5 (14-20)	16.9 (13-20)	17.4 (15-19)
CrMSCs + bFGF	10.7 (8-13)	11.2 (9-15)	9.7 (8-12)
Self-healing	23.9 (22-25)		

Note: The limit values of the parameter in the corresponding group of animals are shown in parentheses.

overall histological picture on the 45th day, which corresponded to mild changes according to the semi-quantitative scale with almost complete restoration of fibrous ring structure in the case of use of cartilage-derived CrMSCs.

Morphometric analysis. The height of IVD Co6-Co7 did not increase in the comparison group of animals with self-healing. Therapy with untreated and stimulated CrMSCs led to a significant increase in the height of IVD Co6-Co7 compare to the self-healing group with the most pronounced effect after application of cartilage-derived CrMSCs potentiated with bFGF (Table 2).

Fibrochondrocyte density in the dorsal part of IVD Co6-Co7 and also its height increased in all groups of animals with therapy compared to the self-healing group, but in varying degrees (Table 3).

In animals of the comparison group, the average density of fibrochondrocytes was low due to the large size of the hypo- and acellular areas of the fibrous ring. The growth of this indicator relative to the group with administration of untreated and TGFβ-stimulated CrMSCs was due to increased cell density in the outer parts of the fibrous ring. At the same time, the wide central areas remained cell-free or hypocellular. When CrMSCs were stimulated with bFGF, the cellularity of the central areas increased, and the difference in the average density of fibrochondrocytes between the parts of the fibrous ring was smoothed. At the same time, using cartilage-derived CrMSCs potentiated with bFGF led to a more pronounced increase in cell density of fibrous ring than other variants of MSC therapy.

DISCUSSION

Currently, degenerative disc injuries are treated with conservative methods or surgical interventions aimed at relieving symptoms and muscle stabilization, without clinical therapy targeting its restoration. Recent advances in tissue engineering provide an exciting potential regeneration approach focused on the delivery of cells capable of restoring disc structure and function [19]. It is known that the result of cell therapy is influenced by a number of

Table 2. Height (M ± m, mm) of IVD Co6-Co7 after local administration of CrMSCs (day 45)

Animal group	Source of CrMSCs		
	Bone marrow	Adipose tissue	Cartilage tissue
CrMSCs	0.70 ± 0.03*	0.66 ± 0.02*	0.72 ± 0.02*
CrMSCs + TGFβ	0.77 ± 0.03*	0.69 ± 0.03*	0.81 ± 0.02*
CrMSCs + bFGF	0.93 ± 0.03*,**	0.90 ± 0.05*,**	1.01 ± 0.03*,**
Self-healing	0.57 ± 0.02		

Notes: here and in Table 3 *statistical significance compared with self-healing group index ($P < 0.05$); **statistical significance compared to the index of the group with administration of untreated CrMSCs from the same source ($P < 0.05$).

Table 3. Cell density (M ± m, cells/mm^2) in the dorsal part of fibrous ring of IVD Co6-Co7 after local administration of CrMSCs (day 45)

Animal group	Source of CrMSCs		
	Bone marrow	Adipose tissue	Cartilage tissue
CrMSCs	769.28 ± 30.21*	683.95 ± 37.58*	873.30 ± 30.16*
CrMSCs + TGFβ	819.33 ± 48.88*	708.96 ± 37.62*	847.62 ± 24.38*
CrMSCs + bFGF	1050.42 ± 70.74*,**	1025.68 ± 58.52*,**	1195.15 ± 47.11*,**
Self-healing	469.65 ± 37.58		

factors. So, depending on the degree of IVD degenerative changes, local administration of MSCs can have an inhibitory or blocking effect on the further progression of the pathological process. The method of introducing the cell suspension can also affect mechanotransduction into the IVD and stimulate the degenerative process rather than the reparative one. The type and functional state of administrated cells are also one of the key factors determining the effectiveness of treatment. Therefore, choice of cell source, delivery methods, pretreatment and preconditioning as well as genetic modifications are considered among the main approaches that amplify the therapeutic potential of MSCs [20].

Soluble biochemical factors such as growth factors and cytokines are multifaceted in their ability to modulate cell behavior and function: they can stimulate growth, proliferation, migration, differentiation and modulation of cellular substrate synthesis or its release. Factors affecting MSCs include TGFβ, bFGF, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) [21]. Since growth factors strongly regulate the biological response of cells, their exogenous addition can further stimulate stem cells, which is especially important in the case of MSC application with reduced functional activity.

Presented in this work experimental data showed that the application of bone marrow-, adipose- or cartilage-derived CrMSCs on gelatin sponge contributed to the normalization of degenerative damaged IVD structural organization versus the comparison group. The intensity of regenerative processes in IVD with the use of bFGF + CrMSCs was more pronounced than in untreated or TGFβ-potentiated cells. Our results are consistent with those that suggest that bFGF may be useful for tissue engineering by accelerating MSC proliferation in vitro [22] and modulating their mobility in a dose-dependent manner [23].

As for TGFβ, it regulates the chondrogenic differentiation of adult MSCs stimulating the early phase of proliferation and differentiation with inhibition of the late phase of hypertrophy and mineralization [24]. Moreover, TGFβ is the crucial growth factor involved in the biological regulation of extracellular matrix protein synthesis. So, its addition to the culture medium activates type II collagen and glycosaminoglycans synthesis by CrMSCs from bone marrow and adipose tissue [16]. Based on these we can assume that the reparative effect of CrMSCs locally transplanted in degenerated IVD cartilage tissue, is primarily due to their mitotic activity which allows them quickly penetrate into the damaged area and replace the missing cellular elements.

CONCLUSIONS

1. Administration of cryopreserved MSCs from bone marrow, adipose and cartilage tissues promotes the activation of regenerative processes in degenerated intervertebral discs of rats.

2. The use of bone marrow-, adipose- and cartilage-derived cryopreserved MSCs potentiated with bFGF increases the fibrochondrocyte density, accelerates the restoration of histostructure and height of degenerated intervertebral discs of animals in comparison with untreated ones.

3. The application of TGFβ for cryopreserved MSCs potentiation did not lead to significant changes in their regenerative potential, regardless of the tissue source.

4. The obtained results can be used for the improvement of approaches to cell therapy of degenerative intervertebral disc damage in clinical practice.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ЕФЕКТ ПОТЕНЦІЙОВАНИХ КРІОКОНСЕРВОВАНИХ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН У ВІДНОВЛЕННІ МІЖХРЕБЦЕВОГО ДИСКА

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Мета дослідження – вивчити вплив кріоконсервованих мезенхімальних стовбурових клітин (КрМСК) з різних тканин (кістковий мозок, жирова тканина, хрящ), потенційованих деякими факторами росту та диференціювання (TGFβ, bFGF), на відновлення хряща міжхребцевих дисків (МХД) з використанням компресійної моделі їх дегенеративного пошкодження. У групі із самовідновленням спостерігали виражені гістологічні зміни хрящової тканини МХД. Введення нестимульованих КрМСК (незалежно від їхнього походження) на 45-ту добу відновлювало структуру МХД до ступеня помірних змін. Після терапії клітинами, потенційованими TGFβ, гістологічна структура диска залишалася подібною до впливу непотенційованих КрМСК, що за інтегрованою напівкількісною шкалою відповідало помірним дегенеративним змінам. Тоді як введення bFGF-потенційованих клітин сприяло відновленню структури диска до легкого ступеня пошкоджень. В останньому випадку на 45-ту добу після терапії збільшувалася клітинність фіброзного кільця, регенерація тріщин і щілин, відновилася фібрилярна структура колагену. При цьому середня щільність клітин фіброзного кільця збільшувалася в 1,35, 1,50 та 1,39 раза, а висота МХД – у 1,33, 1,36 та 1,37 раза при введенні потенційованих bFGF КрМСК відповідно з кісткового мозку, жирової тканини та хряща порівняно з нестимульованим аналогом. Таким чином, введення кріоконсервативних МСК з усіх досліджених джерел активує регенеративні процеси у дегенеративно змінених міжхребцевих дисках щурів, швидкість яких зростає при застосуванні клітин, потенційованих bFGF.

Ключові слова: кріоконсервування; культивування; мезенхімальні стовбурові клітини; b-фактор росту фібробластів; трансформуючий фактор росту β; міжхребцевий диск; гістологічна структура.

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