

Abstract

J. A. Bodnarchuk³,
M. V. Khyzhnjak¹,
O. O. Potapov²,
N. G. Chopik¹,

¹SI "Romodanov Institute of Neurosurgery of the NAMS of Ukraine", Kyiv, Ukraine;

²Sumy State University, Medical Institute, Sumy, Ukraine;

³Vinnitsa City Clinical Emergency Hospital, Vinnitsia, Ukraine

BIOCHEMICAL AND BIOMECHANICAL SUBSTANTIATION OF REPARATIVE REGENERATION OF INTERVERTEBRAL DISCS IN PATIENTS WITH DEGENERATIVE DISC DISEASES

Degenerative disc diseases occupy the second place in the overall structure of morbidity with temporary disability. In 40% of patients with spinal osteochondrosis, diseases of the locomotor apparatus and connective tissue cause primary disability. Disc degeneration is a pathological process that is the main cause of low back pain and is observed in the vast majority of people at some point in their lives. The influence of mechanical stress leads to degenerative changes in the tissues of the nucleus pulposus of the intervertebral disc. Limited transport and low cellular saturation of the discs hinder recovery, make the intervertebral disc particularly vulnerable to injury, and contribute to the appearance of morphological tissue damage associated with the processes of biological aging. The pathological process involves all structural elements of the intervertebral disc. The earliest manifestations of disc degeneration usually occur in the nucleus pulposus, where a reduced content of proteoglycans disrupts mechanical function, which leads to progressive morphological degeneration of the entire intervertebral segment. Existing treatment methods (both surgical and conservative) are not able to adjust the number of cells in the nucleus pulposus and are unable to stop the pathological process in the intervertebral disc. Prevention of degeneration or repair of the intervertebral disc is a potential treatment for lumbar pain syndromes. Cell therapy has become a subject of great interest, as new research reports significant regenerative potential for many cellular sources, including the regeneration of the nucleus pulposus region of the intervertebral disc. The use and implementation of modern cell therapy in practical neurosurgery allows us to approach the problem of intervertebral disc degeneration at a new qualitative level with the use of multipotent cells, biochemical peptides in the reparative processes of the nucleus pulposus, as a possibility of treatment and prevention of vertebrogenic pain syndromes in the future.

Keywords: intervertebral disc, nucleus pulposus, cell therapy, transplantation, degenerative changes, reparation.

Corresponding author: jurabod@bigmir.net

Резюме

Ю. А. Боднарчук³⁾,
М. В. Хижняк¹⁾,
О. О. Потапов²⁾,
Н. Г. Чопик¹⁾,

¹⁾ДУ «Інститут нейрохірургії
ім. А. П. Роговського НАМН
України», м. Київ, Україна;

²⁾Сумський державний універ-
ситет, медичний інститут,
м. Суми, Україна;

³⁾Вінницька міська клінічна
лікарня швидкої медичної допо-
моги, м. Вінниця, Україна

БІОХІМІЧНІ ТА БІОМЕХАНІЧНІ ОБҐРУНТУВАННЯ РЕПАРАТИВНОЇ РЕГЕНЕРАЦІЇ МІЖХРЕБЦЕВИХ ДИСКІВ У ХВОРИХ З ДЕГЕНЕРАТИВНИМИ ЗАХВОРЮВАННЯМИ ХРЕБТА

Дегенеративні захворювання хребта займають друге місце в загальній структурі захворюваності з тимчасовою втратою працездатності. Захворювання опорно-рухового апарату та сполучної тканини у 40 % пацієнтів з остеохондрозом хребта викликають первинну інвалідність. Дегенерація диску – це патологічний процес, який є основною причиною виникнення болю в попереку та турбує переважну більшість людей в певний момент їхнього життя. Вплив механічного навантаження призводить до дегенеративних змін в тканинах пульпозного ядра міжхребцевого диску. Обмежений транспорт і низька клітинна насиченість дисків перешкоджають відновленню, роблять міжхребцевий диск особливо вразливим до травм та сприяють появі морфологічних ушкоджень тканин пов'язаних з процесами біологічного старіння. Патологічний процес поширюється на всі структурні елементи міжхребцевого диску. Найбільш ранні прояви дегенерації диску зазвичай відбуваються в пульпозному ядрі, де знижений вміст протеогліканів порушує механічну функцію, що призводить до прогресуючої морфологічної дегенерації всього міжхребцевого сегменту. Існуючі методи лікування (як хірургічні, так і консервативні) не здатні корегувати кількість клітин пульпозного ядра та не можуть зупинити патологічний процес у міжхребцевому диску. Профілактика дегенерації або репарація міжхребцевого диску є потенційним лікуванням для поперекових больових синдромів. Клітинна терапія стала предметом великого інтересу, оскільки нові дослідження повідомляють про великий регенеративний потенціал для багатьох клітинних джерел, в тому числі і для регенерації області пульпозного ядра міжхребцевого диску. Використання і впровадження в практичну нейрохірургію сучасної клітинної терапії, дозволяє підійти до вирішення проблеми дегенерації міжхребцевого диску, на новому якісному рівні із застосуванням мультипотентних клітин, біохімічних пептидів в репаративних процесах пульпозного ядра, як можливості лікування і профілактики в майбутньому вертеброгенних больових синдромів.

Ключові слова: міжхребцевий диск, пульпозне ядро, клітинна терапія, трансплантація, дегенеративні зміни, репарація.

Автор, відповідальний за листування: jurabod@bigmir.net

Introduction

Epidemiology. In Ukraine, degenerative disc diseases occupy the second place in the overall structure of morbidity with temporary disability, second only to respiratory diseases. In the structure of peripheral nervous system diseases, the specific weight of spinal osteochondrosis (degenerative-dystrophic changes of the spine accompanied by pain syndrome) it takes up approximately 70% [1]. Every year, more than 320 thousand new cases of spinal osteochondrosis are registered in Ukraine,

which is about 4% in the structure of locomotor apparatus diseases [2]. In 40% of patients with spinal osteochondrosis, diseases of the locomotor apparatus and connective tissue cause primary disability [3].

Intervertebral disc (IVD) injuries and age-related degeneration contribute to lower back pain [4]. Disc degeneration is a pathological process that is a common cause of lower back pain, a painful condition that occurs in about 85% of all people at a certain period of their life [1, 2]. Intervertebral

disc degeneration (IVDD) is one of the most common causes of persistent disability associated with debilitating pain in the elderly [2, 3].

Anatomical features of the vertebral-motor segment. Being located between two adjacent vertebrae, the discs consist of an external fibrous ring (FR) that surrounds the central gelatinous nucleus pulposus (NP). The fibrous ring is populated by fibroblast-like and stem cells, in the outer part it consists mainly of type I collagen, while type II collagen is contained mainly in the inner part (closer to the NP). Along with this type of collagen, it also contains a small amount of type V (3%), VI (10%) and IX type (1-2%) collagen. The FR's main function is to contain the circular loads that occur when the NP swells, and the extensional forces that occur during bending or twisting. NP consists mainly of negatively charged proteoglycans and type II collagen fibers, irregularly located in the hydrated matrix, rich in proteoglycans (about 80% of water by dry weight). Type II collagen amounts for about 80% of the nucleus pulposus collagen, 15% is type VI collagen, 1–2% is type IX collagen, close to 3% – type XI collagen, and 1% – type III collagen. The NP functions mainly to counteract and distribute compressive loads at high swelling pressure [5].

Limited transport and low cellular saturation of the discs prevent recovery and make IVD particularly sensitive to injuries, contribute to the appearance of morphological tissue damage associated with the processes of biological aging. [6, 7].

IVD degeneration is a widely recognized factor for lower back pain and is characterized by an early decrease in the number of cells in the NP area and associated changes in the extracellular matrix, decreased hydration, and progressive degeneration. Pathological changes in the IVD affect all its structures. The earliest manifestations of disc degeneration usually occur in the NP, where reduced proteoglycan content disrupts mechanical function, which leads to progressive structural deterioration of the entire intervertebral joint [7,8]. Prevention or reparation of IVDD is a potential treatment for lumbar pain syndromes. While the main cause of IVDD is usually associated with a decrease in the number of NP cells, modern treatment strategies (both surgical and conservative) cannot replenish NP cells or stop the pathological process and are mostly aimed at relieving symptoms, rather than restoring the structure and function of the disc [4, 9]

Cellular therapy and biochemical substantiation. Cellular IVD therapy has been the subject of extensive research, as recent research reports great regenerative potential for many cellular sources, namely autologous or halogen chondrocytes, primary IVD cells, and stem cells. There is considerable interest in cellular strategies for the regeneration of the NP region of the IVD [4,10].

NP cells originate from notochordal cells, which either disappear or are replaced by chondrocytic NP cells during development and differ from neighboring intervertebral disc cells in the expression of phenotypic markers and morphology. It is proved that NP cells retain some notochordal molecular markers. Adult human NP cells (nucleus pulposus) lose this phenotype and morphology with age, which contributes to progressive disc degeneration and the occurrence of pathological changes [10].

Cellular therapy by transplantation of progenitor cells / notochordal NP cells has been proposed as a way to stop the progression of disc degeneration. It was found that the number of CD24-positive NP cells significantly decreases with increasing degree of disc degeneration. In addition, CD24-positive NP cells have been shown to retain their multipotency before differentiation and self-renewal potential *in vitro*, suggesting that CD24-positive NP cells are progenitor cells in NP. Further *in vivo* experiments have shown that CD24-positive cell transplantation can restore degenerative discs, as evidenced by the increase in disc height detected by magnetic resonance imaging, the restoration of the T2-weighted signal intensity and the NP structure [11].

The influence of mechanical stress leads to degenerative changes in the tissues of the NP of IVD. As a result of *in vitro* study, it was found that prolonged IVD compression significantly reduces the expression of n-CDH markers (N-cadherin) and specific NP markers (Brachyury, Laminin, Glypican-3 and Keratin 19), reduces the level of glycosaminoglycan (GAG) and hydroxyproline (HYP), as well as the expression of matrix macromolecules (aggrecan and type II collagen) [12].

It is proved that the NP cells are located in an environment rich in laminin, which changes significantly with age, including loss of water content and changes in the structure of the extracellular matrix, which can lead to the development of IVD degeneration. There is a great interest in methods of reactivation of healthy

biosynthetic active cells of the NP using biomimetic peptides derived from laminin, in order to use autologous cell sources for tissue regeneration [4, 12].

It has been proved that the interaction of NP cells with laminin promotes their adhesion and biosynthesis, as a result of which the biomaterial induced by laminin can be used to promote or maintain the phenotype of NP cells [4]. The selected laminin-mimetic peptide ligands were studied for their ability to regulate the phenotype and biosynthesis of human NP cells by expressing the NP-specific markers agrecan, N-cadherins, type I and II collagen, and GLUT1 (Glucosetransporter 1) [12].

As a result of further research, laminin-111 (PEG-LM111) hydrogel was developed. The mechanical properties of the PEG-LM111 hydrogel may be adjusted in the range of dynamic loads previously set for the human IVD [4]. The substrates conjugated with the peptide demonstrated the ability to stimulate the expression of specific markers of healthy NP, as well as increased biosynthetic activity [12, 13].

The use of human umbilical cord mesenchymal stromal cells (HUCMSCs), which have their origin in Wharton jelly, remains a priority due to their ability to differentiate into multiple lines. Mesenchymal stem cells (MSCs) have been studied as a potential source for disc tissue regeneration. The data showed that pseudo-three-dimensional culture conditions (enriched with laminin-1) in the absence of serum promote differentiation of HUCMSCs. Starting from day 1 of the study, HUCMSCs demonstrated a cell clustering morphology similar to that of immature NP cells in a similar laminin-rich culture system [13]. It was found that differentiated HUCMSCs contain glycosaminoglycans that express the extracellular matrix proteins collagen II and laminin $\alpha 5$, laminin receptors (integrin $\alpha 3$ and $\beta 4$ subunits), and therefore have the potential to differentiate into

cells with general properties identical to immature cells in a laminin-rich environment, and therefore may be used for cellular therapy of IVD [10, 13].

Many studies have shown that growth factor injections and mesenchymal stem cell (MSC) transplantation are promising biological treatments for IVD degeneration. Growth factors stimulate cell proliferation and matrix synthesis by IVD cells, stimulate the differentiation of MSCs in the direction of an NP-like phenotype, and therefore increase the number of functional cells in the IVD or enhance the function of endogenous disc cells, which leads to the regeneration of degenerative discs. Among many factors, the isolated growth and differentiation factor-5 (GDF-5), which increases anabolism in IVD cells and induces differentiation of MSCs in the direction of NP-like cells. In the experiment, it was found that defects in the GDF-5 gene lead to abnormalities of collagen and proteoglycan in the discs of mice, which suggests the role of GDF-5 in the structural and functional support of IVD. Thus, GDF-5 is a promising therapeutic agent in the treatment of IVD pathology [8].

There are known studies where the introduction of IVD cells, chondrocytes, or MSCs into various models of degenerative discs is often not successful. As an alternative to the above methods of cellular therapy, pluripotent stem cells (PSCs) may be used, including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), which have great potential for regenerative medicine. NP precursors derived from the embryonic chord may not only exist in the harsh hypoxic environment of IVD, but also effectively differentiate into NP-like cells. The ability to induce differentiation of human iPSCs in the direction of NP-like cells can give an insight into the processes of differentiation of NP cells and provide a source of cells for the development of new methods for the treatment of IVD diseases [13, 14].

cells, biomimetic peptides in the reparative processes of NP, as a possibility of treatment and prevention of vertebrogenic pain syndromes.

Conclusions

Thus, the use of modern cellular therapy allows us to approach the problem of IVD degeneration at a new qualitative level with the use of multipotent

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Conflict of interest

The authors declare no conflict of interest.

Відомості про авторів

К. мед. н. Боднарчук Юрій Анатолійович, лікар-нейрохірург Вінницької міської клінічної лікарні швидкої медичної допомоги, Україна, 21032, м. Вінниця, вул. Київська, 68 (e-mail: jurabod@bigmir.net; tel.: +390962385546);

Д. мед. н. проф. Хижняк Михайло Віталійович, завідувачий відділенням малоінвазивної лазерної спинальної нейрохірургії з рентгеноопераційною ДУ «Інститут нейрохірургії ім. А. П. Ромоданова НАМН України», Україна, 04050, м. Київ, вул. П. Майбороди, 32;

Д. мед. н. проф. Потапов Олександр Олександрович, завідувач кафедри неврології та нейрохірургії Сумського державного університету, медичний інститут, Україна, 40000, м. Суми, вул. Римського-Корсакова, 2;

Чопик Наталія Григорівна, провідний науковий співробітник відділу нейробіохімії ДУ «Інститут нейрохірургії ім. А. П. Ромоданова НАМН України», Україна, 04050, м. Київ, вул. П. Майбороди, 32