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The influence of the culture of fibroblastic cell elements on the indicators of the metabolism of connective tissue in experimental animals

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The number of patients with degenerative tendon disease affects millions of people both among athletes and the general population, causing significant socio-economic consequences. Despite the availability of various methods of conservative and surgical treatment, more than a third of patients experience constant pain. Objective. To study the indicators of the metabolism of connective tissue in animals with a model of degenerative damage to tendons against the background of the introduction of a culture of fibroblastic cell elements. Methods. Therefore, the development of methods for restoring the structure of tendons using cell cultures, in particular fibroblasts, will allow to optimize the course of reparative processes, reduce the risk of complications during surgical intervention and accelerate healing, and at the molecular level — to improve the structure of collagen fibers. Laboratory studies of biochemical markers of a tendon with a degenerative-dystrophic lesion and against the background of the introduction of cell culture can help in the differential diagnosis of its extracellular matrix. Results. The experimental data obtained by us indicate the presence of differences in the biochemical markers of tendons with degenerative-dystrophic lesions in rats 7, 21, and 45 days after the introduction of culture of fibroblastic cell elements. However, 45 days after the introduction ofthe culture of fibroblast cell elements, the normalization of metabolic processes in the extracellular matrix of connective tissue occurs, namely, the activity of collagenase and the concentration of protein-bound hydroxyproline approaches normal values. This indicates the predominance of the synthetic phase over the catabolic one in collagen metabolism. Conclusions. In this context, the introduction of culture of fibroblastic cell elements, as an alternative anti-inflammatory method, may provide another potential opportunity in the treatment of chronic degenerative-dystrophic lesions of the Achilles tendon.

Кількість хворих із дегенеративним ураженням сухожилків постійно зростає як серед спортсменів, так і між населення взагалі, спричиняючи водночас значні соціально-економічні наслідки. Незважаючи на наявність різноманітних способів консервативного та хірургічного лікування, більше третини пацієнтів відчувають постійний біль. Мета. Вивчити показники метаболізму сполучної тканини у тварин із моделлю дегенеративного ушкодження сухожилків на тлі введення культури фібробластних клітинних елементів. Методи. Розробка способів відновлення структури сухожилків із використанням клітинних культур, зокрема фібробластів, дозволить оптимізувати перебіг репаративних процесів, знизити ризик ускладнень під час хірургічного втручання та прискорити загоєння, а на молекулярному рівні — покращити структуру колагенових волокон. Лабораторні дослідження біохімічних маркерів сухожилків із дегенеративно-дистрофічним ураженням на тлі введення культури клітин можуть допомогти в диференціальній діагностиці його позаклітинного матриксу. Результати. Експериментальні дані вказують на наявність у щурів відмінностей біохімічних маркерів сухожилків із дегенеративно-дистрофічним ураженням через 7, 21, і 45 діб після введення культури фібробластних клітинних елементів. Проте через 45 діб після введення відбувається нормалізація метаболічних процесів у позаклітинному матриксі сполучної тканини, а саме активність колагенази та концентрація білковозв'язаного гідроксипроліну наближується до нормальних величин. Це свідчить про переважання в метаболізмі колагену синтетичної фази над катаболічною. Висновки. Введення культури фібробластних клітинних елементів, як альтернативний протизапальний спосіб, може надати ще одну потенційну можливість для лікування хронічних дегенеративно-дистрофічних уражень ахілового сухожилка. Ключові слова. Колагеназа, гідроксипролін, глікозаміноглікани, фібробласти, ураження сухожилків.

Keywords. Collagenase, hydroxyproline, glycosaminoglycans, fibroblasts, tendon damage

Introduction

The development of new high-tech methods of treating patients with degenerative tendon damage is now very important, since the existing methods cannot provide full and stable treatment of the complications that accompany their lesions — the biological and biomechanical functions of the connective tissue are not fully restored [1, 2]. Treatment difficulties are explained by the small number of cellular elements in tendon tissues in general, and especially in the case of degenerative changes.

New strategies for treating patients are focused on transplantation or *in situ* mobilization of mesenchymal precursors or stem cells, which activate reparative processes [3, 4]. The development of techniques for restoring the structure of tendons using cell cultures, in particular fibroblasts, will allow to optimize the course of reparative processes, reduce the risk of complications in case of surgical intervention and speed up healing, and at the molecular level — to improve the structure of collagen fibers. From this point of view, it is important to conduct experimental studies, in particular biochemical ones [5–7].

In vertebrates, tendons are mostly composed of closely spaced bundles of parallel type I collagen fibrils linked by small proteoglycan molecules. A specific spatial organization ensures their mechanical properties [8]. Degenerated tendons have significantly more type III collagen. In addition, collagen disorganization occurs in them with the separation of collagen fibers, increased cellularity, neovascularization, and focal necrosis [9].

Laboratory studies of biochemical markers of tendons with degenerative-dystrophic damage and the introduction of cell culture can help in the differential diagnosis of their extracellular matrix. Thus, determination of collagenase activity in biological fluids is necessary for evaluating the metabolism of collagen in them in diseases accompanied by destructive processes in the connective tissue. Hydroxyproline is a biomarker for major tendon collagens type I and III. Its content in the blood reflects the balance of the rate of collagen catabolism. The ratio of free and protein-bound fractions of hydroxyproline, respectively, indicates the predominance of collagen synthesis or breakdown processes [10]. A characteristic feature of degenerative-dystrophic damage to tendons is the loss of glycosaminoglycans (GAG) in the matrix, which leads to excess hydration and splitting of the matrix with subsequent dehydration and rupture of collagen fibers [9, 10].

Purpose: to study the indicators of the metabolism of connective tissue in animals with a model of degenerative tendon damage secondary to administration of a culture of fibroblastic cell elements.

Material and methods

The study was performed on 47 sexually mature male rats, weighing (300 ± 12) g. All procedures on animals were carried out in accordance with the requirements of bioethics and the principles of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [11]. The materials of the study were considered at the meeting of the Bioethics Committee at the Institute of Traumatology and Orthopedics of the National Academy of Sciences of Ukraine (Protocol No. 5 dated 07.12.2023).

All the animals underwent a modeling of a degenerative-dystrophic lesion of the Achilles tendon according to the developed method [12]. 7 days after receiving this injury, 0.1 ml of a culture of fibroblastic cell elements taken from the autologous dermis (concentration — 0.25 × 106 cells in 1 ml) was once injected into the thickness of the Achilles tendon of rats, 0.25 cm proximal to the heel hills. The indicators of 10 intact animals were considered the physiological norm. The control group of rats was injected with sodium chloride.

Cell cultures of fibroblasts of autologous skin were used, developed according to the methods of the Department of Cryobiology of Reproductive Systems of the State Establishment Institute of Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Kharkiv) [13]. Rats were removed from the experiment by decapitation on days 7, 21, and 45.

The following indicators were determined in the blood serum of these animals: collagenase activity, hydroxyproline fractions, and glycosaminoglycans. Collagenase activity was determined according to Lindy [14]. Fractions of hydroxyproline were isolated according to Frey [15]. Hydroxyproline (HP) in the fractions was determined according to Stegemann [16]. The total content of glycosaminoglycans was determined according to the method of Klyatskin and Lifshits [17].

Statistical processing of the obtained results was carried out using the Origin Pro 8.5 software package. The average values of the obtained indicators (x) with standard deviations (SD) were studied. Student's t-test was used to assess the significance of the difference, in the presence of a normal distribution of the research results.

Results and their discussion

Data obtained during the study of blood serum in animals with a model of degenerative tendon damage, which were injected with fibroblast culture, indicated that the activity of the collagenase enzyme on the 7th day of observation was (5.40 ± 0.12) μmol/l·h and exceeded the norm by more than 1.9 times, reaching 193 %. Along with the high activity of this enzyme, the content of the free fraction of hydroxyproline also increased, indicating a high catabolic activity of the metabolism of collagen, the main protein of connective tissue. The concentration of protein-bound hydroxyproline exceeds the physiological norm by 1.6 times and is (14.80 ± 0.50) μmol/l h (normal (9.14 ± 0.16) μmol/l h). The GAG content fell to normal values — 21 %.

The values on the 7th day after the administration of the culture of fibroblasts indicate the activation of metabolic processes in the connective tissue, which can be explained by the initial transient inflammatory reaction to the introduction of platelets. An *in vitro* study by Hudgens [18] demonstrated that one of the early reactions to the use of fibroblasts in rats is periodic flares of inflammation. They observed activation of pro-inflammatory tumor necrosis factor-alpha and NFkB pathways after exposure to fibroblasts, as well as gene expression related to cell proliferation and tendon collagen remodeling.

The results of blood serum research on the 21st day of observation show that the activity of collagenase decreases compared to the data on the 7th day from 195 to 132 %, and in absolute terms to (3.70 ± 0.12) μmol/l·h. At the same time, it should be noted that these indicators are still higher than the physiological norm. During these periods, the content of the free fraction of hydroxyproline decreases by up to 150 % compared to the markers of the 7th day. The concentration of protein-bound hydroxyproline increases to (16.90 ± 0.40) μmol/l•h. The GAG content also increases (table, Fig. 2). In the case of chronic tendinopathies, an acute flare of inflammation may be a key element in triggering the subsequent regenerative response. It can also partially support the positive result of the introduction of fibroblasts in chronic tendon degeneration [9, 18].

Data obtained on the 45th day of observation indicated a further decrease in collagenase activity to normal values. The concentration of the free fraction of hydroxyproline was lower than the physiological norm and is 85 %, and in absolute terms it was (7.29 ± 0.35) μmol/l•h.

At the same time, the content of protein-bound hydroxyproline approaches the norm, which indicates the predominance of the synthetic phase

Table

Biochemical indicators of blood serum of rats injected with fibroblasts, (M ± m)

Indicators	Physiological norm (n = 10)	Control group (n = 10)	Day 7 (n = 10)	Day 21 (n = 9)	Day 45 (n = 8)
Collagenase, μmol/l h	2.80 ± 0.15	4.90 ± 0.10*	5.40 ± 0.12*	3.70 ± 0.12*	2.90 ± 0.11*
Free HP fraction, μmol/l•h	8.59 ± 0.43	12.70 ± 0.35*	13.70 ± 0.17*	12.88 ± 0.40*	7.29 ± 0.35
Protein-bound fraction of HP, μmol/l•h	9.14 ± 0.16	3.00 ± 0.35*	14.80 ± 0.50*	16.90 ± 0.40	10.70 ± 0.50*
GAG, g/l	0.057 ± 0.003	0.087 ± 0.01*	0.012 ± 0.005*	0.032 ± 0.004*	0.035 ± 0.006*

Notes. * — P < 0.05 in relation to the norm. The norm is the indicators of intact animals.

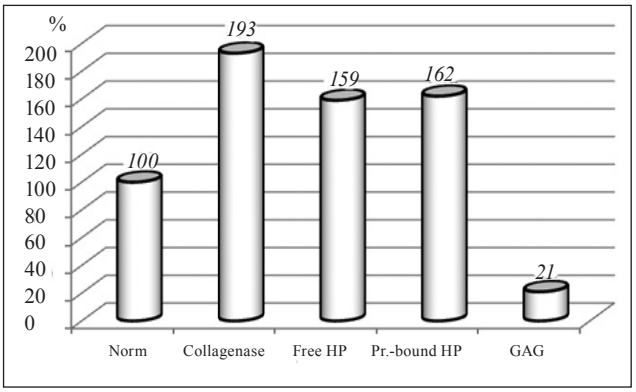

Fig. 1. Biochemical indicators of blood serum of animals injected with fibroblast culture (7 days)

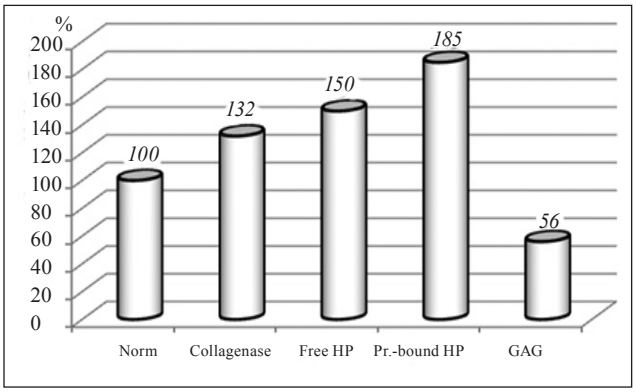

Fig. 2. Biochemical indicators of blood serum of animals injected with fibroblast culture (21 days)

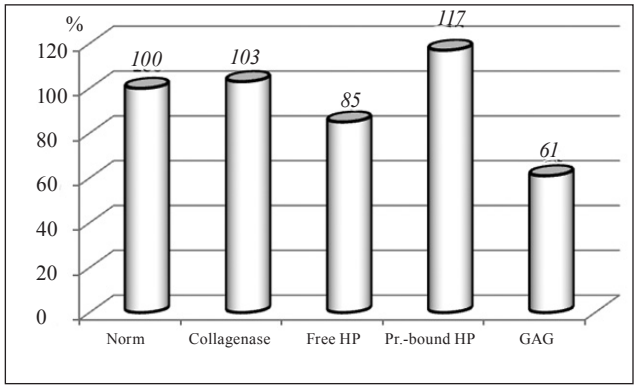

Fig. 3. Biochemical indicators of blood serum of animals injected with fibroblast culture (45 days)

over the catabolic collagen metabolism. The content of GAG reaches (0.035 ± 0.006) g/l for normal (0.057 ± 0.003) g/l (table, Fig. 3).

Taken together, these data indicate the presence of differences in biochemical markers of tendons with degenerative-dystrophic lesions in rats 7, 21, and 45 days after the introduction of culture of fibroblastic cell elements. However, 45 days after the introduction of these cellular elements, the normalization of metabolic processes in the extracellular matrix of connective tissue occurs, namely, the activity of collagenase and the concentration of protein-bound hydroxyproline approaches normal values. This indicates the predominance of the synthetic phase over the catabolic one in collagen metabolism. In this context, administration of culture of fibroblastic cell elements, as an alternative anti-inflammatory method, may provide another potential opportunity in the treatment of chronic degenerative-dystrophic lesions of the Achilles tendon.

Conclusions

The obtained data indicate the presence of differences in biochemical markers of tendons with degenerative-dystrophic lesions in rats 7, 21, and 45 days after administration of culture of fibroblastic cell elements.

The study showed that normalization of metabolic processes in the extracellular matrix of the connective tissue of the tendons occurred 45 days after the introduction of the culture of fibroblast cell elements.

Determination of biochemical markers of tendons with degenerative-dystrophic damage can help in the differential diagnosis of its extracellular matrix.

Conflict of interest. The authors declare no conflict ofinterest.

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THE INFLUENCE OF THE CULTURE OF FIBROBLASTIC CELL ELEMENTS ON THE INDICATORS OF THE METABOLISM OF CONNECTIVE TISSUE IN EXPERIMENTAL ANIMALS

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