



Research Article

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Use of Fetal Stem Cells for Slowing Down Aging of the Spine

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Abstract

The article outlines the principal processes of human locomotor apparatus attrition; it dwells upon importance of this problem for contemporary medicine development.

Objective: analyzing the effects of fetal stem cells (FSCs) on the patients suffering from age-related vertebral column changes.

Material and methods: The study group was composed of 16 male patients in age from 55 to 60 years, who were performed Thomayer's test to evaluate overall mobility of the spine, whereas flexibility in the lumbar spine was determined with the help of Schober's test. We also investigated effect of problems with spine on activities of daily living in the patients by means of the Oswestry questionnaire. All patients of the study group completed one course of treatment using FSCs.

Results: Assessment of the results of treatment we made over 6 and 12 months following FSCs transplantation. All results received pertaining to evaluation of spine flexibility by Schober's and Thomayer's test prove positive influence of FSCs on mobility of vertebral column, resulting in elevation of physical activity of the patients who underwent stem cell treatment in accordance with the Oswestry questionnaire.

Conclusion: FSCs therapy is the only maximally effective contemporary method directed at prevention of vertebral column senescence. This treatment mode allows maintaining physical properties of the cartilaginous tissue, preserving its strength and elasticity of intervertebral disks, contributes to strengthening of the pectoral muscle sling and, eventually, supports the human spine. Slowing down locomotor apparatus aging tends to enhance life quality of any individual.

Keywords

Fetal stem cells, Vertebral column, Aging, Thomayer's test, Schober's test, Oswestry questionnaire

Introduction

Aging of the spine is dystrophic changes of joints and ligaments of the spine, including degenerative disc disease, spondylosis,

and spondylarthrosis. Aging or involutonal processes of the musculoskeletal system start from the puberty period, and all people suffer from them. We do not take into account genetic disposition, infectious factors, the influence of smoking, radiation, etc.

Movement in the spine and all joints is impossible without muscles. The condition of the joints depends on muscles strength, elasticity, and ability to strain and relax. It is well-known fact that after the age of 50 years and every subsequent 10 years, a person loses 10% of muscle weight, and in case of sedentary life style - up to 35%. During the process of age-related changes, the volume of blood in the blood stream decreases due to arteries induration and narrowing, leading to impairment of blood circulation and deterioration of trophic processes in the articular cartilage.

Cartilage is an important anatomical substrate of joints; its special properties allow joints to perform all assigned to them functions. Accordingly, in the spine, this function is performed by the cartilaginous tissue of the intervertebral discs, connecting the individual vertebrae to each other in a vertebral column.

Intervertebral discs make 1/3 of the spinal column height. They perform depreciation function and take the entire load, providing both flexibility and elasticity, which determines the motor activity of the spine. The disk consists of cartilaginous tissue and is anatomically divided into three parts: *nucleus pulposus* - a gel-like mass, solid fibrous structure - fibrous ring and a "closing" plate - a thin layer of hyaline cartilage that separates the disc from the vertebral body. Disc trophism is provided by adjacent vertebral vessels. Trophic disorder leads to the degenerative changes in the disk. With aging, the core of the disc loses water, and becomes harder. The closing plate is gradually getting sclerosed and thickened [1].

The most primitive theory of the human body aging is the theory of its wear due to long-term functioning and exercising during life. According to this mechanical theory, body organs and tissues lose their cellular elements, change their physico-chemical composition, morphological structure, partially degrade, die and become incapable of fulfilling the functions assigned to them. But according to the theory of biochemical aging, living matter has a great ability to continuously restore and regenerate cellular elements. A living body struggles against wearing with the help of its ability to constantly restore its biochemical properties, which are being changed during life. The way of constant biochemical restoration of the chemical composition and biological properties of cellular plasma colloids is significantly important for cartilage tissue [2].

Cartilage refers to tissues with low metabolism, slightly involved in the overall metabolic processes. Cartilaginous tissue is deficient of its own blood system and the trophic function is performed by diffusion and osmosis through intertissue surfaces. Cartilaginous tissue starts aging very quickly. It loses its valuable physical properties, becomes unstable, inferior, and reduces endurance as well as resistance to physical stress.

The rate of natural involution can be slowed down by applying preparations of fetal material, since, the depletion of the stem cell pool leads to muscle mass decrease in old age and contributes to appearing of typical age-related musculoskeletal disorders [3].

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The main objective of our study was to analyze the impact of fetal stem cells on patients with age-related changes in the spine.

Materials and Methods

The study group consists of 16 males aged from 55 to 60 years who consider themselves to be almost healthy, without bad habits (alcohol, smoking), and do not take any medication. All patients lead ordinary lifestyle, are not focused on diet, do not exercise and go to the swimming pool. Patients of the study group are not overweight, and their professional activities are not related to physical exercises.

At examination, patients reported recurrent heaviness and fatigue in the back, discomfort, pain in different parts of the spine during movements and static load. The physiological curves of kyphosis and lordosis were preserved in all patients during simple examination of the spine in a standing position. Some people from the study group had focal palpatory tenderness with deep palpation of the lumbar spine. The involvement of muscles in the pathological process was not found.

To assess the overall mobility of the spine, Thomayer's test was performed. The test was carried out by measuring the distance in centimeters from the third finger of outstretched arms to the floor with the maximum bending and knees straight. Naturally, this distance is zero and increases with the limitation of spine flexion.

Thomayer's test: to evaluate the overall mobility of the spine. Determined by measuring the distance in centimeters from the end of the middle fingers outstretched arms to the floor with a maximum slope ahead. This distance is normally "0" and increases when restricted bending the spine [4].

Mobility of the lumbar spine was determined by Schober's test: the distance of 10 cm was measured from acantha V of the lumbar vertebra upwards along the spinous spine line, and was marked. Patient in position "legs together" maximally flexed the back in the lumbar region, and in this position from the point V of the lumbar vertebra, a distance of 10 cm was measured again. Naturally, the difference between the upper points is 4-5 cm.

Schober's test to identify limitation of motion in the lumbar spine. Find LV of the lumbar vertebrae, put a dot, measure upwards 10 cm and make second mark. At maximum lean forward in healthy subjects, this distance increases for 4-5 cm [4].

We used the Oswestry questionnaire to understand how the identified problems with the back affect the daily life of patients [5]. The Oswestry questionnaire provides an opportunity to assess the impact of pain intensity on self-care, walking, movement over a distance, the ability to lift weights, stand and sit, sleep, social and sexual life. The questionnaire consists of 10 sections with 6 points each. Depending on the ordinal number of the section (1,2,3,4,5,6), the answer is in scores (0,1,2,3,4,5). The answer index (ODI) in percentage is the sum of the points scored / the maximum possible score (50) and multiplied by 100. The results of the questionnaire: 0% -20% - minimal disability, 21% -40% - moderate disability, 41% -60% - severe disability, 61% -80% - crippled, 81% -100% - these patients are either bed-bound or exaggerating their symptoms.

All patients of the study group underwent one course of treatment with medicinal preparations of fetal material [6]. Stem cell preparations were made of 5-12 weeks of gestation embryonic fetuses [7]. Embryos were obtained after an artificial interruption of pregnancy in medical institutions according to social indications

from healthy women, who were previously examined for viral and hemic infections. All work with fetal material was conducted in accordance with current Ukrainian legal and ethical standards [8-10].

Prior to stem cells transplantation all patients were orally acquainted with the plan of treatment and procedures for diagnosis; later the patients reported about their health state and we informed them on the results of laboratory and instrumental investigations. All patients were informed in details on FSCs suspensions and methods for stem cells transplantation. As soon as doctors presented a clarified treatment description, all patients signed their written informed consent for treatment.

FSCs preparations were individually selected for every patient from clinic cryobank, where they were stored in liquid nitrogen at -196°C. All FSCs suspensions stored in cryobank for clinical use were tested for bacterial and viral infections (HIV-1, HIV-2, HBV, HCV, HGV, HPV, CMV, EBV, HHV6, HSV-1,2, Rubella, Parvovirus B19, *Treponema pallidum*, *Toxoplasma gondii*, *Chlamidia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma Parvum*, *Urealyticum*); suspensions had a definite number of nucleated cells and CD34+, colony forming units and cell viability prior to cryopreservation.

Suspension defrost was carried out in accordance with standard protocols. The containers were taken out the liquid nitrogen immediately before transplantation, immersed in a water bath at +37°C and kept till the liquid phase. Further procedures were performed at indoor temperature following strict compliance with aseptic requirements. Additional control of suspension quality, including microscopic studies and calculation of viable cells were conducted before transplantation; trypan blue staining method was applied for visualization under the microscope by means of counting chamber and flow cytofluorimeter.

Several different types of FSCs suspensions were used to treat one patient which allowed getting a more pronounced positive effect. The preparation was administered intravenously via blood transfusion system on the top of 200 ml 0.9% saline solution after previous intravenous jet pre-medication with dimedroli 10 mg, and prednisolone 30 mg. The rate of administration was 20-40 drops per minute. The amount of administered suspension was from 3.0 ml up to 5.0 ml per one course of treatment, the number of nucleated FSCs – from 1 to 50x10⁶/ml, the percentage of living cells - not less than 70%, colony forming units - >0,001x10⁶, CD34+ cells – >0,01x10⁶, CD133+ cells – >0,05x10⁶. Every additional suspension was administered subcutaneously and paravertebrally during 2-3 days in the amount from 2.1 ml up to 8.0 ml of preparation.

Results and Discussion

All patients observed manifestations of early post-transplantation syndrome, which were represented by burst of energy, increased physical and mental capacity, better sleep and appetite, improved mood. These effects were presented throughout treatment course.

No phenomenon of adverse reaction, allergy manifestation or complications after administration of suspensions with FSCs was observed. All our patients followed their routine mode of living.

The treatment results were evaluated in 6 and 12 months after FSCs treatment, using a standardized quantitative scale: the Oswestry Disability Index (ODI) questionnaire [5]. The results of our patients' responses in dynamics are presented in the Table 1. At the initial

Table 1: Oswestry Disability Index (ODI).

Patient No	Pain Intensity			Lifting			Walking			Sitting			Standing			Sleeping			Social life			Travelling			Total score							
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3					
1	0	0	0	0	0	0	2	1	2	1	1	1	1	1	0	0	0	2	1	1	1	2	1	1	16	8	6					
2	2	1	0	2	0	0	3	2	1	2	1	1	2	1	1	1	3	1	1	1	3	1	1	25	10	6						
3	2	1	0	1	0	0	4	2	1	2	1	3	2	1	1	1	1	1	0	0	1	1	1	16	9	5						
4	3	2	1	2	1	1	4	3	3	2	1	3	2	1	1	1	1	1	0	0	2	2	1	24	17	11						
5	2	1	0	2	1	0	4	3	2	2	1	3	2	1	1	1	1	1	0	0	2	1	1	20	12	8						
6	2	1	1	1	0	4	3	2	3	2	1	3	2	1	1	1	1	1	0	0	2	1	1	19	12	9						
7	2	1	1	1	0	3	2	1	2	1	1	2	1	1	1	1	1	1	0	0	2	1	1	18	10	8						
8	1	1	1	1	0	4	2	1	3	2	1	3	2	1	1	1	1	1	0	0	3	2	1	20	12	8						
9	2	1	1	1	0	4	2	1	3	2	1	3	2	1	1	1	1	1	0	0	3	2	1	21	12	6						
10	2	1	0	1	0	3	2	1	2	1	1	2	1	1	1	1	1	1	0	0	2	1	0	17	10	4						
11	3	2	1	1	0	4	2	1	3	2	1	3	2	2	2	1	1	1	0	0	3	2	2	24	16	11						
12	3	2	1	2	1	0	4	2	1	3	2	1	3	2	1	1	1	1	0	0	3	2	1	25	16	10						
13	2	1	0	2	1	0	4	2	1	3	2	2	3	2	1	1	1	1	0	0	3	2	1	24	14	7						
14	3	2	1	2	1	1	4	2	1	3	2	1	3	2	1	1	1	1	0	0	3	2	1	25	15	9						
15	3	2	1	2	1	1	4	2	1	3	2	1	3	2	1	1	1	1	0	0	3	2	1	25	15	8						
16	3	2	1	2	1	1	4	2	1	3	2	1	3	2	1	1	1	1	0	0	3	2	0	24	15	6						
Total score	35	21	10	23	13	4	61	35	20	45	29	17	39	23	13	38	23	18	18	16	13	6	0	40	24	14	35	19	12	343	203	122
Average	2,2	1,3	0,6	1,4	0,8	0,25	3,8	2,2	1,3	2,8	1,8	1,1	2,4	1,4	0,8	2,4	1,4	1,1	1,1	1,0	0,8	0,4		2,5	1,5	0,9	2,2	1,2	0,8	21,4	12,7	7,6

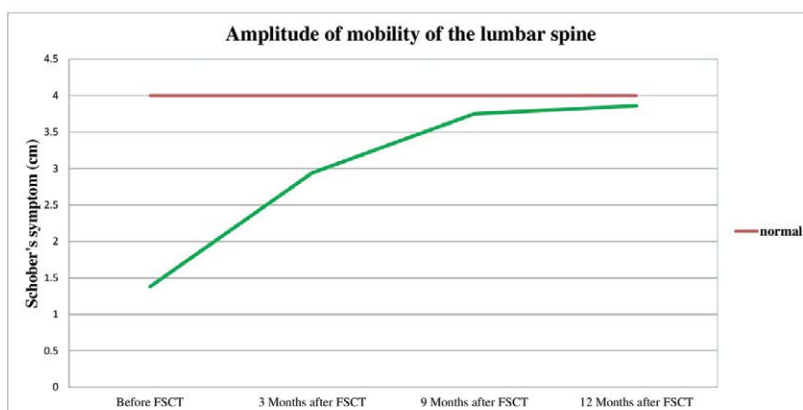
Note:
In horizontal direction the measuring points are as follows:
1 – before treatment, 2 – over 6 months after treatment, 3 – over 12 months after treatment

point before the treatment, the degree of inability varied from 32% to 50%, namely: 9 patients fell into the category of severe disability ODI 41% -60%, i.e. pain for them is the main problem that interferes with daily life activities; according to the results of the questionnaire, 7 people relate to the category of moderate disability ODI 21-40% - due to severity of pain, difficulties occur when walking, standing, sitting, and especially when lifting weights. Six months after the treatment with fetal stem cells preparations, according to the results of the questionnaire, the degree of pain decreased significantly and the ODI level varied from 16% to 34%, namely, 5 patients had a minimum level of lifestyle abnormalities ODI 0-20%, and 11 people remained in the category of moderate disability ODI 21-40%. The highest inability was recorded in the section of the lifting of items -18.75% of the respondents noted that "pain prevents me from lifting heavy weights, but I can manage light to medium weights if they are conveniently positioned", but 81.25% of the respondents indicated that "pain prevents me from lifting heavy weights off the floor, but I can manage if they are conveniently placed e.g. on a table. The next place for the severity of pain refers to the walking section, in which 81.25% of the respondents indicated that "pain prevents me from walking more than 1/2 mile" and only 18.75% could walk a distance

of 1 km (pain prevents me from walking more than 1 mile). 56.25% of respondents in 6 months after treatment noted that pain does not affect social activity, but continues arising with increased activity, namely, workout, dancing. After 12 months of observation, most patients (87.5%) did not have pain, which might interfere with their daily activities. Two patients had the level of activity ODI that was 22% and they remained in the category of moderate disability (21-40%).

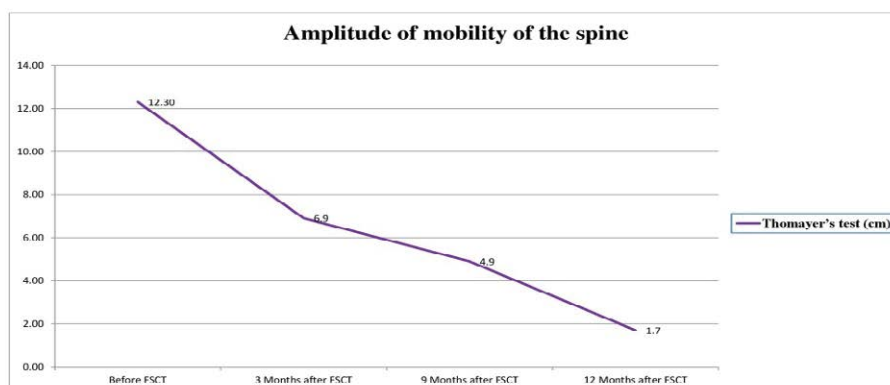
The results of the evaluation of the lumbar spine movement (Schober's test) are shown in Figure 1. At the initial observation point, the limitation of lumbar spine mobility was detected in all patients of the study group by 65.5%. Taking into account that we considered the distance of 4.0 cm as a standard, in the study group it was 1.38 ± 0.62 cm. After 3 months of observation, this distance increased and was 2.94 ± 0.91 cm. After 9 months, on average for the group, this indicator was only 6.25% lower than the standard and it was 3.75 ± 0.33 cm. After FSCs treatment we did not notice significant differences between the numbers obtained after 9 and 12 months of follow up, although the lumbar spine mobility in 12 months rose to 3.86 ± 0.21 cm.

Figure 2 shows the dynamics of the spinal mobility parameters



Legend: FSCT – fetal stem cell treatment

Figure 1: FSCT- Fetal stem cell treatment and mobility of lumbar spine.



Legend: FSCT – fetal stem cell treatment

Figure 2: FSCT- Fetal stem cell treatment and mobility of spine.

obtained during the Thomayer's test. At the initial observation point, the limitation of flexion of the spine in all patients of the study group was revealed and the average distance of maximum bending with knees straight was 12.30 ± 1.53 cm. But only 3 months after the stem cell therapy, the improvement of the spinal mobility was observed and, according to Tomayer's test, the distance to the floor is reduced by 5.4 cm, which is 6.90 ± 1.33 cm. After 9 months of observation, the distance due to Tomayer's test reduced by 60.2% from the distance at the starting point of observation. In 12 months after application of fetal preparations, Thomayer's distance was as close as possible to the standard and took 1.70 ± 0.83 cm.

The results of the spine mobility evaluation on Schober's and Tomayer's tests confirm the positive effect of FSCs treatment on the increase of spinal mobility.

We did not find any limitation of activities in everyday life due to pain syndrome or motor constraints in the spine while examining patients in 1.5 years after stem cell therapy. All patients in the study group were in an optimistic mood, with high physical endurance. Some part of them included physical exercises and swimming in the schedule of their lives.

Conclusion

Use of fetal stem cells had a positive effect on the metabolic processes in the intervertebral discs for the 55-60 years old patients, who reported initial age-related changes in the spine during examination and led to the increase in the physical activity of patients (according to Oswestry's questionnaire), which to a certain extent was limited due to pain.

The results of Schober's and Thomayer's tests during the observation period indicate that the injected stem cells contributed to the restoration of fibrous rings and pulp nuclei of the spine, which positively affected the cushioning function of the intervertebral discs and the mobility of the spine.

Stem cell therapy is the only most effective way to prevent the aging of the today. It allows to maintain the physical capacity of the cartilage tissue, maintain the strength and elasticity of the intervertebral discs, strengthen the muscular corset, which supports the spine. High activity, mobility and flexibility increase the quality of life.

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Competing Interests

The paper is intended to be an original paper; all authors of the manuscript are members of Cell Therapy Center EmCell, Kyiv, Ukraine. The authors have approved the manuscript and agree to its submission.

All authors emphasize absence of financial and other conflict interests in respect of the submitted manuscript. The content of the manuscript is original and it has not been published or accepted for publication, either in whole or in part, in any form. No part of the manuscript is currently under consideration for publication elsewhere. There are no matters relevant to the conflict of interests among the authors who contributed to manuscript submission.

All works with fetal material were conducted in accordance with current Ukrainian legal and ethical standards.

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