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Research Article

Indicators of Osteogenic Activity of Bone Marrow Stromal Stem Cells in Patients with Rheumatoid Arthritis in the Presence of Frontal Deformities of the Knee Joint

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Abstract

Purpose: To study the indicators of osteogenic activity of stromal (mesenchymal) bone marrow (BM) cells of the bones forming the knee joint (lateral and medial areas of the distal femur and proximal tibia) removed during arthroplasty in patients with roaring - matoid arthritis in the presence of frontal deformities.

Methods: Cancellous bone was taken during surgery (knee arthroplasty) from the lateral and medial sections of the distal femur and proximal tibia. Using the technique of cloning fibroblast colony-forming units (CFU) BM, 37 patients with rheumatoid arthritis with lesions of the knee joint were examined. Investigated 168 samples of KM, grown 204 cultures of stem stromal cells (SSC) KM.

Results: A significant slowdown in the regenerative processes in bone tissue in rheumatoid arthritis was revealed. A significant difference in the number of CFU per unit volume (1 cm^3) was found in different parts of the knee joint of patients with rheumatoid arthritis. In the medial process of the femur, this indicator was (0.2307 ± 0.16) x 104, which is 11 - 20 times higher than the values obtained in other areas under study. It was shown that the SCC of the BM of the lateral condyle of the tibia have much worse conditions for the implementation of the regenerative potential in the conditions of development of hallux valgus in comparison with varus. More than 4 times less bone marrow CFUF was isolated from his spongiosa, and the efficiency of their cloning was 2.6 times lower.

Conclusion: There were significant differences in the parameters of the parameters of the osteogenic activity of the SCC of the BM of the bones forming the knee joint (lateral and medial condyles of the distal femur and proximal tibia).

Keywords: *Osteogenic Activity; Frontal Deformities; Knee Joint*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of the connective tissue, which is characterized by a progressive chronic course with predominant joint damage, the development of severe irreversible deformities and significant dysfunction that leads to disability and disability [1].

Usually within 5 years from the onset of the disease up to 60% of patients lose their ability to work, and after 20 - 90%, 35% of patients become disabled.

The disease occupies a prominent place among rheumatic pathology. According to the WHO, the prevalence of rheumatic diseases reaches up to 1.0%. In Ukraine, there are about 118 thousand patients with RA, of which 54 thousand - people of working age who are under dispensary supervision.

According to various authors [2-4], lesions of the knee joints in patients with RA are observed from 10 to 30% within 5 years from the onset of the disease, accompanied by the formation of contractures and the development of discordant deformities of the lower extremities, which, in turn, lead to partial or complete loss of limb function.

Numerous deformities and restoration of musculoskeletal function of the knee joints and lower extremities in patients with RA in the late stages of the disease can be eliminated only with the help of reconstructive surgery. Based on published works in domestic and foreign literature, the issue of patho- and mechanogenesis of knee deformities in patients with RA is not given enough attention. The mechanism of development of contractures and deformations of the lower extremities is very complex. According to the results of clinical-radiological and bio-mechanical studies, it is caused by changes in the joints caused by the underlying pathology and the corresponding adaptive reactions of the motor system.

In the early stages of the pathological process, significant exudative phenomena and synovitis lead to overstretching of the capsular ligament and an increase in intra-articular pressure. During flexion and extension of the thigh, the pressure of the exudate on the synovial membrane, which is densely innervated, decreases. The flexion-drive installation of the thigh in the hip joint, as a manifestation of the analgesic reaction, allows the patient to reduce pain.

In the vertical position of the patient and especially during walking, the flexion-drive installation leads to the movement of the center of gravity of the body, changes in the distribution of functional load on bones, joints and muscles and the further development of compensatory changes in the knee joint.

The development of deformity in the knee joint depends on the structures that suffer the primary lesion: the distal femur or proximal tibia, lateral or medial areas. Accordingly, if the lateral parts of the knee joint are affected, then there is a valgus deformity, in the case of disease of the medial parts - varus.

Persistent autoimmune systemic inflammatory process in RA conditions steadily and irreversibly destroys the skeletal system [5,6]. The development of chronic inflammation, in turn, leads to a violation of the rate of bone remodeling [2,3,5]. Our previous studies have shown a significant inhibition of regenerative processes in the bone tissue of patients with RA.

Objective of the Study

To study the indicators of osteogenic activity of stem stromal (mesenchymal) bone marrow cells that form the knee joint (lateral and medial areas of the distal femur and proximal tibia) removed during endoprosthesis on RA in the presence of frontal deformations.

Materials and Methods

The studies were performed in the laboratory of immunology (Certificate of certification № PT-77/15 dated 12.03.2015 issued by SE "Ukrmetr test-standard") SI "Institute of Traumatology and Orthopedics NAMS", Kyiv.

Cloning of stem stromal cells (SSC) of the bone marrow (BM) was performed according to the method of O. Ya. Fridenstein [7], modified by VS Astakhova [8].

The material for the study was a spongy bone, which was taken during surgery (knee arthroplasty) from the lateral and medial areas of the distal femur and proximal tibia. Using the method of cloning colony-forming units of fibroblasts (CFU) of the bone marrow, 37 patients with RA with a lesion of the knee joint were examined. 168 samples of CM were studied, 204 cultures were grown.

Tours SSK. Bacterial-fungal growth was detected in 12 Petri dishes (5.9%).

Cloning was performed without changing the culture medium 199 under standard conditions for 14 days in Petri dishes at 37°C in a gas mixture with 5% CO_2 content in atmospheric air using lethally irradiated KM rabbit cells as a feeder.

The osteogenic activity of SSC KM was evaluated by the following indicators: the total number of nucleated cells and SSC - CFU CM in 1 cm^3 and the efficiency of their cloning among 105 nucleus-containing cells.

The cloning efficiency was determined by the formula:

$$\text{ЕКУОф} = \frac{\text{К}}{\text{N}} \times 10^5, (1)$$

Where K is the number of colonies that grew in the Petri dish; N is the number of cells planted in the cup.

The amount of CFU in 1 cm^3 was determined by the formula:

$$\text{Кількість КУОф в 1 см}^3 = \frac{\text{К} \times \text{n}}{\text{N} \times \text{V}}, (2)$$

Where K is the number of colonies that have grown in the cup; n is the number of cells washed from the spongy bone sample; N is the number of cells planted; V is the volume of the spongy bone sample.

Statistical processing of the obtained material was performed using the software package Statistica 6.0. The differences of the mean values were estimated using the nonparametric Wald-Wolfowitz series criterion for two independent sets.

Results and Discussion

It is well known that the number of nucleated cells in 1 cm^3 x 10^7 is an indicator that is determined before the start of the study, because it is calculated in the initial suspension of bone marrow cells, which is obtained from surgical bone marrow material. The other two indicators reflect the functional status of osteogenic CM progenitor cells and are calculated from cloning results. If bacterial-fungal germination is registered in the culture, the data of such experiments are not involved for further analysis and statistical processing.

It is important to note that out of 44 cultures of stromal fibroblasts of the CM of the lateral condyle of the femur, only 11 cups (25%) found colonies, and in 33 (75%) their growth was not recorded, single fibroblasts were found that did not form colonies. Out of 47 cultures of SSC KM of the medial condyle of the femur in 14 cultures (30%) colony growth was registered, and in 33 (70%) only single fibroblasts were detected. Regarding cultures from the lateral and medial condyles of the tibia, the growth of SSC colonies - in 17 (33%) and 13 (26%) cups, respectively. In 67% (34 of 51 cups) and 74% (37 of 50) colony growth is absent, respectively. These results indicate a significant slowdown of regenerative processes in bone tissue and are a characteristic feature of RA.

Studies (Table 1) have shown that there is a large difference in the parameters of the number of CFU per unit volume of different parts of the knee joint. In particular, in the medial condyle of the femur it is (0.2307 ± 0.16) x 104, which is 11 - 20 times higher than the parameters of the same indicator in other study areas, but the difference is statistically insignificant.

Place of material extraction, condyle	The total number of nucleated cells in 1 cm^3 x 10^7	Number of CFU in 1 cm of spongiosis 10^4	Efficacy of CFU cloning among 10^5 nucleus-containing bone marrow cells
Lateral Femur	0,24 ± 0,05 n = 48	0,0132 ± 0,0060 n = 44	4,49 ± 1,77 n = 44
Medial Femur	0,30 ± 0,09 n = 50	0,2307 ± 0,1588 n = 47	6,25 ± 2,02 n = 47
Lateral Tibia	0,35 ± 0,05 n = 54	0,0206 ± 0,0074 n = 51	3,41 ± 1,05 n = 51
Medial Tibia	0,41 ± 0,15 n = 52	0,0116 ± 0,0044 n = 50	1,91 ± 0,80* n = 50

Table 1: *Indicators of osteogenic activity of SSC KM of bones, which form the knee joint, patients with gonarthrosis on the background of RA.*

**: The difference is significant (p < 0,05) between lateral and medial condyles.*

Regarding the efficiency of cloning SSK KM spongiosis of this area, the difference in parameters was not significant and ranged from 1.4 to 3.3 times, and reliable - only for the medial condyle of the tibia.

These results were obtained by us in the general group of patients with RA with lesions of the knee joint without taking into account frontal deformities.

As is known from the literature, valgus deformities of the knee joint develop approximately 3.5 - 4 times more often than varus deformities, which completely coincides with the data obtained by us. Of the 37 patients with gonarthrosis on the background of RA, 29 (78%) had valgus deformity, and 8 (22%) had varus deformity.

As you can see (Figure) from the diagram, the greatest load falls on the lateral structures of the knee joint with valgus and on the medial - with varus deformity, so it is advisable to compare the osteogenic activity of stem stromal cells of the bone marrow of patients with gonarthrosis on the background of RA in the case of frontal deformations in the relevant areas.

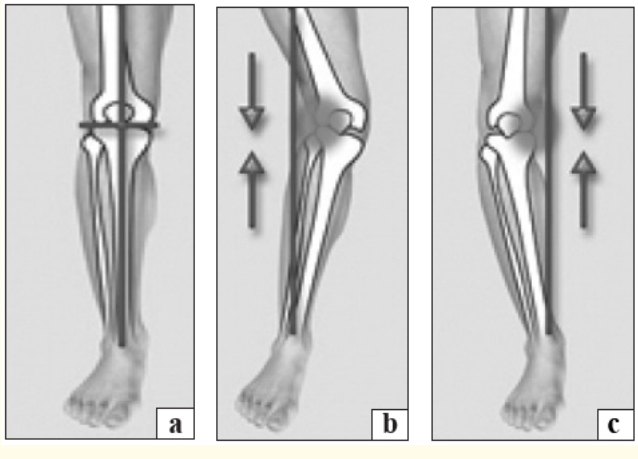

Figure: *Scheme of physiological axis (a) of the lower extremity and its displacement and redistribution of loads on the articular ends under the development of valgus (b) or varus (c) frontal deformities of the knee joint.*

As can be seen from table 2, the parameters of osteogenic activity of the SSC KM of the lateral condyle of the femur are radically different in terms of valgus deformation from those under conditions of varus deformity. Thus, out of 34 cultures with valgus deformity, only 11 cups (32%) had colonies, and in 23 (68%) their growth was not recorded, single fibroblasts were located separately from each other. In contrast, in the case of varus deformity, in no case out of 10 (100%) growth of colonies of SSC KM of the lateral condyle of the femur was registered, i.e. the cloning efficiency of CFU was zero.

The place of material extraction is the lateral condyle of the femur	The total number of nucleated cells in 1 cm^3 x 10^7	Number of CFU in 1 cm of spongiosis x 10^4	Efficacy of CFU cloning among 10^5 nucleus-containing bone marrow cells
With Valgus Deformity	0,25 ± 0,07 n = 37	0,0170 ± 0,0077* n = 34	5,78 ± 2,24* n = 34
At Varus Deformation	0,20 ± 0,07 n = 11	0 n = 10	0 n = 10

Table 2: *Indicators of osteogenic activity of SSK KM of a lateral condyle of a femur in the presence of frontal deformations].*

**: The difference is statistically significant (p < 0.05) between valgus and varus deformities.*

The results of studies indicate a loss of restorative properties of bone tissue in this area of the knee joint with the development of frontal varus deformity.

Analyzing the table 3 we see that per unit volume of spongiosis of the medial condyle of the femur under conditions of valgus deformity contains more than 350 times more CFU compared to cases of varus deformity. In terms of the efficiency of CMC CM cloning in this area, the difference is more than 29 times, but it turned out to be insignificant according to Wald-Wolfowitz.

The place of extraction of the material is the medial condyle of the femur	The total number of nucleated cells in 1 cm^3x 10^7	Number of CFUf in 1 cm of spongiosis x 10^4	Efficacy of CFU cloning among 10^5 nucleus-containing bone marrow cells
With Valgus Deformity	0,31 ± 0,10 n = 41	0,2851 ± 0,1959 n = 38	7,66 ± 2,44* n = 47
At Varus Deformation	0,25±0,11 n = 9	0,0008±0,0006 n = 9	0,26±0,18 n = 9

Table 3: *Indicators of osteogenic activity of the SSC KM of the medial condyle of the femur in the presence of frontal deformities.*

**: The difference is statistically insignificant between valgus and varus deformities.*

In 12 (32%) cups with cultures of SSC KM of the medial condyle of the femur in the presence of valgus deformity, colony growth was registered, and in the rest - not detected, there are only a few fibroblasts. In the case of varus deformation, only in 2 (22%) cups from 9 cultures from this area the growth of SSC colonies was noted.

A comparison of the regenerative potential of the SSC KM of the lateral tibial condyle under conditions of frontal deformities of the knee joint in patients with RA is given in table 4.

The place of material extraction is the lateral condyle of the tibia	The total number of nucleated cells in 1 cm^3 x 10^7	Number of CFU in 1 cm of spongiosis x 10^4	Efficacy of CFU cloning among 10^5 nucleus-containing bone marrow cells
With Valgus Deformity	0,29 ± 0,05 n = 41	0,0114 ± 0,0046 n = 38	2,42 ± 0,87 n = 38
At Varus Deformation	0,52 ± 0,15 n = 13	0,0475 ± 0,0250 n = 13	6,30 ± 3,18* n = 13

Table 4: *Indicators of osteogenic activity of SSK KM of a lateral condyle of a tibia in the presence of frontal deformations.*

**: The difference is statistically significant (p < 0.05) between valgus and varus deformities.*

It should be noted that only in 11 (29%) cups of 38 cultures of stromal fibroblasts of the CM of the lateral condyle of the tibia in valgus deformity colonies were found, and in the presence of varus deformity - in 6 (46%) cups of 13 cultures. As a result of research, it was found that SSC KM of the lateral condyle of the tibia have much worse conditions for the realization of regenerative potential in the case of valgus deformity compared to varus, as found more than 4 times less KUOf KM and 2, 6 times probably lower efficiency of their cloning.

Characteristics of indicators of osteogenic activity of SSC KM of the medial condyle of the tibia with frontal deformations are given in table 5.

The place of extraction of the material is the medial condyle of the tibia	The total number of nucleated cells in 1 cm^3 x 10^7	Number of CFU in 1 cm of spongiosis x 10^4	Efficacy of CFU cloning among 10^5 nucleus-containing bone marrow cells
With Valgus Deformity	0,48 ± 0,18 n = 41	0,0145 ± 0,0054 n = 40	2,38 ± 0,98 n = 40
At Varus Deformation	0,13 ± 0,03 n = 11	0,00007 ± 0,000005 n = 10	0,06 ± 0,05* n = 10

Table 5: *Indicators of osteogenic activity of the stem SSC KM of the medial condyle of the tibia in the presence of frontal deformities.*

**: The difference is statistically insignificant between valgus and varus deformities.*

It is shown that varus deformation is characterized by a more than 20-fold reduction in the number of CFUs in the spongiosis of the medial condyle of the tibia and a reduction in almost 40 times their cloning efficiency compared to the conditions of valgus deformity.

At the same time, in the presence of valgus deformity, in 11 (27%) of 40 SSC cultures colony growth was registered, and in varus deformation - only in 2 (20%) cups out of 10 cultures.

Thus, as a result of the research, a significant difference in the number of CFUs per unit volume in different parts of the knee joint was revealed. In particular, in the medial condyle of the femur it was equal to (0.2307 ± 0.16) x 104, which is 11-20 times higher than in other studied areas. The difference in the parameters of the cloning efficiency of SSC KM of spongiosis ranged from 1.4 to 3.3 times and was significant only for the medial condyle of the tibia.

The decrease in the parameters of indicators (number of CFUs per unit volume and cloning efficiency of stem stromal cells (SSC) of the bone marrow) of the lateral condyles of the femur and tibia of patients with RA can be explained by the redistribution of load on the

lateral parts of the knee joint and accordingly, in the medial condyles of the femur and tibia with varus deformity, significant inhibition of the rate of bone remodeling on the background of persistent chronic autoimmune inflammation.

As mentioned above, the type of frontal deformity at the level of the knee joint depends on which joint was affected first - the hip or knee. It is impossible to definitively determine whether the primary development of deformity or impaired osteogenic activity of mesenchymal (stromal) stem cells of KM in the affected areas, because the nature of the course of RA is due to many factors, including the severity of inflammatory reactions in general and in a particular patient.

However, these processes occupy a prominent place in the pathogenesis of RA with a predominant lesion of the large joints of the lower extremity and are so closely interconnected that they resemble a vicious circle, where it is difficult and sometimes impossible to separate from each other and determine the sequence of pathological changes.

Conclusion

Significant differences in the parameters of osteogenic activity of stem stromal (mesenchymal) cells of the KM bones, which form the knee joint (lateral and medial condyles of the distal femur and proximal tibia). According to the ability to regenerate bone tissue, the studied areas of the knee joint of patients with RA can be arranged in descending order as follows: medial, lateral femoral condyles, lateral and medial tibial condyles bones.

The lowest osteogenic activity was found in the condyles of the tibia. The cloning efficiency of SSC KM from its lateral condyle was equal to (3.41 ± 1.05), which is 1.3 times less than the lateral femoral, and in the medial was (1.91 ± 0.8), which in 3.27 times less than the medial condyle of the femur.

The obtained results of cultural studies expand the idea of the ability of bone tissue (bone) affected by the pathological process to repair; encourage the continuation of complex (along with biomechanical) study of the condition of the bone in the places of the future location of the components of the endoprosthesis.

The studies make it possible to objectively assess the structural and functional condition of the bones (in situ) that form the knee joint of patients with RA, to develop a differential approach to the choice of orthopedic treatment and predict its results.

Conflict of Interest

The authors declare no conflict of interest.

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