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Treatment of pseudoarthrosis of the upper limb using expanded mesenchymal stem cells: a pilot study

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Abstract. – BACKGROUND: In orthopedic field is growing interest in the use of stem cells: this mesenchymal multipotent line (MSCs) can lead to differentiation into osteocytes and thus the formation of bone tissue. In literature applications of this line are described in injuries of tendons and ligaments, small bony avulsions, nonunion fractures and cartilage defects.

AIM: Utilize MSCs expanded in laboratory in case of atrophic pseudoarthrosis of the upper limb.

MATERIALS AND METHODS: We obtain the amount of cell necessary for the implant by the collaboration with the UO Haematological Department.

For the procedure we make a blood sample from the iliac crest bone marrow and a subsequent phase of selection and cultivation of mesenchymal line for 3 weeks, to get a sufficient amount of tissue to be used, which is presented at the time of surgery on a scaffold made by autologous plasma gel and $CaCl_2$.

We reassessed our experience in 8 different types of upper limb fractures result in pseudarthrosis and delayed of consolidation: 4 women and 4 men, average 44 years old followed with a follow-up of 50.3 months.

In all cases the site of non-union has been revitalized (by microfractures and drilling) and a synthesis was performed with a rigid plate. So we fill the bone gap with autologous bone and mesenchymal stem cells expanded in the laboratory.

RESULTS: We have a radiographic healing in 8 cases and no adverse events were highlighted.

CONCLUSIONS: Using this cells line we obtained encouraging but certainly not conclusive impressions, according to the limited number of cases and lack of adequate comparative studies. In tissue engineering are also certainly needed further investigations and developments.

Key Words:

Mesenchymal stem cells (MSCs), Nonunion, Radiographic healing.

Introduction

The great interest in mesenchymal stem cells (MSG) is due to their unique properties. This cell type is multipotent and can give tissue regeneration. These cells have a pleiotropic differentiation capacity, can give rise to different cell lines based on specific biochemical contact[1].

The most recent international literature has shown that these cells may also have a trophic local action; in case of tissue injury secrete in a paracrine way bioactive molecules, growth factors, cytokines and chemokines specific for environment and type of lesion[2].

Due to international regulations and costs, currently, the majority of orthopedics experience using mesenchymal stem cell is to obtain cell's concentrate in a extemporaneous tecnique during the surgical procedure.

The use of mesenchymal stem cells expanded in the laboratory and then using a procedure which allows cell manipulation is much more limited in the context of orthopedic surgery. In the literature highlight the experiences in tendon and ligaments injuries[3-6], small bone avulsion[7-9], in case of bone gap[10-12] in nonunion fractures (pseudarthrosis)[13-18] cartilagine injuries[19-24]. In order to make a scientific contribution on the use of mesenchymal stem cell line expanded in the laboratory in this study we wanted to re-evaluate our caseload in the nonunion of the upper limb focusing our attention on the results in terms of radiographic healing and safety of the implant in the long period.

Materials and Methods

We re-evaluated 8 patients 4 males and 4 females with a mean age of 44 years (min 18-max

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Table I. Type of fracture with the corresponding type of nonunion (Müller AO classification).

Patient (age)	Fracture's type	Failed surgical tecnique	Nonunion type
F. 45	12-A1	Internal flexible nailing	Atrophic pseudoarthrosis
M. 27	12-B3	External fixation	Atrophic pseudoarthrosis
F. 73	12-C1	Intramedullary nail	Atrophic pseudoarthrosis
M. 61	21-B1	Orif stainless steel wires	Atrophic pseudoarthrosis
M. 51	21-B1	Orif stainless steel wires	Atrophic pseudoarthrosis
M. 46	22-A1	Orif (plates and screw)	Atrophic pseudoarthrosis
F. 18	22-A2	Orif (plates and screw)	Atrophic pseudoarthrosis
F. 31	22-C3	Orif (plates and screw)	Atrophic pseudoarthrosis

73 years). The mean follow-up was 50.3 months (min 35 months-69 months max). We used the classification Müller AO[25] and Table I shows the types of nonunion fracture in which we use the MSCs expanded in the laboratory.

Procedure to Obtain Mesenchymal Stem Cells Expanded in the Laboratory

The method is standardized and requires the cooperation of the Haematology Unit. Is performed under local anesthesia a blood sample of 80 ml from the iliac crest bone marrow. We use a needle model Steri Perfect heparin (heparin 15 IU/ml). We perform also a peripheral blood sample of 100 ml to obtain serum required for the preparation of the culture medium.

The blood taken from the bone marrow is diluted 1:3 with saline, layered by density gradient (Ficoll) and centrifuged at 900 g for 25 minutes. The mononuclear cells obtained are washed twice with saline, counted and plated (0.2-0.4 cells/cm^2 × 10^6) in cell culture flasks in complete medium [Dulbeccoís Modified Eagle Medium (DMEM) LG, gentamicin, 2 mM L-glutamine, 6-8% autologous serum]. The cells are then incubated at 37°C, 5% CO_2. After 48-72 hours the culture medium is completely removed, cells washed with saline and complete culture medium added again. Since then the land is half renewed every 2-3 days. We perfom a further sampling of peripheral blood (100 ml approximately) needed to carry forward the culture until use of these cells.

Using protease (select Triple, animal free) cells are counted and plated in double of flasks.Aliquots of cells are used to run a test of osteogenic induction and a phenotypic assessment. The cells are subjected to osteogenic induction by a treatment with 50 microgr/ml of ascorbic acid and hydrocortisone 10-6 M. At the third week of culture, the day before or the day of surgery is perform peripheral blood sample in EDTA (8 tubes), necessary to obtain autologous plasma. The cells are then added to plasma (1-4 × 10^6 cellule/2 ml) to which is added $CaCl_2$.

At the time of surgery the MSCs are placed in a gelled suspension of autologous plasma and sterilized $CaCl_2$ called "clot" (Figure 1). This represents a real scaffold for stem cells and provides good surgical manipulation at the site of lesion[26].

In all cases, in the site of nonunion we make revitalization with drillling, microfractures, opening the medullary and then we performed a synthesis with a plate and screws. We fill the bone gap with autologous or homologous bone tissue and then implanted mesenchymal stem cells.

In all cases the patient have 30 days of immobilization in a plaster cast or brace.

Results

Radiographic healing was seen in all patients with a median time of 6 months (min 3.5 months-up to 10 months) (Figure 2, Table II). There were no complications. In all cases there was a functional recovery of the district and treated patients

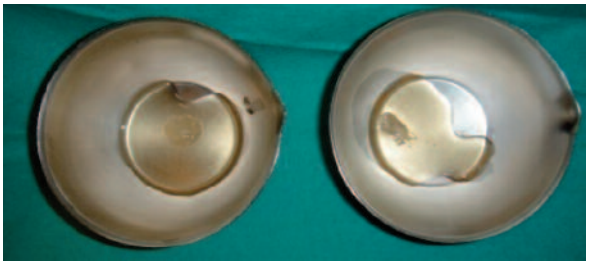

Figure 1. Blood clot at the time of surgery.

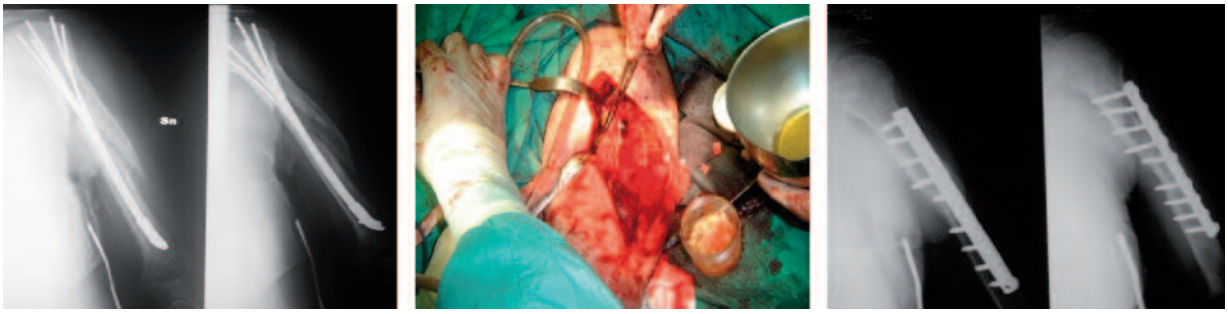

Figure 2. A case report of a female 73 years with fracture type 12-C1 hesitated nonunion after treatment with intramedullary nail, during engagement of MSCs expanded in the laboratory, radiographic healing at 5 months.

Table II. Radiographic healing time by type of fracture.

Fracture's type	Time of the radiografical healing (months)
12-A1	5
12-B3	3.5
12-C1	5
21-B1	10
21-B1	7.5
22-A1	6
22-A2	5
22-C3	6

have resumed their daily activities and sports. Considering the follow-up period of 50.3 months no local or systemic adverse event was highlighted in the long term.

Discussion

The series is limited and heterogeneous and does not allow conclusive judgments. We can say that the results obtained are encouraging and the technique appears safe. Surgical approach still remains the most important point by revitalizing, drilling, opening of the intramedullary canal and the synthesis. The graft of MSCs should be seen as the catalyst for bone healing and not the only determining factor for the resolution of the disease.

To validate the use of MSCs is, therefore, necessary to perform comparative studies for age, sex, general condition, location and method of injury, as a further clinical validation of the efficiency of this cell line. This will lead to new strategies such as preload the cells on biodegradable scaffolds for osteogenic address.

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