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Bone marrow mesenchymal stem cell aspirates from alternative sources: Is the knee as good as the iliac crest?

Javier Narbona-Carceles[a,*], Javier Vaquero[a], Susana Suárez-Sancho B.S[b], Francisco Forriol[c], Maria Eugenia Fernández-Santos[b]

[a]Orthopaedics Department, Hospital General Universitario Gregorio Marañon, Madrid, Spain
[b]Regenerative Medicine and Cellular Therapy Unit, Hospital General Universitario Gregorio Marañon, Madrid, Spain
[c]School of Medicine, University San Pablo CEU, Campus Monteprincipe, Boadilla del Monte, Madrid, Spain

KEYWORDS

Mesenchymal stem cells
Bone marrow
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ABSTRACT

Introduction: The most common method to obtain human mesenchymal stem cells (MSCs) is bone marrow aspiration from the iliac crest, but MSCs have also been isolated from different bones. The main purpose of this study was to compare bone marrow MSCs aspirated from the metaphysis of the distal femur and the proximal tibia with those obtained from the iliac crest, and to determine whether these locations represent potential alternative sources of MSCs for research and clinical application.
Materials and methods: Bone marrow was aspirated from the iliac crest and the metaphysis of the distal femur and the proximal tibia during total knee arthroplasty in 20 patients. The aspirates were centrifuged by density gradient, then mononucleated cell (MNC) concentration in the different aspirates was determined using a Coulter counter. MSCs were isolated, cultivated and characterised by their immunophenotype and by their *in vitro* potential for differentiation into osteoblasts, chondroblasts and adipocytes in specific media. Expansion and cell viability were quantified using trypan blue staining and cell counting with a haemocytometer (Neubauer chamber). The three sources were compared in terms of MNC concentration, viability of the cultures and presence of MSC using the Wilcoxon test.
Results: MNC concentration was significantly higher in the iliac crest (10.05 Millions/ml) compared with the femur (0.67 Millions/ml) and tibia (1.7 Millions/ml). Culture success rates were 90%, 71% and 47% for MSCs from the iliac crest, femur and tibia, respectively. Flow cytometry analysis showed the presence of CD90+, CD105+, CD73+, VEGF+, CD71+, HLA-DR-, CD45-, CD34-, CD19-, and CD14- cells. The immunophenotype pattern of MSCs was similar for the three locations. Trilineage differentiation was achieved with all samples.
Conclusions: MSCs can be found in bone marrow from the metaphysis of both the distal femur and the proximal tibia. The phenotype and differentiation potential of these cells are similar to those of bone marrow MSCs from the iliac crest. Bone marrow aspiration from these locations is a relatively easy and safe alternative to that from the iliac crest for obtaining MSCs. Further study is required to assess whether the concentrations of MSCs obtained from these sources are sufficient for one-step therapeutic purposes.

Introduction

Mesenchymal stem cells (MSCs) were first described in bone marrow by Friedenstein and Owen in 1988 [1] and have since been the subject of increasing research interest. MSCs can differentiate into many tissue types. They have paracrine and immunosuppressive effects and reparative and regenerative properties. These characteristics, and the ease with which they can be isolated, mean that MSCs are useful therapeutically and in tissue engineering [2-5]. One of the earliest and most common uses of MSCs is in the treatment of musculoskeletal pathologies, such as pseudarthrosis, osteochondral defects and avascular necrosis of the bone [6]. Several studies report the efficacy of MSCs in treating such pathologies, and the use of MSCs has extended to other fields of medicine [7-10].

Traditionally, the most common source of MSCs has been bone marrow from the iliac crest, but there are several reports showing these cells are also present in bone marrow from the vertebral body, humeral head and sternum [11-14].

Metaphyseal trabecular bone of the distal femur and proximal tibia has a similar structure to that in the previously mentioned locations [15]. The knee is a relatively straightforward and safe anatomical area for aspiration of bone marrow by bone puncture because of the ease of identification of anatomical landmarks and the well-established nature of the procedures involved.

* Corresponding author at: Orthopaedics Department, Hospital General Universitario Gregorio Marañon, C/ Doctor Esquerdo, 46 - 28007 Madrid, Spain. Tel.: +34 915868426; fax +34 915868425.
E-mail address: franciscojavier.narbona@salud.madrid.org (J. Narbona-Carceles).

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However, as far as we could determine after a thorough literature search, only a few studies investigate the existence of MSCs in this area [16,17].

The aim of this study was to compare aspirates from the metaphysis of the distal femur and proximal tibia with the assumed gold-standard aspirates of the iliac crest from the same patient. The study was approved by the Ethical Committee of our hospital. The primary hypothesis of the study was that MSCs with the same characteristics as those found in bone marrow from the iliac crest would be present in bone marrow from the metaphysis of the distal femur and proximal tibia. A secondary hypothesis was that the concentration of MNCs at the three locations would be similar. A further objective of this study was to determine whether bone marrow from the metaphysis of the distal femur and the proximal tibia represents a potential alternative source of MSCs.

Materials and methods

We obtained bone marrow aspirates through puncture of the iliac crest, distal metaphysis of the femur and proximal metaphysis of the tibia from a group of volunteer patients during total knee arthroplasty (TKA). MSCs were isolated from the aspirates, characterised and compared. Samples were taken from the limb undergoing surgery. The study was conducted on patients undergoing TKA to minimise iatrogenesis. Patients with knee osteoarthritis assigned to a TKA procedure were enrolled in a prospective, non-randomised way. Subjects were invited to participate and in all cases gave prior written informed consent. Exclusion criteria were age over 75 years, previous treatment with corticosteroids or cytostatic drugs, alcoholism, skin lesions in lower extremities, previous radiotherapy affecting the pelvis or knee, active infection, anaemia (Hb < 10.0 g/dl), leucopenia (< 4000/ml), and/or an active tumoural process.

There were 20 subjects in the study: 4 males and 16 females, with a mean age of 70.9 years (range 64-75 years). Twenty bone marrow aspirates were collected from the iliac crest, 17 from the distal femur and 16 from the proximal tibia. In three patients, metaphyseal aspirates were obtained under tourniquet ischaemia to the limb; these samples were not taken in accordance with the protocol and so were not included in the study. In one patient, for unknown reasons, puncture and aspiration of the tibia failed to provide bone marrow.

Bone marrow aspiration technique

All samples were obtained by the same investigator in the operating room just before TKA surgery. Patients were supine, under spinal anaesthesia, and with deflated ischaemic tourniquet. The operating field was sterile. Puncture and bone marrow aspiration were performed with an 11-gauge cannulated trocar with lateral holes (Bone Marrow Aspiration System, Synthes. Umkirch, Germany).

Puncture of the iliac crest was performed first. The skin puncture point was located over the iliac crest, approximately 5 cm posterior to the anterior superior iliac spine. The tip of the cannulated trocar was directed towards the widest part of the ilium. Local infiltration of this area with Bupivacaine (0.5%) reinforced postoperative analgesia.

Puncture of the distal femoral metaphysis was made via the anterior face of the knee. The entry point was located 1 cm proximal to the superior pole of the patella. The trocar was directed through the quadriceps and into the anterior femoral cortex at a caudal angle of 30° (Figure 1).

Finally, puncture of the proximal tibia metaphysis was conducted over the anterior tibial tubercle in a parallel direction

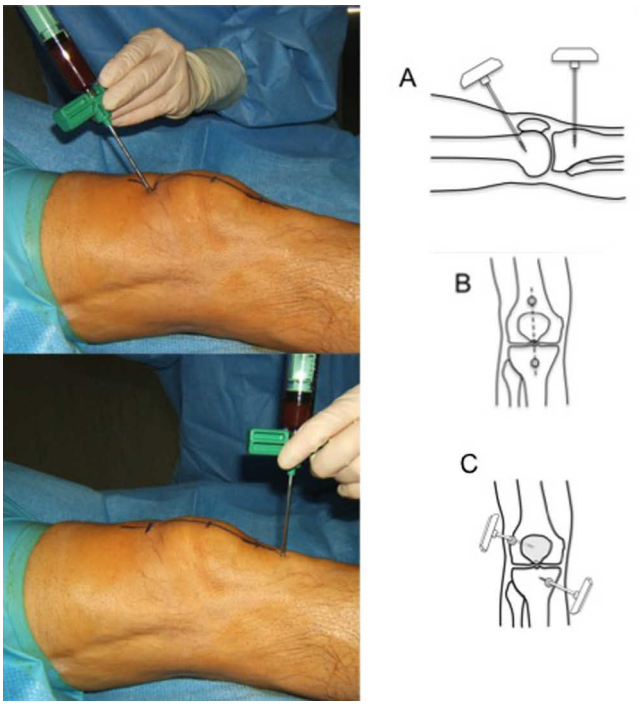

Fig. 1. Technique for puncture-aspiration of bone marrow from distal femoral and proximal tibial metaphysis. A. Entry point in femur and tibia in this study. B. Punctures made in line with a programmed total knee arthroplasty (TKA) skin incision. C. Preferred entry points for actual clinical practice.

to the articular surface. Entry points at the knee were in line with the planned skin incision for TKA surgery.

A minimum of 5 ml bone marrow aspirate was obtained from each location. To avoid haemodilution of the aspirate, the depth and angle of the trocar was changed after each 2 ml of material had been aspirated [13-18]. Samples were placed in heparinised tubes, labelled and sent immediately to the laboratory. TKA surgery was then performed without modification to standard procedure. Postoperative analgesia and early rehabilitation for TKA were as per the hospital's usual protocols.

Isolation of MSCs

The minimal criteria described recently by the International Society for Cell Therapy (ISCT) were used to define MSCs in our study [19]. These criteria include adherence to plastic, specific surface antigen expression and potential to differentiate into adipocytes, osteoblasts and chondroblasts. Laboratory processing techniques followed Good Manufacturing Practice (GMP) [20].

Samples were filtered using a standard 100 µm filter to remove clots, fat and bone debris. Preliminary immunophenotypic analysis of bone marrow samples was conducted to rule out contamination with peripheral blood. Mononucleated cell (MNC) count was made with a Coulter counter.

The MNC fraction was obtained by density gradient centrifugation in Ficoll-Paque (GE Healthcare. Freiburg, Germany). Centrifugation was performed at 400 g (1200 rpm) and 22°C for 30 minutes, after which the MNC layer was aspirated and washed twice in phosphate buffered saline (PBS) to remove remains of Ficoll. Osmotic shock was used to remove any remaining erythrocytes.

MNCs were seeded at a density of 160,000-200,000 cells/cm^2 in culture flasks and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with glutamine, 10% bovine foetal serum (BFS), and penicillin-streptomycin 1% (MACS medium, Miltenyi Biotec, Germany) and incubated.