

Treatment of osteonecrosis of the femoral head by core decompression and implantation of fully functional ex vivo-expanded bone marrow-derived mesenchymal stem cells: a proof-of-concept study

Rodrigo Mardones¹
Daniel Camacho^{1,2}
Francisco Monsalvo¹
Nicolás Zulch¹
Claudio Jofre³
José J Minguell³

¹Department of Orthopedics, Clínica Las Condes, Santiago, Chile;

²Department of Orthopedics, Instituto Traumatológico, Santiago, Chile; ³Centro de Terapia Regenerativa Celular, Clínica Las Condes, Santiago, Chile

Background: Based on several attributes involved in bone formation, bone marrow-resident mesenchymal stem cells (MSCs) have been employed in the treatment of patients suffering from femoral head osteonecrosis. Due to the low content of MSCs in the bone marrow, ex vivo expansion procedures are utilized to increase the cell number. Customarily, before administration of the resulting expanded cell product MSCs to the patient, its cellular identity is usually evaluated according to a set of “minimal phenotypic” markers, which are not modified by ex vivo processing. However, MSC functional (“reparative”) markers, which are severely impaired along the ex vivo expansion routine, are usually not assessed.

Patients and methods: In this proof-of-concept study, a cohort of five avascular osteonecrosis patients received an instillation of ex vivo-expanded autologous MSCs, manufactured under controlled conditions, with an aim to protect their functional (“reparative”) capacity.

Results and conclusion: Outcomes of this study confirmed the safety and effectiveness of the MSC-based therapy used. After a follow-up period (19–54 months), in all patients, the hip function was significantly improved and pain intensity markedly reduced. As a corollary, no patient required hip arthroplasty.

Keywords: avascular necrosis, femoral head, osteonecrosis, mesenchymal stem cell-based therapy

Introduction

Avascular necrosis (AVN), also called osteonecrosis, aseptic necrosis or ischemic bone necrosis, is a condition that occurs when blood supply to a bone is interrupted and/or reduced. Therefore, the involved bone might collapse and frequently progress to osteoarthritis. In the early stages of the disease, most treatments are focused on preventing further bone collapse. Among them, nonsteroidal anti-inflammatory drugs and/or other medications are used with an aim to relieve pain and inflammation associated with AVN. In turn, when the disease is fairly advanced, treatment options include total hip arthroscopy (THA) and femoral head core decompression. Given that AVN typically affects young patients, the above-indicated methods do not represent outstanding curative options.^{1–4}

Bone regeneration,^{5,6} a rather complex physiological process, involves the participation of several components and mesenchymal stem cells (MSCs) are one among them. These have the potential to differentiate into bone-forming cells as well as to

Correspondence: José J Minguell
Centro de Terapia Regenerativa
Celular, Clínica Las Condes, Av. Las Condes 1283, Torre B-303, CP 7590992, Santiago, Chile
Tel +569 9501 7675
Email jose.minguell@bioscell.cl

produce specific signaling molecules (growth factors, cytokines, others) and extracellular matrix molecules.⁷⁻¹⁰ As a result, several studies have been performed to assess in AVN patients the clinical effectiveness elicited by the instillation of diverse bone marrow products containing MSCs. The latter include bone marrow aspirates,^{11,12} bone marrow-derived mononuclear cell fraction^{13,14} and/or bone-marrow-derived ex vivo-expanded autologous MSCs.¹⁵⁻¹⁷

In almost all reported clinical studies employing MSCs as the “reparative cell product”, the standard criteria for MSC characterization include the assessment of a “minimal set” of phenotypical attributes, including morphology, in vitro differentiation and expression/no expression of a set of surface molecules.¹⁸ However, the latter in any case validates MSC functional (“reparative”) status, which is modified alongside ex vivo processing,¹⁹⁻²¹ as well as by patient conditions at inclusion, comprising age, gender, comorbidities and/or concomitant medication.²²⁻²⁸

Consequently, the safety and effective use of an ex vivo-manipulated MSC product is associated with several factors. Among them are the following factors: 1) the assurance that its functional “reparative” attributes are intact and impinged neither by patient conditions nor by ex vivo manipulations and 2) the implementation of procedural maneuvers assuring the proper delivery as well as the permanence (homing) of the cell product in the damaged zone.

Based on the above remarks, in this proof-of-concept study, a cohort of five AVN patients received an instillation of ex vivo-manipulated autologous bone marrow-derived MSCs manufactured under conditions proficient to protect and sustain their phenotypic and functional characteristics.²⁹⁻³²

The above comprises an invitation to pay attention to a group of relevant issues involved in the formulation of an MSC-based therapeutic approach. Among them are the following: 1) the preparation by means of ex vivo expansion procedures of an optimum number of fully functional reparative MSCs; 2) the feasibility to perform a core decompression procedure aimed to reduce pressure, increase blood flow and

slow down bone and/or joint damage^{14,16} and 3) an attempt to prolong the permanence (homing?) of the cell product in the osteonecrotic area, by depositing a plug immediately after cell instillation.¹³

Patients and methods

Patient population

Five patients with diagnosis of AVN of the femoral head, which met the following criteria, were included in this proof-of-concept study: 1) AVN at stage 2 or 3 of the Ficat classification system² and 2) <50% of the articular area compromised. The clinical characteristics of the study population are presented in Table 1. An X-ray as well as a magnetic resonance imaging (MRI) preoperative image (patient 2) are shown in Figure 2.

Preparation and characterization of the ex vivo-expanded autologous bone marrow-derived cell product

All study patients had a bone marrow aspiration, and the samples were sent to the adjoining Good Manufacturing practices facility for the isolation and ex vivo expansion of autologous bone marrow-resident MSCs.^{33,34}

At the end of each expansion passage, cell aliquots were taken for the assessment of 1) cell number, morphology and viability; 2) “minimal phenotypical markers criteria”¹⁸ and 3) functional markers, including fast forward-scattered (FFS) light and cumulative population doubling (CPD), as predictive indicators of MSC-replicative senescence.¹⁹⁻²¹

Instillation of ex vivo-expanded autologous MSCs in patients

In this proof-of-evidence study, all five AVN patients received the instillation of a unique dose (40×10^6) of ex vivo-expanded autologous bone marrow-derived MSCs into the necrotic zone and through the canal of a preceding core decompression process. In an attempt to further prolong the permanence (homing) of MSCs in the bone-damaged area,

Table 1 Clinical characteristics of the study population

Patient ID	Age (years)	Gender	AVN feature: side	Ficat class	Comorbidities
#1	23	Male	Left	3	No
#2	44	Female	Left	2	Lupus erythematosus
#3	59	Male	Right	2	Hypertension, hypothyroidism
#4	43	Male	Right	2	Insulin resistance
#5	37	Male	Left	2	No

Abbreviation: AVN, avascular necrosis.

a hydroxyapatite or a calcium phosphate plug^{35,36} was placed immediately after cell instillation (Figure 1).

Ethics statement

All procedures performed in this study have been carried out according to the ethical guidelines outlined by the Ethics Committee (Institutional Review Board) of Clinica Las Condes, Santiago, Chile (July 27, 2016). Clinica Las Condes is a medical center affiliated with John Hopkins Medicine International and accredited by the Joint Commission International (<http://www.jointcommissioninternational.org>). In addition, written informed consent was provided by the patients, in accordance with the Declaration of Helsinki.

Results

Characterization of the ex vivo-expanded autologous bone marrow-derived MSC products

The ex vivo-expanded autologous MSC product, obtained at the end of each expansion cycle, was assessed for 1) cell number, morphology and viability; 2) expression of a set of “minimal phenotypical markers”¹⁸ and 3) flow cytometry analysis to calculate FFS and CPD values. The latter are usu-

ally considered predictive indicators of cellular functionality and thus replicative senescence.^{19–21,29,30} The results of these studies are shown in Table 2.

Patients' clinical outcomes after the infusion of ex vivo-expanded autologous bone marrow-derived MSCs

As shown in Table 3, at different times after instillation of the ex vivo-expanded autologous cell product, all five patients exhibited an improvement in the modified Harris hip score, from a preoperative mean value of 73.6 (range 47–95) to a postoperative mean value of 98.2 (range 95–100). In turn, the visual analog scale values improved from a preoperative mean value of 4.6 to a postoperative mean value of 0.4. Remarkably, the above results matched with a sustained change observed in the X-ray and MRI images of the pelvis of patient #3, after an extended follow-up period (Figure 2). The above findings are representative of similar results observed in patients #1, 2, 4 and 5 (not shown).

The above data reinforce the notion that the instillation of a “minimally manipulated” ex vivo-expanded autologous bone marrow-derived MSC product^{30,31} emerges as an outstanding therapeutic option for AVN patients.

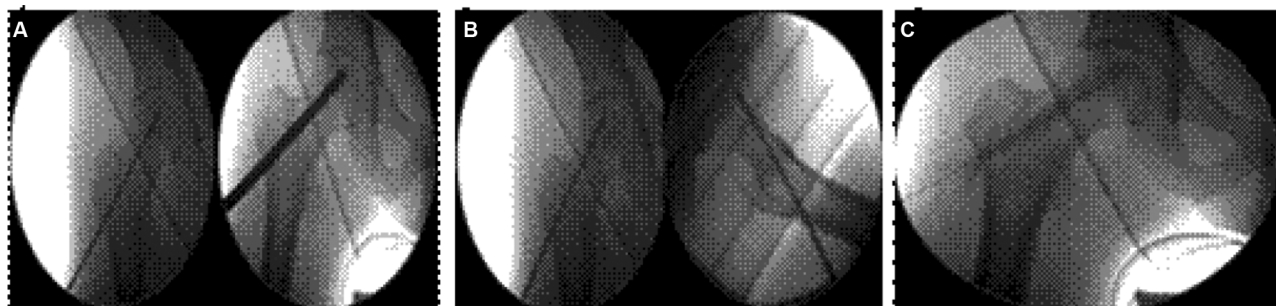


Figure 1 Intraoperative radioscopic views of (A) passing of the 10 mm cannulated drill to the center of the necrotic area in both planes (AP and axial view), (B) the infusion of ex vivo-expanded autologous MSCs into the necrotic area and (C) the subsequent syringe filling of the drilling canal. In all cases, the guide wire was stopped at the subchondral bone of the necrotic area.

Abbreviation: AP, anteroposterior.

Table 2 Assessment of phenotypical and functional attributes of ex vivo-expanded bone marrow-derived MSCs

Patient ID	Expansion cycles ^a	Fulfillment of “minimal phenotypical” markers ^b	Functional markers ^c	
			CPD	FFS
#1	2	Yes	1.2/1.2	92/94
#2	3	Yes	1.5/1.5	93/95
#3	2	Yes	1.8/1.7	92/95
#4	3	Yes	1.6/1.5	93/95
#5	2	Yes	1.4/1.4	92/94

Notes: ^aRequired to manufacture the cell product. ^bDominici et al.¹⁸ ^cAs assessed²⁰ at the end of the first and last ex vivo expansion cycle.

Abbreviations: CPD, cumulative population doubling; FFS, fast forward scatter.

Discussion

As indicated in the “Introduction” section, in the last 10 years, several clinical studies have been initiated to treat AVN patients by the instillation of different types of “reparative

cell products” containing MSCs. The clinical use of MSCs is reinforced by a set of attractive cellular and molecular attributes, including among many others a differentiation potential into bone-forming cells, as well as competence to produce and release a variety of growth factors and extracellular matrix components.^{5,7,9,30}

Table 3 Patients’ clinical outcome after instillation of the ex vivo-expanded autologous MSC-based product

Patient ID	Time elapsed from cell infusion (months)	Modified Harris hip score		Visual analog scale	
		Before	After	Before	After
#1	50	90	100	6	0
#2	36	95	100	3	0
#3	37	71	96	5	0
#4	31	65	100	4	1
#5	15	47	95	5	1

Despite the abundance of tissue sources of MSCs, in a vast majority of clinical studies, patients have received instillation of diverse bone marrow-derived MSCs products. Some of them are as follows: 1) a bone marrow suspension, 2) a bone marrow-derived mononuclear cell fraction and 3) ex vivo-expanded bone marrow-derived MSCs. In the first two cases, despite the absolute number of MSCs obtained being extremely low, their cellular and functional attributes are intact.^{7,8,30,31} Consequently, ex vivo expansion procedures

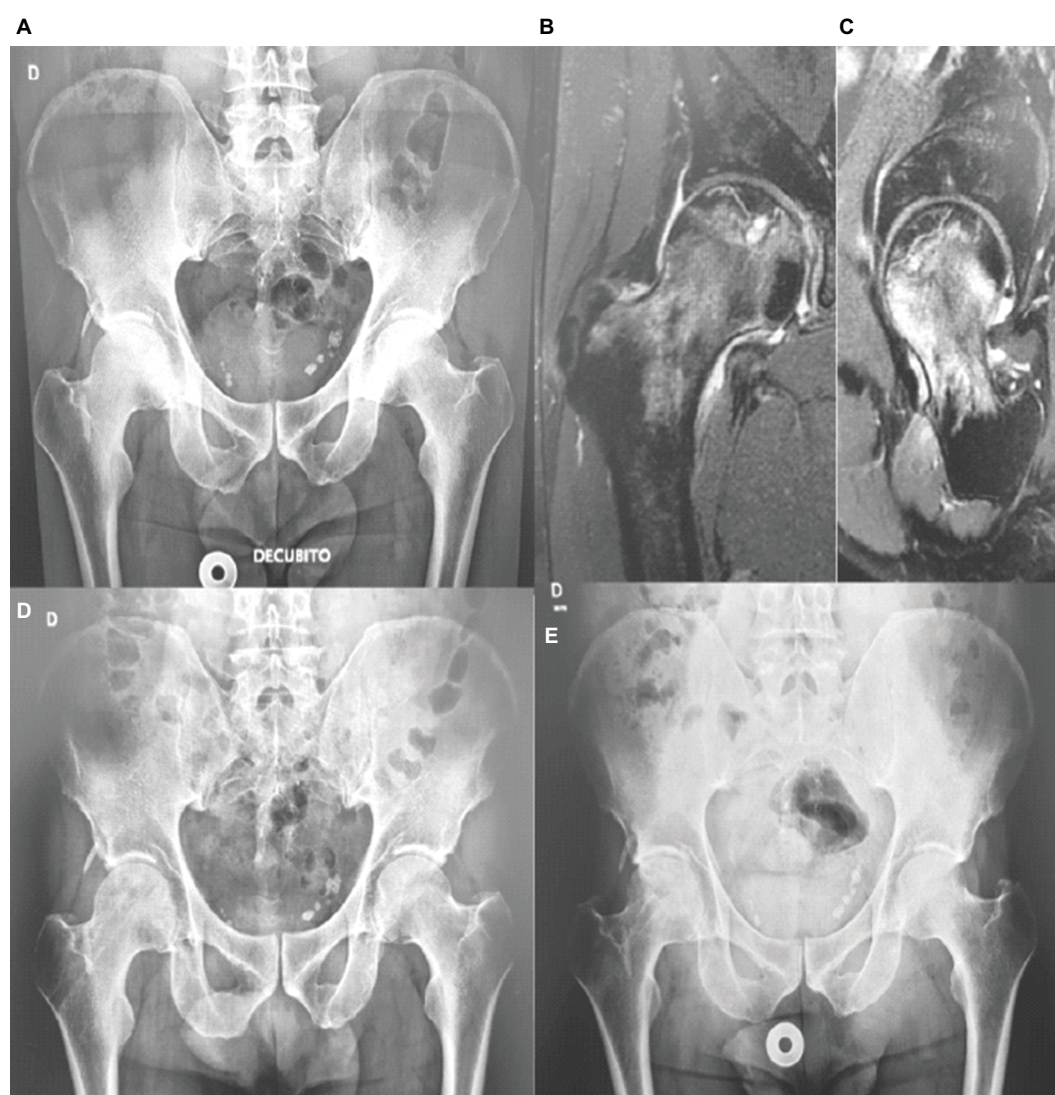


Figure 2 X-ray and/or MRI images of the pelvis of an AVN patient (#3) at inclusion in the study (A, B, C), immediately after the cell infusion (D) and after a 3-year follow-up period (E).

Abbreviation: AVN, avascular necrosis; MRI, magnetic resonance imaging.

have been developed to increase the cell number, thus allowing the instillation of an appropriate number of ex vivo-expanded autologous MSCs to patients. The resulting expanded MSCs are usually assessed for the expression of conventional phenotypic markers,¹⁸ but not for functional (reparative) markers, which are known to be affected by ex vivo expansion.^{19,20,30–32}

In this proof-of-evidence study, a cohort of five AVN patients received the infusion of a unique dose of ex vivo-expanded autologous bone marrow-derived MSCs into the necrotic zone and through the canal of a preceding core decompression process.^{14,16} The “reparative cell product” was manufactured under well-controlled conditions (less than three expansion cycles) which, as seen in Table 2, sustain and warrant the full expression of “minimal phenotypic lineage-associated” markers,¹⁸ as well as of CPD and FFS values, two predictive indicators of senescent events.^{11,19}

In addition to the above-mentioned provisions and in an attempt to further prolong the permanence (homing) of instilled cells in the proximity of the bone damaged region, a hemostatic matrix and/or a calcium phosphate cement^{35,36} was instilled nearby the damaged area.

We presume that the procedural provisions taken along this study as well as others^{37–40} emerged as important contributors to the successful clinical outcome that was observed in all five study patients (Table 3).^{14–16}

Beyond the appealing clinical outcomes of this study, it is interesting to mention that before the setting up of the Stem Cell Laboratory, the conventional therapy for AVN patients in use at this institution comprised femoral core decompression followed by the infusion of a bone marrow-derived mononuclear cell grafting.^{14–16} Results indicated that ≥80% of patients treated with the cell grafting had a treatment failure requiring THA (R Mardones et al, 2018; unpublished data).

Disclosure

The authors report no conflicts of interest in this work.

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