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Treatment of early stage osteonecrosis of the femoral head with autologous implantation of bone marrow-derived and cultured mesenchymal stem cells

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ABSTRACT

Background: Treatment of early-stage osteonecrosis of the femoral head (ONFH) with autologous implantation of iliac crest bone marrow-derived mononuclear cells, which contain tens of thousands of bone marrow mesenchymal stem cells (BMMSCs), recently achieved a promising outcome.
Methods: One hundred patients with early-stage ONFH were recruited and randomly assigned to BMMSC treatment or core decompression (CD) treatment. Each BMMSC-treated hip received femoral head (FH) implantation of 2×10^6 autologous subtrochanteric bone marrow-derived and *ex vivo* expanded BMMSCs. The radiographic stage of ONFH according to the Association Research Circulation Osseous classification, Harris hip score (HHS), and the volume of the necrotic lesion or the low signal intensity zone (LowSIZ) in the FH were assessed before and 6, 12, 24, and 60 months after the initial operation.
Results: Sixty months after the operation, only 2 of the 53 BMMSC-treated hips progressed and underwent vascularized bone grafting. In CD group, 7 hips lost follow-up, and 10 of the rest 44 hips progressed and underwent vascularized bone grafting (5 hips) or total hip replacement (5 hips). Compared with the CD group, BMMSC treatment significantly improved the HHS as well as decreased the volume of femoral head LowSIZ of the hips preoperatively classified at stage IC, IIB, and IIC ($P<0.05$, respectively; stage IIA, $P=0.06$, respectively). No complication was observed in both treatment groups.
Conclusions: Ex vivo expansion of autologous BMMSCs can reliably provide a greater number of BMMSCs for FH implantation. This intervention is safe and effective in delaying or avoiding FH collapse, which may necessitate total hip replacement.

Introduction

Osteonecrosis of the femoral head (ONFH) is a disease in which necrotic bone lesions usually progress to femoral head collapse and symptomatic hip arthritis; the disease mainly affects individuals in their thirty to sixty years of age [1,2]. ONFH is always associated with one or more risk factors, such as trauma to the hips, alcohol abuse, excessive use of corticosteroid, hemoglobinopathy, Gaucher's disease, pregnancy, coagulopathies, Caisson disease, organ transplantation, hyperbaric exposure, inflammatory or autoimmune diseases, and other idiopathic mechanisms. However, the pathophysiology of ONFH remains uncertain [3–6].

Core decompression (CD) has been widely used to delay the progress of osteonecrotic lesions destroying the femoral head. However, a number of factors may influence the prognosis of such treatment, including alcohol abuse and corticosteroid use, as well as the size and location of the necrotic lesion [6–10]. Although vascularized or nonvascularized autologous bone grafts and osteotomies have also been employed in treating ONFH [4,6,8,9], these procedures remain complicated, expensive, and not widely reproducible.

Recent pioneer studies by Hernigou et al. and Gangji et al. have demonstrated the efficacy of autologous bone marrow cell implantation into the femoral head during early-stage ONFH [11–13]. In such procedures, several tens of thousands of bone marrow stem cells, which were isolated and concentrated from anterior iliac crest-aspirated bone marrow, were implanted into the osteonecrotic zone in the femoral head right after CD. A novel protocol has been developed in which subtrochanteric bone marrow was directly aspirated through the CD tunnel and bone marrow-derived mesenchymal stem cells (BMMSCs) were cultured *ex vitro* for about two weeks to obtain millions of cultured BMMSCs for femoral head implantation. The concentration of BMMSCs in harvested autologous bone marrow is relatively lower than that in the *ex vivo* cultured MSC suspension. To obtain a larger number of BMMSCs without *ex vivo* expansion, a higher volume of autologous bone marrow must be aspirated. In

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the current study, about 10 mL of bone marrow were harvested and about 2 mL of concentrated BMMSC suspension were obtained by *ex vivo* expansion. Transplanted BMMSCs are believed to directly differentiate into osteoblasts or into vascular endothelial cells to promote the repair process *in vivo* [7,11]. The present study aims to assess the safety and efficacy of the above-mentioned novel procedures in the treatment of early-stage ONFH.

Methods

Study design

This single-center randomized clinical trial was conducted in a university-affiliated hospital in China between May 2004 and July 2006. The objective of this study was to assess the efficacy of cultured bone marrow-derived mesenchymal stem cell implantation into the femoral head as treatment against early stage osteonecrosis of the femoral head. The protocol of the present study was approved by the Institutional Review Board of Dalian University and the Ethics Committee of the City of Dalian under the authorization of the Ministry of Public Health of China. Written informed consent was obtained from each patient before enrollment.

Participants and randomization

Patients with ONFH were recruited at the Dalian University Zhongshan Hospital. The inclusion criteria were age between 18 and 55 years, presence of osteonecrotic stages from IC to IIC according to the Association Research Circulation Osseous (ARCO) classification [14], and risk factors, such as trauma, corticosteroid use, alcohol abuse, Caisson disease, and other idiopathic mechanisms. The exclusion criteria were pregnancy, current and previous infections, skeletal immaturity, immunosuppressive drug therapy, a history of inflammatory arthritis, cardiovascular diseases, prior systemic corticosteroid treatment, and mental health problems. The patients who met the inclusion criteria were randomly divided into two groups following the randomization sequence created by a third party not involved in this study at the time of patient admission (Fig. 1). Patients in one group were treated with core decompression (CD treatment group) and patients in the other group were treated with femoral head autologous implantation of cultured BMMSCs (BMMSC treatment group).

Procedures

BMMSC treatment

With the aid of a Stryker's Navigation System, a decompression tunnel was made using a trephine through the trochanter and femoral neck into the necrotic region in the femoral head, 2–3 mm away from the cartilage (Fig. 2A). The medial part of the bone core withdrawn from the trephine was sent for pathological examination (Fig. 2B) and the lateral part was bored with a 1 mm diameter Kirschner wire along its central axis (Fig. 2C). After 10 mL of subtrochanteric bone marrow was aspirated through the decompression tunnel (Fig. 2D), the necrotic segment was removed by a custom-made trephine with a collapsible scraping end (Fig. 2E). Next, the bored bone core was plugged into the decompression tunnel (Fig. 2F) before the outlet of the decompression tunnel was sealed with bone wax followed by layer closure. The subtrochanteric bone marrow-derived BMMSCs were subjected to proliferation *in vitro* for two weeks, after which about 2×10^6 BMMSCs were harvested and prepared in 2 ml normal saline solution later injected into the osteonecrotic site in

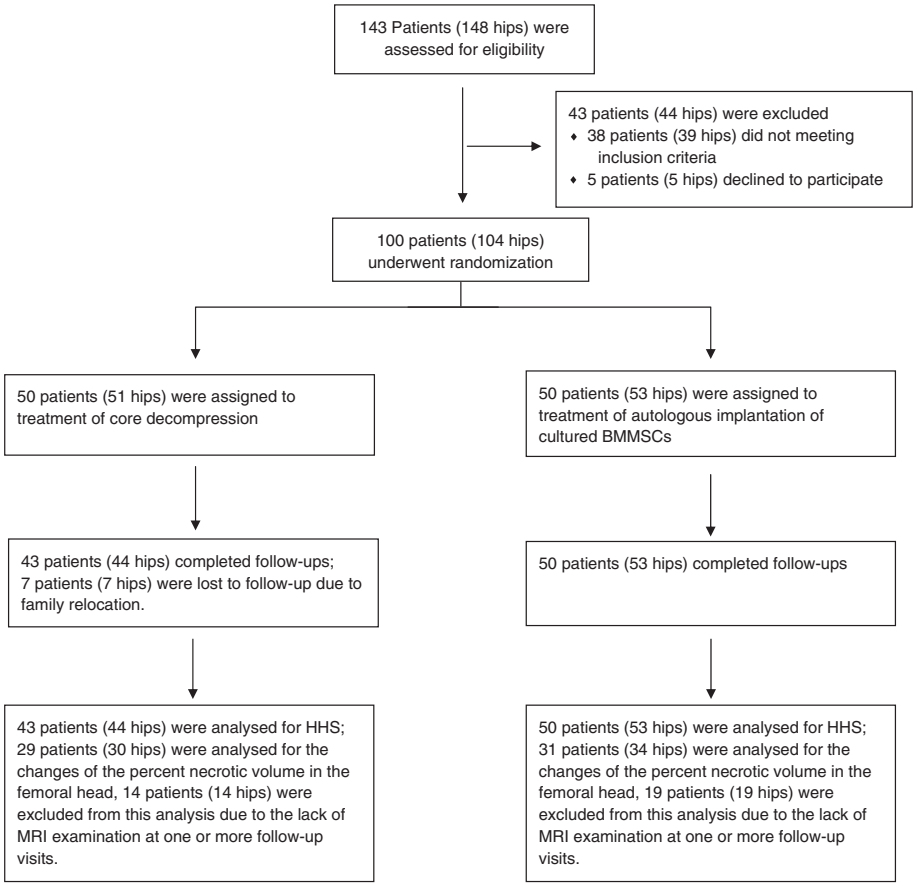

Fig. 1. Enrollment, randomization, and follow-up.