ELSEVIER

Cotransplantation of Bone Marrow Mononuclear Cells and Umbilical Cord Mesenchymal Stem Cells in Avascular Necrosis of the Femoral Head

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ABSTRACT

Objective. We sought to investigate the therapeutic effects of cotransplantation of autologous bone marrow mononuclear cells (BMMNCs) and allogeneic umbilical cord mesenchymal stem cells (UC-MSCs) on avascular necrosis of the femoral head (ANFH).

Methods. In all, 30 patients (49 hips; 24 males and 6 females) with ANFH were enrolled. According to the system of the Association Research Circulation Osseous, there were 24 hips in phase II and 25 hips in phase III. Blood supply to the femoral head was evaluated by using digital subtraction angiography. Generally, 60 to 80 mL of autologous BMMNCs and 30 to 50 mL of UC-MSCs were infused into the femoral head artery. Harris scores including pain and joint function were used to evaluate the effects before and 3, 6, 9, and 12 months after transplantation. Computed tomography and radiographs were performed before and 12 months after the treatment.

Results. Clinical symptoms of pain and claudication were gradually improved. After the treatment, 93.3% (28/30), 86.7% (26/30), and 86.7% (26/30) of patients showed relief of hip pain, improvement of joint function, and extended walking distances, respectively. The Harris scores were increased significantly at 3, 6, and 12 months posttransplant compared with those pretransplant. In addition, the bone lesions in 89.7% of hips (44/49) were improved as showed on computed tomography after transplantation.

Conclusion. Cotransplantation of autologous BMMNCs and allogeneic UC-MSCs showed therapeutic effect on ANFH without severe adverse effects.

BEING A DIFFICULT MEDICAL ISSUE, therapy for avascular necrosis of the femoral head (ANFH) at present is to alleviate the patients' suffering, improve blood supply of the femoral head, retard continuing necrosis, and promote the repair and reconstruction of the necrosis region, ultimately achieving restoration of the anatomy and organizational structure of the femoral head [1]. Previous experimental and clinical studies have shown that transplantation of autologous bone marrow mononuclear cells (BMMNCs) was able to improve the blood flow to ischemic tissues and repair damaged tissues. Several studies used autologous BMMNCs to cure ANFH by direct injection into necrotic area [2–4] and achieved inspiring effects. However, traumatic core decompression was often required and the effects were limited. With the development of tissue engineering, stem cell transplantation becomes a good alternative for tissue repair and an applicable methodology for ANFH [5] owing to its ability to differentiate into osteoblasts, chondrocytes, and muscle and fat cells. Gangji et al [6] showed that stem cell intervention therapy for ANFH had curative effects. To improve effects and reduce trauma, we have introduced a novel method to treat ANFH with cotransplantation of autologous BMMNCs and allogeneic umbilical cord mesenchymal stem cells (UC-MSCs) by arterial intervention.

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METHODS AND MATERIALS

Subjects

This pilot study was conducted at Organ Transplant Center, Fuzhou General Hospital, Xiamen University, from December 2009 to August 2011. We included 30 patients (24 males and 6 females; average age, 41.6 years; range, 19–63), with 12 cases of steroid-induced ANFH, 9 cases of alcoholic, and 9 cases of unknown etiology. All patients were diagnosed with criteria including clinical manifestation such as hip pain and limitation of activity, hip radiography, and computed tomography. There were 19 bilateral and 11 were unilateral cases. The mean history was 1.1 years (range, 4 months to 2 years). According to the Association for Research Circulation Osseous classification [7], there were 24 hips in phase II and 25 in phase III. Patients were divided into subgroup phase II group (n = 15; the patients had only phase II hips) and phase III group (n = 15; each patient had ≥1 phase III hip) for further analysis. Results of conservative treatment (anticoagulants, physical therapy, consultations, and/or symptomatic treatments) for 3 months were ineffective. Informed consent was obtained from all patients. The trial was approved by the local ethics committee. Patients with mild (phase I) or severe (phase IV) ANFH, hip skin lesions, active infectious diseases, coagulation dysfunction, anemia, leukopenia, or malignancy were excluded.

Mononuclear Cell Preparation

Bone marrow (BM) was aspired from both iliac crests under local anesthesia with 2% lidocaine. A minimum of 300 mL and a maximum (target) of 375 mL of BM [8] were mixed with 20,000 U heparin and preserved in the primary bag of a Quadruple Collection Bag (Terumo Medical Products Co. Ltd, Changchun, China). The primary bag was placed upside down and centrifuged (Thermo Scientific, Waltham, MA; Sorvall RC3BP+) at 900×*g* for 20 minutes. The bottom layer red cells were gravitated into the second bag and discarded, the median layer buffy coat was collected in the third bag, and the upper layer plasma and fat were discarded with the primary bag. The buffy coat was washed and resuspended in isotonic normal saline in the third bag, which was about 500 mL in volume. The bag was centrifuged again at 600×*g* for 10 minutes to remove the residual plasma and fat from the buffy coat. After the procedure, BMMNCs were transported for immediate transplantation simultaneously with MSCs.

UC-MSCs Preparation

A piece of human UC (5 cm) from a full-term newborn (blood type O) was harvested at the time of delivery in the Department of Obstetrics and Gynecology, Fuzhou General Hospital. Written consent was obtained from the donor's mother. The mesenchymal tissue (in Wharton's jelly) was diced into cubes of about 0.1 cm^3 and centrifuged at 250×*g* for 5 minutes. After removal of the supernatant fraction, the precipitate (mesenchymal tissue) was washed with serum-free Dulbecco's Modified Eagle Medium (Hyclone, Logan, UT) and centrifuged at 250×*g* for 5 minutes. The mesenchymal tissue was treated with 0.1% collagenase (Sigma, St Louis, MO) at 37°C overnight, washed twice, and further digested with 0.25% trypsin (Gibco, Grand Island, NY) at 37°C for 60 minutes. Fetal bovine serum (Hyclone) was added to the mesenchymal tissue to neutralize the excess trypsin. Cells were plated in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Hyclone), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine (Gibco) at a density of 1×10^6 cells/mL. The medium was renewed every 2 to 3 days and suspended cells were discarded. After reaching 80% to 90% confluence, cells were harvested with 0.25% trypsin and 0.02% EDTA and replated at 1:3 under the same condition. When the number of cells was enough for infusion, confluent MSC in flasks were washed with isotonic normal saline, incubated with medium 199 (Gibco) for 60 minutes, and detached with trypsin-EDTA. MSCs were resuspended in medium 199 + 1% human serum albumin, and cells were cryopreserved using a rate controlled freezer at a final concentration of 10% DMSO (Sigma) and 5% HSA. On the day of infusion cryopreserved units were thawed at the bedside in a 37°C water bath, transferred into 50-mL syringes, and transported to the clinical team for infusion [9]. Cells were phenotypically characterized by flow cytometry and their differentiation potential evaluated following the 2006 International Society of Cellular Therapy's criteria [10]. Bacteria, mycoplasma, and Fungi contamination testing and endotoxin test were performed before each batch of cells was released from the laboratory to the clinical facility for transplantation.

Transplantation Procedures

Patients underwent angiography of the femoral head artery. Digital subtraction angiography was performed using an angiographic system with a 30 × 30-cm flat-panel detector (Innova 3100; GE Healthcare, Port Washington, NY). In all subjects, one of the most predominant vessels of the following 3 arteries: The medial circumflex femoral artery, the lateral circumflex femoral artery, or obturator artery was identified. When the artery was cannulated, 30 to 50 mL of UC-MSCs and 60 to 80 mL of BMMNCs were allowed to infuse in 30 minutes.

Endpoints Assessment

Harris scores were used to evaluate pain relief, joint function, walking distance, and image changes were observed before and at 3, 6, and 12 months after transplantation.

Statistical Methods

All statistical analyses were performed with the use of the software SPSS13.0. Data were presented as mean values ± standard deviation; differences of data before and after treatment were compared with repeated measures analysis of variance. $P < .05$ was considered significant.

RESULTS

The characteristics of osteonecrosis were described in Table 1. All patients successfully underwent arterial

Table 1. Detailed Characteristics of Osteonecrosis in 49 Hips

	Location			Quantitation		
Phase	Medial	Central	Lateral	Minimal <15%	Moderate 15–30%	Extensive >30%
II	25.0% (6/24)	33.3% (8/24)	41.7% (10/24)	25.0% (6/24)	33.3% (8/24)	41.7% (10/24)
III	28.0% (7/25)	36.0% (9/25)	36.0% (9/25)	28.0% (7/25)	36.0% (9/25)	36.0% (9/25)