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Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee osteoarthritis: a prospective study

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SUMMARY

Objective: Cartilage regenerative procedures using the cell-based tissue engineering approach involving mesenchymal stem cells (MSCs) have been receiving increased interest because of their potential for altering the progression of osteoarthritis (OA) by repairing cartilage lesions.
The aim of this study was to investigate the clinical and magnetic resonance imaging (MRI) outcomes of MSC implantation in OA knees and to determine the association between clinical and MRI outcomes.
Design: Twenty patients (24 knees) who underwent arthroscopic MSC implantation for cartilage lesions in their OA knees were evaluated at 2 years after surgery. Clinical outcomes were evaluated according to the International Knee Documentation Committee (IKDC) score and the Tegner activity scale, and cartilage repair was assessed according to the MRI Osteoarthritis Knee Score (MOAKS) and Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score.
Results: The clinical outcomes significantly improved ($P < 0.001$ for both). The cartilage lesion grades (as described in MOAKS [grades for size of cartilage-loss area and percentage of full-thickness cartilage loss]) at follow-up MRI were significantly better than the preoperative values ($P < 0.001$ for both). The clinical outcomes at final follow-up were significantly correlated with the MOAKS and MOCART score at follow-up MRI ($P < 0.05$ for all).
Conclusions: Considering the encouraging clinical and MRI outcomes obtained and the significant correlations noted between the clinical and MRI outcomes, MSC implantation seems to be useful for repairing cartilage lesions in OA knees. However, a larger sample size and long-term studies are needed to confirm our findings.

Introduction

Osteoarthritis (OA) is characterized by degeneration of the articular cartilage and is accompanied by subchondral bone sclerosis and synovial inflammation[1]. Restoration of the diseased articular cartilage in patients with OA is a challenging problem for researchers and clinicians[2]. Recently, some clinical studies involving the use of mesenchymal stem cells (MSCs) as a potential cell-based treatment for OA have been reported[3–9]. MSCs could play a role in cartilage repair by generating new cartilage, releasing factors that stimulate cartilage formation by resident chondrocytes or other cells in the joint, and inhibiting joint inflammation[10].

For MSC-based therapies to emerge as a viable therapeutic alternative, the unique challenges associated with using MSCs to treat patients with OA must be identified and addressed. Therefore, the appropriate delivery of MSCs to the cartilage lesion site is crucial for durable cartilage repair in MSC-based treatment of OA[5]. In a previous recent study, Kim *et al.*[5] performed MSC implantation for cartilage lesions in patients with OA knees and found that the clinical and second-look arthroscopic outcomes of MSC implantation were encouraging. Furthermore, the second-look arthroscopic findings showed that cartilage repair was better in patients who underwent implantation of MSCs loaded in fibrin glue as a scaffold

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than in patients who underwent MSC implantation without a scaffold. However, a significant limitation of their prior research was that, using second-look arthroscopic evaluation, it is difficult to examine the full thickness of repaired cartilage and integration of repaired cartilage with adjacent native cartilage. Moreover, the second-look arthroscopy was performed at 1 year after surgery, and therefore it is not known how the repaired cartilage will behave after the first year. Therefore, we considered that another valid tool for evaluation of repaired cartilage for longer follow-up periods after MSC implantation is needed.

Magnetic resonance imaging (MRI) has rich image contrast variability, thereby providing the ability to discriminate articular tissues; therefore, it holds great potential as a tool for whole-organ imaging of the OA joint[11]. The relevance of MRI for evaluating structural changes during the development and progression of knee OA has been demonstrated[12]. Among several methods[13–16], MRI Osteoarthritis Knee Score (MOAKS), which has been used in several studies[16–18], is advocated to be the tool of choice for semiquantitative analyses of knee OA[17]. Therefore, we used the MOAKS for MRI evaluation of cartilage lesions after MSC implantation in this study. Furthermore, Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scoring system was also used for the evaluation of repaired cartilage.

The aims of this study were (1) to investigate the clinical outcomes of MSC implantation with fibrin glue as a scaffold in patients with OA knees, (2) to assess cartilage regeneration after MSC implantation by using MRI evaluation, and (3) to determine the association between clinical and MRI outcomes.

Materials and methods

Study subjects

In this prospective cohort study, the inclusion criterion was an isolated articular cartilage lesion in OA knees (Kellgren–Lawrence[19] grades 1–2) with symptoms of knee joint pain and/or functional limitations, despite a minimum of 3 months of nonsurgical treatments. Nonsurgical treatment options included rest, physical therapy, and nonsteroidal anti-inflammatory drugs. Intra-articular injections of viscosupplements or steroids were not given in all patients. Patients were excluded if they had a history of surgical treatments, as were patients with multiple cartilage lesions, knee instability, varus or valgus malalignment of 5° or more of the knee joint, metabolic arthritis, joint infections, or large meniscal tears which might result in the mechanical symptom of knees or might be required the surgical treatment. We requested the patients to undergo follow-up MRI for evaluation of the cartilage lesion. From January 2012 to October 2012, 20 consecutive patients (24 knees) with cartilage lesions in the knees underwent arthroscopic MSC implantation with fibrin glue as a scaffold for cartilage regeneration. These patients underwent follow-up MRI at a mean of 24.2 months after surgery (range, 18–29 months). The study population included 11 men and 9 women, with a mean age of 57.9 years (SD, 5.9; range, 48–69). The mean follow-up period was 27.9 months (SD, 3.2; range, 24–34), and the mean preoperative body mass index (BMI) was 26.6 kg/m^2 (SD, 3.2; range, 22.2–31.2). This study was approved by the institutional review board of our hospital, and all patients provided written informed consent prior to treatment.

MSC preparation

Adipose-derived MSCs were isolated as described previously[5,9]. In brief, 1 day before arthroscopic surgery, adipose tissue was harvested from patient buttock. We aimed to collect 140 cc liposuctioned adipose tissue: 120 cc was used for the implantation, and the remaining 20 cc was analyzed to examine the plastic-adherent cells that form fibroblast colony-forming units (CFU–F) and confirm the multilineage differentiation of adipose-derived stem cells. In the operating room, 120 cc adipose tissue was suspended in phosphate-buffered saline (GIBCO BRL, Life Technologies, Carlsbad, CA, USA), placed in a sterile box, and transported to the laboratory. Mature adipocytes and connective tissues were separated from the stromal vascular fraction by centrifugation as described by Zuk *et al.*[20] The remaining 20 cc adipose tissue was processed in the same manner and used for cell analysis.

Epitope profiles and multilineage differentiation were assessed to characterize the MSCs. We investigated the immunophenotype of the adipose-derived stem cells using CD14 (BD Biosciences), CD34 (BD Biosciences), CD90 (BD Biosciences), and CD105 (BD Biosciences) antibodies by flow cytometry (i.e., FACS) analysis, as described previously[21]. After culture expansion using specific inductive media, the adipogenic, osteogenic, and chondrogenic differentiation potentials of adipose-derived stem cells were assessed, as reported previously[21]. Adipogenic induction medium contained 100 nM dexamethasone (Sigma–Aldrich), 0.5 mM isobutyl-methylxanthine (Sigma–Aldrich), and 50 mM indomethacin (Sigma–Aldrich), osteogenic induction medium contained 1 nM dexamethasone, 10 mM b-glycerol phosphate (Sigma–Aldrich), and 50 mg/mL ascorbate-2-phosphate (Sigma–Aldrich), and chondrogenic induction medium contained 10 ng/mL transforming growth factor-β3 (TGF-β3) (Sigma–Aldrich, St. Louis, MO, USA), 1 × Insulin transferrin selenium + premix (Gibco BRL), and 100 nM dexamethasone (Sigma–Aldrich). The capacity of human subcutaneous adipose tissue to generate mesenchymal progenitors was evaluated according to CFU-F. The adipose-derived stem cells represented a mean of 9.0% of the stromal vascular fraction cells (range, 7.6–12.3) after isolation. After the stromal vascular fractions were isolated, a mean of 4.4 × 10^6 stem cells (9.0% of 4.9 × 10^7 stromal vascular fraction cells; range, 3.7–6.0 × 10^6) were prepared. A mean of 4.9 × 10^7 stromal vascular fraction cells, which contained a mean of 4.4 × 10^6 stem cells, were used for MSC implantation. FACS characterization indicated positive expression of the surface markers CD90 (97.83%) and CD105 (95.76%) and negative expression of CD34 (3.52%) and CD14 (2.14%), which represented the characteristics of MSCs. Adipose-derived stem cells treated with conditioned media exhibited adipogenic, osteogenic, and chondrogenic differentiation after staining.

Surgical technique

Before the MSC implantation, accurate debridement of all unstable and damaged cartilage in the lesion was performed [Fig. 1(A)]. We used the fibrin glue product from the commercially available Greenplast kit (Greencross, Seoul, Korea) as a scaffold. The product was administered using two syringes—one contained lyophilized human plasma fibrinogen (71.5–126.5 mg/mL) dissolved in 1 mL of aprotinin solution (1100 kallikrein inhibitor units [KIU/mL]) and the other contained thrombin (4.9–11.1 mg/mL) dissolved in 1 mL of calcium chloride solution (13.9–15.6 mg/mL) in sterile packaging. In general, the fibrin glue product is designed to form a gel instantaneously when the two solutions from each syringe are mixed. First, the cell suspension (stromal vascular fraction cells containing MSCs) was loaded into the thrombin solution in a 1:1 mixture ratio (volume of cell suspension to the volume of thrombin solution). Then, the cell-thrombin suspension was mixed with the fibrinogen solution in a 1:1 ratio by using a Duploject syringe support system (included in the Greenplast kit), which were simultaneously added to each well on the surface of the cartilage lesion. Implantation of this cell thrombin–fibrinogen suspension (i.e., MSCs mixed with the fibrin glue) was performed

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