Intra-articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee

A 2-Year Follow-up Study

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Background: The intra-articular injection of mesenchymal stem cells (MSCs) into the knee has shown a potential for the treatment of generalized cartilage loss in osteoarthritis (OA). However, there have been few midterm reports with clinical and structural outcomes.

Purpose: To assess the midterm safety and efficacy of an intra-articular injection of autologous adipose tissue–derived (AD) MSCs for knee OA at 2-year follow-up.

Study Design: Cohort study; Level of evidence, 3.

Methods: Eighteen patients with OA of the knee were enrolled (3 male, 15 female; mean age, 61.8 ± 6.6 years [range, 52-72 years]). Patients in the low-, medium-, and high-dose groups received an intra-articular injection of 1.0×10^7, 5.0×10^7, and 1.0×10^8 AD MSCs into the knee, respectively. Clinical and structural evaluations were performed with widely used methodologies including the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and measurements of the size and depth of the cartilage defect, signal intensity of regenerated cartilage, and cartilage volume using magnetic resonance imaging (MRI).

Results: There were no treatment-related adverse events during the 2-year period. An intra-articular injection of autologous AD MSCs improved knee function, as measured with the WOMAC, Knee Society clinical rating system (KSS), and Knee injury and Osteoarthritis Outcome Score (KOOS), and reduced knee pain, as measured with the visual analog scale (VAS), for up to 2 years regardless of the cell dosage. However, statistical significance was found mainly in the high-dose group. Clinical outcomes tended to deteriorate after 1 year in the low- and medium-dose groups, whereas those in the high-dose group plateaued until 2 years. The structural outcomes evaluated with MRI also showed similar trends.

Conclusion: This study identified the safety and efficacy of an intra-articular injection of AD MSCs into the OA knee over 2 years, encouraging a larger randomized clinical trial. However, this study also showed potential concerns about the durability of clinical and structural outcomes, suggesting the need for further studies.

Clinical Trial Registration: NCT01300598

Keywords: osteoarthritis; mesenchymal stem cell; intra-articular injection; knee

Osteoarthritis (OA) is a chronic degenerative disease of articular cartilage and is one of the leading causes of disability among noninstitutionalized adults.[13] In the United States, 27 million people have clinical OA, with an associated treatment cost of $185.5 billion per year.[11] Its incidence has doubled in women and tripled in men over the last 20 years.[20] The prevalence and incidence of OA are presumed to accelerate according to the increase in life expectancy and sports activity of the general population as well as the progressive nature of OA.[10] Nonetheless, conventional pharmacological therapies are not effective at preventing the progression of OA, which pushes us to seek alternative treatment options.[15,36] Recent advances in cell therapy with mesenchymal stem cells (MSCs) show potential in the treatment of OA.[3,4,6,9,21,24,26,34,35]

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The delivery of MSCs for the treatment of OA could be achieved via either surgical implantation[23,35] or an intra-articular injection.[4,9] Cartilage lesions in OA are generally observed as multiple, large, and unconfined lesions with various degrees of destruction and are often opposed (or kissing), all of which could be major hurdles or even relative contraindications for successful surgical implantation.[8,9,26] Meanwhile, several recent experimental studies showed the potential of a direct intra-articular injection of MSCs into the knee. MSCs have the capability to home in on and attach to diseased tissue,[1,31,32] participate in the regeneration of articular cartilage,[14,17,28] decrease prostaglandin E2 synovial fluid concentration,[7] and retard the progression of OA experimentally.[2,19] Recently, this strategy was translated into clinical trials for patients with OA and showed promising results.[9,12,24]

In 2014, we reported a proof-of-concept clinical trial of the intra-articular injection of autologous adipose tissue–derived (AD) MSCs for the treatment of knee OA.[9] The study demonstrated the safety and efficacy of an intra-articular injection of 1×10^8 AD MSCs with clinical, radiological, arthroscopic, and histological evidence at 6-month follow-up. Recently, several authors reported studies of an intra-articular injection of MSCs.[12,16,18,25,34] Most of these studies utilized bone marrow–derived MSCs and had a short-term follow-up of 6 or 12 months. The midterm clinical and structural outcomes after an intra-articular injection of autologous AD MSCs are currently unknown.

Therefore, the purpose of this study was to evaluate the clinical and structural midterm results of an intra-articular injection of autologous AD MSCs in patients with severe knee OA using the same evaluation methods of the original study.[9]

METHODS

Patient Data and Follow-up

This study is a follow-up study of a phase I/II clinical trial of an intra-articular injection of autologous AD MSCs for the treatment of 18 patients with severe OA of the knee (NCT01300598).[9] The study was approved by the institutional review board of our institute. The inclusion and exclusion criteria are listed in the Appendix (available in the online version of this article). The phase I study consisted of 3 dose-escalation cohorts, low (1.0×10^7 cells), medium (5.0×10^7 cells), and high dose (1.0×10^8 cells), with 3 patients in each cohort. Details of the dose-escalation method are described in the Appendix. The phase II study included 9 patients receiving the high dose. Analysis was performed according to the level of cell doses (low, medium, and high dose) and according to the intention-to-treat principle in clinical and structural outcomes. The baseline characteristics of the patients are listed in the Appendix.

In the original trial, patients were followed up at 1, 2, 3, and 6 months after the injection. After the 6-month follow-up, patients were asked to visit at 1 year and 2 years after the injection for a midterm safety and efficacy evaluation of an injection of AD MSCs. The follow-up was conducted using the same clinical and structural evaluation methodology that was used in the original trial (Figure 1).

Preparation and Injection of AD MSCs

AD MSCs were isolated from abdominal subcutaneous fat by liposuction and cultured under current good manufacturing practice (GMP) conditions as previously described. Cells were tested for cell number, viability, purity (CD31, CD34, and CD45), identity (CD73 and CD90), sterility, endotoxin, and mycoplasma before being injected.

An arthroscopic examination of the patient was performed under spinal anesthesia. After fluid was expressed from the knee joint before the injection, AD MSCs in 3 mL of saline were injected into the knee joint through the medial portal using a 22-gauge spinal needle. Debridement, synovectomy, and meniscectomy were not performed during arthroscopic surgery, and no drainage was conducted.

Physical exercise emphasizing range of motion and quadriceps strengthening was advised before surgery and started on the first day after the injection. Nonweightbearing was recommended for 2 months after the injection, and the use of crutches was encouraged during this period. A stepwise increase in load bearing was encouraged over 1 month, and full weightbearing was achieved by 3 months after the injection.

Clinical Outcomes

Clinical outcomes included the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC),

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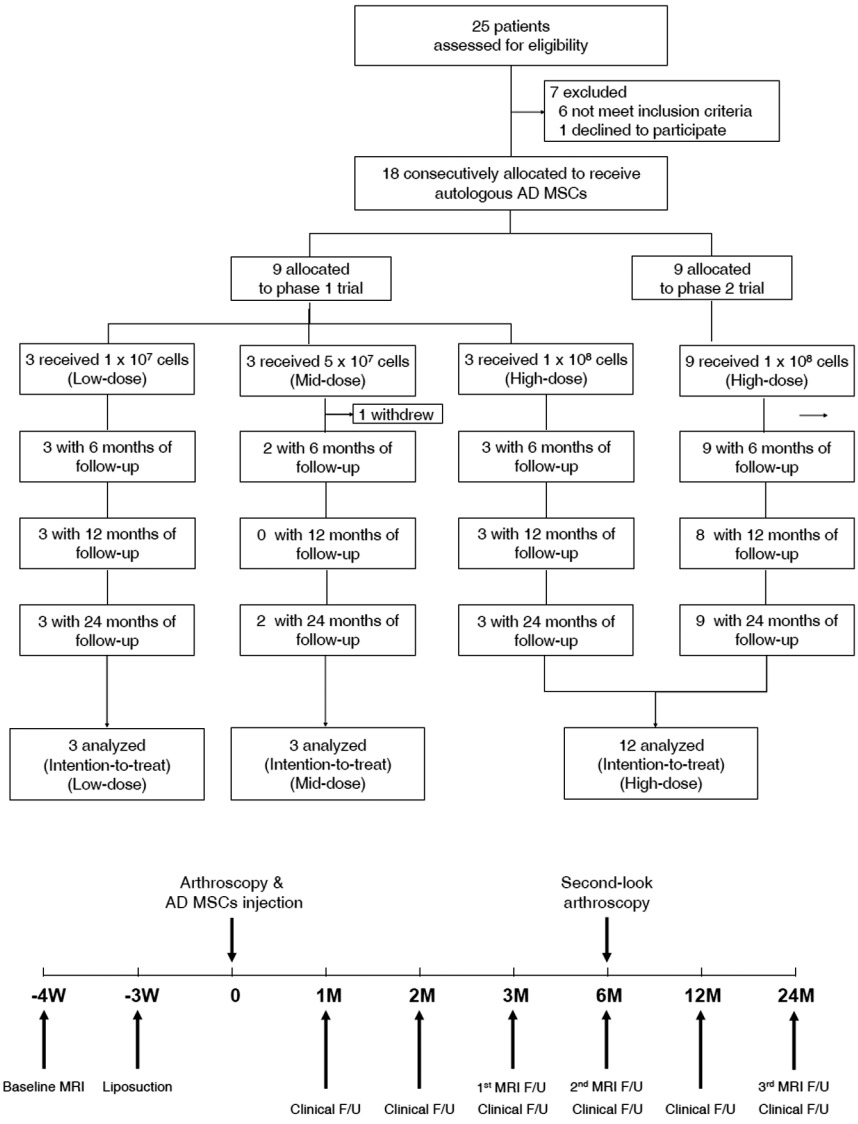

Figure 1. Study flow diagram. AD MSCs, adipose tissue–derived mesenchymal stem cells; F/U, follow-up; MRI, magnetic resonance imaging.

visual analog scale (VAS) for pain (0-10), and Knee Society clinical rating system (KSS) subscales of knee and function. In addition, we included the Knee injury and Osteoarthritis Outcome Score (KOOS) in this study, which had not been part of the original protocol but was conducted as a routine follow-up procedure. The failure of an injection of AD MSCs was defined by the following: (1) any kind of surgery including knee arthroscopic surgery and arthroplasty, and (2) the return of the primary functional outcome, the WOMAC, to its baseline value or below once past 6 months after the injection.

Structural Outcomes

Structural outcomes included the Kellgren-Lawrence grade, joint space width of the medial compartment, mechanical axis using the weightbearing line, and anatomic axis using weightbearing radiography. The size and depth of the cartilage defect and signal intensity of regenerated cartilage were also measured using magnetic resonance imaging (MRI) by a blinded musculoskeletal radiologist as previously described (see the Appendix).[9] Changes in the cartilage volume of the knee joint were measured using a semiautomated segmentation method by a blinded researcher (see the Appendix).

Statistical Analyses

Missing data were replaced with multiple imputation (10 sets) using the fully conditional specification method under a missing-at-random assumption.[33] Ten imputed datasets were generated, analyzed separately for each outcome measure, and then combined into a single set of estimates according to Rubin's[27] rules to incorporate the between-

imputation variability. For sensitivity testing, single imputation using the last observation carried forward method and complete case analysis were additionally performed. Because all 3 methods did not yield meaningful changes in each measurement, we presented only the imputation analyses. Changes from baseline of scale variables were determined by a paired *t* test. The Kellgren-Lawrence grade and depth of the cartilage defect measured by MRI were determined with a Wilcoxon signed-rank test. Three group comparisons at each follow-up time point were conducted with analysis of variance or analysis of covariance after adjusting the baseline outcome value. Analyses were performed using SAS version 9.4 (SAS Institute Inc) and R version 3.3.1 (R Foundation for Statistical Computing). Multiple outcomes were tested without adjusting for type I error rates. For all tests, the significance level was set at $P \leq .05$.

RESULTS

Follow-up of Patients

The patients in each group had similar baseline characteristics (see the Appendix). There were 15 female and 3 male patients, with a mean age of 61.8 ± 6.6 years (range, 52-72 years), with a mean body mass index of approximately 26 kg/m^2 (range, 22-30 kg/m^2) and a Kellgren-Lawrence grade of 3 (n = 12) or 4 (n = 6). Patients were symptomatic for more than 5 years and participated in a sporting activity from time to time but were restricted because of the knee joint before the injection. The mean size of the defect in the medial femoral condyle with MRI was approximately 400 to 500 mm^2.

Fourteen and 17 of the 18 patients who participated in the previous study were followed up at 1 and 2 years, respectively. Three patients in the medium-dose group and 1 patient in the high-dose group were not available for follow-up at 1 year. One patient in the medium-dose group was also unavailable at the 2-year follow-up. None of the patients underwent any kind of knee surgery including arthroscopic surgery and arthroplasty at the 2-year follow-up.

Clinical Outcomes

There were no clinically important adverse events according to physical examinations, vital signs, or laboratory tests during the follow-up of 2 years. The WOMAC scores in the high-dose group significantly decreased by 39.4%, 70.0%, and 64.9% at 6 months, 1 year, and 2 years, respectively, compared with baseline (Table 1). However, no further improvement was noted after 1 year (Figure 2A). Patients in the low- and medium-dose groups showed similar trends over 2 years, but these had no statistical significance.

The VAS score for pain in the high-dose group significantly decreased by 44.5%, 57.4%, and 42.5% at 6 months, 1 year, and 2 years, respectively, compared with baseline (Table 1). Again, no further improvement was observed after 1 year (Figure 2B). The VAS score in the low- and medium-dose groups exhibited similar patterns during the 2 years after the injection. However, these were without statistical significance, except for a 42.9% decrease in the low-dose group at the 2-year follow-up (Figure 2B).

The knee subscore of the KSS at 6 months, 1 year, and 2 years significantly increased by 91.3%, 117.9%, and 71.9%, respectively, from the baseline values for the low-dose group and increased by 50.4%, 78.6%, and 68.0%, respectively, for the high-dose group (Table 1). The function subscore of the KSS at 6 months, 1 year, and 2 years also increased by 38.8%, 50.0%, and 44.5%, respectively, from the baseline values for the low-dose group and increased by 9.5%, 18.5%, and 17.7%, respectively, for the high-dose group (Table 1). Both scores reached a plateau at the 1-year follow-up and then appeared to be maintained or decreased in both groups (Figure 2, C and D).

The pain, symptoms, and activities of daily living subscores of the KOOS in the high-dose group continued to increase until 1 year after the injection compared with baseline and then slightly decreased at 2 years but still significantly improved by 79.3%, 50.3%, and 95.4%, respectively (Table 1 and Figure 2, E-G). The sports subscore of the KOOS improved until 2 years for the high-dose group (Figure 2H). In the low-dose group, the symptoms subscore of the KOOS improved at 2 years, and the activities of daily living subscore of the KOOS improved at 1 year and 2 years. No statistically significant improvements were found in the quality of life subscore of the KOOS for any of the dose groups (Figure 2I).

There was no failure of an injection of AD MSCs during 2 years of follow-up.

Structural Outcomes

For all of the dose groups, there was no significant change in the Kellgren-Lawrence grade, joint space width, mechanical axis, or anatomic axis over 2 years (see the Appendix). Nonetheless, for some patients in the high-dose group, narrowing of the medial compartment was improved at 6 months, and this remained relatively stable over the next one and a half years (Figure 3, A and B).

Serial MRI examinations demonstrated a gradual regeneration of articular cartilage in the medial femoral and tibial condyles during the first 6 months, while signs of destruction of the regenerated cartilage were observed at 2 years (Figure 4A).

Cartilage defects in the medial femoral condyle (green arrows) and in the medial tibial condyle (yellow arrows) were identified as signal voids between the 2 condyles. In the low-dose group, no significant changes were observed after the injection until 2 years. In the medium-dose group, thin and irregular regenerated cartilage appeared at 3 months and remained without any significant change at 6 months. However, most of the regenerated cartilage disappeared at 2 years. In the high-dose group, regenerated articular cartilage could be found in both the medial femoral and tibial condyles at 3 months. They were still thin but relatively smooth compared with those in the medium-dose group. At 6 months, the regenerated cartilage became thicker, smoother, and mature with isointensity with surrounding cartilage in both the condyles. At 2 years, the regenerated cartilage appeared partially destroyed, especially in the medial tibial condyle.

TABLE 1
Changes in the WOMAC, VAS, KSS, and KOOS Scores between Baseline and Post Injection Time Points[a]

	Low-Dose Group (n = 3)	*P* Value	Medium-Dose Group (n = 3)	*P* Value	High-Dose Group (n = 12)	*P* Value
WOMAC						
Baseline	43.3 ± 12.7		69.0 ± 5.9		54.2 ± 5.2	
6 mo	25.3 ± 19.5	.339	48.5 ± 9.5	.339	32.8 ± 6.3	.003
1 y	14.7 ± 12.7	.124	13.1 ± 10.0	.208	16.0 ± 4.4	<.001
2 y	17.0 ± 9.8	.083	25.1 ± 11.0	.210	19.0 ± 5.5	<.001
Pain subscale of WOMAC						
Baseline	8.7 ± 1.5		12.0 ± 1.7		10.7 ± 1.1	
6 mo	4.7 ± 3.7	.383	10.2 ± 2.4	.610	5.8 ± 1.1	.002
1 y	2.7 ± 2.2	.122	3.5 ± 3.2	.317	3.1 ± 1.1	<.001
2 y	3.0 ± 2.5	.161	5.1 ± 2.2	.258	3.7 ± 1.1	.001
Stiffness subscale of WOMAC						
Baseline	3.3 ± 1.3		6.7 ± 0.7		4.8 ± 0.6	
6 mo	2.0 ± 1.2	.529	4.3 ± 1.1	.378	2.6 ± 0.6	.007
1 y	0.7 ± 0.7	.270	1.3 ± 1.2	.227	1.6 ± 0.4	<.001
2 y	1.7 ± 0.9	.370	1.9 ± 0.8	.186	1.9 ± 0.5	.001
Physical function subscale of WOMAC						
Baseline	31.3 ± 11.1		50.3 ± 4.4		38.8 ± 3.9	
6 mo	18.7 ± 14.7	.315	35.3 ± 7.7	.399	24.4 ± 4.8	.006
1 y	11.3 ± 9.8	.124	12.8 ± 6.7	.183	11.2 ± 3.0	<.001
2 y	12.3 ± 6.6	.093	15.3 ± 7.3	.163	13.4 ± 4.1	<.001
VAS						
Baseline	70.0 ± 10.0		78.3 ± 1.7		79.6 ± 2.2	
6 mo	48.3 ± 14.8	.069	67.0 ± 15.8	.610	44.2 ± 6.3	<.001
1 y	33.3 ± 14.5	.053	46.0 ± 19.1	.500	33.3 ± 7.8	<.001
2 y	40.0 ± 15.3	.035	66.0 ± 14.7	.601	45.8 ± 8.1	.002
Knee subscale of KSS						
Baseline	41.3 ± 6.8		35.3 ± 9.8		47.2 ± 2.6	
6 mo	79.0 ± 12.5	.025	47.4 ± 6.6	.307	71.0 ± 4.4	<.001
1 y	90.0 ± 10.0	.005	82.9 ± 12.4	.232	84.3 ± 4.5	<.001
2 y	71.0 ± 12.1	.031	70.8 ± 12.8	.241	79.3 ± 4.7	<.001
Function subscale of KSS						
Baseline	60.0 ± 5.8		56.7 ± 6.7		70.8 ± 2.6	
6 mo	83.3 ± 8.8	.020	70.0 ± 6.6	.236	77.5 ± 2.5	.120
1 y	90.0 ± 10.0	.035	84.3 ± 12.9	.434	83.9 ± 4.1	.034
2 y	86.7 ± 3.3	.015	73.3 ± 11.3	.439	83.3 ± 3.8	.026
Pain subscale of KOOS						
Baseline	49.1 ± 4.0		30.6 ± 12.1		42.6 ± 4.2	
6 mo	66.7 ± 21.0	.423	53.3 ± 12.0	.249	63.4 ± 5.2	.001
1 y	82.4 ± 12.0	.059	77.4 ± 16.2	.319	78.4 ± 5.1	<.001
2 y	69.4 ± 12.7	.148	61.0 ± 9.9	.220	76.4 ± 5.4	<.001
Symptoms subscale of KOOS						
Baseline	61.9 ± 7.2		39.3 ± 16.4		48.5 ± 5.3	
6 mo	73.8 ± 13.7	.346	57.9 ± 8.8	.303	64.9 ± 5.0	.030
1 y	91.7 ± 8.3	.107	80.1 ± 12.2	.332	77.8 ± 5.2	<.001
2 y	72.6 ± 5.2	.035	76.9 ± 10.5	.214	72.9 ± 5.2	.003
Activities of daily living subscale of KOOS						
Baseline	58.8 ± 10.0		22.5 ± 6.0		41.1 ± 5.1	
6 mo	72.5 ± 21.6	.374	50.3 ± 11.2	.325	64.0 ± 7.0	.001
1 y	83.3 ± 14.5	.047	87.4 ± 10.2	.165	84.2 ± 4.4	<.001
2 y	81.9 ± 9.7	.001	73.1 ± 12.7	.237	80.3 ± 6.0	<.001
Sports subscale of KOOS						
Baseline	23.3 ± 10.1		5.0 ± 2.9		7.9 ± 2.9	
6 mo	33.3 ± 16.7	.368	14.7 ± 10.2	.559	20.8 ± 5.9	.050
1 y	43.3 ± 22.4	.339	9.5 ± 12.2	.796	27.3 ± 7.8	.022
2 y	21.7 ± 10.9	.808	26.8 ± 14.5	.354	30.0 ± 5.4	<.001
Quality of life subscale of KOOS						
Baseline	29.2 ± 13.7		20.8 ± 2.1		28.6 ± 3.6	
6 mo	41.7 ± 14.6	.225	35.2 ± 9.6	.357	31.8 ± 4.0	.477
1 y	43.8 ± 6.3	.192	39.0 ± 5.5	.277	36.7 ± 2.6	.174
2 y	54.2 ± 11.0	.057	41.5 ± 6.5	.181	33.9 ± 3.0	.312

[a]Data are presented as mean ± standard error. KOOS, Knee injury and Osteoarthritis Outcome Score; KSS, Knee Society clinical rating system; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

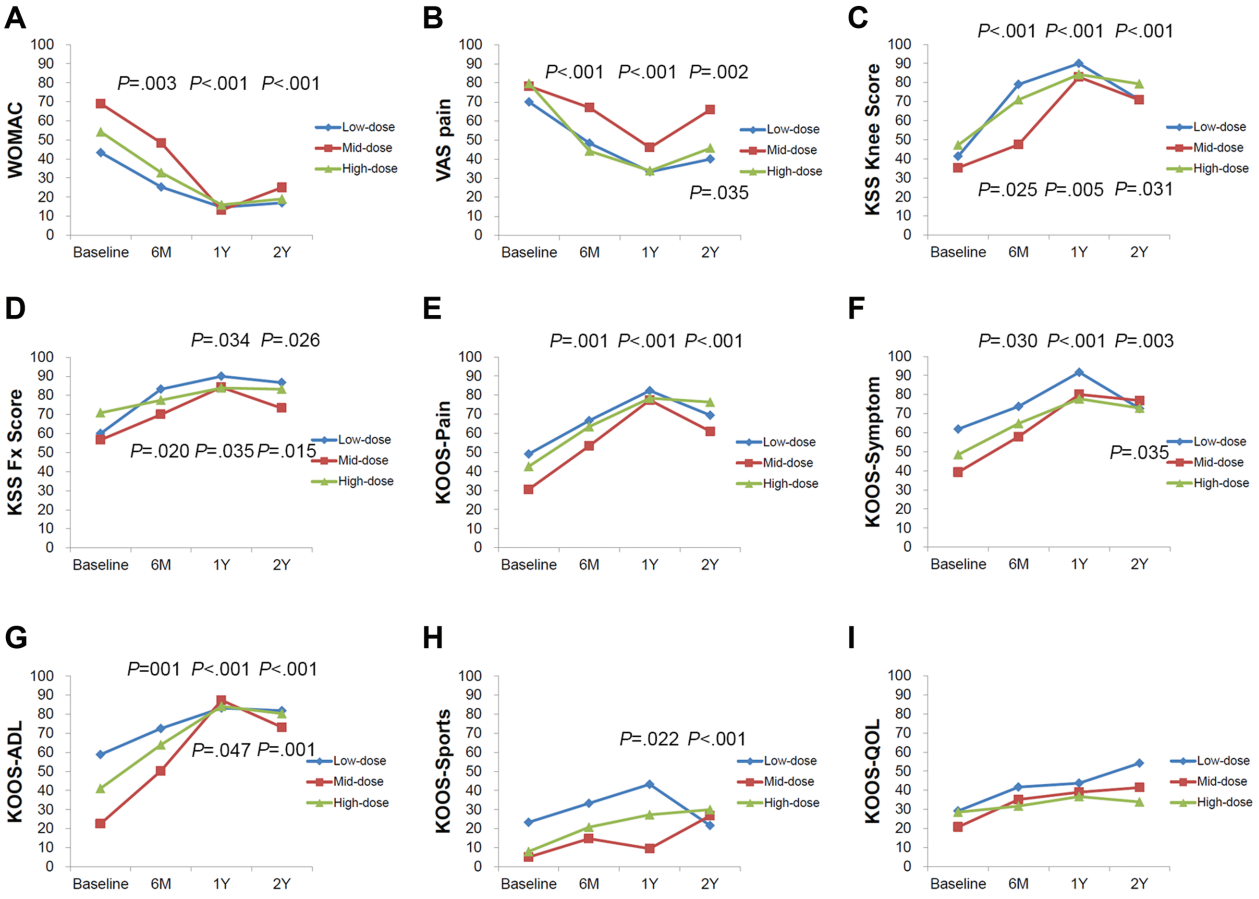

Figure 2. Changes in the (A) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC); (B) visual analog scale (VAS) for pain; Knee Society clinical rating system (KSS) subscales of (C) knee and (D) function; and (E) pain, (F) symptoms, (G) activities of daily living (ADL), (H) sports, and (I) quality of life (QOL) subscales of the Knee injury and Osteoarthritis Outcome Score (KOOS) after an intra-articular injection of adipose tissue–derived (AD) mesenchymal stem cells (MSCs). *P* values above the graph are for the high-dose group, and those below are for the low-dose group. Data up to 6 months are reprinted with permission from Jo et al.[9]

In the high-dose group, the size of the cartilage defect measured with MRI decreased from baseline in all 5 compartments of the knee over 2 years (Table 2 and Figure 4A). However, a significant size reduction was found in the medial femoral, medial tibial, lateral femoral, and lateral tibial condyles at 6 months and in the medial femoral (49.4% decrease) and lateral tibial condyles (64.4% decrease) at 2 years. There were no significant changes found in the other dose groups.

There were no significant changes in the depth of the cartilage defect and signal intensity of regenerated cartilage until 2 years after the injection (see the Appendix). The cartilage volume of the high-dose group significantly increased from baseline in both the medial femoral and tibial condyles at 6 months (9.7% increase and 13.9% increase, respectively) but remained stable without a significant difference from baseline at 2 years (see the Appendix).

DISCUSSION

This study is a midterm follow-up report of our previous study, a first-in-human clinical trial with autologous AD MSCs for the treatment of knee OA. The trial was conducted between March 2009 and September 2011, with AD MSCs manufactured in current GMP conditions under the regulation of the Ministry of Food and Drug Safety of Korea. The AD MSCs showed CD markers specific for MSCs: positive for CD73 and CD90 and negative for CD31, CD34, and CD45. The most important findings of this follow-up study are the following: (1) An intra-articular injection of autologous AD MSCs for OA was not associated with apparent adverse events including, but not limited to, tumor formation and ectopic cartilage or bone formation. (2) It improved knee function, as measured with the WOMAC, KSS, and KOOS, and reduced knee pain as measured with the VAS for up to 2 years regardless of the cell dosage; however, statistical significance was found mainly in the high-dose group. (3) Clinical outcomes tended to deteriorate after 1 year in the low- and medium-dose groups, whereas those in the high-dose group plateaued until 2 years. (4) The structural outcomes evaluated with MRI also showed similar trends. The WOMAC scores of the high-dose group significantly declined at 6 months (39.4% decrease), declined at 1 year (70.0% decrease), and plateaued at 2 years (64.9%

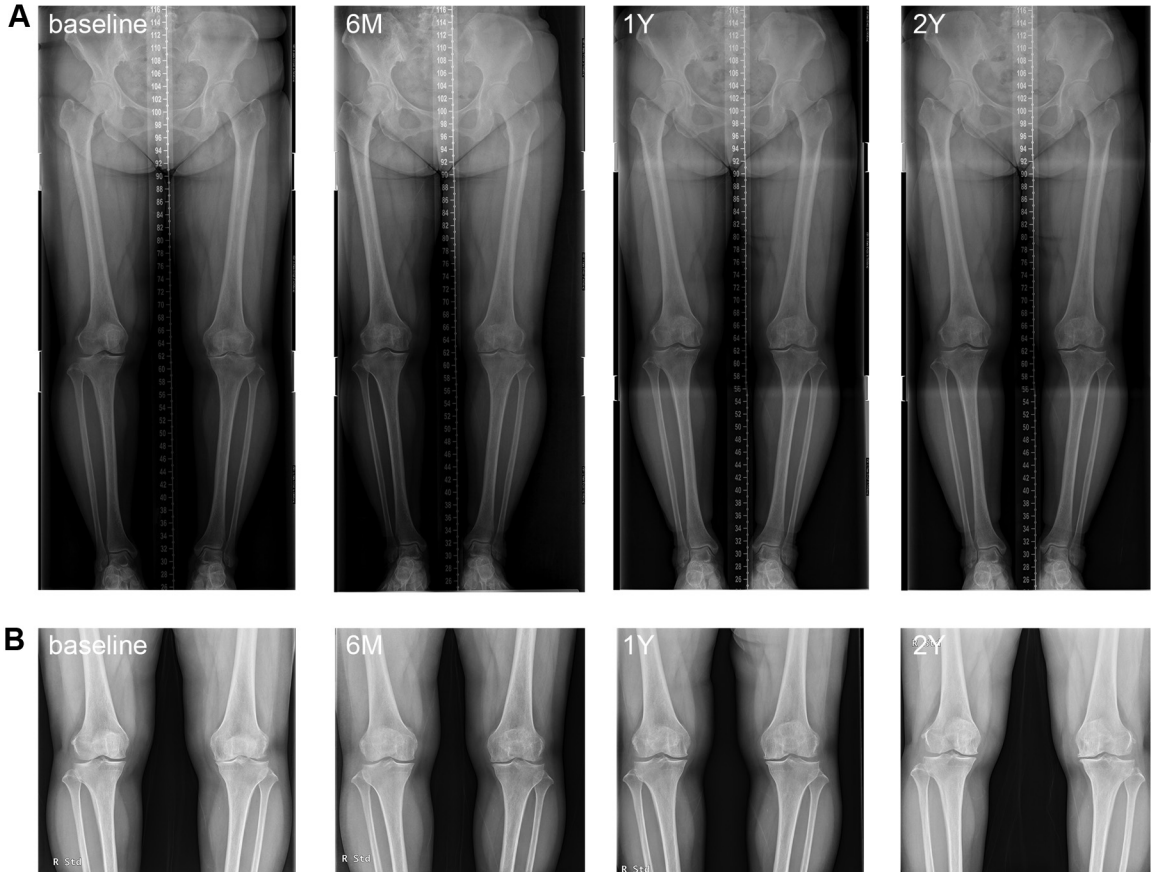

Figure 3. Changes in the appearance of the knee after an intra-articular injection of adipose tissue–derived (AD) mesenchymal stem cells (MSCs) (A) on standing teleradiography of the lower extremity and (B) on standing anteroposterior radiography of the knee. The Kellgren-Lawrence grade was determined as 3 at baseline and did not change over 2 years. The joint space width measured 2.6 mm at baseline and 3.5 mm, 1.8 mm, and 2.2 mm at 6 months, 1 year, and 2 years, respectively. The mechanical axis was 19.9% at baseline and 22.2%, 19.1%, and 20.1% at 6 months, 1 year, and 2 years, respectively. The anatomic axis was –1.7° at baseline and –2.1°, –0.5°, and –0.6° at 6 months, 1 year, and 2 years, respectively. All the measures were not significantly different between the time points.

decrease). Patients in the low- and medium-dose groups showed similar trends over 2 years, but these were without statistical significance. Structural outcomes evaluated with MRI at 2 years were in accordance with clinical outcomes. In the high-dose group, the size of the cartilage defect decreased in all 5 compartments of the knee, while statistically significant results were found in the medial femoral (49.4% decrease) and lateral tibial condyles (64.4% decrease). Patients in the low-dose group showed improvement in some clinical outcomes including the VAS, KSS, and symptoms and activities of daily living subscales of the KOOS. However, patients in the medium-dose group did not show improvement in most clinical and structural outcome measures. These results at 2 years are consistent with those of our previous report at 6 months in which clinical and structural outcomes improved in all 3 groups, whereas significance was mostly found in the high-dose group. These results suggest that outcomes could be closely related to the number of injected AD MSCs and that the direct regeneration of articular cartilage by injected cells might outweigh paracrine effects, although both mechanisms would concurrently work.

Another important finding of the study is that it demonstrated solid structural as well as clinical evidence with respect to the durability of regenerated articular cartilage after an intra-articular injection of AD MSCs. There have been few follow-up studies that could provide information about durability. Davatchi et al[4] reported an original study of an intra-articular injection of autologous bone marrow–derived MSCs in 4 patients with knee OA in 2011. In 2016, they reported a 5-year follow-up study with 3 patients.[5] In this study, the beneficial effect of an injection of MSCs started to decline after 6 months. However, clinical outcomes were still better at 5 years compared with baseline, and there was no patient who underwent knee arthroplasty. Orozco et al[21,22] reported another study of an intra-articular injection of autologous bone marrow–derived MSCs for 12 patients with knee OA in 2013 and then reported a 2-year follow-up study in 2014. They described that the improvement of the algofunctional indexes at 1 year was maintained during the second year and that the quality of cartilage measured by T2 relaxation on MRI was further improved at 2 years. More recently, they reported a 4-year follow-up study for 15 patients in

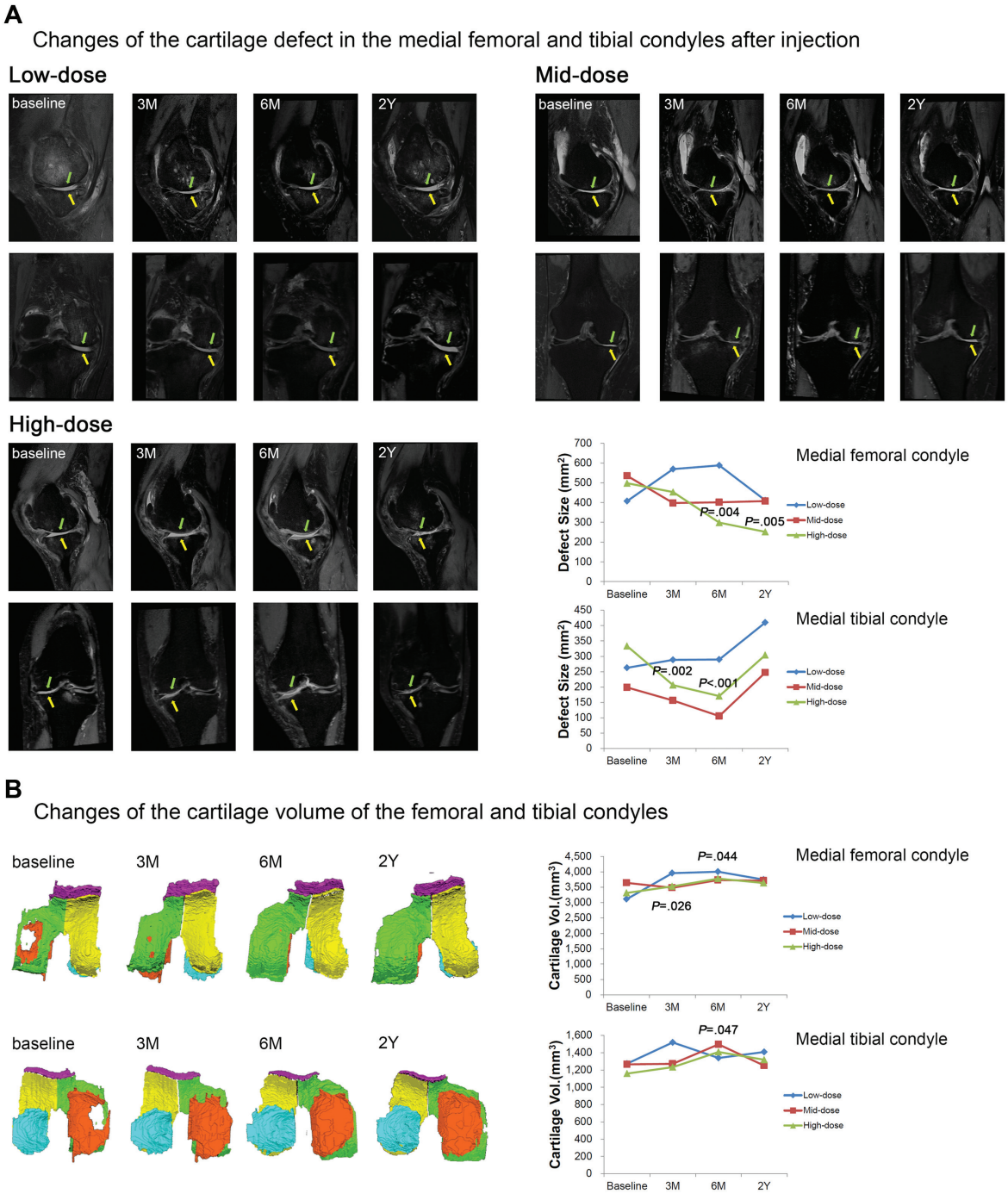

Figure 4. Changes in the articular cartilage defect and regeneration in the medial and femoral condyles on magnetic resonance imaging (MRI) after an intra-articular injection of adipose tissue–derived (AD) mesenchymal stem cells (MSCs). (A) Sagittal and coronal MRI scans of the medial femoral and tibial condyles before and 3 months, 6 months, and 2 years after an injection of AD MSCs. The cartilage defect significantly decreased both in the medial femoral and tibial condyles at 6 months in the high-dose group because of regenerated cartilage. However, at 2 years, the cartilage defect in the medial femoral condyle remained stable, whereas that in the medial tibial condyle increased again probably because of the destruction of regenerated cartilage. (B) Changes in the articular cartilage volume before and 3 months, 6 months, and 2 years after an injection of AD MSCs in the medial femoral condyle (green; right knee, caudal view) and in the medial tibial condyle (orange; right knee, cephalad view) in the high-dose group. The void seen at baseline before the injection was gradually filled over the first 6 months. While grossly no significant changes were observed, the cartilage volume decreased both in the medial femoral and tibial condyles at 2 years. *P* values above the graph are for the high-dose group, and those below are for the low-dose group. Data up to 6 months are reprinted with permission from Jo et al.[9]

TABLE 2
Changes in Cartilage Defect Size Measured on Magnetic Resonance Imaging between Baseline and Post Injection Time Points

	Low-Dose Group (n = 3)	*P* Value	Medium-Dose Group (n = 3)	*P* Value	High-Dose Group (n = 12)	*P* Value
Medial femoral condyle, mm^2						
Baseline	407.0 ± 100.5		535.0 ± 18.0		497.9 ± 29.7	
6 mo	587.8 ± 304.9	.517	400.9 ± 99.0	.420	297.9 ± 51.2	.004
2 y	411.0 ± 200.9	.977	407.8 ± 166.6	.617	252.1 ± 66.5	.005
Medial tibial condyle, mm^2						
Baseline	262.7 ± 136.9		198.8 ± 49.5		333.2 ± 51.2	
6 mo	289.7 ± 152.5	.252	105.6 ± 40.2	.121	170.6 ± 48.2	<.001
2 y	409.9 ± 155.6	.058	247.2 ± 108.7	.735	303.8 ± 85.3	.478
Lateral femoral condyle, mm^2						
Baseline	45.4 ± 45.4		25.6 ± 19.6		103.6 ± 27.1	
6 mo	36.1 ± 36.1	.423	19.1 ± 10.0	.808	51.1 ± 24.9	.011
2 y	107.0 ± 55.1	.151	14.5 ± 6.3	.690	59.4 ± 36.1	.061
Lateral tibial condyle, mm^2						
Baseline	12.0 ± 12.0		3.6 ± 1.9		19.4 ± 7.3	
6 mo	77.0 ± 77.0	.423	11.3 ± 7.0	.523	10.4 ± 4.1	.041
2 y	65.0 ± 43.9	.238	8.1 ± 4.4	.538	6.9 ± 3.6	.037
Patella, mm^2						
Baseline	18.1 ± 18.1		12.3 ± 6.6		93.3 ± 33.3	
6 mo	18.1 ± 18.1	.423	12.1 ± 13.4	.992	79.1 ± 27.5	.340
2 y	117.5 ± 88.8	.297	19.4 ± 10.6	.678	74.1 ± 32.7	.236

[a]Data are presented as mean ± standard error.

2016.[30] This study had only one clinical measure, the VAS for pain, which revealed a further reduction at 4 years. There was 1 patient who underwent knee arthroplasty during the 4 years.

Differences between the results of the above 2 groups may arise from the small number of patients and outcome measures, relatively less systematic and inconsistent evaluation methodology, and different follow-up periods. For the avoidance of these errors, we used the same measures that were used in the original study, as we believed that this would enhance the validity of the follow-up study. Our results showed that most clinical outcomes appeared to either plateau or deteriorate after 1 year; that is, the outcomes of the low- and medium-dose groups tended to deteriorate after 1 year, while those of the high-dose group tended to plateau after 1 year until 2 years. The structural outcomes evaluated with MRI also showed similar trends, as most measures did not improve further but rather plateaued or began to deteriorate after 6 months. The most notable deterioration was found in the medial tibial condyle, in which the size of the cartilage defect increased by 78.1% at 2 years compared with that at 6 months (P = .040) (Figure 4B). However, we are not aware of the exact mechanism of the deterioration. The quality of regenerated cartilage, concomitant injuries around the joint (eg, in the synovium, bone, ligaments, fibrocartilagenous structures, and musculature as a "whole joint" disease), and inappropriate patient selection might all contribute to these uncertainties.[26,29] Therefore, the results suggest that, although regenerated cartilage after an intra-articular injection of AD MSCs appeared to be both clinically and structurally durable until 2 years, concurrent or subsequent treatment strategies need to be considered—arthroscopic surgery for the management of intra-articular injuries, osteotomy for knee alignment, procedures for the enhancement of MSC engraftment such as hyaluronic acid and platelet-rich plasma injections, optimized postinjection rehabilitation, and possibly repeated injections of AD MSCs—in addition to further basic studies for obtaining and processing MSCs of superior quality for cartilage regeneration.

There are some limitations of the study. First, the number of participants was small, and no control was included in the study. A larger randomized controlled trial would be necessary before clinical application. Second, no patient in the medium-dose group visited for a checkup at 1 year, which could affect statistical procedures for managing missing data. Third, there were no structural outcomes with MRI at 1 year. Otherwise, these could provide more valuable information about the durability of regenerated cartilage. Fourth, although a variety of clinical and structural outcome measures were used, they might not be specific enough for evaluating patients after an intra-articular injection of AD MSCs. Fifth, while OA has been known as a whole joint disease, the intra-articular injection of AD MSCs seemed to treat cartilage loss mainly without affecting alignment and other structures. Sixth, the exact mechanism of action of the injection of AD MSCs was not clarified. While there was a strong relationship of dose-dependency, we are still not sure how the regenerated cartilage was made. A clinically applicable nontoxic technique for MSC tracking would be necessary. Seventh, arthroscopic exploration with lavage might be a potential confounding factor. It could influence outcomes in positive ways or prime the joint so that the joint is more responsive to cell injections. Finally, the optimal period of nonweightbearing after an

injection was not studied. However, as a proof-of-concept study, we chose a conservative protocol and focused on the regeneration of articular cartilage.

In summary, we demonstrated the continued safety and promising efficacy of an intra-articular injection of AD MSCs into the OA knee over 2 years, encouraging a larger randomized clinical trial. However, this study also showed potential concerns about the durability of clinical and structural outcomes with current protocols both before and after the injection, suggesting further studies.

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