Clinical Efficacy and Safety of the Intra-articular Injection of Autologous Adipose-Derived Mesenchymal Stem Cells for Knee Osteoarthritis

A Phase III, Randomized, Double-Blind, Placebo-Controlled Trial

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Background: Intra-articular injection of autologous culture-expanded adipose-derived mesenchymal stem cells (ADMSCs) has introduced a promising treatment option for knee osteoarthritis. Although the clinical efficacy and safety of ADMSCs have been reported, the treatment remains controversial owing to the small sample sizes and heterogeneous osteoarthritis grades in previous studies.

Purpose: To assess the efficacy and safety of intra-articular injection of ADMSCs as compared with placebo in alleviating pain and improving functional capacity in a large sample of patients with knee osteoarthritis of Kellgren-Lawrence (K-L) grade 3.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: This phase III multicenter clinical trial was a double-blind randomized controlled study that included 261 patients with K-L grade 3 symptomatic knee osteoarthritis who were administered a single injection of autologous culture-expanded ADMSCs or placebo. Clinical data were assessed at baseline and at 3 and 6 months after the injection. The primary endpoints were improvements in 100-mm visual analog scale (VAS) for pain and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) for function at 6 months after the injection. The secondary endpoints included clinical and radiologic examinations and safety after injection. The changes in cartilage defects after injection were assessed by magnetic resonance imaging at 6 months.

Results: The ADMSC and control groups included 125 and 127 patients available for follow-up, respectively. At 6 months, the ADMSC group showed significantly better improvements in 100-mm VAS (ADMSC vs control, 25.2 vs 15.5; $P = .004$) and total WOMAC score (21.7 vs 14.3; $P = .002$) as compared with the control group. The linear mixed model analysis indicated significantly better improvements in all clinical outcomes in the ADMSC group after 6 months. At 6 months, the ADMSC group achieved significantly higher proportions of patients above the minimal clinically important difference in 100-mm VAS and WOMAC score. Radiologic outcomes and adverse events did not demonstrate significant differences between the groups. No serious treatment-related adverse events were observed. Magnetic resonance imaging revealed no significant difference in change of cartilage defects between the groups at 6 months.

Conclusion: Intra-articular injection of autologous culture-expanded ADMSCs provided significant pain relief and functional improvements in patients with K-L grade 3 osteoarthritis. Long-term results are needed to determine the disease-modifying effects of ADMSCs, such as structural changes, and the duration of effect of intra-articular injection of ADMSCs in knee osteoarthritis.

Registration: NCT03990805 (ClinicalTrials.gov identifier).

Keywords: osteoarthritis; mesenchymal stem cell; intra-articular injection; knee; adipose tissue; culture expansion

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Recent advancements in the study of osteoarthritis have brought a new perspective of its pathophysiology, in which inflammation, oxidative stress, growth factor, and the

cytokine-mediated signaling pathway have been shown to play a crucial role in its progression as the balance of anabolic and catabolic activities is compromised.[5,51] In this regard, the suppression of the inflammatory process in the knee joint has gained attention. Moreover, mesenchymal stem cell (MSC)–based therapy has recently attracted interest as an effective disease-modifying treatment for knee osteoarthritis, given its promising effects.[28,31] The paracrine effects of MSCs include chondroprotective, anti-inflammatory, and immune-modulatory effects via the secretion of cytokines and exosomes by the signaling of MSCs, which may skew the biochemical environment of osteoarthritis into regenerative and anti-inflammatory conditions.[11,23,34,38,40] Given the advantages of easy accessibility and abundance, adipose-derived MSCs (ADMSCs) have become an attractive option.[1,31,51] In experimental models, ADMSC-based therapies have shown consistent evidence of disease-modifying effects for the treatment of osteoarthritis based on a recent systematic review of preclinical studies.[42] However, clinical evidence of ADMSC-based therapies remains limited.[18,22]

In 2019, a phase IIb clinical randomized controlled trial (RCT) of an intra-articular (IA) injection of autologous high-dose ADMSCs (1×10^8 cells) demonstrated the safety and effectiveness of this treatment for knee osteoarthritis at 6-month follow-up in an outpatient setting, with results of pain relief and functional improvement without structural aggravation.[35] Recent meta-analyses also showed that an IA injection of autologous ADMSCs without adjuvant therapy showed remarkable clinical efficacy and safety in short-term follow-up.[12,16,31] Furthermore, a 5-year follow-up study reported that an IA injection of the ADMSCs provided safe and remarkable clinical improvements without radiologic aggravation for 5 years.[32] It additionally suggested that an IA injection of ADMSCs is a potential disease-modifying treatment for knee osteoarthritis, based on serial magnetic resonance imaging (MRI) evaluations showing significant improvements in structural changes in knee osteoarthritis up to 3 years after the injection.[32] However, a definite conclusion regarding the efficacy and safety of an IA injection of ADMSCs remains elusive because most studies had small sample sizes (<30 patients) and heterogeneous osteoarthritis grades among the inclusion criteria.[15,21,26,31,35,37]

Therefore, we performed a phase III clinical trial through a randomized, double-blinded, placebo-controlled study to assess the efficacy and safety of the IA injection of autologous high-dose ADMSCs (1×10^8 cells) in patients with knee osteoarthritis of Kellgren-Lawrence (K-L) grade 3 with a large sample size in the outpatient setting. The study hypothesis was that, in patients with K-L grade 3 osteoarthritis, those receiving an IA injection of ADMSCs would show safe and clinically superior pain relief and functional improvement when compared with the placebo group.

METHODS

Study Design and Patient Selection

This prospective phase III clinical trial was conducted at multicenter institutions from June 2019 to February 2021 and was based on a randomized, double-blind, placebo-controlled design. The study was approved by the institutional review board of Kyung-Hee University Hospital at Gangdong (KHNMC 2019-04-017-001) and registered at ClinicalTrials.gov (NCT03990805) before enrollment of the first patient. Informed consent was obtained from all patients.

Eligible patients were aged >20 years with K-L grade 3 knee osteoarthritis based on the American College of Rheumatology criteria,[4] a pain intensity ≥50 on a 100-mm visual analog scale (VAS),[19] and functional impairment ≥40 on the total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[8] at the time of screening despite nonoperative treatment for >3 months. The detailed inclusion and exclusion criteria are listed in Appendix Table A1 (available in the online version of this article).

Randomization and Study Protocol

Clinicians assessed patient eligibility, and research coordinators introduced and explained the study to the patients using a standardized script. The patients underwent physical examination and laboratory tests after screening and informed consent. After study enrollment, patients received identification numbers and were assigned to the ADMSC or control group in a 1:1 ratio according to a schedule based on a stratified random permuted block design with a block size of 4 to 6.[10] Lipoaspiration was performed in all patients in the outpatient clinic 1 week after the screening and baseline MRI. Three weeks after lipoaspiration, an IA injection, which contained either autologous ADMSCs (1×10^8 ADMSCs; normal saline, 2.1 mL; autologous serum, 0.9 mL) or saline (normal saline, 2.1 mL; autologous serum, 0.9 mL) (Figure 1), was administered in the outpatient clinic. Ice pack application and limited physical activity were recommended on the day after the injection. The patients were followed up at 1, 3, and 6 months after the injection, and the clinical and radiologic outcomes and safety were assessed at 12 and 24 weeks

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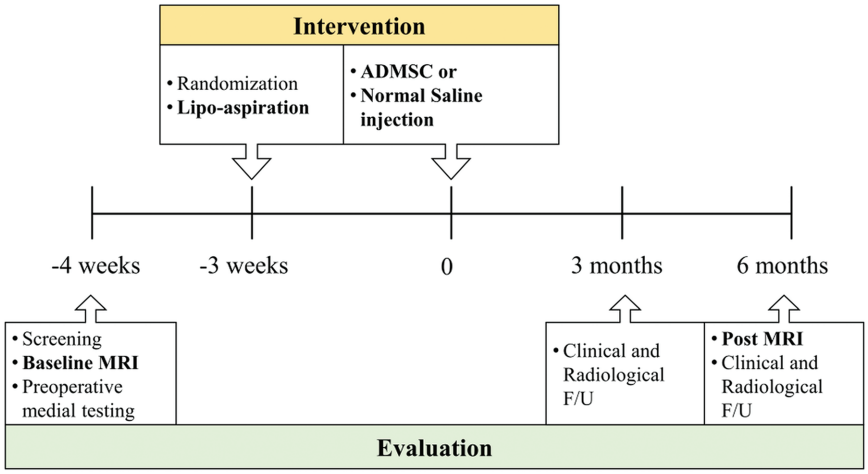

Figure 1. Details of the study protocol. ADMSC, adipose-derived mesenchymal stem cell; F/U, follow-up; MRI, magnetic resonance imaging.

after the injection. Follow-up MRI was performed at 6 months after the injection. Rescue medicine (acetaminophen, $\leq$3000 mg/d) was allowed when the participants required analgesics for knee pain. Other analgesics and nonoperative treatments including physical therapy and injections were not allowed.

ADMSC Preparation and Intervention

Autologous adipose tissue was obtained in both groups from abdominal subcutaneous fat by lipoaspiration via the tumescent technique as previously described (see Appendix Table A2, available online).[27,44] ADMSCs were isolated from this tissue and cultured under good manufacturing practice conditions, as previously described.[30,32,35,44] All adipose tissues collected from multiple institutions were transported to a single manufacturer (Jointstem; R-Bio) for the preparation of ADMSCs under strict and homogeneous conditions per good manufacturing practices.[30,32,35,44] All ADMSCs were collected at passage 3 (mean, 4-5 days per passage) and tested for cell number, viability, purity (CD31, CD34, and CD45), identity (CD73 and CD90), sterility, and endotoxin and mycoplasma contamination, as recommended by the Code of Federal Regulations, title 21, before being injected.[30,32,35,44]

IA injection was performed under ultrasound guidance by a specialized physician not involved in the evaluation of the participants. As the study was designed to be double-blinded, neither the physician nor the patients were aware of who was receiving ADMSCs. In the ADMSC group, 1×10^8 ADMSCs in 3 mL of saline (normal saline, 2.1 mL; autologous serum, 0.9 mL) were injected, while the control group received an injection of 3 mL of saline (normal saline, 2.1 mL; autologous serum, 0.9 mL). The dose of cells for injection was based on the results of previous studies. These studies reported that an IA injection of high-dose ADMSCs (1×10^8) had better clinical efficacy and safety as compared with the outcomes of administering intermediate or low doses of ADMSCs (5×10^7 or 1×10^7).[27,35]

Outcome Measurements

The primary outcomes were improvement in the 100-mm VAS for pain and total WOMAC score for function at 6 months. The secondary outcomes of patient-reported outcome measures (PROMs) were improvements in the Knee injury and Osteoarthritis Outcome Score (KOOS),[45] 36-Item Short Form Health Survey score,[3] and International Knee Documentation Committee subjective knee score.[24] The patients were assessed 3 and 6 months after the injection. As the trial followed a double-blinded design, all clinical evaluations were performed by an independent physician and clinical research coordinator at each institution, who were blinded to the treatment. Further analysis of the clinical efficacy of the treatment was performed to evaluate the number of patients who achieved the minimal clinically important difference (MCID) for the primary outcomes. Based on previous literature, the MCID of the 100-mm VAS was set at 14 points and 20% relative improvement, and the MCID of the total WOMAC was set at 9 points and 33% relative improvement.[17,41,48,52]

The secondary outcomes included radiologic evaluation of change in K-L grade,[29] joint space width of the medial and lateral compartments,[35] and hip-knee-ankle angle[13] with simple radiographs. MRI evaluations were performed using a 3.0-T scanner before and 6 months after the injection. All MRI scans were sent to a dedicated workstation (Qmetrics Technology) and evaluated in a blinded manner. The modified whole-organ MRI score (WORMS) was used to evaluate MRI changes in cartilage status, including cartilage defect depth, defect surface area, and signal intensity.[20]

Safety was assessed per adverse events (AEs), serious AEs, vital signs, physical examination, electrocardiography, and laboratory tests. AE severity was determined according

to the National Cancer Institute–Common Terminology Criteria for Adverse Events (NCI-CTCAE).[7] Causality assessment for AEs attributed to the treatment was determined and recorded per the World Health Organization–Uppsala Monitoring Centre causality assessment system.[39]

Statistical Analysis

Sample Size Calculation. A priori sample size calculation was determined according to previous studies[26,35] to detect a 15.2-point difference in total WOMAC score based on a 1-tailed test, an SD of 30.2, an α value of .025, and a power (ß) of 0.90, resulting in 104 participants required per group. Accordingly, the current trial was designed to include 130 participants per group to account for a potential dropout rate of approximately 20%.

Statistics. For the current study, statistical analyses were performed on the full analysis set (n=252). For missing data, the "last observation carried forward" method was performed.[33] For sensitivity testing, multiple imputation with the fully conditional specification method under a missing-at-random assumption was additionally performed for the primary outcomes.[49] All continuous data were expressed as mean and standard deviation, while categorical data were expressed as frequency and percentage. The normality of continuous data was confirmed by Kolmogorov-Smirnov tests. The baseline demographic characteristics and mean improvement in clinical outcomes from baseline to follow-up visits were compared between the study cohorts using the Student *t* or Mann-Whitney *U* test according to the normality of the continuous variables and Pearson chi-square tests for categorical variables. A linear mixed model (LMM) was used to detect the difference between the groups during the 6 months and included patients as random effects and treatment groups, visit, and visit × treatment interaction as fixed effects. Data were analyzed with SAS Version 9.4 (SAS Institute). Statistical significance was set at $P < .05$.

RESULTS

The current RCT screened 334 patients, of which 73 were excluded. Thus, 261 patients were enrolled and randomly allocated to the ADMSC (n = 131) or control (n = 130) group (Figure 2). At the 6-month assessment, follow-up was discontinued in 9 patients because of loss to follow-up (ADMSC, n = 1) and failure to collect an adequate amount of adipose tissue (ADMSC, n = 5; control, n = 3). Finally, 125 and 127 patients in the ADMSC and control groups, respectively, completed the follow-up. The 2 groups showed no significant differences in age, sex, body mass index, duration of osteoarthritis diagnosis and symptoms, radiologic data, and clinical outcomes, except for WOMAC stiffness and KOOS Symptoms subscores at baseline (Table 1).

Clinical Outcomes

At 6 months, the improvements in primary outcomes (VAS and total WOMAC) were significantly higher in the ADMSC group (VAS, 25.2 ± 24.6; total WOMAC, 21.7 ± 18.6) than the control group (VAS, 15.5 ± 23.7 [P = .004]; total WOMAC, 14.3 ± 19.2 [P = .002]). The results of the LMM analysis revealed that the ADMSC group had significantly higher improvements in primary outcomes than the control group at 6 months after treatment (VAS, $P < .001$; total WOMAC, $P < .001$) (Table 2). Regarding MCID achievement for the VAS, the ADMSC group achieved a significantly higher proportion of patients above the MCID at 6 months for 14 points of absolute improvement (ADMSC, 68.5%; control, 53.7%; P = .019) and 20% relative improvement (ADMSC, 75.0%; control, 60.5%; P = .021). Regarding MCID achievement for total WOMAC score, the ADMSC group achieved a significantly higher proportion of patients above the MCID at 24 weeks for 9 points of absolute improvement (ADMSC, 73.4%; control, 47.6%; P = .001) and 33% relative improvement (ADMSC, 61.3%; control, 45.2%; P = .015) (Figure 3).

Improvements in the secondary outcomes were significantly higher in the ADMSC group than the control group. Specifically, results of the LMM analysis revealed that the ADMSC group had significantly higher improvements in PROMs than the control group at 6 months after treatment (Table 3).

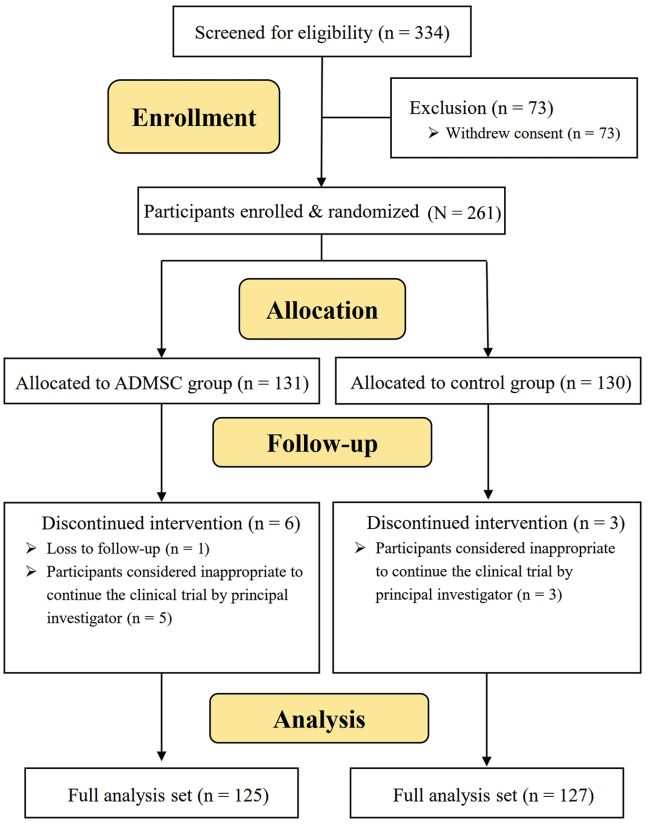

Figure 2. CONSORT (Consolidated Standards of Reporting Trials) flow diagram. ADMSC, adipose-derived mesenchymal stem cell.

TABLE 1
Demographics and Baseline Characteristics: Full Analysis Set[a]

	ADMSC (n = 125)	Control (n = 127)
Age, y	63.7 ± 7.1	63.8 ± 7.1
Sex, male:female, No.	39:86	26:101
Body mass index, kg/m^2	26.3 ± 3.2	25.9 ± 3.1
Smoking, No. (%)	7 (5.6)	5 (3.9)
Duration of osteoarthritis diagnosis, mo	84.1 ± 68.1	85.7 ± 66.5
Symptom duration, mo	113.1 ± 79.1	108.3 ± 84.6
Radiologic data		
K-L grade 1:2:3:4, No.	0:0:125:0	0:0:127:0
HKA angle, deg[b]	–3.8 ± 5.3	–3.3 ± 4.7
Joint space width, mm	3.5 ± 1.3	3.6 ± 1.5
Clinical data		
100-mm VAS for pain	57.7 ± 17.1	60.9 ± 16.6
WOMAC index		
Pain	10.7 ± 3.3	11.3 ± 3.2
Stiffness	4.5 ± 1.3	4.9 ± 1.5
Function	39.8 ± 9.4	41.8 ± 10.3
Total	55.0 ± 13.4	58.0 ± 14.4
KOOS		
Symptoms	55.7 ± 15.9	51.7 ± 15.9
Pain	50.1 ± 13.9	46.9 ± 16.2
Activities of Daily Living	53.7 ± 14.8	50.2 ± 17.0
Sport and Recreation	23.6 ± 18.3	21.5 ±19.0
Quality of Life	32.9 ± 14.3	31.8 ± 16.1
SF-36		
PCS	38.0 ± 5.9	37.9 ±6.2
MCS	46.6 ± 10.1	45.9 ± 9.6
IKDC subjective score	38.5 ± 11.7	37.0 ± 13.1

[a]Data are presented as mean ± SD unless noted otherwise. There was no significant difference between the groups for any outcome measure except for WOMAC stiffness and KOOS Symptoms subscores at baseline. ADMSC, adipose-derived mesenchymal stem cell; HKA, hip-knee-ankle; IKDC, International Knee Documentation Committee; K-L, Kellgren-Lawrence; KOOS, Knee injury and Osteoarthritis Outcome Score; MCS, Mental Component Summary; PCS, Physical Component Summary; SF-36, 36-Item Short Form Health Survey; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[b]Negative values indicate varus alignment of the knee joint.

Radiologic Outcomes

Radiologic outcomes, including K-L grade, hip-knee-ankle angle, and joint space width, did not change significantly at 6 months in the 2 groups. Modified WORMS on MRI evaluation did not significantly differ between the groups at baseline and 6 months after the treatment, and changes in modified WORMS showed no significant difference at 6 months between the groups regarding the cartilage defect depth, surface area, and signal intensity (see Appendix Table A3, available online).

Safety

AEs occurred in 48 (38.4%) and 41 (32.3%) patients in the ADMSC and control groups, respectively, with no significant difference in their frequency. No patients experienced grade 3, 4, or 5 AEs, according to the scale of the NCI-CTCAE. Serious AEs occurred in 1 (0.8%) patient in the ADMSC group (pneumonia) and 3 (2.4%) patients in the control group (COVID-19, herpes zoster, and spondylolisthesis), which were not related to the treatment. Meanwhile, 3 patients (2.4%) in the ADMSC group reported procedure-related joint pain and swelling, and 1 patient (0.8%) in the control group reported procedure-related joint pain, with no significant differences in frequency between the groups (Table 4; also see Appendix Table A4, available online). No complications related to lipoaspiration occurred.

DISCUSSION

The current phase III RCT with a large sample size investigated the efficacy and safety of the IA injection of autologous, culture-expanded, high-dose ADMSCs in patients with K-L grade 3 osteoarthritis. The results demonstrated that the treatment provided significant improvements in knee pain and function at 6 months after the injection. The improvements in all PROMs in the ADMSC group were significantly better than those in the control group at 6 months in terms of pain relief, functional improvement, and enhanced quality of life. Moreover, the ADMSC group achieved a significantly higher proportion of patients above the MCID at 6 months for VAS and total

TABLE 2
Mean Improvements in Primary Outcomes From Baseline to the Follow-up Visits: Full Analysis Set[a]

Outcome: LMM[b] or Time	ADMSC (n = 125)[c]	Control (n = 127)[c]	95% CI of the Difference	*P* Value
Δ 100-mm VAS on pain				
LMM	11.8 (2.9)[b]		6.4-17.4	**<.001**
3 months	22.2 ± 24.6	13.2 ± 23.7	0.6-12.7	**.030**
6 months	25.2 ± 24.6	15.5 ± 23.7	3.0-15.3	**.004**
Δ WOMAC				
Δ Pain subscore				
LMM	2.0 (0.5)[b]		1.0-3.0	**<.001**
3 months	3.8 ± 4.1	2.7 ± 3.8	0.1-2.1	**.027**
6 months	4.3 ± 4.0	2.7 ± 4.4	0.6-2.7	**.003**
Δ Stiffness subscore				
LMM	0.8 (0.2)[b]		0.3-1.2	**<.001**
3 months	1.4 ± 1.8	1.3 ± 1.6	-0.3-0.5	.620
6 months	1.8 ± 1.9	1.3 ± 1.9	0.1-1.0	**.017**
Δ Function subscore				
LMM	6.1 (1.7)[b]		2.8-9.4	**<.001**
3 months	13.3 ± 13.6	9.7 ± 12.1	0.4-6.8	**.030**
6 months	15.7 ± 13.4	10.3 ± 14.1	2.0-8.9	**.002**
Δ Total score				
LMM	8.9 (2.3)[b]		4.3-13.4	**<.001**
3 months	19.1 ± 18.7	13.5 ± 17.2	0.35-9.2	**.024**
6 months	21.7 ± 18.6	14.3 ± 19.2	2.8-12.4	**.002**

[a]Data are presented as mean ± SD unless noted otherwise, and bold indicates statistical significance ($P < .05$). ADMSC, adipose-derived mesenchymal stem cell; LMM, linear mixed model; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

[b]An LMM was used to detect differences between the groups during the 6 months and included patients as the random effect and treatment groups, visit, and visit × treatment interaction as the fixed effects. Data are presented as least squares mean difference (standard error).

[c]Detailed sample size: 3 months, ADMSC (n = 112), control (n = 103); 6 months, ADMSC (n = 110), control (n = 109).

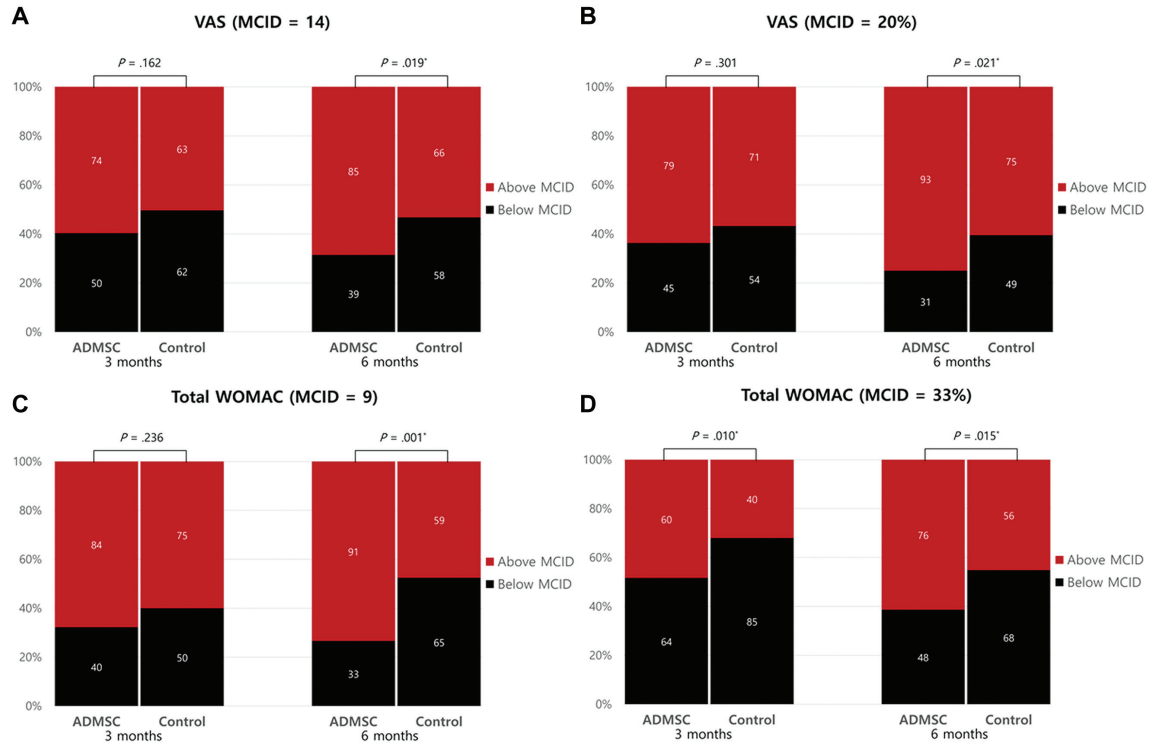

Figure 3. Proportions of patients achieving the MCID for (A, B) VAS for pain and (C, D) total WOMAC score for function. The proportion of patients who achieved scores above the MCID was significantly higher in the ADMSC group than the control group in all assessments at 6-month follow-up. ADMSC, adipose-derived mesenchymal stem cell; MCID, minimal clinically important difference; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.*A *P* value of <.05 indicates statistical significance

TABLE 3
Mean Improvements in Secondary Clinical Outcomes From Baseline to Follow-up Visits: Full Analysis Set[a]

Outcome: LMM[b] or Time	ADMSC (n = 125)[c]	Control (n = 127)[c]	95% CI of Difference	*P* Value
Δ KOOS				
Δ Symptoms				
LMM	7.8 (2.0)[b]		3.7 to 11.8	**<.001**
3 months	11.0 ± 18.3	8.8 ± 17.0	–2.1 to 6.6	.481
6 months	15.0 ± 17.9	9.8 ± 17.5	0.5 to 9.9	**.031**
Δ Pain				
LMM	7.7 (2.0)[b]			**<.001**
3 months	15.3 ±18.2	11.1 ± 17.2	–0.6 to 8.9	.054
6 months	16.3 ± 17.0	10.0 ± 17.1	1.8 to 10.8	**.008**
Δ Activities of Daily Living				
LMM	6.4 (2.0)[b]		2.4 to 10.4	**<.001**
3 months	15.7 ± 18.6	10.4 ± 16.5	0.6 to 10.0	**.029**
6 months	15.7 ± 17.3	11.0 ± 17.0	0.1 to 9.2	**.046**
Δ Sport and Recreation				
LMM	9.8 (2.7)[b]		4.5 to 15.1	**<.001**
3 months	13.5 ± 21.7	4.6 ± 21.6	3.2 to 14.8	**<.001**
6 months	15.7 ± 22.4	5.9 ± 20.0	4.2 to 15.4	**<.001**
Δ Quality of Life				
LMM	5.5 (2.0)[b]		1.6 to 9.5	**.006**
3 months	12.6 ± 16.4	5.9 ± 14.9	2.5 to 10.9	**<.001**
6 months	13.4 ± 17.3	8.5 ± 15.6	0.5 to 9.2	**.024**
Δ SF-36 PCS				
LMM	1.9 (0.7)[b]		0.5 to 3.4	**.008**
3 months	3.6 ± 6.5	2.1 ± 5.8	–0.1 to 3.2	.069
6 months	4.1 ± 6.6	1.9 ± 6.1	0.4 to 3.8	**.014**
Δ SF-36 MCS				
LMM	3.1 (1.1)[b]		1.0 to 5.2	**.004**
3 months	2.6 ± 10.1	1.0 ± 7.2	–0.8 to 3.9	.179
6 months	3.3 ± 10.0	0.6 ± 8.9	–0.2 to 5.2	.090
Δ IKDC subjective score				
LMM	7.3 (1.8)[b]		3.9 to 10.7	**<.001**
3 months	11.2 ± 14.9	7.1 ± 13.0	0.5 to 7.9	**.021**
6 months	13.0 ± 15.7	6.3 ± 13.0	2.9 to 10.6	**<.001**

[a]Data are presented as mean ± SD or n (%) unless noted otherwise, with bold text indicating statistical significance ($P < .05$). ADMSC, adipose-derived mesenchymal stem cell; IKDC, International Knee Documentation Committee; LMM, linear mixed model; KOOS, Knee injury and Osteoarthritis Outcome Score; MCS, Mental Component Summary; PCS, Physical Component Summary; SF-36, 36-Item Short Form Health Survey.

[b]A linear mixed model was used to detect differences between the groups during 24 weeks and included patients as a random effect and treatment groups, visit, and visit × treatment interaction as fixed effects. Data are presented as the least squares mean difference (standard error).

[c]Detailed sample size: 3 months, ADMSC (n = 112), control (n = 103); 6 months, ADMSC (n = 110), control (n = 109).

WOMAC score when compared with the control group, which were the primary outcomes of the current study. The safety profile showed that the treatment was safe, and most treatment-related AEs were mild and resolved during the study period. However, 6 months after treatment, there was no significant difference in structural improvement on MRI.

Among the various sources of MSC-based therapies, adipose tissue has been an attractive option given its easy accessibility, abundance, and safety.[25,50] Moreover, the delivery of ADMSCs by IA injection is not only less invasive but also an effective procedure that can target the degenerated tissue via a paracrine effect, making the procedure an attractive option, especially for elderly patients with morbidities.[2,46] Thus, clinical trials of IA injection of ADMSCs have been recently increasing[28]; however, clinical evidence remains weak because most studies have had small sample sizes (mostly <20 cases) and heterogeneous inclusion relating to the degree of osteoarthritis.[1,12,31] The current study describes a prospective double-blind RCT in multiple centers with an adequate sample size after a priori sample size calculation. Meanwhile, recent studies inferred that the treatment was more effective in patients with K-L grade 3 than others.[1,47,52] In this regard, to avoid possible bias concerning the degree of osteoarthritis, the study included only knees with K-L grade 3 to evaluate the clear potency of the IA injection of ADMSCs because the degree of osteoarthritis reflects the severity of inflammation and destruction of the knee joint, which would affect the ADMSC efficacy.[1,47]

TABLE 4
Treatment-Emergent Adverse Events in the Safety Set[a]

	ADMSC (n = 125)	Control (n = 127)	*P* Value
Patient summary			
Patients with TEAE	48 (38.4)	41 (32.3)	.310
Patients with SAE	1 (0.8)	3 (2.4)	.622
Patients with fatal SAE	0	0	>.999
Procedure-related joint pain	3 (2.4)	1 (0.8)	.337
Procedure-related joint swelling	3 (2.4)	0	.198
Event summary			
Total TEAEs	72	65	
Severity by NCI-CTCAE scale			
Grade 1	50	36	
Grade 2	22	29	
Grade 3	0	0	
Grade 4	0	0	
Grade 5	0	0	
Relationship between the treatment and TEAEs			
Certain	0	0	
Probable/likely	8	2	
Possible	17	2	
Unlikely	42	58	
Conditional/unclassified	3	0	
Unassessable/unclassifiable	1	0	
Not applicable	1	3	
Result of TEAEs			
Recovered/resolved	54	43	
Recovering/resolving	16	21	
Not recovered/not resolved	2	1	
Recovered or resolved with sequelae	0	0	
Death	0	0	
Unknown	0	0	

[a]Data are presented as No. of patients or cases (%). ADMSC, adipose-derived mesenchymal stem cell; NCI-CTCAE, National Cancer Institute–Common Terminology Criteria for Adverse Events; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

The primary outcomes of the current RCT demonstrated significantly better improvements in VAS and total WOMAC score in the ADMSC group at 6 months after the injection. Furthermore, the LMM analysis showed significantly greater improvements in primary and secondary outcomes (PROMs including KOOS, International Knee Documentation Committee subjective score, and 36-Item Short Form Health Survey), with significantly greater improvements in the ADMSC group as compared with the control group after the injection. These findings are consistent with those of previous studies showing that the IA injection of ADMSCs significantly improved clinical outcomes in short-term follow-up.[26,31,35,37] In addition, a recent midterm study showed that a single IA injection of ADMSCs provided safe and effective clinical improvements in the VAS and WOMAC score, with safety for up to 5 years.[32] This midterm result may support the evidence on the clinical efficacy of ADMSCs, suggesting its potential as a disease-modifying treatment for patients with knee osteoarthritis.[32] Despite the promising clinical efficacy of the IA injection of ADMSCs in previous studies,[26,31,35,37] there remain some concerns that prevented robust conclusions because the studies had small sample sizes and heterogeneous study inclusion relating to the degree of osteoarthritis. Accordingly, the results of the current RCT provide information that a single IA injection of ADMSCs could be a therapeutic option for pain reduction and functional improvement in knee osteoarthritis, because it was performed with a large sample size and included osteoarthritic knees with only K-L grade 3 to clearly reflect the clinical efficacy of ADMSCs in homogeneous osteoarthritis conditions.

Meanwhile, we utilized the MCID to evaluate clinically significant differences in primary outcomes between the groups. Specifically, we selected the absolute and relative values of the MCID for primary outcomes that had been applied in previous studies regarding the IA injection of ADMSCs or biologic therapy for knee osteoarthritis.[15,17,52] Yokota et al[52] reported that 76% and 50% of patients receiving IA injections of ADMSCs had responses greater than the MCID of 14 for the VAS at 3 and 12 months, respectively. Garza et al[17] also reported that 62% of patients in the treatment group (IA injection of adipose-derived stromal vascular fractions) had a response greater than the MCID of 33% for the total WOMAC score, in contrast to only 38% of patients in the placebo group at 6 months. Our results regarding the MCID were similar to those of previous studies, with 68.5% and 75% of patients

in the ADMSC group achieving MCID values >14 points and >20% for the VAS score, respectively, while 73.4% and 61.3% in the ADMSC group achieved MCID values >9 points and >33% for the total WOMAC score. Similar to the reports of previous studies, the treatment and placebo groups showed improvements during the first 3 months; however, the improvement began to decline after the 3-month period.[9,17,36] It might be postulated that autologous serum was injected in the ADMSC and control groups for the stabilization of ADMSCs because, according to previous studies, IA injection of autologous-conditioned serum was clinically effective for patients with knee osteoarthritis.[6,43,53] Nevertheless, all primary outcomes were significantly superior to those in the control group at 6 months, and these findings suggest that the IA injection of ADMSCs can provide statistically and clinically significant improvements in pain and function in patients with knee osteoarthritis.

Our MRI evaluation results did not reveal a significant structural change in cartilage status at 6 months after treatment. A recent meta-analysis of RCTs reported controversial cartilage change on MRI evaluations, with limited evidence for cartilage regeneration after the IA injection of ADMSCs in short-term follow-up.[31] Meanwhile, preclinical models have documented evidence of the disease-modifying effects of ADMSCs for the treatment of knee osteoarthritis in macroscopic, histologic, and immunohistochemical evaluations.[42] According to preclinical studies, the promising effects of ADMSCs could skew the biochemical environment of osteoarthritis into regenerative and anti-inflammatory conditions via paracrine effects.[11,23,40,42] A recent midterm clinical study showed significant improvement in cartilage changes between 2 and 3 years after a single IA injection of ADMSCs based on serial MRI evaluation.[32] Thus, structural cartilage improvement requires a longer follow-up to reflect the regenerative and chondroprotective effects of ADMSCs.

The current RCT has several limitations. First, the follow-up period of 6 months seems short for conclusive findings regarding the efficacy and safety of the IA injection of ADMSCs. As this study was a phase III clinical trial, the follow-up period was a priori set identical to that of the phase IIb clinical trial.[35] A mid- or long-term follow-up of the current RCT will provide stronger evidence of the effectiveness and safety of the treatment. Second, some patients were unable to complete the follow-up period; however, the rates of follow-up loss were only 4.6% and 2.3% in the ADMSC and control groups, respectively. Moreover, some patients were not able to complete the PROMs at the planned visits. Yet, these losses are inevitable when an RCT is performed at a multiple institutions and has a large sample size. In addition, missing data imputation was performed through valid statistical methods.[33] Third, this study was conducted in a Korean cohort of patients with a lower body mass and female predominance when compared with those in Western cohorts with knee osteoarthritis,[14] which may limit its generalizability. This limitation should be noted before these results are extrapolated to other populations. Last, there were minor differences between the groups, such as the proportion of female participants and clinical scores at baseline, although these were not statistically significant.

CONCLUSION

The IA injection of autologous culture-expanded ADMSCs provided significant pain relief and functional improvements in patients with K-L grade 3 osteoarthritis. Long-term results are needed to determine the disease-modifying effects of ADMSCs, such as structural changes, and the duration of effect of IA injection of ADMSCs in knee osteoarthritis.

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