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Intra-articular knee implantation of autologous bone marrow–derived mesenchymal stromal cells in rheumatoid arthritis patients with knee involvement: Results of a randomized, triple-blind, placebo-controlled phase 1/2 clinical trial

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Abstract

Background. In this study, we intend to assess the safety and tolerability of intra-articular knee implantation of autologous bone marrow–derived mesenchymal stromal cells (MSCs) in patients with rheumatoid arthritis (RA) and to determine the preliminary clinical efficacy data in this population. The trial registration numbers are as follows: Royan Institute Ethics Committee: AC/91/1133; NCT01873625. *Methods.* This single-center, randomized, triple-blind, placebo-controlled phase 1/2 clinical trial randomized RA patients with knee involvement to receive either an intra-articular knee implantation of 40 million autologous bone marrow–derived MSCs per joint or normal saline (placebo). Patients were followed up for 12 months to assess therapy outcomes. *Results.* A total of 30 patients, 15 in the MSC group and 15 in the placebo group, enrolled in this study. There were no adverse effects reported after MSC administration or during follow-up. Patients who received MSCs had superior findings according to the Western Ontario and McMaster Universities Arthritis Index (WOMAC), visual analogue scale (VAS), time to jelling and pain-free walking distance. However, this improvement could not be significantly sustained beyond 12 months. The MSC group exhibited improved standing time ($P = 0.01$). In addition, the MSCs appeared to contribute to reductions in methotrexate and prednisolone use. *Conclusion.* Intra-articular knee implantation of MSCs appeared to be safe and well tolerated. In addition, we observed a trend toward clinical efficacy. These results, in our opinion, have justified the need for further investigations over an extended assessment period with larger numbers of RA patients who have knee involvement.

Key Words: *bone marrow, mesenchymal stromal cells, osteoarthritis, rheumatoid arthritis*

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Introduction

Rheumatoid arthritis (RA) is an inflammatory polyarthritis with a worldwide prevalence of approximately 0.5–1% in adults 40–50 years of age [1]. Knee involvement is one of the major consequences of this disease that causes chronic pain and disability. Adaptive immune responses mediated by B and T cells have an important role in pathogenesis of autoimmune diseases, such as RA, in which joint fibroblast activation contributes to joint destruction [1,2]. Knee involvement in RA occurs because of a chronic inflammatory process that results in tissue destruction due to leukocyte infiltration into the synovial compartment and secretion of inflammatory cytokines [1,2]. Recommendations for three primary treatments to be used in RA patients with knee joint pain include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying anti-arthritic drugs (DMARDs) such as methotrexate (MTX). Unfortunately, these medications exhibit numerous adverse effects that may cause additional problems (e.g., osteoporosis) for patients [3,4]. In addition, a significant number of patients do not respond to these drugs and need new therapies. Although total knee arthroplasty (TKA) is the last option for RA patients with knee involvement, there exists a higher risk for systemic complications, infections and further replacement after TKA in these patients [5,6]. Medication adverse effects and younger age justify the use of TKA. Researchers are motivated to find a less-invasive treatment method with decreased adverse effects for RA patients with knee involvement.

In the previous year, mesenchymal stromal cells (MSCs) have been proposed as a possible biological therapy for various diseases [7]. MSCs not only have the potential to differentiate into diverse cell lineages, they also mediate a wide spectrum of immunoregulatory activities that usually modulate innate and adaptive immune responses. These properties have led to interest in the prospect for developing novel cell therapies for autoimmune disease. The preclinical results have been promising in experimental models of autoimmune/inflammatory disorders such as RA [8–10], systemic lupus erythematosus (SLE) [11–13], Crohn's disease (CD) [14,15] and multiple sclerosis (MS) [16,17]. Clinical trials show encouraging results for autoimmune/inflammatory disorders such as knee osteoarthritis [18–22], SLE [23,24], CD [25], MS [26,27] and graft-versus-host disease (GVHD) [28,29].

There are a limited number of clinical trials that evaluated MSCs for RA patients. Until now, two articles have been published that discussed the treatment of RA patients with intravenous infusion of MSCs [30,31]. A nonrandomized comparative trial of 172 RA patients considered unresponsive to classical medications assigned 136 patients to receive umbilical cord MSCs and vehicle for 36 patients [30]. Treatment with MSCs induced significant disease remission for 3–6 months. Repeated infusions administered twice at 3-month intervals enhanced therapeutic efficacy. Alvaro-Gracia *et al.* [31] recently reported results from a multicenter, dose escalation, randomized, single-blind, placebo-controlled, phase 1/2 clinical trial that enrolled 53 patients with RA who received adipose-derived MSCs. The results showed the safety of MSCs. The treatment was well-tolerated and they observed a trend for clinical efficacy during 6 months of follow-up.

Therefore, there is a need to explore the effect of MSCs in a randomized trial to gain insight into efficacy for RA patients with knee involvement. In this triple-blind placebo-controlled clinical trial, we have sought to randomly assess the safety and tolerability of intra-articular knee injection of autologous bone marrow MSCs in RA patients with knee involvement. We also obtained preliminary clinical efficacy information in this controlled study.

Materials and methods

Study design

This was a triple-blind, single-center, placebo-controlled phase 1/2 clinical trial of intra-articular knee implantation of MSCs into the knee joints of RA patients with knee involvement. We used autologous bone marrow MSCs that fulfilled the International Society of Cellular Therapy (ISCT) criteria [32]. A single, trained physician evaluated eligible patients to collect baseline characteristics and select one knee joint for intervention. In this study, a statistician calculated a sample size of 60 patients. We randomly assigned patients to the study (MSC) or placebo (normal saline) groups based on the block (size 4) randomization method. For this purpose, the statistician used a random process to generate the sequences.

Patients could continue taking DMARDS during the study. However, we excluded the use of NSAIDs to avoid confounding effects with MSCs. Patients with postimplantation or injection pain for less than 1 month could take NSAIDs for pain relief under physician supervision.

Ethics

The Royan Ethics Committee approved this study, which we conducted according to good clinical practice standards and the amended Declaration of Helsinki (Seoul, October 2008). All patients signed a written informed consent form for study participation.

Patients and procedures

This study included patients 18–65 years of age. After we evaluated patients' medical histories, each patient underwent a physical examination to confirm the preliminary diagnosis of RA according to the American College of Rheumatology (ACR) 2010 criteria. Serum and urine biochemical tests were conducted to evaluate the presence of any acute and/or chronic underlying diseases. Exclusion criteria consisted of any history of uncontrolled chronic diseases other than RA, any injections into the studied knee in the last 3 months and any congenital or acquired diseases that resulted in knee malformations that would affect the results of the trial. Supplementary Table S1 lists the inclusion and exclusion criteria. Then, 30 patients (29 women and 1 man) were enrolled in this trial after completion of the eligibility assessments. Patients had a mean age of 48.9 ± 1.7 years and confirmed diagnosis of RA with knee involvement. Enrolled patients had Kellgren-Lawrence grades 2 and 4 based on upright position radiology of the knees.

Intervention

All study personnel and participants, with the exception of the clean room director, were blinded to the treatment assignments. Each patient received an injection of MSCs/joint (study group) or normal saline (placebo group). The Good Manufacturing Practice (GMP) cell product facility prepared 10-mL identical covered syringes with 22-gauge needles that contained either MSCs or normal saline. According to each patient's code, this ready-to-inject syringe was transported to the operating room at Royan Institute. The patient's knee was prepped and draped using povidone iodine by an experienced nurse. The orthopedic surgeon who administered the injection used a superomedial approach to the selected knee joint under sterile conditions.

Assessments

The patients returned for follow-up visits at 1, 3, 6 and 12 months after the injection. The same physician recorded all safety and efficacy outcomes.

Safety profile

We used the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 to assess any potential adverse events, whether related or not to the treatment. Major related adverse events included pulmonary embolism and severe allergic reactions to the intervention.

Efficacy profile

We used a 1.5 Tesla magnetic resonance imaging (MRI) unit (VB33D Vision Plus; Siemens) for radiological evaluations as previously described [33].

Efficacy outcomes were assessed based on the patients' baseline conditions and after intervention. Assessment criteria included Disease Activity Score 28 (DAS 28) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores in addition to subscales of pain, stiffness, physical function and time to jelling (minutes). Other assessments included a visual analogue scale (VAS; numbered), walking distance (meters), pain-free walking distance (meters), standing time (minutes) and doses of prednisolone (mg per day) and MTX (mg per week). Biochemical tests, as previously described (except for viral markers), were obtained at 3, 6 and 12 months after the intervention.

Statistical analysis

Data are presented as mean ± standard error (SE) or 95% confidence interval (CI). Demographic characterization data are presented as mean ± standard deviation (SD). We used analysis of covariance (ANCOVA) for repeated measures to determine the effects of both groups over a defined period of time. If the main effect F ratio was significant, we performed Tukey post-hoc analysis to identify differences among time points. The statistical software program Stata version 14 was used for data analysis. In addition, GraphPad Prism version 6 was used to produce graphs. $P \leq 0.05$ was considered statistically significant.

Results

Figure 1 describes the study outline. We assessed 150 RA patients with knee involvement from October 2011 to December 2013. From these patients, 119 individuals did not complete the screening process: 79 individuals did not meet the inclusion criteria and 40 individuals declined to participate. We randomly assigned the remaining 30 patients to either the MSC (n = 15) or placebo (n = 15) groups. There were two patients in the MSC group who were excluded according to predefined criteria. Consent withdrawal was the main reason for exclusion from the MSC-treated group. Demographic and baseline characteristics of the trial population did not significantly differ between the two groups (Table I). Participants were mostly women (96%) with a mean age of approximately 50 years and were moderately overweight (average body mass index [BMI] of approximately 29). Kellgren-Lawrence radiological grades 3 and 4 were evenly distributed. Patients' RA knee symptoms were moderate to severe. Average pain based on VAS score of the affected knee was 40 mm.

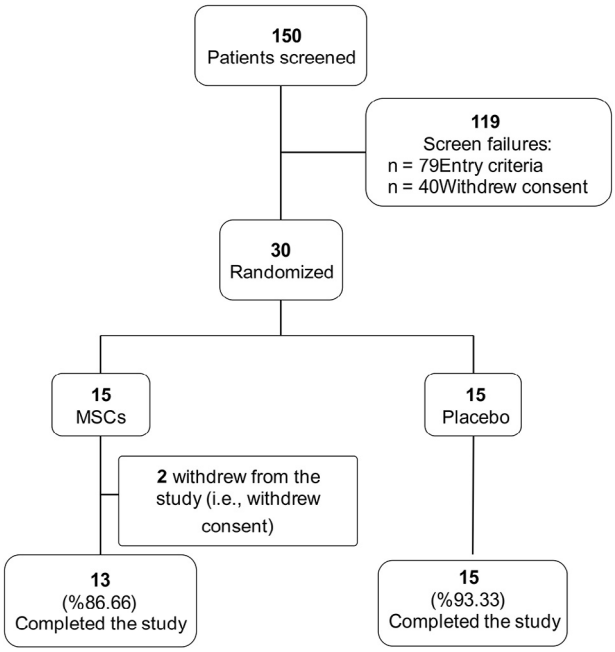

Figure 1. Study description.

All recruited patients in the MSC and placebo groups underwent bone marrow aspiration (150 mL) from their iliac crests. Bone marrow aspiration as well as MSC isolation and preparation were performed as previously described [22]. Cells obtained from patients were relatively homogenous and had a fibroblastic appearance at 7–10 days after culturing. This morphology did not change until use. All cells showed MSC surface antigens according to the ISCT criteria (Supplementary Figure S1) [32]. The cells were suspended in 5 mL normal saline supplemented with 2% autologous serum. In the placebo group, patients received vehicle (5 mL) composed of normal saline supplemented with 2% autologous serum. Each patient in the MSC-treated group received an injection of $42 \pm 4 \times 10^6$ cells in 5 mL of normal saline into the affected study knee joint (Supplementary Table S2).

The results of this trial showed that the MSCs and placebo injections were safe and well-tolerated. There were no adverse effects observed after MSC administration or during follow-up. We noted the presence of only pain and/or articular swelling at 1 month after the injection, which resolved with NSAIDs administration (Table II).

MSCs showed superiority in a number of secondary endpoints to the placebo group. However, improvement could not be significantly sustained beyond 12 months. In both groups, we observed changes in the WOMAC pain subscale from baseline (Figure 2). MSC-treated patients showed improvements during the first month after treatment, which was maintained until the end of the study. However, the trend in the placebo-treated group was

Table I. Participants' demographic characteristics[a].

	MSCs (n = 13)	Placebo (n = 15)
Age (y)	50.4 (8.5)	48.1 (10.8)
Women (%)	13 (100)	13.0 (86.6)
BMI (kg/m^2)	28.9 (10.4)	28.6 (5.0)
Kellgren-Lawrence, N (%)		
Grade 2	0 (0)	0 (0)
Grade 3	7.0 (53.8)	7.0 (46.6)
Grade 4	6.0 (46.1)	7.0 (46.6)
WOMAC (0–100 scale)		
Total index	62.7 (10.4)	52.4 (18.4)
Pain subscale	62.8 (14.0)	52.3 (22.1)
Physical function subscale	64.2 (9.6)	54.2 (18.7)
Stiffness subscale	50.0 (26.6)	35.8 (34.3)
VAS for affected knee (0–10 cm)	3.3 (0.6)	3.8 (0.7)
Time to jelling (min)	22.1 (20.5)	28.7 (31.1)
Standing time (min)	10.6 (8.9)	11.9 (20.2)
Walking distance (m)	1137.5 (1231.1)	1016.0 (1320.1)
Pain-free walking distance (m)	396.9 (447.3)	669.4 (1271.8)
Knee flesam (cm)		
Right	2.4 (4.4)	1.5 (1.8)
Left	2.4 (3.6)	2.2 (3.1)
Knee flexion limitation (degrees)		
Right	125.6 (13.1)	127.1 (19.8)
Left	125.3 (17.2)	126.7 (12.9)
Heel to femur length (cm)		
Right	16.2 (6.9)	16.4 (6.9)
Left	19.7 (7.9)	16.7 (6.6)
Swollen joint (numbers)	0.4 (0.61)	0.4 (0.7)
Time of onset (min)	127.8 (100.9)	153.9 (109.3)
Prednisolone intake (mg) per day	8.2 (2.6)	6.5 (2.8)
MTX intake (mg) per wk	16.2 (11.9)	14.4 (13.0)

Data are mean (SD).
BMI, body mass index.
[a]There were no significant differences between groups in demographic characteristics as evaluated using the paired *t* test.

that the patients did not experience consistent improvement during the entire trial. At the 12-month follow-up, WOMAC pain score in the MSC-treated group decreased to -16.5 ± 13.5 compared with -6.7 ± 13.6 for the placebo group (Table III). Knee pain, as assessed using VAS, decreased by more than 50% in the MSC-treated group at the 12-month follow-up point. This reduction was less marked in the placebo group. We observed the same degree of effectiveness in terms of the pain and physical function WOMAC subscales, and for the WOMAC total score in the MSC-treated versus placebo-treated groups. Although there were no statistically significant differences observed in these findings between the two groups, the MSC-treated group had a better trend (Figure 2). The MSC group had superior results according to time to jelling and pain-free walking

Table II. Number and proportion of patients with adverse events.

Minor adverse events	MSCs (%)	Placebo (%)	Overall (%)
Postimplantation pain and/or articular swelling within 1 mo after implantation (expected/study related), responded to NSAIDs	9 (69.2)	10 (66.7)	19 (67.8)
Unexpected articular pain and/or swelling in each joint, (unexpected, unrelated)	0 (0)	0 (0)	0 (0)
Other: menstrual disorders, influenza, migraine, toothache, restlessness, memory loss, testicular pain, rhinitis, sensitive hand alteration, sleepiness, allergic reaction, tinnitus, dental implant, lipoma, skin tumor (unexpected, unrelated)	2 (15.4) migraine 12 (92.3) influenza 10 (76.9) rhinitis 5 (38.5) sleepiness 2 (15.4) allergic reaction	14 (93.3) influenza 13 (86.7) rhinitis	2 (7.1) migraine 26 (92.8) influenza 23 (82.1) rhinitis 5 (17.8) sleepiness 2 (7.1) allergic reaction

distance; however, this improvement could not be significantly sustained beyond 12 months (Figure 3; Table III). On the other hand, the MSC group exhibited an improvement in standing time ($P = 0.01$; Figure 3; Table III). There were no significant changes in DAS 28 scores and laboratory analyses (erythrocyte sedimentation rates [ESR] and C-reactive protein [CRP]) between both groups (Table III; Supplementary Figure S2). The MSCs appeared to contribute to reductions in MTX ($P < 0.05$) and prednisolone intake during the first 6 months of follow-up (Supplementary Figure S2) but not after 1 year (Table III). The MRI imaging score of the knee did not reveal any differences from baseline and the placebo group, but showed a trend toward improvement in some of the patients who received MSCs (Supplementary Figure S3).

At the 12-month follow-up, patients reported improved physical and mental subscales (Short Form Survey-36) in the treatment group, but this did not significantly differ from that reported by the placebo-treated subjects (data not shown).

Discussion

To the best of our knowledge, this was the first randomized, triple-blind, placebo-controlled trial with

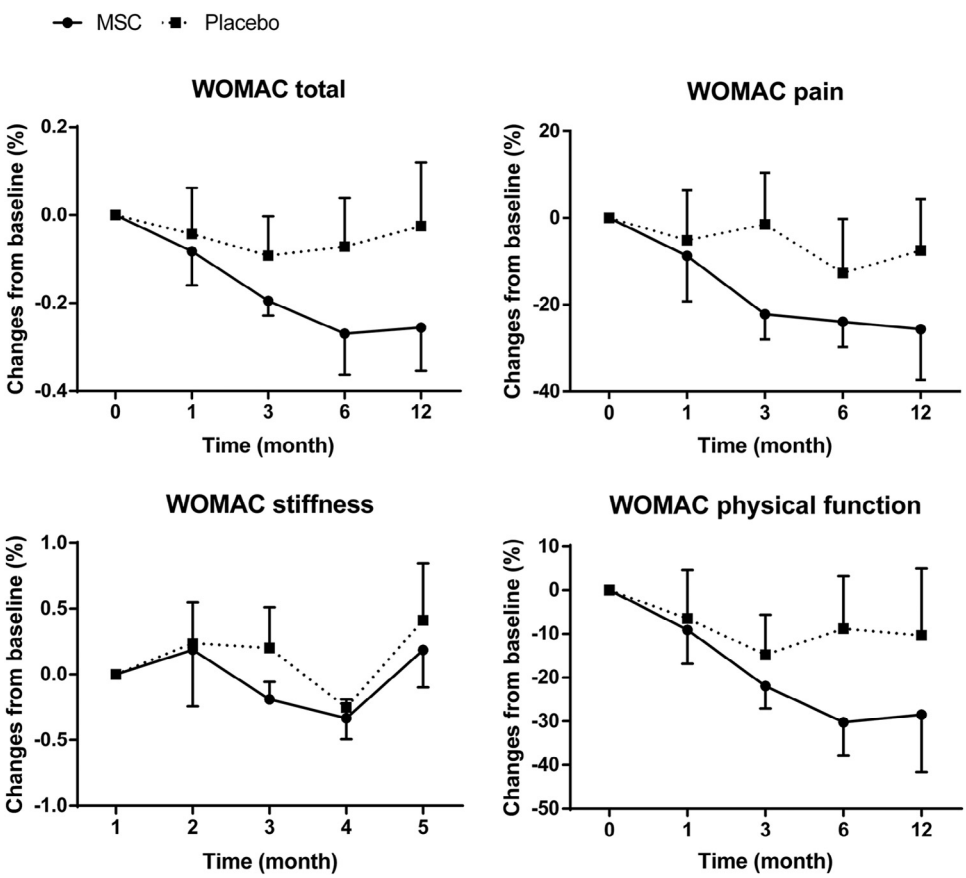

Figure 2. The WOMAC subscale changes in MSC- and placebo-treated groups.

Table III. Mean changes and 95% CI from baseline to the study endpoint (12 mo after implantation) for clinical and paraclinical parameters in MSC- and placebo-treated groups.

	MSC (n = 13)	Placebo (n = 15)	Difference	*P*[a]	Effect size
WOMAC pain	−16.5 (−30.5—2.6)	−6.7 (−20.3–6.9)	−9.88 (−29.5–9.7)	0.31	0.04
WOMAC function	−16.5 (−30.4—2.6)	−9.6 (−20.8–1.6)	−6.9 (−24.6–10.8)	0.43	0.02
WOMAC stiffness	−8.6 (−26.4–9.1)	3.3 (−14.1–20.8)	−12.00 (−37.0–13.0)	0.33	0.03
WOMAC total	−16.1 (−27.7—4.4)	−6.9 (−17.7–3.9)	−9.2 (−25.0–6.7)	0.25	0.05
VAS	−2.2 (−3.6—0.9)	−1.7 (−4.0–0.6)	−0.5 (−3.3–2.3)	0.72	0.005
Time to jelling	42.1 (15.9–68.3)	24 (−5.9–53.6)	18.1 (−22.1–58.2)	0.36	0.03
MTX	−2.4 (−6.9–2.1)	−0.8 (−10.9–9.3)	−1.6 (−13.3–10.1)	0.78	0.003
HF	−0.8 (−2.2–0.6)	−0.13 (−2.5–2.2)	−0.6 (−3.5–2.2)	0.65	0.01
PND	−0.9 (−3.6–1.9)	0.4 (−1.6–2.4)	−1.3 (−4.6–2.1)	0.44	0.02
Pain FWD	2434.6 (1276.9–3592.4)	790.6 (−473.0–2054.2)	1644.0 (−93.0–3381.0)	0.06	0.12
ESR	−5.9 (−14.5–2.7)	−6.1 (−17.0–4.7)	0.2 (−14.0–14.4)	0.97	0.00003
CRP	−0.2 (−0.5–0.2)	−0.3 (−0.6—0.1)	0.2 (−0.2–0.6)	0.37	0.03
DAS 28	−0.4 (−0.7—0.1)	−0.4 (−0.8–0.1)	−0.01 (−0.6–0.5)	0.96	0.00008
Standing time	22.9 (5.4–40.4)	−1.3 (−11.9–9.4)	24.2 (4.3–44.1)	0.02[a]	0.19
WD	1707.7 (338.9–3076.5)	764 (−347.2–1875.2)	943.7 (−804.8–2716.9)	0.28	0.04

The data presented as mean. The data in parentheses represent 95% CI.
HF, heel to femur; PND, prednisolone; Pain FWD, pain-free walking distance; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WD, walking distance.
[a]Significant at $P < 0.05$.

bone marrow MSCs in RA patients who had knee involvement. The most important findings of our work were the safety and feasibility of intra-articular knee injections with bone marrow-derived MSCs in patients with RA in addition to the superiority of clinical outcomes in patients who received the MSCs. However, due to the small number of participants, these findings were not significant compared with the placebo-treated group, with the exception of standing time.

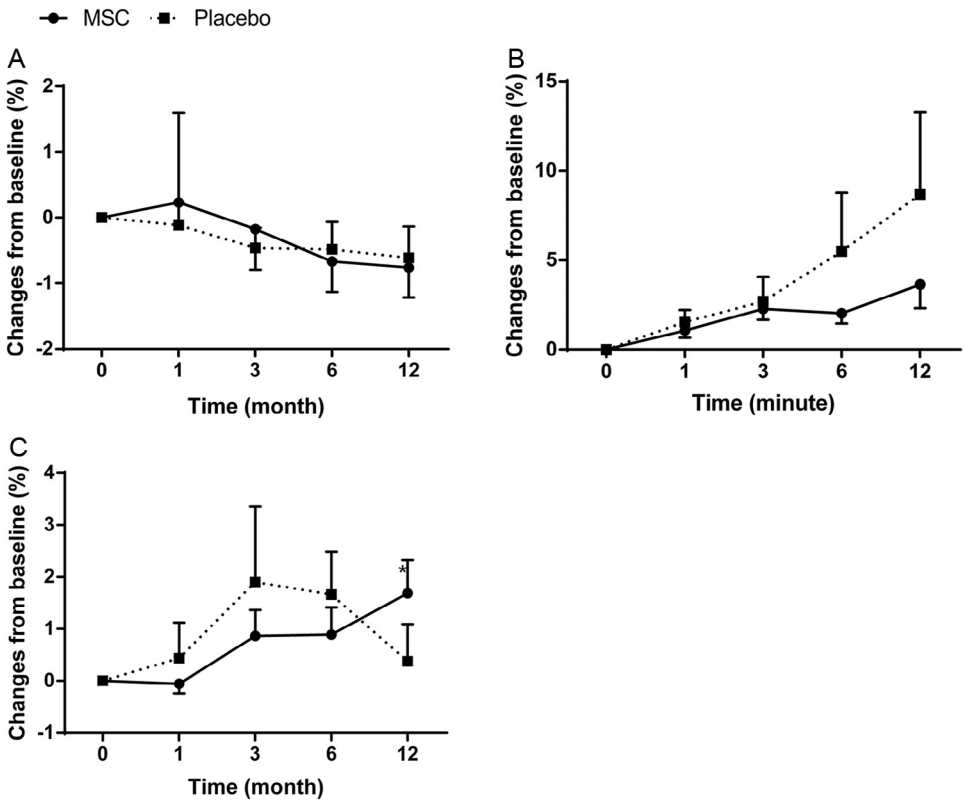

Figure 3. Clinical parameter changes in the MSC- and placebo-treated groups. (A) VAS in mm. (B) Time to jelling. (C) Standing time. *$P < 0.05$.

The exact mechanism by which MSCs exert their therapeutic effects is not fully understood. However, these cells exhibit multilineage differentiation into osteocytes, adipocytes and chondrocytes [32] and mediate a wide spectrum of immunoregulatory activities that usually modulate innate and adaptive immune responses [14]. These cells inhibit the pro-inflammatory activities of neutrophils and proliferation, cytokine production and cytotoxic activity of resting natural killer (NK) cells. MSCs also inhibit T-cell proliferation while promoting the development of regulatory T (Treg) cells. In this regard, a clinical trial for RA with MSCs has demonstrated that the patient group that received MSCs showed significant remission of the disease. This result correlated with an increased numbers of Treg cells in their peripheral blood [30]. Finally, MSCs have been shown to inhibit proliferation and expansion of T-cell populations at the edge between adaptive and innate immunity [34].

The therapeutic dosage of MSCs remains unclear and depends on the therapeutic application. In the current study, we have used approximately 40 million autologous MSCs. The number of clinical randomized trials that compare cell dosage is limited. In an interesting study, Orozco *et al.* [18] have reported improvements in pain and function with the use of a single intra-articular injection of 40 million autologous MSCs. In another randomized clinical trial that used allogeneic MSCs, Vega *et al.* [19] reported good clinical outcomes in pain control and function after a single intra-articular injection of 40 million allogeneic MSCs. In a recent clinical study, Gupta *et al.* [20] compared the administration of different dosages of allogeneic MSCs (25, 50, 75 and 150 million cells) in the knee of osteoarthritis patients. They observed that one injection of 25 million cells was safe. A trend toward improvement was seen in all evaluated parameters although the findings were not statistically significant compared with the placebo. Adverse events (knee pain and swelling) were predominant complaints in the higher-dose groups [20]. On the other hand, Yubo *et al.* reviewed 11 eligible trials with 582 knee osteoarthritis patients who received injections of MSCs intra-articularly into the knee; MSCs induced pain relief and functional improvement [35]. In these trials, the patients received cell infusions that ranged from 1–150 million MSCs [35]. In a clinical trial for RA that used 10–100 million autologous MSCs, the researchers noted improvements in the high-dose group [21]. Wang *et al.* [30] administered 40 million MSCs in RA patients. Alvaro-Gracia *et al.* [31] used doses of 1, 2, and 4 million adipose-derived MSCs/kg on days 1, 8 and 15 intravenously. Patients were followed up for therapy assessments for 6 months. There was no apparent relationship between dose and tolerability. Therefore, the heterogeneity in the methodology used in the different studies with different cell production methods and dosage precluded these authors from obtaining solid conclusions.

The limitations of the current study include the limited number of patients. In contrast, the randomized placebo-controlled design and the triple-blind evaluation of efficacy are the main study strengths. Our results have suggested that an intra-articular knee injection of MSCs is generally safe and well tolerated at the dose and time period studied. The preliminary clinical efficacy data of these cells have been demonstrated in a limited population number of RA patients with knee involvement. To strengthen the conclusion derived from this study, we propose that further investigations over an extended period of time with larger numbers of participants are necessary to clarify the therapeutic potential of MSCs in RA patients with knee involvement.

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Disclosure of interests: We declare that we have no conflicts of interest.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jcyt.2017.12.009.