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ORIGINAL ARTICLE

Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients

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Abstract

Aim: Osteoarthritis is a degenerative joint disease characterized by the destruction of joint cartilage. Mesenchymal stem cells (MSCs) are found in low numbers in normal cartilage, mainly in the superficial layer, acting as repairing agents. In OA, MSCs are seen in larger numbers, but act chaotic and are unable to repair the cartilage. The synovial membrane becomes inflamed and interacts with the cartilage. Transplanted MSC have the ability to normalize them, redirecting them to their normal function. In a preliminary study, we showed that MSC could improve knee OA in four patients at 6 months. This report shows their long-term follow-up at 5 years.

Methods: One patient was lost to follow-up at 2 years and three were followed for 5 years. They were aged 55, 57, 65 and 54 years, and had moderate to severe knee osteoarthritis. The worse knee of each patient was injected with 8–9 $\times$ 10^6 MSC.

Results: As previously reported, all parameters improved in transplant knees at 6 months (walking time, stair climbing, gelling pain, patella crepitus, flection contracture and the visual analogue score on pain). Then, they started gradually to deteriorate, but at 5 years they were still better than at baseline. PGA (Patient Global Assessment) improved from baseline to 5 years. The better knee at baseline (no MSC), continued its progression toward aggravation and at 5 years became the worse knee.

Conclusion: Transplant knees were all in a rather advanced stage of OA. Earlier transplantation may give better results in long-term follow-up. This is what future studies have to demonstrate.

Key words: bone marrow, intra-articular injection, knee osteoarthritis, mesenchymal stem cell, stem cell transplantation, tissue culture.

INTRODUCTION

Osteoarthritis (OA) is a biomechanical disease progressing gradually to the degradation of joint cartilage. Knee OA is one of the most frequent forms of osteoarthritis. It has a high prevalence in Asian countries (from 4.1% to 11.3%),[1] especially in Iran (from 16% to 21%).[2–6]

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Unfortunately, the available treatments are more symptomatic than preventive. Although some of them may slow down the process, there is no hope for a reversal, leading gradually to more cartilage loss and finally to joint replacement.[7] There are some treatment options that have shown some disease-modifying response, but are not yet proven and remain controversial.[8]

In OA, obesity, trauma and overuse,[9] malalignancy,[10] aging, genetic factors[11] autoimmune-inflammatory arthritis are among factors[12] leading gradually to joint destruction.[9] They produce pro-inflammatory cytokines and metalloproteinases (local inflammatory reactions).[7]

Subchondral bone seems to have a role in the nutrition of cartilage and in case of malfunction influences the progression of OA lesions.[13] It has been shown that the presence of subchondral bone marrow lesions, and especially the presence of subchondral cysts, can predict the progression of joint cartilage lesions.[14]

OA will produce alterations and changes of subchondral bone (thickening, cyst, osteophyte production)[13,15,16] and synovial tissue (inflammation, production of inflammatory cytokines, and metalloproteinases).[16–18] The synovium becomes inflamed, cellular phenotype changes with infiltration of CD4+ T cells and CD68 macrophages,[19] leading to degeneration of cartilage, and then aggravation.[7] OA seems to be more a synovial disease than a pure degeneration of cartilage, as believed before. Resident mesenchymal stem cells (MSC) change phenotypes and instead of repairing, act chaotically toward activation of chondrocytes for production of metalloproteinases instead of matrix production, leading toward articular destruction.[7]

In animal models, MSC transplantation can prevent or improve experimental osteoarthritis.[20–23] The cells were taken from the animal's bone marrow or knee adipose tissue. MSCs were injected into induced OA and evaluated a few weeks to 6 months later. The results were satisfactory and bring new hope for the treatment of OA. At the beginning, it was supposed that implanted MSCs will transform to chondrocytes, replacing the missing cells and repairing the cartilage. More studies have shown the contrary. MSCs do not transform into chondrocytes, but suppress synovial activation and indirectly ameliorate cartilage damage.[24] They establish a repair microenvironment and stimulate the tissue repair by recruitment of local endogenous stem cells.[25]

In humans, the first trial was in a male patient with moderate to severe knee osteoarthritis. He had an autologous MSC transplantation (bone marrow origin) with 22.4 million cells. Results at 6 months were improved visual analogue score (VAS) on pain, walking distance, and joint space width on magnetic resonance imaging (MRI).[26,27]

The second human experience was in our group of four patients; two men and two women[28] with moderate to severe OA of both knees. They received 8–9 million autologous MSC in their worse knee. The preliminary results at 6 months showed a high improvement of subjective parameters, but less on physical parameters. We concluded that the results were encouraging, but not excellent and proposed earlier treatment.

The third human experience was on six female patients with moderate to severe knee OA.[29] They received each 20–24 million autologous MSCs. All parameters (subjective and objective) improved at 6 months, but at 12 months the majority of parameters started to decline.

The aim of this study is to present a 5-year follow-up of the patients of the second study.[28]

PATIENTS AND METHODS

This section is the same as in our preliminary report.[28]

Ethics

The research performed on the four human subjects was in compliance with the Helsinki Declaration. The project was first approved by the Research Committee of the Rheumatology Research Center, and then approved by the Research Committee of the Tehran University of Medical Sciences (TUMS). It was finally approved by the Ethics Committee of TUMS and was registered under ID 3087.

Registration

The project was also registered at ClinicalTrials.gov (ID: NCT00550524).

Patients

The two male subjects were AA (55 years old) and HM (65 years old). The two female subjects were PZ (57 years old) and MS (54 years old). All patients had moderate to severe OA of both knees. They had mechanical pain of their knee joints, which was aggravated by walking or stair climbing. They also complained of gelling pain (gelling phenomenon). On physical examination, they had crepitus and limitation of the joint range of motion with joint bony hypertrophy. X-rays showed narrowing of joint space and osteophyte formation. The procedure was fully explained to the patients, and their signed written consent was obtained. Walking time to produce pain, the number of stairs to climb to produce knee pain, the amount of pain calculated on a VAS of zero (no pain) to 100 (maximum pain), how much time to rest to induce the gelling pain, the range of motion, the presence of a possible instability (due to lateral and cruciate ligaments tear), patellae crepitus, and possible joint swelling (by the presence of synovial fluid) were checked at baseline and at successive controls, mainly 6 months, 1 year, 2 years and 5 years. In calculating the VAS for pain, the patient was told what was the precedent VAS and asked "according to the precedent VAS, how do you evaluate your pain today"? The Patient Global

Assessment (PGA) was concluded from that (better, the same, and worse).

Sample collection from bone marrow and MSC culture and expansion

As explained in the first report, 30 mL of bone marrow was obtained from the patients, 3–5 weeks prior to the knee joint MSC transplantation.[28] The mononuclear cells of bone marrow were separated by ficoll hypaque density gradient. Vented flasks (75 cm^2) with 21 mL MSC medium, consisting of Dulbecco's modified Eagle's medium (DMEM) with 10% of fetal bovine serum (FBS), were seeded with 1 × 106 MSC/mL for primary culture. Flasks were incubated at 37°C in a humid chamber containing 5% CO_2 and were fed by complete medium replacement every 4 days, until the confluence of fibroblast-like cells at the base of flasks. Thereafter the adherent cells were re-suspended using 0.025% trypsin and re-seeded at 1 × 104 cells/mL. When cells reached confluence by the end of the first passage, they were incubated only with M199 medium for one more day. Cells were detached with trypsinization and washed with normal saline supplemented with 2% human serum albumin three times, then re-suspended at a density of 1–2 × 106 cells/mL.

Immunophenotyping

The expression of CD105, CD44, CD13 (MSC markers), CD34, CD45 (HSC markers), and CD31 (endothelial cell marker) were determined in culture-expanded MSCs using flow cytometry.[28] Anti-CD44, CD45 and CD34 fluorescein isothiocyanate (FITC), anti-CD13 and CD31 phycoerythrin (PE) were all purchased from Dako (Glostrup, Denmark), along with anti-CD105, PE from Serotec (Space Company, Milano, Italy). Flow cytometry was performed on a FacScan (Becton Dickenson, Franklin Lakes, NJ, USA). Data were analyzed with cellquest software (www.bdbiosciences.com/documents/15_cellquest_prosoft_analysis.pdf).

Safety assessment

Bacteriological tests were performed on samples after each passage and before any injection (to make sure of non-contamination of samples).[28] Before injection the viability of cells was assessed by methylene blue dye exclusion test.

Injection of MSCs

A mean volume of 5.5 mL containing approximately 8–9 × 106 cells were prepared and injected into the selected knee of the patient.[28] In each patient, the most painful knee, or the worse knee on physical examination, was selected as the site of injection. No previous preparation or premedication was given. All inflammatory or analgesic drugs were stopped at the entry to the study, 3–4 weeks before the injection of MSCs. Glucosamine was permitted, if the patient was taking it before selection for the study. During the procedure, no joint fluid was aspirated and no steroid was injected in the knee joint. Patients were not hospitalized for the procedure, and went back home half an hour after the procedure. No analgesics, anti-inflammatory drugs or immunosuppressive drugs were given or allowed after the procedure.

Follow-up

The patients were checked 1 week after the procedure. They were then controlled every month up to 1 year. All the parameters as explained above were checked at each visit. Knee X-rays in standing position were taken at baseline, 6 months and at 1 year.

After the follow-up of 1 year, as in our preliminary report,[28] patients were seen as reported in Tables 1–2. Patient AA was lost to follow-up after the 2-year check. Patients PZ, HM and MS had their last follow-up at 5 years.

RESULTS

Patients' genders and ages were given earlier in Materials and Methods. They were overweight with a body mass index (BMI: kg/m^2) of 28.5 (AA), 29.7 (PZ), 30.2 (HM), and 37.1 (MS). They complained of knee pain, respectively, for 7, 15, 10 and 8 years. The different parameters checked at baseline, 6 months, 1 year, 2 years and 5 years are given in Tables 1 (AA), 2 (PZ), 3 (HM) and 4 (MS).

All parameters were checked at each visit. The baseline parameters of all patients are shown in Table 1. The change of major parameters during the follow-up is shown in Table 2. The parameters were: walking time (WT) for the pain to appear (in minutes of walking on a flat surface), the number of stairs to walk up, climbing stairs (CS) for the pain to appear (in number of stairs to go up), rest time to produce a gelling pain (GP) in minutes of resting on a chair, for the pain to appear when getting up to walk, flexion contracture (in degrees of loss of the range of motion: RM) checked for both knees, the absolute range of flexion (in degrees, disregarding the flexion contracture) for both knees, patella crepitus (by Likert-type scale from 1 to 4) for both knees, and the pain on VAS, to be pointed at on a scale of 0–100.

Table 1 Baseline characteristics of the four patients

	AA†	HM	PZ	MS
Gender	Male	Male	Female	Female
Age (years)	55	65	57	54
BMI (kg/m^2)	28.5	30.2	29.7	37.1
Osteoarthritis duration (years)	7	10	15	8
Knee transplanted with MSC	R	L	R	R
X-ray deterioration grading (0 to 4+) R/L knee	2–3/2–3	2–3/2–3	2–3/2–3	2–3/2–3
Walking time for pain to appear (minutes)	20	1	0	10
Number of stairs climbed for knee pain to appear	5	1	3	7–8
Rest time for gelling pain to appear (min)	15	5–10	15	15
VAS for pain (1 to 100)	90	90	80	85
Flexion contracture (degree) R/L knee	0/0	5/10	15/10	0/0
Flexion range (degree) R/L knee	Full/Full	120/90	90/80	Full/Full
Patella crepitus (0 to 4+) R/L	1/1	2/3	4/0	3/4
Lateral instability (0 to 4+) R/L	0/0	0/0	0/0	0/0
Drawer sign (- or +) R/L	−/−	−/−	−/−	−/−
Swelling (0 to 4+) R/L	0/0	0/1	0/0	1/0

†Defaulted follow up after 2 years. BMI, body mass index; MSC, mesenchymal stem cell; R, right; L, left; VAS, visual analogue score.

No patients had instability of knees at baseline and kept it until the last evaluation. X-rays before the procedure showed a 2+ to 3+ (moderate to moderate-severe) OA, corresponding approximately to Kellgren–Lawrence grade 2 to grade 3. X-rays did not show any improvement of the joint space after 6 months. However, as the X-rays were not taken with flexed knees, the exact joint space could not be evaluated.

Although at 5 years follow-up some parameters declined compared to the 6 months improvement, they were still better compared to the baseline. As announced in the materials and methods, the worse knee was selected for the MSC implant. At 5 years follow-up, no implanted knee was the worse knee, as reported by the patient. Finally, the PGA demonstrated an improvement compared to the baseline.

DISCUSSION

In this pilot open label study of MCS injection for osteoarthritis in four patients, the results at 6 months were good, especially on major parameters such as WT, CS, GP, VAS and RM. At 6 months the WT, CS, GP and VAS improved in all patients. Two patients had limited RM; they improved this (Table 2). At 2 years follow-up, good results started to decline (Table 2). At 5 years, two patients aggravated their GP, two improved the CS and the RM, and three improved the WT and the VAS. If in our patients, in this report, an important decline was seen in some parameters of the last evaluation, compared to those at 6 months, it may be due to the advanced stage of OA in patients. As shown by animal studies and concluded by van Lent in his editorial, one of the main effect of MSC transplantation in the joint is the suppression of the synovial inflammation.[24] For Roelofs, the transplanted MSCs creates a microenvironment to repair the cartilage.[25] This repair may be due to the change of the effect of transforming growth factor (TGF)β on local MSCs and chondrocyte, switching the Smad1/5/8 phosphorylation (due to the presence of A Disintegrin And Metalloproteinase with Thrombospondin Motifs [ADAMTS5], probably because of synovial inflammation) to Smad2/3 phosphorylation which is a protector of joint normal cartilage.[2,30–32] Why after several months this action may be reversed and the process of cartilage degradation starting again is not known. Perhaps, after a while, the transplanted MSCs loses their characteristics and become like the original resident MSCs with chaotic function. If that is what happened, the transplanted MSCs did not have the time to significantly repair the cartilage. However, this is just a presumption and has to be verified scientifically. It is interesting to note that although after a while the improvement started to decline, the transplanted knee joint declined slower than the contralateral non-transplanted knee. This may mean that the repair effect of MSCs has not completely disappeared. For future studies, it may be appropriate to select patients at earlier stages of OA.

Our experiment, when started, was the second experiment on human subjects. The first experiment was reported by Centeno, in May 2008, on one human subject.[26,27] It showed good results at 6 months with

Table 2 Change from baseline value for the transplant knee. "−" for decrease, "+" for increase

	6 months	1 year	2 years	5 years
Change in walking time (WT) in min				
Patient AA†	+5	–	−5	–
Patient HM	+3 to 7	–	–	+4 to 5
Patient PZ	+60	+30 to 45	+30 to 45	+1
Patient MS	+0 to 5	–	−5	+5 to 15
Change in number of stairs climbed				
Patient AA†	+5	–	+35	–
Patient HM	+14	–	–	0
Patient PZ	+67	+27	+67	+2
Patient MS	+12	–	+24	+22
Change in rest time for gelling pain (GP) in min				
Patient AA†	+15	–	+15	–
Patient HM	No GP	–	–	No GP
Patient PZ	+15	+15	+45	−5
Patient MS	−5	–	−13	−5 to 0
Change in VAS for pain (1–100)				
Patient AA†	−40	–	0	–
Patient HM	−35	–	–	−45
Patient PZ	−40	−55	−55	−72
Patient MS	−20	–	−20	−40
Change in flexion range (R/L in degrees)				
Patient AA†	Full/Full	–	Full/Full	–
Patient HM	120/100	–	–	120/90
Patient PZ	100/90	90/80	90/80	100/90
Patient MS	Full/Full		Full/Full	Full/Full
Change in flexion contracture (R/L in degrees)				
Patient AA†	0/0	–	0/0	–
Patient HM	10/5	–	10/10	15/10
Patient PZ	10/5	10/5	10/5	10/5
Patient MS	0/0	–	0/0	0/0
Change in patella crepitus (R/L, from 1+ to 4+)				
Patient AA†	0/0	–	1+/0	–
Patient HM	1+/1+	–	–	2+/2+
Patient PZ	1+/0	2+/2+	2+/1+	2+/1+
Patient MS	1+/3+	–	1+/2+	2+/3+
Change in swelling (R/L, from 1 to 4+)				
Patient AA†	0/0	–	0/0	–
Patient HM	0/0	–	–	0/0
Patient PZ	0/0	0/0	0/0	0/0
Patient MS	1+/0	–	0/0	0/0
Better knee/worse knee				
Patient AA†	Control/MSC			–
Patient HM	Control/MSC			MSC/Control
Patient PZ	Control/MSC			MSC/Control
Patient MS	Control/MSC			MSC/Control

†Defaulted follow-up after 2 years. MSC, mesenchymal stem cell; R, right; L, left; VAS, visual analogue score.

no side-effects. We started our project in June 2008, as a pilot study, to see if MSCs were able to repair the cartilage of an OA joint, the safety and outcome at long follow-up. In 2012 Emadedin reported the results of six patients with knee OA, who had an MSC transplantation.[29] They had a follow-up of 1 year. Their results were reported as the average (mean ± 95% confidence interval) of the parameter in the six patients. They were

good at 6 months, but at 12 months they declined slightly to moderately, compared to 6 months, but were much better than at the baseline. They did not report any local systemic adverse events. It would be interesting to see their long-term follow-up. Lastly, in 2014, Centeno and Freeman reported six cases of carpometacarpal (CMC) OA injected with MSCs.[33] They too report their results as an average, which was improved compared to a series who did not have treatment. At 1 year the improvement was 60% in the treatment group and an aggravation of 19% in the control group. There were no side-effects, local or systemic, reported at 1-year follow-up. Here too, we need to see what will be the case after 5 years of follow-up. Unfortunately, the results at 6 months were not reported and cannot be compared with the result at 1 year, to see if some decline was also noted in them.

Our study has limitations. The most important is the low number of patients, an open labeled study, and the absence of a control arm. The second weak point is the advanced stage of the knee OA in our study; earlier stages of the disease could have given better and longer-sustained results. The third weak point is the absence of different stages of OA in the same study, which would have shown which stage of knee OA would be the optimum time for MSC transplantation. Finally, for future studies, it is interesting to look at what will be the outcome if more than one MSC transplantation were done for the same joint. Another weak point of the study is the technique of knee X-rays, which were not taken with flexed knees and not in weight-bearing positions to show the correct joint space width.

CONCLUSION

MSC transplantation via intra-articular injection seems beneficial for the joint in osteoarthritis of the knee and carpometacarpal joint, without local or systemic adverse events. However, the beneficial effect starts to decline after 6 months, but is still better at 5 years compared to the baseline. It is also important to note that at baseline, the transplant knee was the worse knee, but at 5 years the contralateral knee became the worse knee.

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CONFLICT OF INTEREST

None.

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