International Journal of
Rheumatic Diseases

International Journal of Rheumatic Diseases 2011; **14:** 211–215

ORIGINAL ARTICLE

Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients

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Abstract

Background: Osteoarthritis (OA) is a cartilage degenerative process, involving the immune system, producing local inflammatory reactions, with production of pro-inflammatory cytokines and metalloproteinases. No treatment is still available to improve or reverse the process. Stem cell therapy opened new horizons for treatment of many incurable diseases. Mesenchymal stem cells (MSCs) due to their multi-lineage potential, immunosuppressive activities, limited immunogenicity and relative ease of growth in culture, have attracted attentions for clinical use.

Aim: The aim of this study was to examine whether MSC transplantation could reverse the OA process in the knee joint. The project was approved by the Tehran University of Medical Sciences Research Committee and Ethical Committee.

Patients and Methods: Four patients with knee osteoarthritis were selected for the study. They were aged 55, 57, 65 and 54 years, and had moderate to severe knee OA. After their signed written consent, 30 mL of bone marrow were taken and cultured for MSC growth. After having enough MSCs in culture (4–5 weeks) and taking in consideration all safety measures, cells were injected in one knee of each patient.

Results: The walking time for the pain to appear improved for three patients and remained unchanged for one. The number of stairs they could climb and the pain on visual analog scale improved for all of them. On physical examination, the improvement was mainly for crepitus. It was minor for the improvement of the range of motion.

Conclusion: Results were encouraging, but not excellent. Improvement of the technique may improve the results.

Key words: bone marrow, knee joint, mesenchymal stem cell, osteoarthritis, stem cell transplantation.

Osteoarthritis (OA) is a degenerative process of the joint's cartilage, involving the immune system, and producing local inflammatory reactions with production of pro-inflammatory cytokines and metalloproteinases. Knee OA is a frequent form of the disease with high prevalence in Asian countries,[1] especially in Iran.[2–5] Whatever the cause of cartilage degeneration (aging process, trauma, overuse and overweight, genetic predisposition, inflammatory and autoimmune arthritis, metabolic arthritis, infectious arthritis, etc.)[6,7] the healing process is slow and where damage repair is not possible, a secondary fibrosis process occurs. As a result, the process of degeneration gradually continues. Unfortunately, no treatment is available to improve or reverse the process. Although medications

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based on suppression of interleukin 1 and metalloproteinase, and stimulation of transforming growth factor (TGF)β may decrease, stop, or reverse the process, they are controversial. Mesenchymal stem cell (MSC) transplantation has brought some hope.

In the light of the new research on stem cell therapy during recent years, new horizons have been opened for treatment of many incurable diseases. These multipotent cells exhibit the ability of differentiation to different cell types. This is true for both embryonic and adult stem cells. We can find adult stem cells in different organ and tissues, such as bone marrow, central nervous system, liver, etc.

The procedure used in adult stem cell therapy consists of stem cell isolation from adult tissues, their expansion *in vitro* and transfusion back into the patient. It has been postulated that factors secreted in the micro-environment of damaged tissue recruit stem cells to the site of active disease and facilitate their differentiation into desired cells.

MSCs, due to their multilineage potential, immunosuppressive activities, limited immunogenecity and relative ease of growth and expansion in culture have attracted researchers' attentions for clinical use. Although MSCs were initially believed to be necessary for hematopoietic stem cell (HSC) survival and function, further studies demonstrated their abilities to differentiate into different types of cells (e.g. osteocytes, chondrocytes and skeletal myocytes, as well as the cells of the nervous system).[8,9]

The presence of MSC in an organ initiates the production of immature antigen-presenting cells (APCs). These APCs, by eliminating T-cells or modulating them toward regulatory (CD4+ CD25+) phenotypes induce an anergy state.[10]

The absence of HLA class II antigens, as well as co-stimulatory molecules such as CD80 and CD81, is the main element of low immunogenicity of MSCs.[11] Therefore, due to their immunosuppressive/immunomodulatory potential MSCs play a stimulatory role as a cell factory in injured and inflamed tissues, downregulate the reactivity of activated immune cells and reduce tissue damage. MSCs promote tissue repair by differentiating into the injured cell types, compensating their lost and secreting trophic factors.[12]

In our present study, autologous MSCs were separated from bone marrow of arthritis patients, expanded *in vitro* and then injected into the patients' knee joints.

Animal experiments on MSCs in the prevention and treatment of experimental OA showed encouraging results.[13–14] A human experiment[15,16] by intra-articular injection of autologous MSCs showed good results after 6 months. The pain on a visual analogue scale (VAS) and the range of motion improved. Magnetic resonance imaging (MRI) showed a significant growth of articular cartilage and the regeneration of meniscus.

We present here the preliminary results, at 6 months, of four cases of knee OA treated with intra-articular injection of autologous MSCs.

PATIENTS AND METHODS

Ethics and registrations

The research carried out here with human subjects was in compliance with the Helsinki Declaration. It was approved by the Research Committee and the Ethical Committee of the Tehran University of Medical Sciences, and is registered under the ID 3087. It was then registered at ClinicalTrials.gov under the ID NCT00550524.

Patients

Two middle-aged men (55 and 65 years, AA and HM) and two middle aged woman (57 and 54 years, PZ and MS) were selected for the study. They had moderate to severe bilateral knee OA (mechanical pain of knees, aggravated with walking or climbing stairs, gelling pain, crepitus, restricted range of motion, limitation of joint motion, epiphyseal bony hypertrophy, and X-ray signs of joint space narrowing and osteophyte formation). They were fully explained about the procedure, and after their signed written consent, they entered the study. The following parameters were checked before MSC transplantation and at successive controls: pain on VAS, time to walk to produce pain, number of stairs to climb to produce pain, the resting time to induce the gelling pain, the range of motion, the instability (if existing) due to lateral and cruciate ligaments, patellae crepitus, and the presence of synovial fluid.

Sample collection and MSC expansion

Thirty milliliters of bone marrow were obtained from patients 3–5 weeks prior to injection. Using ficoll hypaque density gradient, the mononuclear cells of bone marrow were separated. Vented flasks (75 cm^2) with 21 mL MSC medium, consisting of Dulbecco's modified eagles medium (DMEM) with 10% of fetal bovine serum (FBS), were seeded with 1×10^6 mononuclear cells (MNCs)/mL for primary culture. Flasks were

incubated at 37°C in a humidity 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 reseeded at 1×104 cells/mL. When cells reached confluence by the end of 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 resuspended at a density of $1–2 \times 10^6$ 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. 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 (Milan, Italy). Flow cytometry was performed on a FacScan (Becton Dickenson, Franklin Lakes NJ, USA). Data were analyzed with cellquest software (http://flowcytometry.ualberta.ca/PDF/FACScan%20%20Setup.pdf).

Safety assessment

Bacteriological tests were performed on samples after each passage, and before any injection (to ensure non-contamination of samples). 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 $8–9 \times 10^6$ cells was prepared and injected in the selected knee of the patient. In each patient, the most painful knee, or the worst knee on physical examination, was selected as the site of injection. No previous preparation or premedication was given. All anti-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 first follow-up after the procedure was at 1 week, then every month up to 1 year. At each visit, all the parameters that were checked before the procedure were checked again. X-rays of both knees in standing position were taken at time 0, 6 months, and 1 year. X-rays were taken in the standard positioning to evaluate the overall state of OA.

RESULTS

Patient gender was respectively male (AA, 55 years), female (PZ, 57 years), male (HM, 65 years), and female (MS, 54 years). All were overweight. Their BMI was 28.5, 29.7, 30.2 and 37.1, respectively. They had their clinical symptoms respectively for 7, 15, 10 and 8 years. The walking time for the pain to appear was respectively 20, 0, 1 and 10 min. It improved to 25, 60 and 6 min for the first three patients. It did not change for the fourth patient. The number of stairs to climb for the pain to appear was respectively 5, 3, 1 and 8 stairs. It improved to 10, 70, 15 and 20 stairs. Another interesting parameter was the time of resting (sitting immobile) for the gelling pain to appear. It was 15, 15, 0 and 15 min respectively for the four patients. It became 30, 30, 0 and 15 min. The amount of pain on VAS (100-mm scale) was 90, 80, 90 and 85. It improved to 50, 40, 55 and 65. The amount of improvement was 44%, 50%, 39% and 24%. On joint examination, the physical parameters improved slightly, in comparison to subjective parameters. PZ and HM had limitation of extension of 15 and 10°. They each improved it by 5°. AA and MS had normal range of motion of their knees. At baseline, flexion was normal for them and remained the same at 6 months. For PZ and HM the flexion was only 90° for each. They each improved their flexion by 10°. Patellar crepitus at baseline was mild at 1+, 4+, 3+, and 4+ respectively. The crepitus disappeared for AA, and improved to 1+ for the three others. No patients had instability of knees at baseline and at 6 months follow-up. HM had a mild swelling of the knee at baseline, which disappeared at 6 months. X-rays before the procedure showed a 2+ to 3+ (moderate, to moderate-severe) OA. 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. Table 1 shows the parameters of the knees (transplanted and the opposite knee) of the four patients at baseline and at 6 months.

Table 1 Baseline and follow-up parameters of the four patients

	Mr. AA (baseline)		Mr. AA (6 months)		Ms. PZ (baseline)		Ms. PZ (6 months)		Mr. HM (baseline)		Mr. HM (6 months)		Ms. MS (baseline)		Ms. MS (6 months)	
Age (years)	55				57				65				54			
Duration of OA (years)	7				15											
BMI	28.5				29.7				25.71				37.1			
Walking time†	20		25		0		60		1		6		10		10	
Number of stairs‡	5		10		3		70		1		15		8		20	
Rest time to gelling pain§	15		30		15		30		0		0		15		15	
Pain on VAS	90		50		80		40		90		55		85		65	
Knee	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
MSC transplant	Yes				Yes				Yes				Yes			
Flexion contracture	–	–	–	–	10°	15°	5	10	10	5	5	10	–	–	–	–
Flexion¶	140°	140°	140°	140°	80°	90°	80°	100°	90°	120°	100°	120°	140°	140°	140°	140°
Crepitus	1+	1+	–	–	–	4+	–	1+	3+	2+	+	+	4+	3+	3+	1+
Swelling	–	–	–	–	–	–	±	–	±	–	–	–	–	–	–	–
Instability	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

†Time to walk for the pain to appear (min). ‡Number of stairs to climb for the pain to appear. §Time to rest for gelling pain to appear (min). ¶Normal flexion: 140°. OA, osteoarthritis; BMI, body mass index; VAS, visual analogue scale; MSC, mesenchymal stem cell.

DISCUSSION

This is the second report of a human clinical trial for knee OA, showing that intra-articular injection of expanded MSCs is a safe procedure without any complication. The first report was on one case of knee OA; this report is on four cases. This project being a pilot study, no controls were selected for comparison. A randomized controlled trial (RCT) will be undertaken after enough clinical experience on more patients is obtained. The results reported here are preliminary reports at 6 months only. Patients will be followed further and results on longer follow-up will be given later.

Results of the four patients, 6 months after MSC transplantation, are encouraging, but not excellent. Perhaps one of the reasons is the stage of the OA. Animal experiments on experimental autoimmune encephalomyelitis (animal model of multiple sclerosis) demonstrated that the best results were obtained at the beginning of the disease, and then, at the peak of attacks,[17] which can be interpreted as essentially preventive action rather than curative action of the procedure.

In our patients, subjective parameters improved highly with MSC transplantation, while physical parameters improved much less. We did not find any improvement on X-rays. The better improvement of subjective parameters may greatly be explained by the placebo effect of the procedure. Future RCT will clarify this matter. However, the mild improvement of objective signs cannot be explained by the placebo effect. The global improvement of subjective parameters was for both knees, also suggesting a possible central role of MSCs.

The results obtained by Centeno *et al.*[16] on their unique case were much better than our results, perhaps due to their procedure (injection of platelets and hematopoietic stem cells as well as MSCs). However, the growth of MSC in culture shows fibroblast-like cells. As Centeno *et al.* report, we should be sure whether this is a fibroblast-like cells or true growth of cartilage.

The main problem is to find the required number of cells for injection, in order to have the optimal response. Whether one injection will be enough, or more than one in a time period to reach the desired result, is another question to be answered in future works.

In conclusion, we can consider that MSC therapy could improve the patient's conditions, as shown by

these preliminary results. Before starting the general use of MSCs as a new way of treatment, the exact role of MSC therapy in the management of OA should be clarified. For that, further investigations are necessary. We certainly need more experience at a large scale to determine: (i) the required cellular dose; (ii) the number and the timing of injections; (iii) the proper use of co-stimulators; (iv) the determination of the best cell subtypes; (v) the stage of the disease to select for MSC transplantation (as shown in other studies, if we treat the patients earlier and in a better clinical condition the results may be much better); and finally (vi) a non-invasive way for labeling and tracing MSC cells after injection.

ACKNOWLEDGMENT

The authors thank Sina Cell for providing the facilities for MSC culture. Grant: Tehran University of Medical Science. ID:3087. ClinicalTrials.gov ID: NCT00550524.

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