

The Effect of Intra-articular versus Intravenous Injection of Mesenchymal Stem Cells on Experimentally-Induced Knee Joint Osteoarthritis

Aya Mostafa, Horeya E. Korayem, Ereny Fekry, Somaya Hosny

Department of Histology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Abstract

Background: Osteoarthritis (OA) is a chronic degenerative debilitating disease, primarily affects joints, particularly weight-bearing areas. The surface layer of the articular cartilage breaks down and wears away leading to rubbing of bones, pain, swelling, and joint stiffness. **Aim and Objectives:** This study investigates the possible therapeutic effects of intra-articular versus intravenous injection of umbilical cord blood mesenchymal stem cells (UCB-MSCs) against mono-iodoacetate-induced OA of the knee joints in male albino rats, using histological and immunohistochemical techniques. **Materials and Methods:** Thirty male adult albino rats were randomized into five groups as follows: Group (I) and (II): Served as control. Group (III): Osteoarthritic group. Group IV: Osteoarthritic and intraarticularly-injected MSCs. Group V: Osteoarthritic and intravenously-injected MSCs. Animals were sacrificed 1 month after stem cell injection, the right knee was prepared for histological techniques (Hematoxylin and Eosin and Toluidine blue stains) and immunohistochemical technique (Bax stain). Prussian blue stain was used to assess homing of MSCs in Groups IV and V. **Results:** Knee joint surface was irregular, fissured, and fragmented in Group III. In Groups IV and V, affected area was filled with newly formed tissue. Toluidine blue showed a decrease in matrix staining in Group III compared to both control and MSCs-treated groups. Chondrocytes in Group III showed strong Bax immunoreactivity and this reaction decreased in Group IV and V; however, Group V immunoreactivity was more than Group IV. Prussian blue stain showed labeled UCB-MSCs in many chondrocytes in Group IV and few chondrocytes in Group V. **Conclusion:** Intraarticularly-injected UCB-MSCs showed better healing of knee OA than intravenously-injected UCB-MSCs.

Keywords: Animal model, mesenchymal stem cells, mono-iodoacetate, osteoarthritis

INTRODUCTION

Osteoarthritis (OA) is characterized by progressive loss of joint motion, deformities, and degenerative changes.^[1,2] The surface layer of articular cartilage breaks down and wears away, which causes rubbing of bone, pain, swelling, and joint stiffness.^[3]

There are surgical and chemical models of OA. Surgical models need skillful surgical intervention.^[4,5] However, chemical models include noninvasive procedures through single knee joint injections of immunotoxins, inflammatory agents, papain, collagenase, or mono-iodoacetate (MIA).^[6,7] Intra-articular injection of MIA disturbs chondrocyte glycolysis by

inhibiting glyceraldehyde-3-phosphatase dehydrogenase. Neovascularization, chondrocyte death, subchondral bone necrosis, and inflammation can also occur.^[8]

Mesenchymal stem cells (MSCs) are an attractive alternative for the treatment of OA due to their trophic, immunomodulatory, and anti-inflammatory effects.^[9] Intra-articular injection of MSCs may become a new cell therapy for OA.^[10] It helps in cartilage regeneration of animals subjected to MIA treatment.^[11]

Address for correspondence: Prof. Somaya Hosny,
Department of Histology, Faculty of Medicine, Suez Canal University,
Ismailia, Egypt.
E-mail: Somaya@med.suez.edu.eg, Somaya@hotmail.com

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Intravenous injection of MSCs showed therapeutic effects, varying from significant improvement to no effect, and hence, its results remain inconclusive.^[12]

Umbilical cord blood-derived MSCs (UCB-MSCs) are a promising alternative to bone marrow as they are abundant, easy to be harvested, and noninvasive.^[13]

Most of the previous studies have investigated articular cartilage repair using bone marrow-derived MSCs and not UCB.^[14] Moreover, up to our knowledge, no available histological studies compared the effect of intra-articular versus intravenous injection of UCB-MSCs on MIA-induced OA.

MATERIALS AND METHODS

Thirty adult male albino rats weighing 180–250 g were used in this study. Rats were randomized into five groups as follows:

Group I (control I): animals of this group received single intra-articular injection of 50 µl of physiological saline in the right knees.^[15] Group II (control II): received single intravenous injection of 50 µl physiological saline in their tails. Group III: osteoarthritic group; animals received single intra-articular injection of 1 mg of MIA dissolved in 50 µl physiological saline in the right knees.^[16] Group IV: (osteoarthritic and intraarticularly-injected MSCs); animals received single intra-articular injection of 1 mg of MIA dissolved in 50 µl physiological saline in the right knee. Seven days later, they received single intra-articular injection of 10⁶ UCB-MSCs in 50 µl of phosphate-buffered saline (PBS) in the right knees.^[17] Group V: (osteoarthritic and intravenously-injected MSCs); animals received single intra-articular injection of 1 mg of MIA dissolved in 50 µl physiological saline in the right knees. Seven days later, they received single intravenous injection of 10⁶ UCB-MSCs in 50 µl of PBS in their tails.

Procedure of intra-articular injection^[16]

1. Animals were anesthetized using ketamine (40 mg/kg)
2. Their right knees were shaved and sterilized using 70% ethyl alcohol
3. The right knees were flexed at a 90° and injected through the infrapatellar ligament using a syringe with a 30-G needle.

Steps of isolation and culture of stem cells

1. Human UCB was collected from normal volunteers after taking their written informed consent at the Obstetric Emergency Room of Suez Canal University Hospitals. Blood collection was made before the expulsion of the placenta. The umbilical vein was cleansed with alcohol followed by betadine. Then, the blood was collected in 50 ml graded sterile plastic Falcon tubes each contains 10 ml of citrate phosphate dextrose anticoagulant^[18]
2. The culture media were prepared as follows; 500 ml MEM + 50 ml FBS + 5 ml Penicillin-Streptomycin^[19]
3. Workplace preparation: The laminar flow and the CO₂ incubator were swabbed with Ethyl Alcohol (70%)

4. Processing of UCB samples: UCB sample was diluted in a proportion of 1:1 in a Dulbecco's PBS(Biowest). The diluted UCB sample was layered into sterile centrifuge tube containing Ficoll-Hypaque solution. After centrifugation, the buffy coat containing the low-density mononuclear cells (MNCs) was isolated
5. MNCs were cultured in flasks, then 5 ml of prepared culture media were added to each flask, incubated in the CO₂ incubator at 37°C and humidified atmosphere containing 5% CO₂. After overnight incubation, nonadherent cells were removed, and fresh media were added to the culture flask, media were changed every 3 days and cellular growth assessed continuously under an inverted microscope
6. For labeling of MSCs, each flask of cultured cells was injected once with 1 ml of superparamagnetic iron oxide (SPIO) contrast agent 25 µg Fe/ml (feridex) (Bayer Health Care Pharmaceuticals). Then, flasks were incubated at 37°C/5% CO₂ for 24 h^[20]
7. For the subculture of MSCs; when the Feridex-labeled MSCs reached 80% confluence, the media were discarded from the flask. Adherent cells were washed twice with pre-warmed PBS to remove any SPIO, and then the wash solution was discarded. Trypsin was added to each flask for cellular detachment. Then, cell suspension was collected in falcon tubes and then was centrifuged.

The cell pellet was counted using automated counting device, where the nucleated cell count/ml = No. × 10 (as No. = The number of nucleated cells counted by the automated counter).^[19]

After 1 month of stem cells injection, the animals in all groups were anesthetized using ketamine and sacrificed by decapitation. Specimens from the right knee (lateral femoral and tibial condyles with subchondral bone) were cut by a sharp blade and then were fixed in 10% neutral buffered formalin for 2 days then put in 10% EDTA for 2 weeks.^[21] The solution was changed every 3 days until the softening of the specimens was achieved. Dehydration through graded alcohol and clearing with xylene was done. Then, specimens were embedded in paraffin and 5 µm sagittal sections were obtained. Sections were stained by Hematoxylin and Eosin (H and E) for demonstration of the general architecture of the articular joint,^[22] and toluidine blue for demonstration of the cartilaginous proteoglycan (PG) content of the articular cartilage of the knee joint.^[23] Immunohistochemistry for downregulation of the protein expressed by Bax (pro-apoptotic protein marker) was assessed to clarify the apoptosis of the chondrocytes.^[24] Qualitative and quantitative assessments were performed. Qualitative assessment was conducted by examining ten serial sections from each animal at high power field (×400 and ×1000) to look for surface irregularity, chondrocytes shape (shrunken – disorganized), nuclei (pyknotic, karyorrhectic, karyolytic), cytoplasm (pale, vacuolated), tidemark (incomplete or absent), and subchondral bone (erosions or cracks).

Quantitative measurements were done on 5- μ m thick sections using an image J-based analysis system. Three sections per animal were used to assess the following: (a) the mean articular cartilage thickness in toluidine blue-stained sections. (b) The mean optical density for the PG content of the articular cartilage matrix in the toluidine blue-stained sections, and (c) the pro-apoptotic immunoreactivity of chondrocytes in BAX-immunohistochemical-stained sections.

To assess the homing of MSCs in cell-treated groups (Groups IV and V), the slides were stained with Prussian blue.

All experimental procedures of animals used for the current study were approved by the Ethical Committee of the Faculty.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 20 software, IBM Corporation). A one-way analysis of variance test was performed to compare between the groups followed by *post hoc* test. The studied groups' differences were only statistically significant when $P < 0.05$.

RESULTS

Examination of H and E stained knee joint sections of the animals of the two control groups showed normal articular cartilage. The articular surface was intact and smooth. The chondrocytes in the superficial zone had a small elongated, elliptic, and flattened shape, with the long axis parallel to the surface. Those located deeper (in the transitional zone) were nearly spherical or rounded [Figure 1a and b]. Some cells were found in small groups forming cell nests [Figure 2a and b]. H and E stained sections of the knee joint from group III showed

marked histopathological changes. The knee joints of all animals (100%) [Table 1] in this group showed thin irregular fibrillated articular cartilage [Figure 1c]. The normally arranged four zones could not be seen in osteoarthritic cartilage. Most of the animals (83%) showed chondrocytes with vacuolated cytoplasm and shrunken/pyknotic or karyorrhectic nuclei [Figure 2c]. The tidemark was incomplete or absent in many animals (67%) [Table 1] and the upper part of subchondral bone showed few erosions and vertical cracks in 83% of the animals [Figure 2c]. Group IV (osteoarthritic and intraarticularly-injected MSCs) showed an almost smooth and intact articular surface in many animals (67%) of this group [Figure 1d], the rest of the animals had some surface irregularities. Chondrocytes, with basophilic cytoplasm and round open face nuclei, were found inside their lacunae in addition to cell nests similar to control groups [Figure 2d]. Few cells with pyknotic nuclei were seen in only 17% of the animals. Tidemark and subchondral bone had nearly normal appearance in all animals of this group [Figure 2d]. In Group V (osteoarthritic and intravenously-injected MSCs), many animals (67%) had irregularities of the articular surface [Figure 1e] and 50% of the animals, had pyknotic nuclei in many chondrocytes [Figure 2e]. Chondrocytes with vacuolated cytoplasm were also seen in some animals (33%). Tidemark was incomplete in some animals (50%) and subchondral bone showed vertical cracks [Figure 2e]. The rest of the animals (33%) showed nearly normal articular cartilage appearance.

Toluidine blue sections of the control group showed homogenously deep blue stained matrix of the articular cartilage. The extracellular matrix (ECM) showed densely stained areas closely surround the chondrocytes (territorial

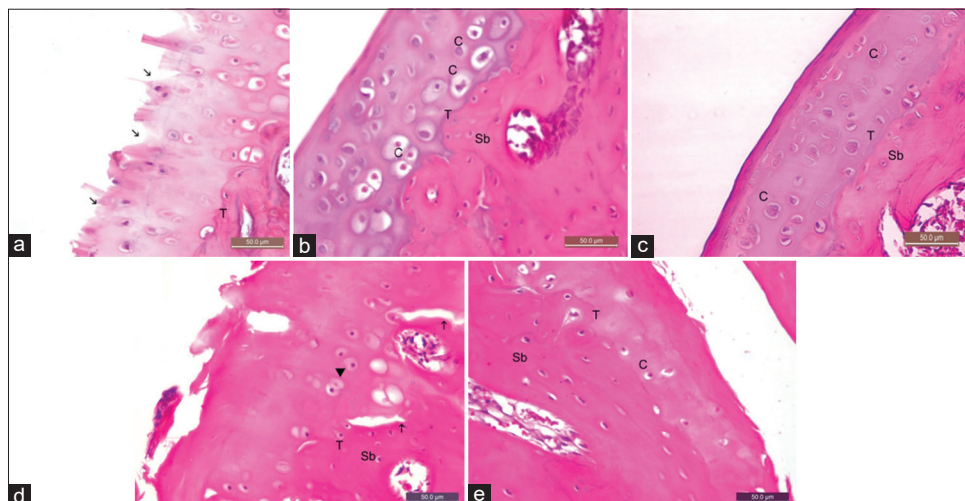


Figure 1: (a) A photomicrograph of knee joint from control Group A showing chondrocytes (C), tidemark (T) and subchondral bone (H and E, $\times 400$). (b) A photomicrograph of knee joint from control Group B showing chondrocytes (C), tidemark (T) and subchondral bone. (H and E, $\times 400$). (c) A photomicrograph of knee joint from osteoarthritic group showing disorganized irregular articular surface (\blacktriangleright). Incomplete tidemark is also shown (T). (H and E, $\times 400$). (d) A photomicrograph of knee joint from osteoarthritic and intraarticularly-injected mesenchymal stem cells group showing almost normal chondrocytes (C), tidemark (T) and subchondral bone. (H and E, $\times 400$). (e) A photomicrograph of knee joint from osteoarthritic and intravenously-injected mesenchymal stem cells group showing irregular surface. Chondrocytes have pyknotic nuclei (\blacktriangle). Tidemark (T) is incomplete and subchondral bone showed vertical cracks [\uparrow] (Hand E, $\times 400$)

zone) and the stain showed decreased intensity in between the chondrocytes (inter-territorial zone) [Figure 3a and b]. Toluidine blue sections of the osteoarthritic group showed thin articular cartilage in comparison to control groups [Table 3]. The articular surface was fibrillated and irregular. The blue-stained matrix of the articular cartilage was less intense than that of the control groups [Figure 3c]. Osteoarthritic and intraarticularly-injected MSCs group showed that the newly formed matrix in the upper part of the cartilage had less blue staining intensity than that in the lower part [Figure 3d]. The same finding was detected in the osteoarthritic and intravenously-injected MSCs group [Table 2 and Figure 3e].

BAX immunostaining of Group I and II showed almost negative immunoreactivity in the cytoplasm of chondrocytes [Figure 4a and b]. Osteoarthritic group showed a strong positive brownish color reaction in the

cytoplasm of chondrocytes [Figure 4c]. In Osteoarthritic and intraarticularly-injected MSCs group, there was a decrease in the brown cytoplasmic immunoreactivity of the chondrocytes compared to osteoarthritic group [Figure 4d]. The immunoreactivity in the cytoplasm of the chondrocytes in the osteoarthritic and intravenously-injected MSCs group was slightly less than osteoarthritic group and more than osteoarthritic and intraarticularly-injected MSCs group [Table 2 and Figure 4e].

Prussian blue stain (of iron oxide-labeled cells) for detecting homing of labeled UCB-MSCs in Group IV and V showed the labeled UCB-MSCs in the form of intracellular blue staining in many chondrocytes in osteoarthritic and intraarticularly-injected MSCs group [Figure 5a], and in few chondrocytes in osteoarthritic and intravenously-injected MSCs group [Figure 5b].

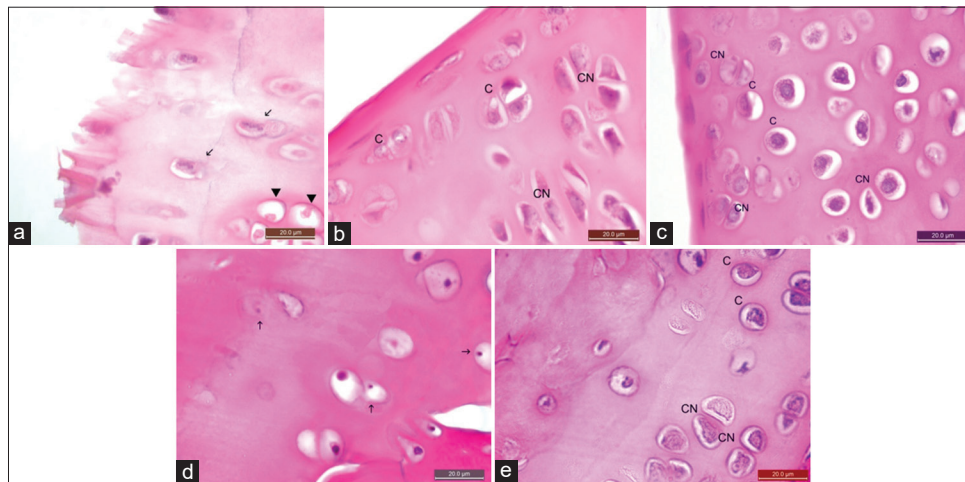


Figure 2: (a) A photomicrograph of knee joint from control (A) group showing many chondrocytes (C), with open face nuclei and clear basophilic cytoplasm, inside their lacunae. Cell nests are also shown (H and E, ×1000). (b) A photomicrograph of knee joint from control (b) group showing many chondrocytes (C), with clear basophilic cytoplasm, inside their lacunae. Cell nests are also shown (H and E, ×1000). (c) A photomicrograph of knee joint from osteoarthritic group showing chondrocytes with karyorrhectic nuclei (↑). Some chondrocytes showing vacuolated cytoplasm with shrunken nuclei (▲) (H and E, ×1000). (d) A photomicrograph of the knee joint from osteoarthritic and intraarticularly-injected mesenchymal stem cells group showing almost normal chondrocytes, with open face nuclei and basophilic cytoplasm, inside their lacunae (C). Cell nests are also shown (H and E, ×1000). (e) A photomicrograph of the knee joint from osteoarthritic and intravenously-injected mesenchymal stem cells group showing many chondrocytes with pyknotic nuclei (↑) (H and E, ×1000)

Table 1: The frequency distribution of the histopathological changes in the articular cartilage in the different experimental groups

Groups	Histopathological changes				
	Surface irregularity (%)	Chondrocytes		Incomplete/absent tide mark (%)	Subchondral bone changes (%)
		Pale/vacuolated cytoplasm (%)	Pyknotic/karyolytic/karyorrhectic nuclei (%)		
Group I (control A)	0	0	0	0	0
Group II (control B)	0	0	0	0	0
Group III (osteoarthritic)	100* ⁺	83* ⁺	83* ⁺	67	83* ⁺
Group IV (OA and intraarticularly-injected MSCs)	33 [#]	17	17	17	0 [#]
Group V (OA and intravenously-injected MSCs)	67	33	50	50	33

OA: Osteoarthritis, MSCs: Mesenchymal stem cells. *Statistically significant compared to control A group, ⁺Statistically significant compared to control B group, [#]Statistically significant compared to osteoarthritic group

DISCUSSION

H and E stained sections in osteoarthritic group, in the current study, showed irregularity and fibrillation of the articular surfaces. Many chondrocytes showed degenerative/necrotic changes. The tidemarks were incomplete/absent and subchondral bone showed erosions with vertical cracks. These results are consistent with those of Kim *et al.*^[25] study in which rats were received 2.5 mg MIA injected into the infrapatellar ligament of the knee joints resulting in severe irregular abrasions of the articular surfaces with rough edges around the femur and the tibia. Furthermore Jacer *et al.*^[26] reported articular surfaces fibrillations, decreased cartilage thickness and loss of tidemarks integrity in the knee joints of osteoarthritic rats, 6 weeks after OA induction by intra-articular injection of collagenase II.

In our study, there was a significant decrease in the cartilage thickness and the optical density of matrix PG in toluidine

blue-stained sections in the osteoarthritic group compared to other groups. These results were supported by Niu *et al.*^[27] who found, after surgical transection of the anterior cruciate ligaments in the femorotibial joints of rabbits that the cartilage thickness decreased in addition to fading or totally disappearance of the toluidine blue stain from the surface to the deep layers as OA grade increases.

The BAX immunohistochemical-stained sections of osteoarthritic group showed the strong brownish color reaction of the chondrocytes compared to other groups.^[28] also found significantly increased levels of BAX-positive cells in rats of the surgically-induced osteoarthritic group than other groups after 16 weeks of modeling. Our BAX results could be explained by the fact that MIA caused apoptosis of chondrocytes via the mitochondrial pathway,^[29] as BAX is a promoting cell-apoptotic factor.^[30,31] The infiltration of inflammatory cytokines into the chondrocytes leads to a decrease of mitochondrial membrane potential due to the

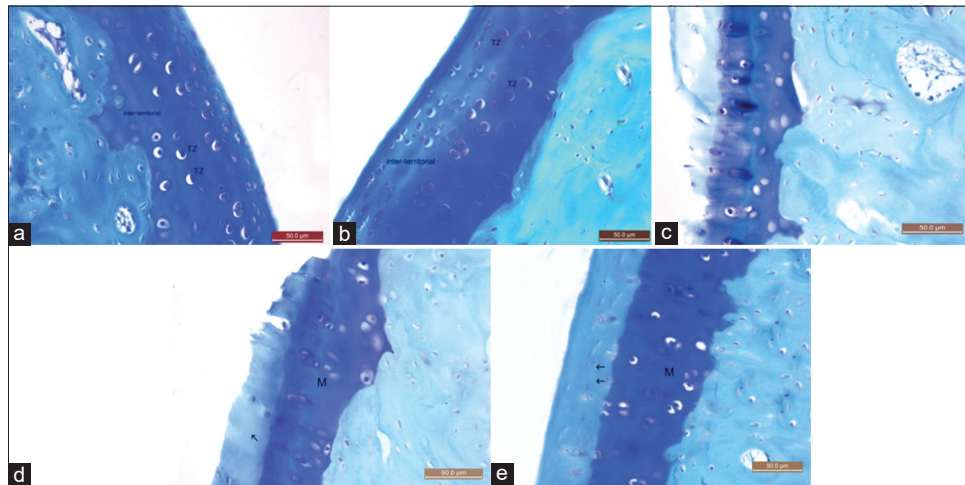


Figure 3: (a) A photomicrograph of knee joint from control (A) group showing homogenously deep blue articular cartilage. The territorial zones are more densely stained than inter-territorial zones (Toluidine Blue, $\times 400$). (b) A photomicrograph of knee joint from control (B) group showing homogenously deep blue articular cartilage. The territorial zones are more densely stained than interterritorial zones (Toluidine Blue, $\times 400$). (c) A photomicrograph of the knee joint from osteoarthritic group showing thinning of the articular cartilage compared to control groups. The blue matrix staining is less than control groups (Toluidine Blue, $\times 400$). (d) A photomicrograph of knee joint from osteoarthritic and intraarticularly-injected mesenchymal stem cells group showing that the blue staining of the newly formed matrix in the upper part (\uparrow) of the cartilage is less intense than that in the lower part (M) (Toluidine Blue, $\times 400$). (e) A photomicrograph of knee joint from osteoarthritic and intravenously-injected mesenchymal stem cells group showing that the blue staining of the newly formed matrix in the upper part (\uparrow) of the cartilage is less intense than that in the lower part (M) (Toluidine Blue, $\times 400$)

Table 2: Mean optical density of proteoglycan content in toluidine blue and immunoreactivity of BAX - immunohistochemical stained sections in different study groups

Group	Mean \pm SD	
	Density of toluidine blue	Density of BAX
Group I (control group A)	73.15 \pm 7.26	0.44 \pm 0.22
Group II (control group B)	67.63 \pm 8.34	0.56 \pm 0.15
Group III (osteoarthritic group)	14.57 \pm 10.14* ⁺	43.96 \pm 9.75* ⁺
Group IV (osteoarthritic and intraarticularly-injected MSCs)	60.80 \pm 9.45 [#] _S	7.86 \pm 3.19 [#] _S
Group V (osteoarthritic and intravenously-injected MSCs)	39.16 \pm 9.37 ^{+,#} _S	23.55 \pm 8.23 ^{+,#} _S

SD: Standard deviation, MSCs: Mesenchymal stem cells, BAX: Bcl-2-associated X protein. *Statistically significant compared to control A group ($P=0.000$). ⁺Statistically significant compared to control B group ($P=0.000$). [#]Statistically significant compared to osteoarthritic group ($P=0.000$). _SStatistically significant difference between intra-articular and intra-venous MSCs groups ($P=0.000$)

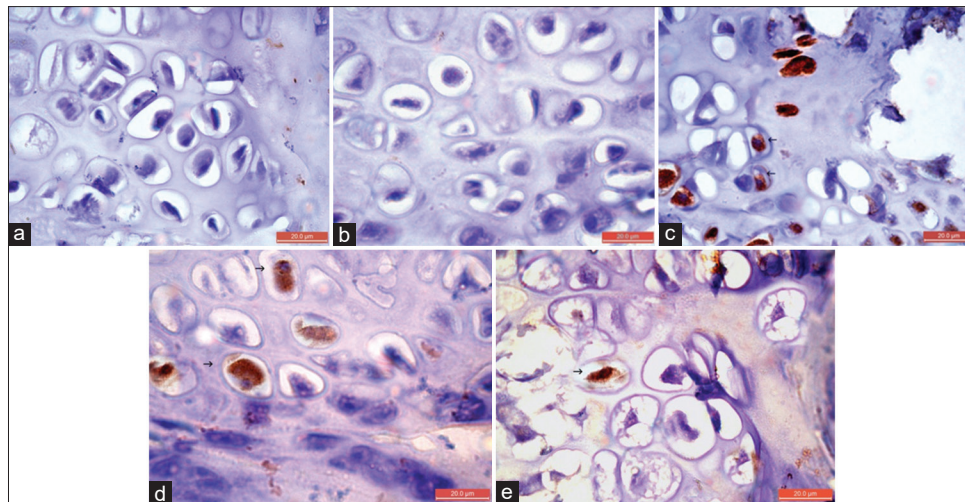


Figure 4: (a) A photomicrograph of knee joint from control (A) group showing almost negative cytoplasmic immunoreactivity of chondrocytes (BAX immunostaining, $\times 1000$). (b) A photomicrograph of the knee joint from control (B) group showing almost negative cytoplasmic immunoreactivity of chondrocytes (BAX Immunostaining, $\times 1000$). (c) A photomicrograph of the knee joint from osteoarthritic group showing strong cytoplasmic immunoreactivity of the chondrocytes in the form of brownish color reaction (\uparrow). (BAX immunostaining, $\times 1000$). (d) A photomicrograph of the knee joint from osteoarthritic and intraarticularly-injected mesenchymal stem cells group showing decreased brownish cytoplasmic immunoreactivity of chondrocytes compared to the osteoarthritic group (\uparrow). (BAX immunostaining, $\times 1000$). (e) A photomicrograph of the knee joint from osteoarthritic and intravenously-injected mesenchymal stem cells group showing brownish cytoplasmic immunoreactivity of chondrocytes slightly less than osteoarthritic group and more than intraarticularly-injected mesenchymal stem cells group (\uparrow) (BAX immunostaining, $\times 1000$)

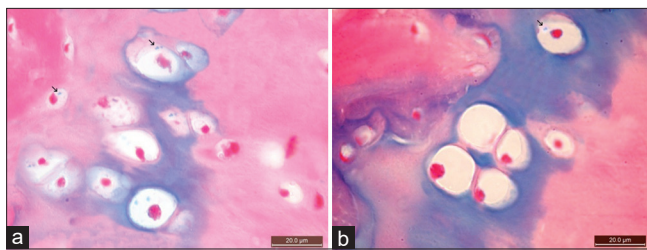


Figure 5: (a) A photomicrograph of the knee joint from osteoarthritic and intraarticularly-injected mesenchymal stem cells group showing iron oxide-labeled human umbilical cord blood-mesenchymal stem cells which appear as intracellular blue staining in the form of dots in many chondrocytes (\uparrow) (Prussian blue, $\times 1000$). (b) A photomicrograph of the knee joint from osteoarthritic and intravenously-injected mesenchymal stem cells group showing iron oxide-labeled human umbilical cord blood-mesenchymal stem cells which appear as intracellular blue staining in the form of dots in few chondrocytes (\uparrow) (Prussian Blue, $\times 1000$)

opening of small holes in the inner mitochondrial membrane. This triggers cyt-c (proapoptotic) migration from mitochondria to the cytoplasm, leading to the formation of complex apoptosome. The formed apoptotic body caused activation of caspases, resulting in chondrocyte apoptosis,^[32,33] which was showed as increased levels of BAX-positive cells in our results.

In osteoarthritic and intraarticularly-injected MSCs (IV) group, H and E stained sections showed almost smooth and intact articular surfaces. Chondrocytes, tidemark, and subchondral bone appeared similar to those of the control groups.^[11] It was demonstrated that the intraarticularly injected HUC-MSCs 7 days after induction assisted MIA-treated chondrocytes in recovering from impaired proliferation and increased apoptosis and helped in regeneration of hyaline cartilage.

In our study, few animals of this group showed surface irregularities and few pyknotic nuclei. These findings could be explained by the possibility that MSCs might deposit in the joint space without attaching to the surface of the injured cartilage. Furthermore, the mobility of the joint might damage MSCs affecting their biological activity and reduce their ability of cartilage repair.^[34,35]

In Toluidine blue-stained sections of this group, we found almost intact articular surfaces and increased blue staining of the matrix. There was a statistically significant increase in the optical density of PG content and articular cartilage thickness compared to the osteoarthritic group.

These results are consistent with those of Miki *et al.*^[36] study in which a surgically-induced cartilage defect was created in the medial femoral condyle of dogs' knees. They found marked PGs accumulation and a considerable amount of chondrocyte-like cells after combined intra-articular injection of synovium-derived MSCs and hyaluronic acid 7 weeks postsurgery. These results could be due to differentiation potential of the intraarticularly-injected stem cells when these cells differentiate into chondrocytes, they change their morphology, express chondrogenic differentiation markers, and produce an ECM that contains acidic PGs, which stain positive for toluidine blue, the positive blue areas and intensity of toluidine blue staining indicated the existence of acidic PGs.^[37]

BAX immunohistochemical-stained sections of this group, in our study, showed a significant decrease in the immunoreactivity of the chondrocytes compared to the osteoarthritic group. This can confirm the decrease in the apoptotic activity observed in the histopathological analysis of the cartilage in this group.

Table 3: Mean articular cartilage thickness in different experimental groups in toluidine blue stained sections

Group	Mean ± SD
Group I (control Group A)	226.4101±33.044
Group II (control Group B)	218.31±31.505
Group III (osteoarthritic group)	85.613±14.337* ⁺
Group IV (osteoarthritic and intraarticularly-injected MSCs)	200.41±38.055 ^{#,§}
Group V (osteoarthritic and intravenously-injected MSCs)	142.46±26.914 ^{+,§,5}

*Statistically significant compared to control A Group ($P=0.000$),

⁺Statistically significant compared to control B Group ($P=0.000$),

[#]Statistically significant compared to osteoarthritic group ($P=0.000$),

[§]Statistically significant difference between intra-articular and intravenous MSCs groups ($P=0.000$). SD: Standard deviation, MSCs: Mesenchymal stem cells

MSCs have been shown to prevent or reduce apoptosis in a variety of *in vitro* or *in vivo* models. Production of stromal cell-derived factor-1 alpha (SDF-1 alpha) and secreted frizzled-related protein 2 was reported to participate in the antiapoptotic function of MSCs.^[38]

In osteoarthritic and intravenously-injected MSCs (V) group, H and E stained sections of some animals showed irregularities of the articular surfaces and many chondrocytes had pyknotic nuclei. However, the rest of the animals showed nearly normal articular cartilage appearance. Our results are in concordance with many studies that used MSCs for the treatment of systemic arthritis.^[39] It was demonstrated that intravenous infusion of MSCs, significantly reduced the severity of systemic collagen II-induced arthritis, 35 days post infusion in the injected mice than PBS-treated mice.^[40] It was mentioned that MSCs can exert systemic anti-inflammatory effects following IV administration in inflammatory disorders such as OA.

However, MSCs have been given intravenously in animal models of autoimmune arthritis and lead to different therapeutic effects, varying from significant improvement to no effect.^[12] This could be explained by some studies which reported that MSCs didn't migrate into the joints, but they found to be located in the spleen.^[41,42]

In Toluidine blue-stained section, in this group of our study, irregularities of the articular surface were seen. The blue staining of the matrix and the optical density of PG content and the articular thickness were slightly increased compared to the osteoarthritic group. This may be explained by the intravenous route of administration and the resulting few cells detected in the affected joints, which lead to chondrogenic differentiation and increase matrix PG content.

BAX immunoreactivity of the chondrocytes in this group was slightly less than the osteoarthritic group and more than intraarticularly-injected MSCs group. Our findings were similar to those of Hussien *et al.*^[43] who showed a significant decrease in Bax immunoreaction in islets of Langerhans of diabetic rats after two successive intravenous injections of MSCs (2 weeks apart) compared to diabetic group. This could

be explained by the antiapoptotic and anti-inflammatory effects of MSCs. Many studies reported similar findings.^[44,45]

CONCLUSION

Intraarticularly-injected UCB-MSCs showed better healing of knee OA than intravenously-injected UCB-MSCs. Therefore, UCB-MSC therapy might be used as a therapeutic agent in osteoarthritic patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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