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Research Article

Induced Knee Osteoarthritis: Preventive Effects of Wharton-Jelly Mesenchymal Stem Cell Conditioned Medium, Captopril and Losartan in Male Rats

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Abstract

Background: Osteoarthritis (OA) is a degenerative joint disease that affect different parts of a synovial joint leading to pain and stiffness.

Methods: Forty male rats (220 ± 20g, aged 10 - 12 weeks), were randomly divided into eight groups (n = 8).

OA: Anterior cruciate ligament transection (ACLT) + PBS.

OA+ Hyaluronic acid (HA): ACLT+ treatment with HA.

OA+ Captopril (Cap): ACLT + treatment with Cap.

OA+ Losartan (Los): ACLT + treatment with Los.

OA+ Wharton-jelly mesenchymal stem cell conditioned medium (WJ): ACLT + treatment with WJ.

OA+ WJ+ Cap.: ACLT + treatment with WJ.

OA+ WJ+ Los.: ACLT + treatment with WJ and losartan.

Sham: No ACLT+ PBS.

Osteoarthritis was induced through transection of the anterior cruciate ligament of both knees in rats. Three months after treatment, the samples were harvested and evaluated by histopathological, radiological and ACE activity analyses.

Result: Histopathological and radiological findings indicated significant differences between the WJ+Cap and WJ+Los treated groups with OA+Cap OA+Los, the control and OA+HA groups ($p \leq 0:001$). Significant differences were observed in the subchondral bone scores between WJ-CM+Cap, WJ+Los and WJ groups and OA+Cap, OA+Los, OA+HA groups ($p \leq 0:001$). compared to WJ group alone, co-treatment of WJ and renin-angiotensin system (RAS) inhibitors (WJ+Cap and WJ+Los) showed better results regarding matrix scores ($p \leq 0:001$).

Conclusion: The group treated with WJ concomitant with RAS inhibitor drugs showed better outcomes than other groups in histopathological, radiological and angiotensin converting enzyme (ACE) activity evaluation.

Keywords: *Osteoarthritis; Mesenchymal Stem Cell; Conditioned Medium; Captopril; Losartan*

Introduction

Osteoarthritis (OA), a degenerative disease of synovial joints [1] is a progressive degradation and erosion of articular cartilage which affect all the compartments of the synovial joint including the synovium, meniscus (in the knee), periarticular ligaments, and subchondral bone [2]. Compared to other forms of joint diseases including rheumatoid arthritis (RA), OA is far more common [3]. There are two forms of osteoarthritis based on its etiology: primary (non-traumatic) and secondary (traumatic). The components of the renin-angiotensin system (RAS), such as renin-angiotensin converting enzyme (ACE) [4-6], angiotensin II (Ang II) [7,8] and angiotensin receptor (ATR) [9,10] serve as regulators of blood pressure [11,12]. Recent studies indicated the expression of major components of RAS, including ACE, AT1R, and AT2R in synovial tissue in humans and animals suggesting their probable engagement in the pathogenesis of OA and rheumatoid arthritis (RA); their expression is in accordance to the degree of inflammation and the severity of arthritis [10,13-15]. It has been shown that inhibition of AT1R or ACE could enhance clinical symptoms through suppression of inflammatory factors [16-20] delaying the progression of OA. captopril, an ACE inhibitor drug and Losartan (inhibitor of AT1R) has shown to have chondroprotective effect through suppression of local RAS in a rat model of osteoarthritis [21]. It is noticeable that still no approved therapy or procedure has been introduced to mitigate destructive effects exerted by the OA to the joints. Current therapeutic options, such as physiotherapy, pain control with anti-inflammatory drugs have just the potential to relieve symptoms. While the ultimate cure for patients is total joint replacement with optimistic outcomes regarding mobility and pain alleviation, it still entails health-related risks such as thrombosis and infection and imposes heavy costs in terms of hospitalization and rehabilitation on the shoulder of these patients [22]. overall, an urgent need has emerged to investigate an effective and financially possible treatment. During the last decade, therapeutic role of mesenchymal stem cells (MSCs) for degenerative conditions, such as OA has attracted scientists attention [23]. In addition, evidence suggested that e trophic factors secreted by MSCs, including cytokines, growth factors, chemokines [24], microvesicles [25], exosomes [26] are responsible for MSCs promising therapeutic potential in tissue repair. Considering the fact that these secretions are presented in MSCs' culture medium (CM), which are also known as conditioned medium (CM) [27], it is better to use cell-free CM instead of MSCs. Umbilical cord stem cells, also known as Wharton's jelly mesenchymal stem cells (WJMSCs), have appeared as the first option for cartilage regeneration due to their availability, low immunogenicity and ease of collection [28]. Famian., *et al*. indicated that Wharton-jelly mesenchymal stem cell conditioned medium (WJ) can be used as a stimulating factor for cartilage regeneration as they increase the expression of cartilage-specific genes [29]. Another study showed that the IGF1-induced are capable to promote chondrogenesis, suggested by enhanced expression of SOX9 and COL2 and declined expression of ADAMTS1, ADAMTS5, MMP3, MMP1, and RANKL [30]. CM of IGF1-WJMSCs could also decrease inflammation in injured joint through interaction with human chondrocyte in an OA model [31]. In this regard, we recently indicated that WJ could alleviate renin-angiotensin system in animal model of diabetic nephropathy (In Press). Thus, in this study we examined preventive and paracrine effects of WJ accompanied by Losartan and Captopril (as inhibitors of renin-angiotensin system) in an ACLT induced osteoarthritis model.

Materials and Methods

Culture of Wharton-jelly mesenchymal stem cells (WJMSCs)

This study was conducted at Shiraz University of Medical Sciences, Shiraz, Iran, from January to April 2021. We used WJMSCs collected from 10 full-term infants after obtaining a written informed consent from parents as the source of MSCs [32]. The tissue samples were transferred to the lab in cold Phosphate-Buffered Saline (PBS) containing 100 U/mL penicillin, 100 µg/mL streptomycin (Sigma Aldrich, UK) and washed three times. Then, the arteries were removed, the umbilical vein was opened, and the endothelium was crushed using a sterile blade. Then, the umbilical cords were cut into small explants about 5 mm each and placed in the dishes. After 15 minutes, α-MEM (Gibco BRL, life technology, Germany) containing 10% Fatal Bovine Serum (FBS) (Gibco BRL), 1% L-glutamine (Sigma Aldrich, UK), and 100 U/mL penicillin, 100 µg/mL streptomycin were added to the culture plates. All procedures were approved by Shiraz University of Medical Sciences ethics committee with approval number: 9101445389.

The characterization of WJMSCs was showed by the International Society of Cell Therapy. The cells suspension was adjusted at a concentration of 1×106 cells/mL in 10% FBS/ PBS as the blocking solution for 20 minutes. Then, the cells were labelled with FITC-conjugated anti-CD90 and CD144, phycoerythrin-conjugated anti-CD34 and CD73 antibodies (all from Abcam, UK) for 30 min. The frequencies of positive cells were assessed by a FACS calibrated instrument (BD, USA) and analysed using FlowJo software (BD Biosciences). WJ-MSCs were induced to differentiate into the osteocytes and adipocytes by being exposed to osteogenic (MACS, Germany) and adipogenic media (Stem Cell Technologies Inc., Canada) for 4 and 3 weeks, respectively. The culture media were replaced twice a week. Then, the WJMSCs differentiated toward osteogenic lineage were fixed with 4% paraformaldehyde and stained with Alizarin Red S (Sigma, USA). To indicate

the adipogenic differentiation, the cells were stained by oil red O (Sigma, USA). For Preparation of Wharton jelly mesenchymal stem cell conditioned medium (WJ), 1×106 WJMSCs at third passage were seeded in T75 tissue culture flask. The confluent cells were fed with serum-free medium and cultured for 72h. The medium was collected and centrifuged at 3000g for 4 minutes at room temperature, and the supernatant was filtered by a 0.22-mm filter, used as conditioned medium (CM) of WJMSCs, and stored at -80°C.

Animal study design

Male Sprague-Dawley rats (n = 40), weighing 200 ± 20g, age eight to 10 weeks, bred in the central animal house of Shiraz University of Medical Sciences, Iran, were distributed in standard vivariums, fed standard chow and water *ad libitum*. The animals were housed under standard conditions (12h light/dark cycle, temperature 20 - 25°C, and humidity 55 ± 5%). the experimental procedures were confirmed by the institutional ethical committee for care and use of animals in Shiraz university of medical science with approval number: 9101445389. OA was induced using the ACLT method [33]. Animals were anesthetized with intraperitoneal injection of ketamine (40 mg/kg) and xylazine (10 mg/kg). After being shaved and disinfected, an incision was made in the left medial parapatellar to expose the knee joint, and the joint capsule was cut to reveal the joint cavity. Next, the knee joint was flexed to expose the anterior cruciate ligament (ACL) as far as possible. Then, the ACL was disconnected under direct vision with a small sharp knife. The drawer test was carried out to determine the ACL rupture. The cartilage surface was not damaged during the operation. In the sham group, the ACL was just exposed through a small medial parapatellar incision, then the joint was washed with saline and the incision site was sutured. Subcutaneously injected Flunixin (2.5 mg/kg/day; Banamine®, Merck Animal Health USA) for three days was given for postsurgical analgesia. The rats were given supplemental heat and were closely monitored until fully recovered from the anaesthesia. The rats were also monitored daily for pain, infection and other complications. 2 weeks after confirmation of osteoarthritis through pathological evaluation, preventive treatments initiated in 6 groups (Figure 1).

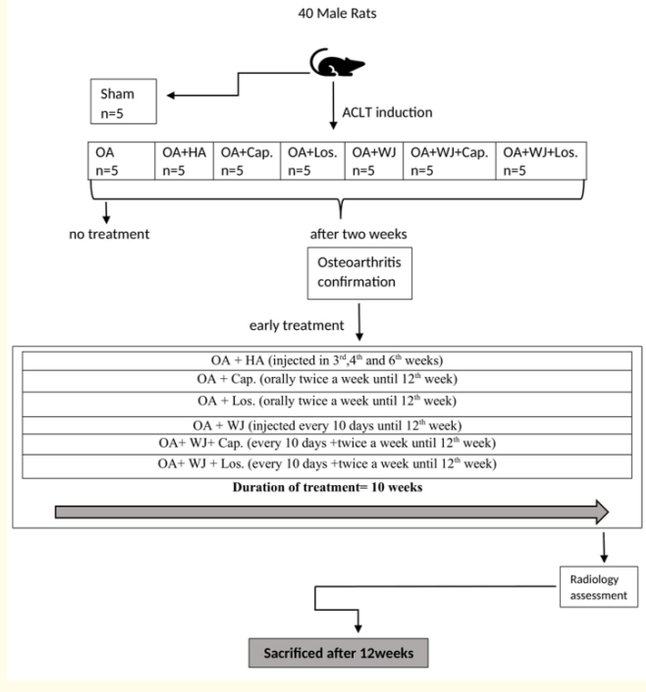

Figure 1: *Graphical scheme. ACLT: Anterior Cruciate Ligament Transection. OA: ACLT + PBS (Negative control). OA+ HA: ACLT+ treatment with Hyaluronic acid (Positive control) OA+ Cap.: ACLT + treatment with captopril (orally at a dose of 50 mg/kg twice a week) OA+ Los.: ACLT + treatment with losartan (orally at a dose of 50 mg/kg twice a week)OA+ WJ: ACLT + treatment with WJMSCs-CM (intra-articular injection of 100 µl conditioned medium every 10 day) OA+ WJ+ Cap.: ACLT + treatment with WJMSCs-CM (intra-articular injection of 100 µl conditioned medium every 10 day and captopril gavage orally at a dose of 50 mg/kg twice a week) OA+ WJ+ Los.: ACLT + treatment with WJMSCs-CM and losartan (intra-articular injection of 100 µl conditioned medium every 10 day and losartan gavage orally at a dose of 50 mg/kg twice a week Sham: No anterior cruciate ligament transection (ACLT) + PBS.*

Male Sprague-Dawley rats (n = 40) were divided into 8 groups (n = 5) as presented below:

- OA: ACLT + PBS (Negative control).
- OA+ HA: ACLT+ treatment with Hyaluronic acid (Positive control) [34].
- OA+ Cap.: ACLT + treatment with captopril (orally at a dose of 50 mg/kg twice a week) [21].
- OA+ Los.: ACLT + treatment with losartan (orally at a dose of 50 mg/kg twice a week) [35].
- OA+ WJ: ACLT + treatment with Wharton-jelly mesenchymal stem cell conditioned medium (intra-articular injection of 100 µl conditioned medium every 10 day) [36].
- OA+ WJ+ Cap.: ACLT + treatment with Wharton-jelly mesenchymal stem cell conditioned medium (WJ) (intra-articular injection of 100 µl conditioned medium every 10 day and captopril gavage orally at a dose of 50 mg/kg twice a week).
- OA+ WJ+ Los.: ACLT + treatment with Wharton-jelly mesenchymal stem cell conditioned medium (WJ) and losartan (intra-articular injection of 100 µl conditioned medium every 10 day and losartan gavage orally at a dose of 50 mg/kg twice a week.
- Sham: No anterior cruciate ligament transection (ACLT) + PBS.

Radiological and pathological evaluations

The X-ray images from knee joints were taken from the lateral aspects of the left knee using the same equipment (Axiom MultixMRadiographic Unit, SiemensTM, Germany). Osteoarthritis was assessed according to a grading system based on ICRS [37]. Scoring subjects were according to radiological indices such as joint space narrowing, presence of osteophytes, subchondral bone sclerosis, and bone ends deformity. The scores included 0 (none), 1 (doubtful), 2 (minimal), 3 (moderate), and 4 (severe). Osteophytes in the medial condyle of tibia, femur, medial fabella, total knee joint, joint space width, and total OA score were evaluated by a blinded radiologist (Figure 5 and 6).

Rats were euthanized with CO_2 70% at the end of the third month. Specimens from the knee joint were obtained and fixed in 10% buffered formaldehyde and then were transferred into paraffin. Serial sagittal sections were provided and stained with haematoxylin and eosin (H&E) for cellular architecture. All pathological specimens were assessed by a pathologist who was blinded from the study data. The degree of cartilage repair of each rat was evaluated based on ICRS scores which consisted of 6 indices including surface, matrix, cell distribution, cell population viability, subchondral bone, and cartilage mineralization (Figure 3 and 4).

Local Angiotensin converting enzyme (ACE) activity assay

Activity of ACE was measured, as previously described by Baudin [38] with minor modification. In brief, ACE activity was determined with an artificial substrate (FAPGG, (N [3- (2-furyl) acryloyl]-L-phenylalanylglycylglycine; Sigma-Aldrich) in a reaction mixture containing 25 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid), 0.5 mM FAPGG, 300 mM NaCl, and the desired dilution of the tissue homogenate at pH 8.2. The reaction rate at 340 nm can be determined using the FAPGG extinction coefficient ($\varepsilon = 0.989\ \mu M^{-1}$). Measurements were performed in 96-well plates at 25°C. Changes in the optical density (340 nm) were measured at 1-minute intervals for 10 minutes with a microplate reader (Epoch2, Biotek, USA). One unit of ACE activity is defined as the amount of enzyme that will cause the oxidation of 1.0µmol of FAPGG to FAP per minute at 25°C. The ACE activity in tissue samples was expressed as µmol of FAPGG oxidized/min/mL mg of protein (Units/mg of protein) by Biorex FarS, ACE kit with product code: BXC0176 (Figure 7).

Statistical analysis

Statistical analysis was performed using the statistical package Graphpad PRISM version 9 (GraphPad software, San Diego, CA, USA). All variables were tested for normal and homogeneous variances by leven's statistic test. All results are presented as Mean + SEM. The statistical differences were applied among the all groups by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis. A calculated P value of less than 0.01 or 0.001 was considered statistically significant.

Results

Characterization and differentiation of hWJMSCs

The flow cytometry analysis indicated that hWJMSCs were positive for the MSC surface markers, such as CD90 (96.7%) and CD73 (98.4%), and negative for CD34 (8.41%) and CD144 (8.30%) markers (Figure 2A). Furthermore, the oil red O and alizarin red S staining confirmed the capability of the cells to differentiate toward adipogenic and osteogenic cell lineages, respectively (Figure 2B and 2C).

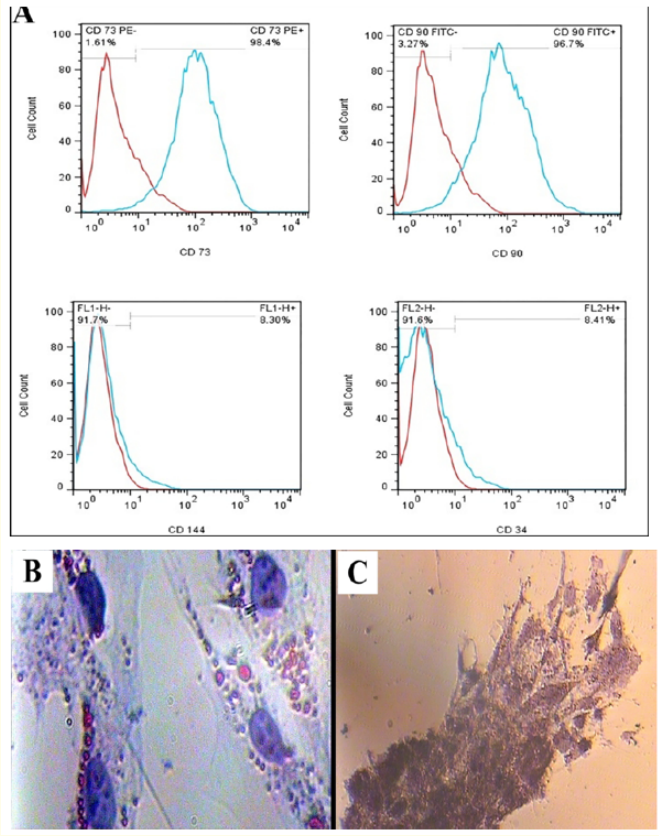

Figure 2: *The flow cytometry confirmed the frequency of WJMSCs, which reacted to CD90 and CD73, was high; whereas, the frequency of the cells, which reacted to CD34 and CD144, was negligible (A). Oil red staining showed that the cells stored lipid droplets in the presence of adipogenic medium (B), and Alizarin red staining showed that the cells deposited Ca2+ in the presence of osteogenic medium (C).*

Histopathological findings

Based on histopathological evaluations, 12 weeks after ACLT, non-treated group (OA) exhibited an apparent increase in osteoarthritis lesions, whereas, all histopathological indices were predominantly closer to the sham group in knees treated with WJ-CM, captopril and losartan groups (Figure 3 and 4). As well indicate in figure 4, OA +WJ, OA+WJ +Cap. and OA+WJ +Los. had significantly higher scores concerning cartilaginous surface, matrix, cell distribution, viability, subchondral bone, and cartilage mineralization, in comparison with other groups (P< 0.0001). specifically, regarding cell distribution, matrix and surface, scores showed better results in these three treated groups compared to non-treated (OA). concerning cell distribution, OA +WJ, OA+WJ +Cap. and OA+WJ +Los. even showed significant statistically difference when compared to treating with either captopril or losartan (OA+Cap) (OA+Los) (P< 0.0001).

In terms of subchondral bone, there was no significant difference among normal rats (Sham) versus OA +WJ, OA+WJ +Cap. and OA+WJ +Los treated groups. Considering matrix scores, while concomitant treatment with WJ and RAS inhibitors drugs could become much closer to sham, yet there was significant difference between OA+WJ and normal groups (Sham) (P< 0.0001).

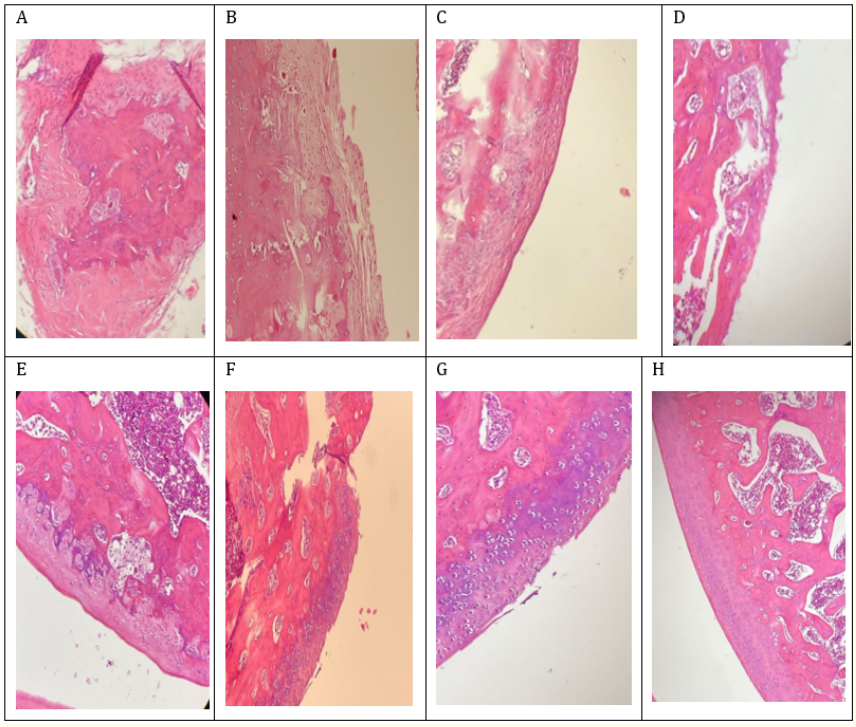

Figure 3: *Representative histopathological sections from the surface of articular cartilage of all groups (H&E. ×200). A: Non-treated group, the surface of articular cartilage was irregular with disorganized fibrous tissues and disorganized cell distribution. B: Hyalgan treated group, the surface of articular cartilage was continuous mainly composed of fibrocartilage with foci of hyaline cartilage cluster. C to F: The thickness and cell distribution in articular surface is gradually increased from group c to group F. The better results belong to group G that shows near normal articular cartilage. H: Normal articular cartilage. C: Captopril treated group, D: Losartan treated group. E: WJ treated group. F: Co-treatment of WJ and captopril. G: Co-treatment of WJ and losartan.*

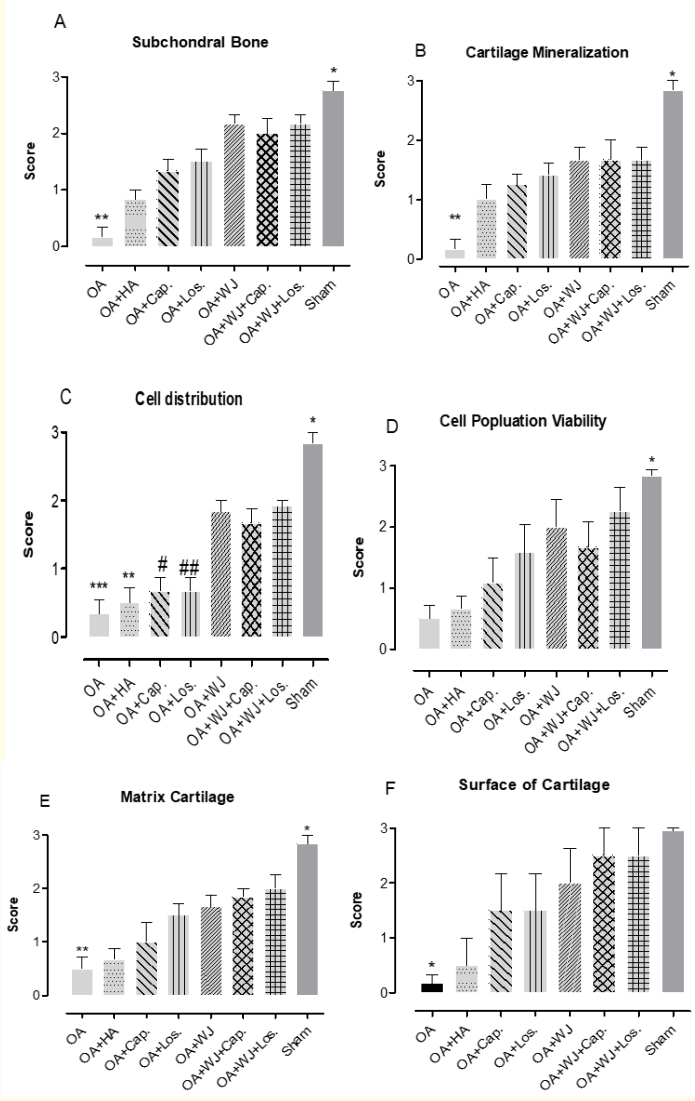

Figure 4: *The effects of Hyalgan, Captopril, Losartan and WJ on pathological scores in synovial tissues of rats with OA. A and B: *Indicates statically difference between Sham vs OA+HA, OA+Cap. OA+Los, OA+WJ, OA+WJ+Cap. OA+WJ+Los. (p < 0.0001) **Indicates statistically difference between: OA vs OA+Cap, OA+Los. OA+WJ, OA+WJ+Cap. OA+WJ+Los and Sham (p< 0.0001). C: *Indicates statistically difference between Sham Vs other groups (p < 0.0001). **Indicates statistically difference between OA+HA vs OA+WJ, OA+WJ+Cap. OA+WJ+Los. (p < 0.0001) ***Indicates statistically difference between OA vs OA+WJ, OA+WJ+Cap. OA+WJ+Los (p < 0.0001). #Indicates statistically difference between OA+Cap vs OA+Los, OA+WJ, OA+WJ+Cap and OA+WJ+Los (p < 0.0001). ##Indicates statistically difference between OA+Los vs OA+WJ, OA+WJ+Cap and OA+WJ+Los. (p < 0.0001). D: Indicates statistically difference between Sham vs OA, OA+HA, OA+Cap p=0.0004. E: *Indicates statistically difference between Sham vs OA, OA+HA, OA+Cap, OA+Los and OA+WJ (p < 0.0001). **Indicates statistically difference between OA vs OA+WJ, OA+WJ+Cap. and OA+WJ+Los (p < 0.0001). F: *Indicates statistically difference between OA vs OA+WJ+Cap, OA+WJ+Los and Sham p=0.0033.*

Radiological findings

Radiographic assessment as presented in figure 5 and 6 showed that, there were a significant enhancement in all treated groups compared with non-treated group (P< 0.0001). However, treatment with Hyalgan showed no significant difference with OA group regarding Medial tibial condyle score. Moreover, the OA+WJ, OA+WJ+ Cap. and OA+WJ+ Los. groups indicated significantly lower scores suggestive of better healing effects regarding medial tibial condyle osteophytes (P< 0.0001), medial femoral condyle osteophytes (P< 0.0001). medial fabella osteophytes (P< 0.0001) and joint space width (P< 0.0001) compared to other groups. In fact, co-administration of Captopril and losartan with WJ resulted in more considerable reduction in all inspected criteria when compared to only treatment with WJ (OA+WJ) (P< 0.0001).

It should also be mentioned that in terms of medial tibial condyle score, OA+WJ, OA+WJ+ Cap. and OA+WJ+ Los. groups showed no significant difference with normal group.

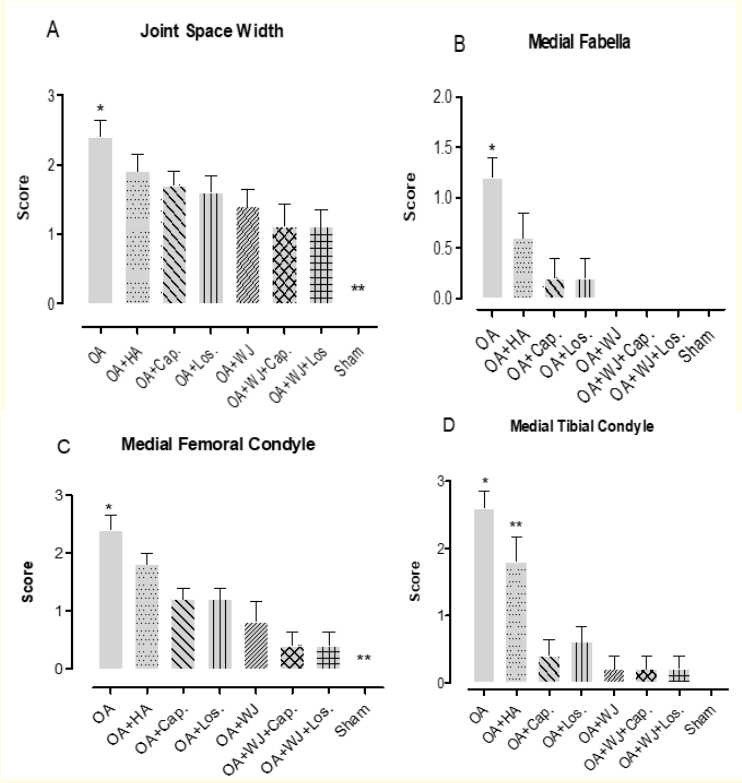

Figure 5: *The effects of Hyalgan, Captopril, Losartan and WJ on radiological scores in stifle joints of rats with OA A: *Indicates statistically difference between OA vs OA+WJ+Cap. and OA+WJ+Los (p < 0.0001). **Indicates statistically difference between Sham vs OA, OA+HA, OA+Cap, OA+Los. And OA+WJ (p < 0.0001). B: *Indicates statistically difference between OA vs other groups (p < 0.0001). C: *Indicates statistically difference between OA vs OA+Cap. OA+Los. OA+WJ, OA+WJ+Cap. OA+WJ+Los (p < 0.0001). **Indicates statistically difference between Sham vs OA, OA+HA, OA+Cap. and OA+Los. (p < 0.0001). D: *Indicates statistically difference between OA vs OA+Cap. OA+Los, OA+WJ, OA+WJ+Cap, OA+WJ+Los, Sham (p < 0.0001). **Indicates statistically difference between OA+HA vs OA+Cap. OA+Los, OA+WJ, OA+WJ+Cap, OA+WJ+Los, and Sham (p < 0.0001).*

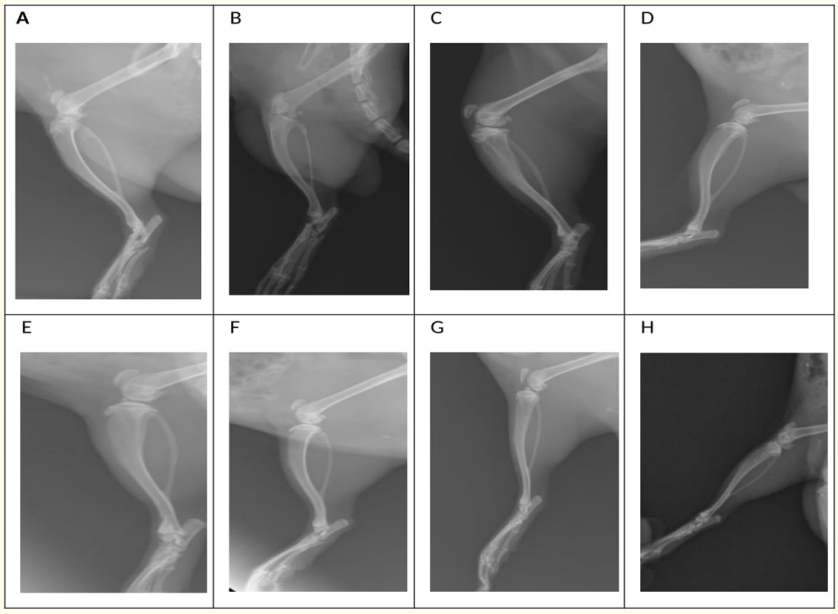

Figure 6: *Lateral view of stifle joints 12 weeks after ACLT-induced osteoarthritis. A: OA: Absent joint space width with severe femoral and tibial condyle osteophytes. B: OA + HA: Absent joint space width with severe femoral and tibial condyle osteophytes. C: Reduced joint space width with moderate femoral and tibial condyle osteophytes. D: Reduced joint space width with moderate femoral and tibial osteophyte. E: Reduced joint space width with small femoral osteophyte. F: Reduced joint space width with very small femoral osteophyte. G: Normal joint space width with very small femoral osteophyte. H: Normal joint space width.*

The effect of WJ, captopril and losartan on the local ACE activity of synovial tissue

To demonstrate the potential role of the local renin-angiotensin system in ACLT-induced osteoarthritis, the activity of ACE was measured in synovial tissue at 12th week. OA rats had higher activity of ACE in the synovial samples when compared to Sham and treated groups ($P< 0.0001$). As shown in figure, the activity of local ACE considerably decreased becoming closer to normal (Sham) after treatment with Captopril, WJ+Cap or WJ+Los. Moreover, treatment with Hyalgan could not affect RAS through inhibition of ACE activity resulting in significant statistically difference with other treated groups ($p < 0.0001$).

Discussion

It is well known that clinical treatment for osteoarthritis comprises drug and surgical intervention, However, surgical manipulation is typically used to treat end-stage osteoarthritis [39]. We chose to evaluate possible preventive effects of captopril, losartan, and WJ on the progression of ACLT-induced osteoarthritis for two reasons. First, mesenchymal stem cells have become a major tool in stem cell therapy in diseases that affect bone and joint function [40]. Second, in recent years, studies have shown that major components of RAS, including ACE, AT1R, and AT2R, are expressed in synovial tissue in humans and animals and participate in the pathogenesis of OA and rheumatoid arthritis (RA); their expression levels are related to the degree of inflammation and the severity of arthritis [10,13,14]. A comparison between the potential of mesenchymal stem cells and their conditioned medium in chondrocyte recovery demonstrated that MSCs can exert an anti-inflammatory effect on chondrocytes through both a paracrine effect and cell-cell contact signaling. However, the

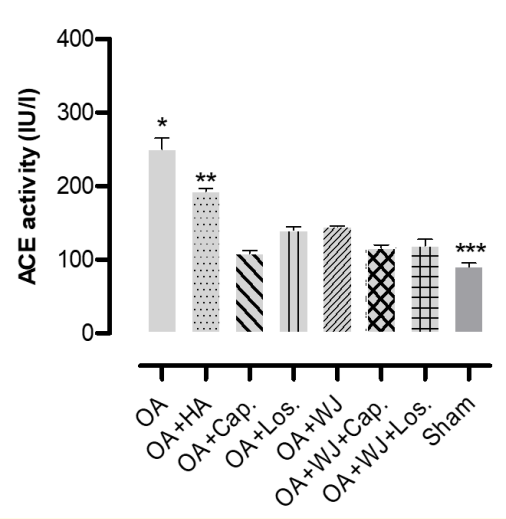

Figure 7: *The effects of Hyalgan, Captopril, Losartan and WJ on ACE activity in synovial joints of rats with OA. Values represent Mean ± SEM of ACE activity (Units/mg of protein).*

****Indicates statistically difference between: Sham vs OA, OA+HA, OA+Los. and OA+WJ ($p < 0.0001$). **Indicates statistically difference between: OA+HA vs, OA+Cap. OA+Los. OA+WJ, OA+WJ+Cap. and OA+WJ+Los. ($p < 0.0001$). *Indicates statistically difference between OA vs OA+HA, OA+Cap. OA+Los. OA+WJ, OA+WJ+Cap. and OA+WJ+Los ($p < 0.0001$).*

anti-inflammation ability of MSC would decrease with incubation time increase, especially in direct cell contact coculture system. Considering using cytokine for OA treatment, mesenchymal stem cell conditioned medium (MSC-CM) could be better choice for OA therapy [41]. Therapeutic inhibition of AT1R or ACE can improve clinical symptoms by reducing the yield of inflammatory factors [16,17,19,20], delaying the development of OA. very few *in vivo* studies were published in this regard, which motivated our interests in examining concomitant use of MSCs-CM and RAS inhibitor drugs in an animal model of OA.

To the best our knowledge, this is the first study that indicates the preventive effect of co-treatment of MSCs-CM and RAS inhibitor drugs in a rat model of OA. According to the outcome of the present study, intra-articular injection of 100-µl conditioned medium every 10 days (total of 7 doses in 10 weeks) exerted more protective impact on the OA in rats when compared to the nontreated group, HA, captopril and losartan treated groups. Interestingly, radiological and pathological evaluation of groups treated with both WJ-CM and RAS inhibitor drugs (Captopril and Losartan) resulted in better scores suggesting the superiority of this method of treatment. We also found that treatment with captopril orally at a dose of 50 mg/kg twice a week and combination of treatments with captopril and WJ-CM reduced the activity of ACE and supressed renin-angiotensin system locally. While Other treatments had no significant impact on RAS.

Tang., *et al*. (2017) evaluated the effects of subcutaneous AMSCs injection in OA rats and showed that the cartilage surface was smooth along with the good distribution of chondrocytes [42]. In line with these results, our previous study also showed that treatment with synovial stem cells accompanied with secreta resulted in columnar-cluster arranged chondrocyte while in the only-stem cell treated group the surface was continuous composed of fibrocartilage tissue [43]. In this study, we also observed that treatment with WJ-CM decreased the thickness and increased the cell distribution in articular surface compared OA, OA+HA, OA+Cap. and OA+Los. groups. histopathological results indicated that administration of RAS inhibitor drugs (captopril and losartan) accompanied by MSCs secreta alleviated patho-

logical changes. It seems that inhibition of synovial renin-angiotensin system synergically affects anti-inflammatory potential of MCs-CM in OA+WJ+Cap and OA+WJ+Los groups. In agreement with our finding, Histological scores of rat ankle joints in a study by Wang., *et al*. (2018) showed that Inhibition of ACE by perindopril (ACE inhibitor drug) mitigates the severity of collagen induced arthritis (CIA) in rats [13]. In this regard, Price., *et al*. reported that in immunohistochemical analysis, both prophylactic and therapeutic administration of 15 mg/kg of losartan reduced knee joint swelling in rats with adjuvant monoarthritic [44]. In contrast, There was not significant difference between the treatment WJ-MSCs (containing 1 × 107 cell suspension) and control group for histopathological findings [45]. Our radiological results were the same as those conducted by Estakhri., *et al*. regarding the effect of synovial membrane derived MSCs (SMMSCs) on the global OA score, where in the study there were also reduction in the severity of OA in treated groups [46]. Another survey by Mehrabani., *et al*. also showed the healing effects of bone-marrow MSC transplantation three month after cutting of ACL in guinea pig based on the repaired lesions in the joints space of treatment group. In consistent with the previous studies [47-49], we also showed that transection of ACLT increased joint space in x-ray images in rats after 12 weeks. There were also increased in osteophyte formation in medial femoral and tibial condyle during this period. Zare., *et al*. (2020) reported that both stem cells derived from synovium and fat pad are able to decrease cartilage degeneration, subchondral sclerosis, and osteophyte formation compared to the nontreated group [34]. We observed that, treatment with hyalgan and RAS inhibitors alone, could not reduce joint space and osteophyte formation considerably. However, orally administration of captopril and losartan accompanied by intraarticular injection of WJ reduced joint effusion and minimized joint space. Indeed, results belong to medial tibial condyle score in OA+WJ, OA+WJ+Cap and OA+WJ+Los showed near normal group. Herein, radiological findings were in agreement with a study by Yuangang (2020) suggesting that the expressions of renin, ACE, and AT1R in synovial tissue of osteoarthritis significantly increase as the K-L (Kellgren-Lawrence X-ray classification) level increased [50]. Wang., *et al*. reported that AT1R, AT2R and ACE in human and rat synovium were up-regulated, and the increased ACE in rat synovial tissues was suppressed by perindopril (an ACE inhibitor drug). They observed significant up-regulation of osteoclastogenesis and downregulation of osteoblastogenesis in the joints of CIA (collagen-induced arthritis) rats, accompanied with the activated RAS [13]. In this study, we also showed that administration of captopril alone (OA+Cap) or co-treatment of WJ and captopril (OA+WJ+Cap.) prevented the progression of OA probably due to reduction in ACE activity and RAS inhibition. Interestingly we noticed that while in OA+Los and OA+WJ+Los there was no significant change in ACE activity, administration of losartan (AT1R inhibitor) and co-treatment of WJ and losartan alleviated the pathogenic progress of OA confirmed by pathological and radiological scores. We speculate that when AT1R is inhibited, the increased Ang II levels may potentially lead to the activation of AT2R. Considering that AT1R and AT2R have opposite effects, continued AT2R activation may induce anti-inflammatory mechanisms that provide potential complementary therapeutic benefits [51].

Conclusion

Overall, the results of the present study indicated that early co-treatment of WJ, captopril and losartan resulted in delaying the progression of osteoarthritis in comparison to non-treated group as well as hyalgan-, captopril- and losartan-treated groups.

Although this study confirmed the promising effect of MSCs secreta and RAS inhibitor drugs in an osteoarthritis animal model, there were still some limitations that should be considered. First, we only examined Wharton-jelly mesenchymal stem cell conditioned medium (WJ) preventive effect and there was no comparison between WJ-MCs and their conditioned medium. Second, the specific effect of losartan on RAS during this period have not been thoroughly elucidated. Thus, conducting further researches would expand our understanding in this regard.

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