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The mechanisms of analgesic effect of mesenchymal stem cells in osteoarthritis pain

Analgesic mechanisms of MSCs in osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common musculoskeletal disease, and it is a major cause of pain, disability and health burden. Pain is the most common and bothersome presentation of OA, but its treatment is still suboptimal, due to the short-term action of employed analgesics and their poor adverse effect profile. Due to their regenerative and antiinflammatory properties, mesenchymal stem cells (MSCs) have been extensively investigated as a potential therapy for OA, and numerous preclinical and clinical studies found a significant improvement in joint pathology and function, pain scores and/or quality of life after administration of MSCs. Only a limited number of studies, however, addressed pain control as the primary end-point or investigated the potential mechanisms of analgesia induced by MSCs. In this paper, we review the evidence reported in literature that support the analgesic action of MSCs in OA, and we summarize the potential mechanisms of these antinociceptive effects.

Key Words: Osteoarthritis; pain; inflammation; mesenchymal stem cells; regeneration; analgesic mechanisms

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Core Tip: Core Tip: Osteoarthritis (OA) is the most common musculoskeletal disease, and it is a major cause of pain, disability and economic burden. Pain is the most common and bothersome presentation of OA, but its treatment is still suboptimal, which highlights the need for new analgesic agents for OA. Mesenchymal stem cells (MSCs) have been extensively investigated as a potential therapy for OA due to their regenerative and anti-inflammatory properties. The administration of MSCs resulted in significant improvement in joint pathology and function, pain scores and/or quality of life in numerous preclinical and clinical studies. Only a limited number of studies, however, addressed pain control as the primary end-point or investigated the potential

mechanisms of analgesia induced by MSCs. So this paper reviews literature for evidence of analgesic actions of MSCs in OA, and summarizes the potential mechanisms of these anti-nociceptive effects

INTRODUCTION

Osteoarthritis (OA) is a disease of movable joints characterized by anatomic and/or physiologic derangements including cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function. It is initiated by micro- and macro-injury of the joint, which activates maladaptive repair responses producing abnormal tissue metabolism (1).

Clinical manifestations of OA include joint pain, tenderness, limitation of movement, coarse crepitus and, occasionally, effusion and mild local inflammation (2). Diagnosis depends on the observation of signs and symptoms through clinical examination (3). X-ray can be performed to help with differential diagnosis and in case of atypical features (4). However, radiologic findings do not always complement the clinical presentation of pain (5).

Pathophysiology of OA is complex and is based on cartilage degeneration preceded by subchondral bone lesions. Historically, OA had been considered a disease of joint wear and tear, but recently, low-grade chronic inflammation has been found to play a key role in OA pathology. Both innate and adaptive central immunological mechanisms are involved in inflammation. Formation of ectopic bone and osteophytes are a characteristic pathologic features of OA (6). Neuroinflammation and central sensitization mechanisms contribute to the development of chronic pain (7).

OA is the most common musculoskeletal disorder worldwide, and it poses a huge health and economic burden. It is considered a major cause of chronic pain, disability -due to diminished joint mobility and function-, and decreased quality of life (8,9). Thus, finding effective and safe therapies for the treatment of OA is a significant clinical need.

Despite the recent progresses in understanding the pathophysiology of OA (7), the treatment of this condition is still suboptimal (10). For low-grade OA, pain management

and lifestyle changes are the only available therapeutic options, with total joint replacement considered the end-stage therapy. However, no effective therapeutic options are available that can stop the progress of the disease (10). Analgesia may be achieved using nonsteroidal antiinflammatory drugs (NSAIDs) (topical or systemic) as a first line, followed by paracetamol or tramadol. Less used medications include duloxetine and topical capsaicin. Intra-articular steroids are effective and recommended if other agents do not provide sufficient pain control, although their effect is short-termed (3). Pharmacological therapies should be always accompanied by physical and psychosocial interventions, such as exercise, weight management and manual therapy. Patients whose joint symptoms are substantially impacting their quality of life and in which non-surgical management is ineffective or unsuitable should be referred for joint replacement surgery (3). **Table 1** summarizes the treatment guidelines for OA as recommended by the American College of Rheumatology and Arthritis Foundation (11).

Pain in OA

Pain is the major manifestation of OA and it significantly affects the function and quality of life of patients (12). Both peripheral and central mechanisms contribute to OA pain. Peripherally, nociceptive signals may arise from the synovium, bone marrow, soft tissues and even cartilage in the advanced stages of the disease (13). These inputs are modulated at the central level through mechanisms involving spinal and cortical sensitization, and the activity of discrete areas of the brain. Central sensitization may explain the poor correlation between pain severity in OA and the extent of cartilage damage, and the persistence of pain after joint replacement in some patients (14).

In the periphery, sensitization of afferent neurons is mediated by cytokines, chemokines and neuropeptides and is associated with low-grade inflammation and innate immunity (15), immune cell infiltration and activation, and damage-associated molecular patterns. These mechanisms involve early post-translational changes to receptor ion channels, followed by late transcription-dependent mechanisms, which

produce changes to the chemical phenotype of the cell (16). Animal models of OA demonstrated the role of nerve growth factor (NGF) in nociceptor sensitization after tissue injury, mainly through the tropomyosin receptor kinase A (TrkA) receptor. In addition, increased NGF levels were found in the synovial fluid of OA patients and were associated with pain (17,18). Other molecules associated with the peripheral component of OA pain include the neuropeptide calcitonin gene-related peptide (CGRP) (19), interleukin (IL)-1β, IL-6 and tumor-necrosis factor α (TNF-α) (20).

Central sensitization occurs both at the spinal and supraspinal levels. In the spinal cord, acute pain is accompanied with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor activation by glutamate, followed by an early phase of sensitization, mediated by substance P (SP) acting on neurokinin 1 (NK1) receptors, in which N-methyl-D-aspartate (NMDA) receptors are activated. The late phase sensitization witnesses gene induction with enhanced synthesis of prostaglandins and other local inflammatory mediators (14). In models of OA, spinal cord exhibits enhanced transient receptor potential cation channel subfamily V member 1 (TPRV1) activity and increased levels of substance P (SP), CGRP, IL-1α/β, IL-13, IL-17, TNF-α, L-selectin, TIMP metallopeptidase inhibitor 1 (TIMP-1) and vascular endothelial growth factors (VEGF) (21). In addition, activation of glial cells was found to contribute to spinal sensitization, indicating a strong neuropathic component of the OA associated pain (22). Supraspinal sensitization occurs in several areas of the brain, including the rostral ventral medulla (RVM) and the periaqueductal gray (PAG) and it involves inflammatory mediators, such as prostaglandins, as well as serotoninergic and noradrenergic facilitation (23).

Treatment of OA pain

Pain management in OA is still considered suboptimal. The use of paracetamol, once recommended as the first-line analgesic for OA pain, has now been reviewed after meta-analysis suggested that monotherapy with paracetamol may be ineffective (24) and that long-term treatment provides no more pain relief than placebo for most

patients (11). NSAIDs are effective on the short term to control OA pain and are thus strongly recommended. Their use, however, is limited by the adverse effect profile (11). Intra-articular injections of steroids also showed short-term effectiveness (2-10 wk) (3), but they are limited in frequency of administration and may cause cartilage damage if used repeatedly (25). The use of other analgesics, such as duloxetine, tramadol, and non-tramadol opioids is not supported by strong evidence of efficacy, as well as other supplements, such as hyaluronic acid, glucosamine, chondroitin, fish oil and vitamin D (11). These factors highlight the need for the development of novel therapies that are both effective and safe on the long term.

MSCs and their therapeutic uses

Mesenchymal stem cells (MSCs) are multipotent adult stem cells likely derived from diverse embryonic lineages and isolated from different sources. They were discovered by Friedenstein in 1970 (26). The International Society for Cellular Therapy has proposed a set of standards to define MSCs: 1) expression of a certain set of cluster of differentiation (CD) markers (CD105, CD73, and CD90), 2) lack of expression of hematopoietic lineage CD markers (CD45, CD34, CD14 or CD11b, CD79α or CD19 and histocompatibility complex (HLA)-DR surface molecules), 3) differentiation into osteoblasts (bone), adipocytes (fat) and chondroblasts (cartilage) *in vitro*, and 4) plastic adherence and ability to form colony-forming unit fibroblasts (CFU-Fs) (27). MSCs from different sources showed differences in differentiation potential, immunophenotype, immunomodulatory activity, proteome, and transcriptome, producing their specific characteristics and features in their application.

MSCs are an attractive option and among the most frequently used stem cell type for clinical application and regenerative medicine due to numerous advantages, including self-renewal and differentiation, mostly due to their secreted trophic factors that mediate cell-to-cell communications. Moreover, immune rejection is an important concern with allogeneic cell-based therapy, but the lack of cell surface ~~histocompatibility complex~~ (HLA) class II molecules and T cell costimulatory molecules

and their paracrine-mediated immunomodulatory activity, and secretion of immunomodulatory factors indicate that the MSCs have broad anti-inflammatory properties and active in tissue repair (28–31).

A large number of studies demonstrated the beneficial effects of MSC-based therapies to treat different pathologies, including neurological disorders, cardiac ischemia, diabetes, and bone and cartilage diseases (32,33), which indicate that MSCs holds great promise for cell therapies and tissue engineering.

Studies showed the ability of MSCs to migrate toward damaged tissues, which functionally influence the repairing of these tissues (34). MSCs act to accelerate healing and reduce inflammation, which is essential to remove dead cells, and facilitate cell migration and proliferation in the injury site (35,36). For instance, a study using a rat model of cardiomyopathy showed that MSC transplantation significantly improved cardiac function by induction of myogenesis and angiogenesis, resulting in decreased left ventricular end-diastolic pressure and increased left ventricular maximum (37). Another study showed that basic fibroblast growth factor (bFGF) can promote the migration and survival of bone marrow MSCs *in vitro*, as the perfusion of the coronary vein with retrograde bFGF can enhance the graft transplantation of MSCs, promote the differentiation of MSCs to cardiomyocytes, and restore cardiac function (38). Some studies used a laboratory-grown cell sheet patches of MSCs to repair large damaged areas instead of intravenous infusion of MSCs. Kim *et al* transplanted adipose-derived stem cells (AD-MSCs) sheet to treat myocardial infarction in a rat model and showed that the stem cell sheet promoted cellular engraftment and upregulated growth factor and cytokine expression (39). An *in vivo* study of neonatal stroke rat model proved that intranasal delivery of MSC reduced ischemic brain damage and reduced white and gray matters loss (40). The study attributed the healing in the injury site to an increase in cell proliferation after transplantation of MSCs.

In skeletal disorders, MSCs could be helpful in tissue repairing and regeneration through several mechanisms, including homing, angiogenesis, differentiation, and response to inflammatory condition (41). Liu *et al* used umbilical cord-MSCs (UC-

MSCs) to treat rheumatoid arthritis in collagen-induced arthritis mice through suppression of T follicular helper cell differentiation partly *via* the production of indoleamine 2,3-dioxygenase (IDO). In addition, MSCs prevented arthritis progression by inhibiting both the number and function of follicular helper cell *in vivo* (42). Another study showed that transplantation of MSCs from allogeneic related donors treated severe progressive systemic sclerosis (43). A third *in vitro* study treated bone marrow derived-MSCs (BM-MSCs) with all-trans retinoic acid, then co-cultured them with CD3/28-activated peripheral blood mononuclear cells derived from ankylosing spondylitis (AS) patients. The results showed that BM-MSCs treated with all-trans retinoic acid significantly decreased pathogenic cytokine, TNF-α, ~~interleukin-17~~ (IL-17A) and interferon-γ (IFN-γ) in AS (44).

MSCs in OA

MSCs are the most studied stem cells for treating bone related diseases and the associated inflammation. The effects of treatment by MSCs on OA pathology and presentation was widely investigated *in vitro* and in different preclinical models of OA using different routes of administration of multiple types and amounts of MSCs. Numerous clinical trials also addressed the potential therapeutic role of MSCs in OA.

MSCs have the ability to differentiate into mesoderm-derived cells, including osteoblasts and chondrocytes. More importantly, MSCs are considered powerful immunomodulators and inflammation combatants rendering them suitable for many immunological and bone diseases where inflammation is a prominent component. OA is the most common form of arthritis and clinically variable in its severity. Patients with symptomatic form of OA suffer from intermittent attacks which are usually associated with joint pain. OA pathology encompasses the inflammation and degeneration of articular cartilage that defects its integrity and leads to subsequent changes in the subchondral bone. Many preclinical and clinical studies have corroborated the therapeutic potential of MSCs in alleviating the inflammation associated with OA and initiating the regeneration of the defective articular cartilage. A pilot study conducted

by Song and co-authors reported positive outcomes of injecting autologous human adipose-derived mesenchymal stem cells (haMSCs) intra-articularly in patients with OA ~~osteoarthritis~~. Song *et al* reported that a dosage of 5 × 107 haMSCs produced the desired improvement in the pain scale and restored the volume and the function of knee cartilage (45). Similarly, Lee and co-authors reported that single intra- articular injection of 1 × 10^8 AD- MSCs for patients with knee osteoarthritis produced a significant reduction in the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index total score and the test related sub-scores for pain, stiffness and physical function at 6 mo post-injection. The range of articular motion remarkably enhanced after MSCs transplantation with no change in the joint space width of medial and lateral compartment and size of the cartilage damage (46). On the other hand, Matas *et al* found that intra-articular injection of two repeated doses of umbilical cord- derived MSCs (UC- MSCs) (20 × 10^6 per dose) at baseline and 6 mo was superior than using a single dose in lowering the WOMAC pain scores. Patients with knee OA who were recruited in Matas *et al* study experienced 86% reduction in pain and 89% reduction in disability with no progression in the chondral damage and intra- articular calcifications examined by MRI at 12 mo (47). Bastos *et al* compared the effect of injecting autologous bone marrow-derived culture-expanded MSCs (hBM-MSCs) intra-articularly with or without the addition of platelet-rich plasma to intra-articular corticosteroid injections in patients with symptomatic knee OA. MSCs alone or in combination with platelet rich plasma were superior to corticosteroids in improving the Knee Injury and Osteoarthritis Outcome Score (KOOS), increasing the range of motion and reducing the expression of IL-10, which is usually increased in OA knees, at 12 mo follow up (48).

The extracellular vesicles (EVs) derived from different MSCs have currently gained a wide interest due to their high therapeutic efficacy and safety profile. Li *et al* injected UC-MSCs-EVs in the articular lumen of a rat model of OA created by the surgical transection of the anterior cruciate ligament (ACLT), and reported that the UC-MSCs derived EVs can deliver therapeutically effective miRNAs, including has-miR-122-5p, has-miR-148a-3p, has-miR-486-5p, has-miR-let-7a-5p, and has-miR-100-5p, which were

able to promote the reprogramming of macrophages into ~~an~~ anti-inflammatory M2-macrophages and increasing the level of inflammation inhibitory cytokine IL-10. Moreover, these miRNAs were effective in inducing the phosphoinositide-3-kinase (PI3K)-Akt signaling pathway, which is integral to prevent further degeneration of the knee cartilage and seize the progression of OA (49). Duan and co-authors conducted an interesting study by isolating synovial MSCs and chondrocytes from the knee cartilage of patients getting total knee arthroplasty (THA). After culturing these cells, they primed the MSCs with lipopolysaccharide (LPS) and isolated EVs from these preconditioned synovial MSCs. The EVs were injected in the right knees of mice model of OA created by surgical removal of anterior cruciate ligament and medial meniscus. It was found that the presence of Let-7b miRNA in the EVs isolated from LPS primed synovial MSCs were effective in reducing the cartilage damage, increasing the thickness of cartilage layer, and decreasing the level of the inflammatory and matrix lysis protein disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) while increasing the levels of aggrecan and type II collagen alpha 1 chain (COL2A1), which are essential for the interaction with hyaluronan and enhancing the load-bearing characteristics of the knee (50). Likewise, Jin and co-authors reported that lncRNA MEG- 3 existed in the exosomes derived from bone marrow MSCs (BM-MSCs) and prevents the apoptosis and senescence of chondrocytes through lowering the level of ~~interleukin 1 beta~~ IL- 1β and decreasing the inflammatory damage of knee cartilage, which helps in restoring the trabecular bone volume and density of the knee joint in OA rat model (51). Mao *et al* observed that miR-92a-3p was significantly less in the chondrocytes isolated from the cartilage of OA patients who underwent total knee replacement. BM-MSCs derived exosomes can deliver miR-92a-3p following their injection in the knees of collagenase-induced OA mouse model, which enhances the differentiation potential of resident stem cells to chondrocytes and increases the synthesis of cartilage matrix. These protective and regenerative effects are mediated by the inhibition of Wnt Family Member 5A (WNT5A) (52). Huang *et al* demonstrated that miR- 206 was downregulated and E74- like factor 3 (Elf3) was upregulated in the

femoral tissues of OA mouse model. The administration of BM-MSCs derived exosomes that have sufficient amount of miR- 206 was effective in downregulating Elf3 and ameliorating the inflammation and apoptosis of resident osteoblasts besides increasing the expression of osteocalcin (OCN) and bone morphogenetic protein 2 (BMP2) and enhancing the deposition of calcium and the activity of alkaline phosphatase (ALP) (53). In the rat model of temporomandibular joint osteoarthritis (TMJ-OA), Zhang and co-authors injected exosomes derived from MSCs. They reported a significant suppression of inflammation mediated by decreasing the level of IL-1β and increasing the levels of nitric oxide and matrix metalloproteinase 13 (MMP13), which promoted the synthesis of glycosaminoglycans (GAGs) required for matrix restoration and regeneration (54).

Genetic modification of MSCs is one of the most studied approaches to enhance their therapeutic and regenerative potential. It has been found that the transplantation of MSCs overexpressing platelet-derived growth factor (PDGF) or heme oxygenase-1 (HO-1) in the surgery-induced canine OA model can considerably suppress the destructive inflammation and increase the levels of aggrecan and collagen type 2 in chondrocytes. PDGF-MSCs were more effective in improving the limb function and reducing pain (55). The accumulative evidence supported by the above mentioned studies highlights the importance of MSCs in mitigating OA inflammation and restoring the integrity and density of volume of articular cartilage and its associated matrix, which indicates that MSCs can be a possible new therapeutic tool for OA.

Effects of MSCs on OA pain

Despite the large number of preclinical studies investigating the effects of MSC administration on OA pathophysiology, only a small part of them included the effects on pain, and even less had pain control as the main outcome. This may be due to the difficulty of pain assessment in animal models of OA (56) and the complexity of the pain phenomenon (57). In addition, reported results were contrasting, probably due to

the variability in animal models and the methods of pain evaluation, as well as types of MSCs used and routes of their administration (56).

A number of *in vivo* studies, however, found an improvement in OA associated pain following treatment with MSCs. TGFβ1-modified MSC-derived exosome was found to attenuate cartilage damage and pain behaviors in the anterior cruciate ligament transection (ACLT) model of OA by inhibiting angiogenesis, suppressing calcification of the cartilage zone and osteoclastogenesis (58). Intra-articular injection of adipose derive MSCs (ADSCs) in rats diminished MIA-induced OA cartilage lesions by paracrine-based mechanisms and restored the OA associated mechanical allodynia and thermal hyperalgesia. In patients, ADSCs also reduced OA pain, as measured by the visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (59).

In a study by Zeng *et al* (60), bone marrow-derived mesenchymal stem cells (BMSCs) were enhanced by kartogenin (KGN) nanoparticles and administered to osteoarthritic rats. In addition to articular cartilage repair and enhanced chondrogenesis, KGN-enhanced BMSCs also ameliorated OA pain, as shown by increased weight bearing on the injured leg and decreased latency period after hot plate exposure. Administration of different concentrations of MSCs into OA rat knees improved both the histological damage and weight bearing distribution in the anterior cruciate ligament transection (ACLT) model (61).

Sakamoto *et al* (62) found that early intra-articular injection of adipose-derived MSCs resulted in significant suppression of inflammation and pain and prevented degenerative OA changes, but did not promote cartilage repair. In another study involving the MIA model of OA, STAT3 signaling pathway was suppressed by treatment of MSCs with STA21. Both intravenous (IV) and intra-articular (IA) administration of STA21-treated MSCs decreased expression of proinflammatory cytokines in the joint, which improved pain severity and cartilage damage (63).

A trial of adult human bone marrow-derived mesenchymal stromal cells in the MIA model of OA caused significant pain reduction, along with articular damage repair. In patients, the same cells did not produce the significant improvement in pain scores (64). Intra-articular injection of a large variety of MSCs in patients with knee OA was found to significantly improve joint pathology, disease symptoms, pain score and quality of life (65). Human umbilical cord MSCs (hUC-MSCs) was found to improve pain scores and quality of life in OA patients. hUC-MSCs increase the expression of chondrocytes and activate anti-inflammatory mechanisms, preventing degradation of cartilage and bone (66). A clinical trial of autologous bone marrow stem cells (BM-SC) concluded that a single IA injection of these stem cells significant reduced knee pain and improved quality of life (67). Other clinical trials reviewed by Hwang *et al* (68) also resulted in improved joint function, reduction of pain severity and improved life satisfaction.

Proposed mechanisms of analgesia of MSCs in OA

Data from literature suggest that the analgesic action of MSCs in OA involves a peripheral component, originating in the joint tissues, and a central component related to central hypersensitization. This reflects the mechanisms of nociception reported in OA (69).

It can be speculated that a major antinociceptive mechanism of MSCs in OA is through inhibition of inflammation. MSCs secrete anti-inflammatory and growth factors that support their immunomodulatory, immunosuppressive and trophic capacities. These properties contribute to the regeneration of damaged cartilage and joint homeostasis, improving inflammatory and catabolic aspects of OA (70,71). Initially, the bioactive substances secreted by MSCs change the inflammatory milieu in the joint from pro-inflammatory to anti-inflammatory, producing analgesia (65). On the longer term, MSCs are inserted in the joint tissues and trigger the repair and regeneration of damaged tissues, including cartilage (72). Numerous molecules have been reported to contribute to the antiinflammatory effects of MSCs in OA, including nitric oxide (NO), inducible nitric oxide synthase (iNOS)-27, IL-10, TGF-β, IL-6, IFN-γ, CCL2, HGF, ~~and~~

NF-κB and TNF-α (73,74,75). COX-2/PGE2 pathway plays a key role in the anti-inflammatory effect of MSCs in OA (76), where MSCs increase the levels of the antiinflammatory mediator PGE2 (75). Similar antiinflammatory results were also produced by MSC EVs (77). Since these molecules and their related pathways are well established contributors to OA pain, their involvement in the analgesic mechanisms of MSCs in OA can be hypothesized.

CCL2/CCR2 signaling is crucial in the development of knee OA pain. Neuronal CCL2 and CCR2 from DRG mediate macrophage infiltration, while local CCL2/CCR2 signaling in the joint directly stimulates intra-articular CCR2 positive sensory nerves, producing knee hyperalgesia (78). Interestingly, the analgesic effect of MSCs in different models of pain was associated with decreased levels of CCL2, produced by the downregulation of the NF-κB and JNK/MAPK pathways (79,80), which suggests that a similar mechanism could be involved in the analgesic effect of MSC in OA.

Other molecules and pathways relevant to OA pain include: 1) NGF / TrkA, 2) CGRP, 3) IL-1β, 4) pyrin domain-containing protein 3 (NLRP3) inflammasome, and 5) Wnt/β-Catenin (69). NLRP3 inflammasome is a component of the innate immune system and is involved in the regulation of active IL-1β. NF-κB increases the expression of inactive NLRP3 and pro-IL-1 mRNA, followed by the assembly of the inflammasome, which results in the activation of caspase 1 and release of IL-1, IL-18, MMP13 and ADAMTS5. In OA, dysregulation of NLRP3 inflammasome in OA contributes to chronic pain (69). MSCs were found to inhibit the NLRP3 inflammasome in macrophages (81), inflammatory cardiomyopathy (82) and inflammatory renal disease (83), which could indicate inhibition of NLRP3 inflammasome as a potential mechanism of MSCs induced analgesia in OA.

In OA, high levels of Wnt are associated with progressive joint damage (84) and hyperalgesia (69).

Since MSCs were found to inhibit the Wnt/β-Catenin pathway in a number of disorders (85,86), this pathway could be involved in MSC analgesic mechanism and should be investigated further. In the study by Lee *et al*, administration of STAT3-inhibited MSCs

reduced the levels of inflammatory mediators and chemokines in the OA joint, and this was associated with improvement in pain behavior and decreased TRPV1 expression in the dorsal root ganglion (63). This effect was more pronounced with IV administration, indicating a systemic immunomodulatory effects on inflammation.

The interaction of MSCs with immune cells, including macrophage, dendritic cells, T lymphocytes, and natural killer (NK) cells, also contributes to MSC antiinflammatory properties (87). MSCs induce polarization of macrophages to an antiinflammatory M2 phenotype through 1) cellular interaction and paracrine factor-mediated mechanisms, and 2) exosome-mediated mechanisms. The first involves cytokines and hormones, and the latter depends on RNAs and other molecules (88). Following an intra-articular injection of BM-MSCs in patients with knee OA, a decrease in synovial fluid levels of pro-inflammatory monocytes/macrophages and IL-12p40 was recorded (89) .

Ai *et al* investigated the effects of MSCs and derived extracellular vesicles (MSC-EVs) on pain behaviors in the destabilization of the medial meniscus (DMM) murine model of OA. It was found that treated OA mice did not display pain behaviors observed in untreated counterparts, and that did not result from reduced joint damage, but rather from a lack of knee-innervating sensory neuron hyperexcitability. MSC-EV treatment also prevented NGF-induced sensory neuron hyperexcitability. These results suggest that MSCs and MSC-EVs may reduce pain in OA by direct action on peripheral sensory neurons (90). Another study (91) found that the intrathecal administration of umbilical cord blood mesenchymal stem cells (UCBMSCs) improved both pain behavior and inflammation in the MIA model of OA. This effect is regulated by LncRNA H19 and involves microRNA-29a-3p/ FOS axis. microRNA29a-3p, the target gene of LncRNA H19, and FOS mRNA were down-regulated after stem cell therapy, suggesting that microRNA-29a-3p and FOS might play a role in pain improvement. c-fos in spinal dorsal horn of rats was significantly down-regulated after UCBMSCs treatment, which may be the reason for pain improvement. Phosphorylation levels of NR1, NR2B, PKCγ, ERK in spinal dorsal horn of rats with OA pain decreased significantly after intervention of UCBMSCs, indicating that the central sensitization of rats with

advanced OA pain decreased and the pain symptoms improved. Similar results were observed in astrocytes.

Intra-articular injection of human adipose tissue-derived MSCs (hAdMSCs) was reported to improve pain behavior in a medial meniscal transection (MMT) rat model of OA (92). These effects were attributed to the recruitment of endogenous cells through paracrine communication (93), and to a lesser extent, to direct engraftment coordinated with the local environment (94). Paracrine factors excreted by MSCs help recruitment of stem and progenitor cells, repair of degraded tissue and, most importantly, counteracting inflammation. A similar result was reported after administration of bone marrow mesenchymal stem cell (BMSC)-derived exosome in the MIA model of OA (95), which was found to inhibit CGRP and iNOS expression in the dorsal root ganglion (DRG), indicating relief of both inflammatory and neuropathic aspects of OA pain (95). BMSC-derived exosome also attenuates the inhibitory effect of IL-1β on the upregulated inflammatory mediators.

In the MNX model of OA, different effects were reported on pain behavior and joint pathology with the use of early and late passage MSCs. Late passage MSCs attenuated established pain behavior, while early passage MSCs exacerbated it. Interestingly, none of them modified MNX-induced joint pathology, which suggests an analgesic mechanism not related to articular pathology (96). SiMAG-labelled MSCs were detected within the synovial cavity at 29 days postinjection, indicating a peripheral site of analgesic action of the MSCs. The recorded decrease in serum TNFα indicated inhibition of systemic inflammation, which is the expected cause of pain relief (97). Similarly, van Buul *et al* reported that intra-articular injection of bone marrow mononuclear cells (BMMNCs) in the MIA model of OA significantly improved pain behavior – measured as weight bearing distribution - but did not affect cartilage damage, subchondral bone changes and synovial inflammation (56). Similar results were observed with the administration of MSCs and MSC-EVs in a mouse collagenase-induced OA model (98). MSCs were also found to downregulate ADAMTS-5 expression, inhibit the expression of anticalcitonin gene related peptide (CGRP) and

increase the expression of TNF-stimulated gene/protein-6 (TSG-6) (99). These changes indicate the suppression of the central sensitization of pain.

Several pain pathways were found to be inhibited by MSCs in other types or models of pain. Small extracellular vesicles from induced pluripotent stem cell-derived mesenchymal stem cells (iMSC-sEVs) alleviated acute pain in tendinopathy by inhibition of mast cell degranulation and infiltration and the expression of proinflammatory cytokines and genes involved in the HIF-1 signaling pathway (100). Human BMSCs relieved pain behavior in rodents by inhibition of neuronal hyperexcitability and primary afferent input, as well as suppression of GluN2A (N-methyl-D-aspartate receptor subunit 2A) tyrosine phosphorylation and protein kinase Cgamma (PKCg) immunoreactivity in the rostral ventromedial medulla (101). In murine chronic constriction injury (CCI) and spared nerve injury models, TGF-β1 was found to suppress spinal synaptic plasticity and DRG neuronal hyperexcitability *via* TGF-β receptor 1-mediated noncanonical signaling. This effect was mediated by paracrine mechanism by which BMSCs target CXCL12-producing DRGs (102). We thus anticipate that MSCs use in OA may exhibit their analgesic effects through the HIF-1, GluN2A tyrosine, PKCg and TGF-β signaling pathways.

Figure 1 summarizes the established and proposed mechanisms of analgesia exerted by MSCs or MSC related EVs in OA.

Figure 1: Graphical summary of proved and anticipated analgesic mechanisms of MSCs in osteoarthritis. MSCs transplantation reverts central sensitization and induces peripheral analgesia due to their anti-inflammatory and immunomodulatory properties and by attenuation of specific pain pathways *via* exosomes-derived microRNAs and MSCs paracrine factors

CONCLUSION

Numerous in vitro, *in vivo* and clinical studies demonstrated the capability of MSCs in halting and/or reversing the progression of joint tissue damage in OA, as well as mitigating joint inflammation, improving pain sensation and enhancing overall patient

quality of life. MSCs were able to produce an analgesic effect in models of OA through both peripheral mechanisms, mainly involving antiinflammatory processes, and on a central level, by preventing or reversing central sensitization. This evidence further reinforces the potential of MSCs as a safe and effective treatment for OA pain. Additional pathways which were observed in other types of pain may contribute to the analgesic mechanisms of MSCs in OA, and require further investigations.

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