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Effect of human adipose-derived mesenchymal stem cell conditioned medium on musculoskeletal pain

M. SANO[1], A. TANABE[2], N. URUSHIHATA[2], X.-L. LIU[1]

[1]Cosmetic and Regenerative Medicine, Sun Field Clinic, Koto-ku, Tokyo, Japan
[2]Research and Development Division, BIOMIMETICS SYMPATHIES Inc., Koto-ku, Tokyo, Japan

Abstract. – OBJECTIVE: Several studies in animal models have shown the safety and effectiveness of mesenchymal stem cell conditioned medium (MSC-CM) in inflammatory lesions involving muscles and joints.

PATIENTS AND METHODS: In this report, we retrospectively evaluated 16 patients who received local administration of the human adipose-derived mesenchymal stem cells conditioned medium (hAMSC-CM) for musculoskeletal chronic pain. Overall, 27 body locations expressing pain have been treated. The local administrated dose was 5 ml in the joint cavity and/or 2 ml in the other locations. The patients were asked to conduct self-evaluation of the degree of pain using a numeric rating scale (NRS) questionnaire and record the severity of pain before administration and at 15 min, 1 day, 1 week, and 4 weeks after administration. A second administration has been performed in 7 locations. The analysis was done considering two conditions: the "current pain status" and the "worst pain status in a week."

RESULTS: The results showed statistically significant differences between before and after administration at each time point for "current pain status" and at 1-week and 4-week time points for "worst pain status in a week" after first administration (Tukey-Kramer test). After second administration, significant differences were found at 1-week and 4-week time points for "current pain status". No serious adverse effect was found.

CONCLUSIONS: It was concluded that local administration of hAMSC-CM appears to be safe and could be expected to have effective therapeutic value against musculoskeletal chronic pain. Further studies are needed to clarify analgesic effects of hAMSC-CM and its underlying mechanism(s).

Key Words:
Human adipose-derived mesenchymal stem cell conditioned medium, Musculoskeletal pain, Safety, Efficacy, Regenerative therapy.

Introduction

Musculoskeletal pain is an unpleasant sensation that is relayed to brain by sensory neurons. Musculoskeletal pain affects roughly 12% of the general population, with a predominance of 2 to 1 in female over male gender[1]. In fact, the most common reason why people visit medical clinics is attributed to the pain. A major type of musculoskeletal pain is the chronic pain which is classified as a pain that lasts for more than 6 months. Chronic pain is projected to affect 1.5 billion people worldwide, with low back pain comprising 23-26% of the population[2]. Of the people at age 65 or older, more than 60% complain from daily pain[3]. It has been estimated that 20-33% of the world's population (1.75 billion people) has some form of chronic musculoskeletal pain[4]. Chronic Musculoskeletal pain impairs the Quality of Life (QOL) and is associated with a huge economic impact in terms of loss of employment and disability payments with a substantial cost for healthcare systems and disability insurance[5]. Therefore, chronic pain is considered as a dynamic disease and a major medical issue with physical, psychological and behavioral consequences. The most common forms of musculoskeletal pain are chronic low back pain, neck and shoulder pain, and the pain associated with osteoarthritis and rheumatoid arthritis[4,6]. Pain is also arising from twisted muscles, fractures and injuries. The frequency of low back pain is 30-40% in adults, while that for rheumatologic problems and rheumatoid arthritis is fairly low at 2%[7]. The frequency of pain in neck and shoulder ranges from 15 to 20%, and in knee from 10 to 15%[8]. Also, the frequency of pain is about 2 times more common in women than in men. The risk of musculoskeletal pain increases with advancing age, although it may occur at any age. Aging can cause deterioration of joints and mus-

Corresponding Author: Xiaolan Liu, MD, Ph.D; e-mail: liuxiaolanjp2000@yahoo.co.jp

cle weakness, leading to musculoskeletal pain. For example, knee pain from osteoarthritis is extremely common in the elderly, accounting for one-third of people over the age 60[8].

Treatment of pain is still unsatisfactory and new treatment concepts have been developed in which pain is evaluated on the basis of underlying mechanism(s). Drug therapy with non-steroidal anti-inflammatory drugs (NSAIDs) is considered as first-line therapy with or without adjuvant therapy[9]. Apart from the analgesic drugs used to relieve pain, several other approaches have been evaluated. In recent years, stem cell therapy has attracted much attention due to their potent immune modulatory effects and encouraging results in treatment of several diseases. The ability of mesenchymal stem cells to improve the inflammatory condition has opened potential avenues for treatment of various painful conditions such as inflammatory pain, neuropathic pain, and cancer pain. Fortunately, some studies[10,11] in animal models and some in humans have reported satisfactory pain relief by treatment with mesenchymal stem cell or its conditioned medium. Since the encouraging results obtained from studies in animal models of pain and in patients suffering from chronic pain both in terms of safety and efficacy, mesenchymal stem cells have entered clinical trials[12]. Besides, the use of stem cells or their conditioned medium has been suggested to provide solutions for treating painful conditions, such as bone and cartilage defects, osteoarthritis, tendon and ligament injuries, and nerve damage[10,13,14]. There are two types of mesenchymal stem cells that have been mostly evaluated for their effects on pain. Bone marrow mesenchymal stem cells (BMSCs) and adipose-derived mesenchymal stem cells (AMSCs). During cell growth and proliferation of mesenchymal stem cells in culture, various substances are released from the cells into the medium, including growth factors, cytokines, chemokines, exosomes, etc. which have potent effects as well as the stem cells. Therefore, mesenchymal stem cell conditioned medium (MSC-CM) has attracted much attention as a cell-free treatment option. In this report, we evaluated the treatment results in a group of patients with chronic musculoskeletal pain who had been administered human adipose-derived mesenchymal stem cells conditioned medium (hAMSC-CM) in order to assess its safety and efficacy and provide clues for potential new therapeutic option. To our knowledge, this is the first report on the safety and efficacy of hAMSC-CM in chronic pain relief in humans.

Patients and Methods

Medical records of patients with chief complain of pain who had been treated with hAMSC-CM were reviewed. Written informed consent and consent to publish have been obtained from the patients. Medical history, physical and orthopedic examinations, treatment, and outcome were assessed, and data related to age, sex, type of disease, and location of pain were collected. A numeric rating scale (NRS) questionnaire, including the validated chronic pain index, was given to patients to self-evaluate the effect of therapy on pain locations. The patients recorded the severity of pain before administration and at 15 min, 1 day, 1 week, and 4 weeks after administration. A second administration has been performed in 7 painful locations. The analysis was done considering two conditions: the "current pain status" and the "worst pain status in a week." The patients' demographic data are presented in the **Supplementary Table I**. There were 16 patients (8 males and 8 females) ranging in age from 39 to 77 years old (mean ± SD = 58.56 ± 11.40). In these 16 patients, there was a total of 27 body locations expressing pain treated by local injection of hAMSC-CM.

Preparation of hAMSC-CM

The hAMSC-CM was prepared basically according to a previously described procedure[15,16]. In brief, human AMSCs were isolated from adipose tissue discards obtained at liposuction therapy and have been grown using an animal origin-free (AOF) medium (sf-DOT; BIOMIMETICS SYMPATHIES Inc., Tokyo, Japan) as reported previously[17,18]. Written informed consent for use was provided by the donors. AMSCs at passages 3-5 were cultured to reach approximately 80% confluent and were incubated for 3 days in the AOF medium. The conditioned medium was then harvested and centrifuged at 3000 x g for 5 min to remove fragments, and then, the supernatant was collected and passed through a 0.22 μm filter to eliminate potential pathogens. This supernatant was used as hAMSC-CM for injection. The pain was treated by local injection of 5 ml hAMSC-CM in the joint cavity and/or 2 ml in the other areas after appropriate disinfection preparation. Overall, the frequency of hAMSC-CM injections

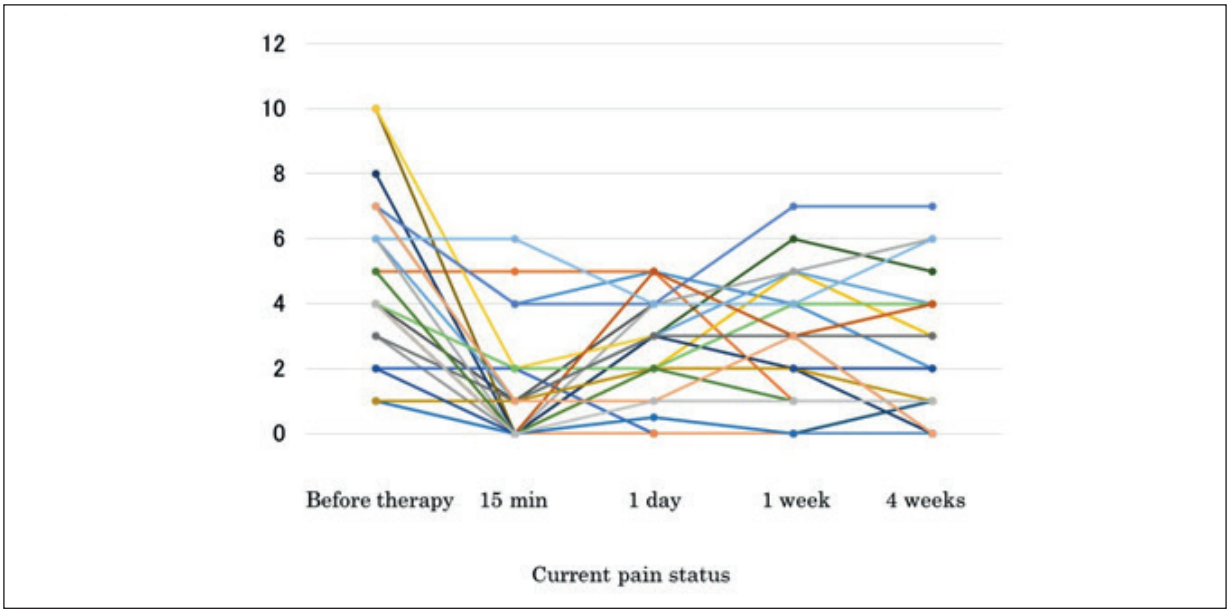

Figure 1. NRS for severity of pain in the patients after administration of hAMSC-CM for the "current pain status" in at the follow-up time points. In most patients, the severity of "current pain status" was significantly declined at 15 min after local injection and was maintained at a lower threshold compared to the before injection.

was at 5 areas in 1 patient, 3 areas in 2 patients, 2 areas in 3 patients and 1 area in the remaining 10 patients.

Evaluation of Pain

Several methods have been used for assessing pain intensity of which the NRS is the most commonly used method in clinical settings due to its ease of application and scoring[19]. For evaluation of the degree of severity of pain, the patients have been asked to conduct self-evaluation of the degree of pain using the NRS questionnaire and we recorded the severity of pain before administration and at 15 min, 1 day, 1 week, and 4 weeks after administration. Also, the patients reported their worst, least and average pain intensity over the week. The NRS was based on a 0 to 10 scale, where 0 represented no pain and 10 the worst pain. In the 16 patients, the pain was evaluated in a total of 27 body locations that has been treated by local injection of hAMSC-CM. The evaluation was done before administration, and at 15 minutes, 1 day, 1 week, and 4 weeks after administration of hAMSC-CM. The analysis was done considering two conditions: the "current pain status" and the "worst pain status in a week."

Statistical Analysis

The comparison between means was performed using one-way analysis of variance followed by the Tukey-Kramer post-hoc comparison test[20]. The significance of differences was considered if the *p*-value was less than 0.05.

Results

Supplementary Table I demonstrates demographic data and results of evaluation of pain in patients who received hAMSC-CM local administration. The NRS for severity of pain in the patients after local administration of hAMSC-CM showed that at the follow-up time points, in most body locations expressing pain, the severity of pain with regard to "current pain status" was significantly decreased at 15 min after local injection and was maintained at a lower threshold compared to before injection (Figure 1). Similarly, the severity of pain with regard to the "worst pain status in a week" was markedly declined at 1 week after local injection and was maintained at a lower threshold compared to the before injection (Figure 2). The severity of pain with regard to "current pain status" (Figure 3A) and "worst pain status in a week" (Figure 3B) was significantly decreased at indicated time points and maintained at a lower threshold compared to the before injection during the follow-up period. In the case of second administration, the severity of pain with regard to "current pain status" and "worst pain status in a week"

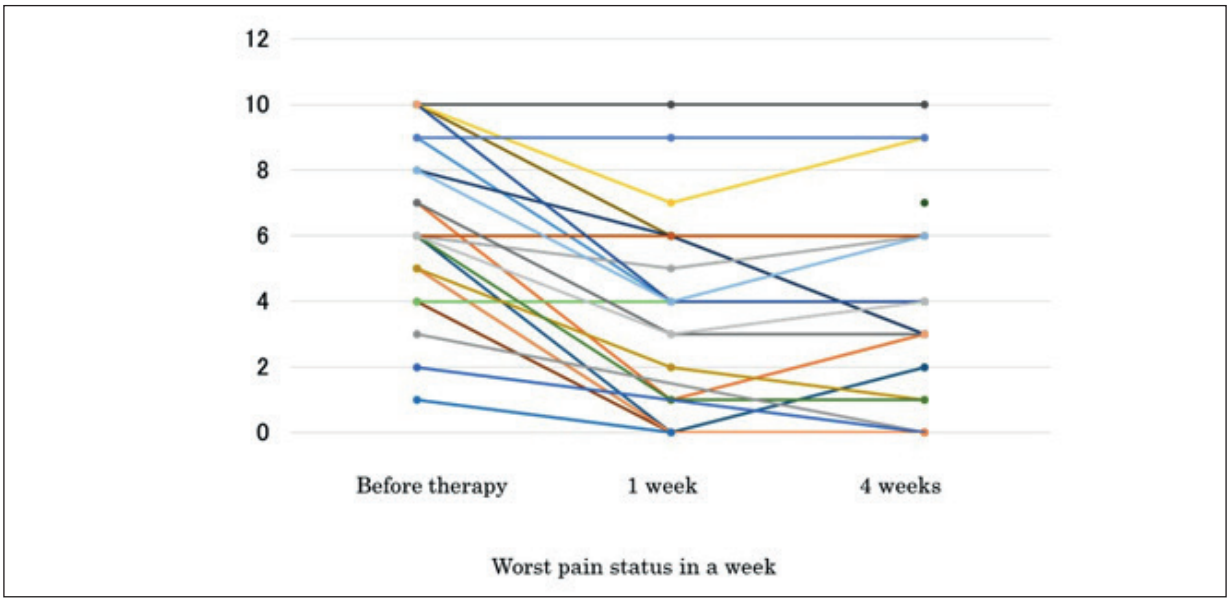

Figure 2. NRS for severity of pain in the patients after administration of hAMSC-CM for the "worst pain status in a week" at the follow-up time points. In most patients, the severity of "worst pain status in a week" was markedly declined at 1 week after local injection and was maintained at a lower threshold compared to the before injection.

was markedly decreased through the 4 weeks follow-up period (Figure 4A and B). In the case of first administration, the peak pain threshold declined at 15 min after administration which was maintained until 4 weeks at a value of about half before the administration (Figure 3A). In the case of the second administration, despite the NRS value before administration being lower than that at the first administration, the NRS value continued to decline significantly with regard to the "current pain status" at 1 week and 4 weeks after administration, and there was a tendency that the NRS value would become lower later on (Figure 4A). Only 1 patient experienced mild pain at the site of injection and one patient had mild degree of fatigue, but in both cases, it was considered as an event due to local injection *per se*. These symptoms were disappeared without treatment, and therefore, no safety problem was experienced.

Discussion

The culture supernatant derived from the human adipose-derived mesenchymal stem cells, also called conditioned medium (hAMSC-CM), contains many biologically active substances. These include growth factors, cytokines, chemokines, and exosomes that are known to be useful for promoting tissue regeneration, angiogenesis, nerve repair, immunomodulation, and bone formation[10,11,13]. Up until now, the beneficial effects of administrating AMSC-CM including hAMSC-CM in chronic musculoskeletal pain have been reported in animal models[13,14]. However, no report has examined the therapeutic potential of hAMSC-CM in humans. In this report, we retrospectively evaluated the safety and efficacy of hAMSC-CM in a group of patients who had a chief complain of chronic pain in different parts of the body. We demonstrated that hAMSC-CM which was obtained from cultured hAMSC, reduced the pain symptom during the 4 weeks follow-up period. The pain significantly decreased shortly after hAMSC-CM injection and was subsequently maintained at 1 day, 1 week and 4 weeks afterwards (Tukey-Kramer test). The continuing administration of hAMSC-CM could reduce the pain symptoms and thus improve the quality of life (QOL) of the patients.

Several studies have been conducted regarding the effect of MSC-CM and hAMSC-CM and explored their mechanism(s) of action. Although not in humans, there are plenty of studies in rodent models of neuropathic pain (NP) supporting the safety and efficacy of MSC-CM in alleviating NP. In a study by Chen et al[10] the effect of neural stem cells-conditioned medium (NSC-CM) on sciatic nerve injury and its repair mechanism were explored in a rat model. It was shown that

NSC-CM facilitated functional recovery of sciatic nerve crush injury at late stage by inhibiting inflammation. Regarding the mechanism(s), NSC-CM markedly downregulated several pro-inflammatory factors, such as TNF-α, IL-6, and IL-18, as well as CD68 inflammatory macrophages in the sciatic nerve tissue. Moreover, *in vitro* lipopolysaccharide (LPS) induced pro-inflammation of macrophages showed that NSC-CM decreased the expression of IL-6, IL-18, TNF-α, and inducible nitric oxide synthase. In the presence of NSC-CM, activation of Sirt-1 signaling in macrophages inhibited inflammation. Also, blocking of Sirt-1 by specific inhibitor, EX527, reduced the anti-inflammatory effect. The authors concluded that NSC-CM facilitates functional recovery after sciatic nerve crush injury *in vivo*. Also, by activating Sirt-1 signaling pathway *in vitro*, it inhibits the inflammation in activated macrophages.

Another study[21] investigated the effect of intraperitoneal injection of rats' BMSC-CM on the expression of NP related purinergic receptors, P2X4 and P2X7, in a rat model of NP induced by chronic constriction injury (CCI) of the sciatic nerve. MSC-CM was administered 1 day before and 7 and 11 days after CCI. In the treated animals, the NP status was evaluated using behavioral tests, such as mechanical allodynia and thermal hyperalgesia, on days 1, 3, 6, 9, 12, and 15 after the treatment. The animals were sacrificed on day 15, and the relative gene expression of P2X4 and P2X7 receptors were measured in the spinal cord using quantitative real-time PCR. It was found that mechanical allodynia and thermal hyperalgesia, as well as the expression levels of P2X4 and P2X7 receptors, were markedly decreased in the group treated with MSC-CM compared with the control group. It was concluded that the pain-relieving effects of MSC-CM in the NP rats were partially mediated by inhibiting the upregulation of P2X4 and P2X7 receptors in the spinal cord.

Furthermore, BMSC-CM was administered to spinal cord injured rats and in parallel, their properties were also assessed *in vitro*[22]. *In vitro* study[22] showed that BMSC-CM protected neurons from apoptosis, activated macrophages and was a pro-angiogenic mediator. *In vivo* study showed that BMSC-CM after spinal cord contusion improved motor recovery. The BMSC-CM characterization identified trophic factors and cytokines that were likely involved in the observed beneficial effects indicating a paracrine-mediated mode of action. These results prompted the possibility of developing a cell-free therapeutic approach.

A study[23] on the therapeutic potential of BMSC and BMSC-CM was conducted in a mouse model of neuropathic pain induced by partial sciatic nerve ligation (PSL). BMSC (1×10^6) or BMSC-CM or vehicle (gabapentin) were adminis-

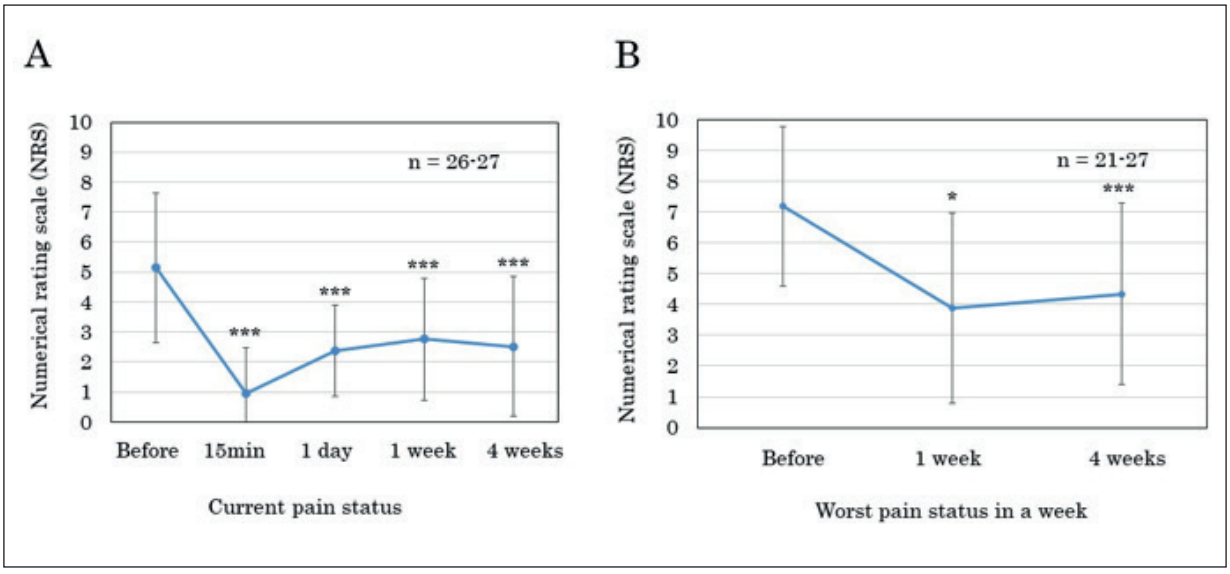

Figure 3. NRS for severity of pain in the patients after 1st administration of hAMSC-CM for the "current pain status" (**A**) and "most painful status" in a week (**B**) at the follow-up time points. The severity of current pain status and "worst pain status in a week" was significantly decreased at indicated time points during the follow-up period. 1 week after local injection and was maintained at a lower threshold compared to the before injection. *; <0.05, ***; <0.001

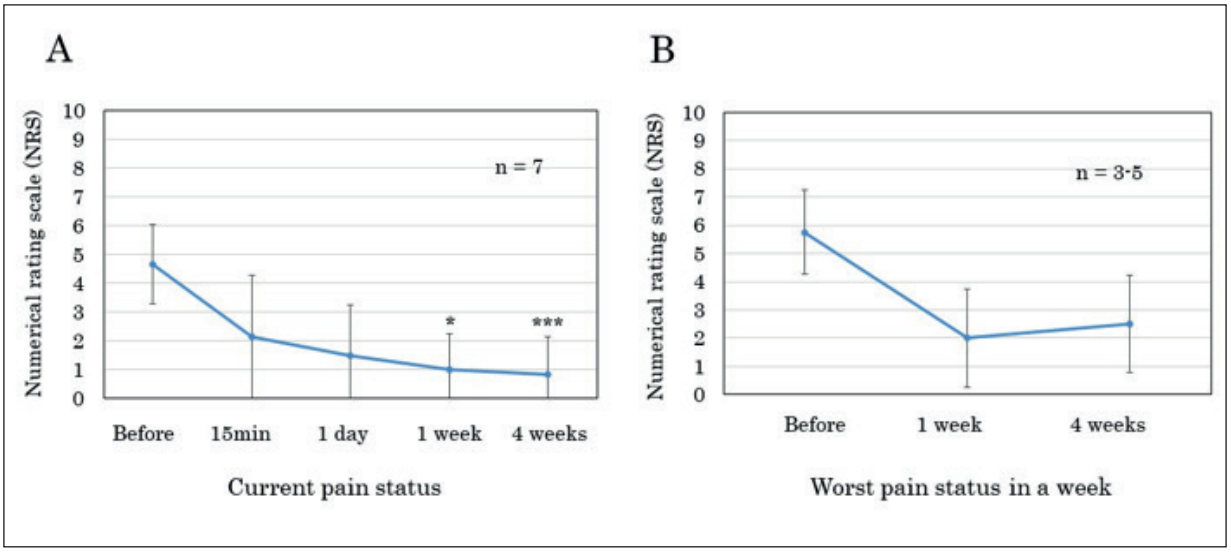

Figure 4. NRS for severity of pain in the patients after 2nd administration of hAMSC-CM for the "current pain status" (**A**) and "worst pain status in a week" (**B**) at the follow-up time points. The severity of "current pain status" was significantly decreased at 1 week and 4 weeks. The "worst pain status in a week" was also decreased at 1 week and 4 weeks (**B**). *; <0.05, ***; <0.001

tered intravenously. Neuropathic mice treated with BMSC-CM showed anti-nociceptive effect 12 hours after administration. Treatment with BMSC also showed non-reversed anti-nociceptive effect. In contrast, vehicle (gabapentin) group showed short-lasting anti-nociceptive effect. Several cytokines including IL-1β, TNF-α, and IL-6 were elevated at sciatic nerve and spinal cord after treatment with BMSC-CM and BMSC. It was concluded that BMSC-CM, similar to injection of live BMSC, produced a powerful and long-lasting anti-nociceptive effect on NP.

In an animal model of osteoarthritis (OA), the efficacy of the secretome (i.e., conditioned medium) from hAMSC to control pain and neuro-inflammation was explored[24]. A fast and long-lasting effect against hyperalgesia and allodynic pain was induced by a single injection with hAMSC-CM. All routes of administration especially intravenous route were effective. The hAMSC-CM was able to reduce inflammatory condition in both the peripheral and central nervous system. In addition, the analysis of the secretome revealed 101 immune regulatory factors. The authors suggested hAMSC-CM as a valid treatment option for OA-related pain. The underlying mechanism was linked to positive modulation of neuro-inflammatory condition in peripheral and central nervous system.

A further study[25] assessed the effect of administration of BMSC-CM on behavioral, cellular and molecular aspects of adjuvant-induced arthritis in rats. BMSC-CM was administered daily intraperitoneally for 21 days after the injection of complete Freund's adjuvant (CFA) to induce arthritis. On days 0, 7, 14 and 21 of the study, hyperalgesia, edema, serum TNF-α levels and p38MAPK and NF-κB activities were measured. It was found that BMSC-CM could reduce inflammatory symptoms and pain, serum TNF-α levels and activity of intracellular signaling pathway factors in CFA-induced arthritis.

Another study[16] evaluated the effect of immortalized hAMSC-CM on lung inflammation and fibrosis in a rat model of Bleomycin (BLM)-induced pulmonary fibrosis. The hAMSC-CM safety and toxicity were evaluated, and no adverse effects were observed. The hAMSC-CM was used at 10 μg protein and 100 μg protein/kg body weight of rats. The hAMSC-CM treatment showed a significant increase in rats' mean body weight compared to the controls. In addition, there was amelioration of lung fibrosis as shown by histopathological staining as compared to controls. Inflammation was significantly reduced in hAMSC-CM-treated mice with reduced inflammatory factors, such as F4/80 macrophage antigen staining, TNF-α mRNA and IL-6 and IL-10 protein levels. Overall, their study showed that the hAMSC-CM is safe and efficient against pulmonary fibrosis, as it significantly reduced inflammation and fibrosis, especially with the larger dose of 100 μg/kg hAMSC-CM.

Data from various animal models support the idea that TNF-α through endothelial cell damage and increase of vascular permeability can induce edema which is prerequisite for developing pain[26]. In general, arthritis involves inflammatory reactions that could be accounted for induction of pain. Studies[25,26] have documented that arthritis causes a marked increase in serum levels of TNF-α which may play important role in continuation of edema and changes in hyperalgesia during arthritis. In this regard, Yew et al[27] showed that treatment with MSC-CM not only reduced the secretion of TNF-α and IFN-γ, but also could increase the secretion of anti-inflammatory cytokines in the site of inflammation. In addition, further studies[28,29] indicated that MSC-CM injection increased expression of anti-inflammatory cytokines, lowered level of pro-inflammatory cytokines and intracellular signaling activity and *via* these immune-modulatory functions could subside spinal neuroinflammatory symptoms. The results of our study also confirmed that hAMSC-CM reduced pain in several cases associated with inflammation including rheumatoid arthritis and osteoarthritis.

Conventional treatment of musculoskeletal pain includes drug therapy using acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) as first-line therapy[30,31]. If the pain sensation is strong, then, anti-inflammatory and strong analgesics such as opioids are administered[32]. However, although these medications induce pain relief and disappearance, they possess known adverse effects[33]. In the present report, we evaluated the effect of hAMSC-CM on pain relief. We assessed 16 patients who had various degrees of chronic pain and received hAMSC-CM. In the case of first administration, the peak pain declined at 15 minutes after administration, and thereafter, the pain-relieving effect was maintained until 4 weeks at a value of about half before administration. In the case of the second administration, despite the NRS value before administration being lower than that in the first-time administration, the NRS value continued to decline further for the "current pain status" at 1 week and 4 weeks after administration. There was also a tendency that the NRS value continue to become lower later. Only 1 patient experienced mild pain at the site of injection and one patient had mild degree of fatigue, but in both cases, it was considered as an event due to local injection *per se*. These symptoms were disappeared without treatment, and therefore, no serious safety problem has been encountered.

Although the number of patients in our report is limited, the results showing a positive effect on pain relief in humans are noteworthy and warrant further larger population study to confirm and establish the role of hAMSC-CM in alleviating pain. It should be noted that in this report, the hAMSCs used for production of CM has been cultured in a proprietary AOF medium (sf-DOT)[15,17,18]. As it is well-known, hAMSCs will behave differently when cultured in different types of medium and environment. In this respect, it is possible that the proprietary AOF medium used has provided superior quality hAMSCs, capable of secreting various biologically active micro-molecules into the medium with a potent effect on chronic pain.

Conclusions

We showed that local administration of hAMSC-CM did not show serious adverse effect in the limited number of patients evaluated. It significantly reduced the chronic pain of several etiologies, including osteoarthritis, herniated disc, meniscus tear and rheumatoid arthritis. In particular, the potent anti-inflammatory effect of hAMSC-CM makes it an attractive candidate for the treatment of inflammatory pain. The hAMSC-CM may provide either a substitute for routine analgesic drugs or serve as an adjunct to reduce the dose of routine analgesic drugs and thus prevent the drug adverse effects. Although not evaluated in our study, a combination of hAMSC-CM with analgesics could also fortify their anti-pain effects and improve the patient's QOL. Further clinical studies are needed to confirm analgesic effects of hAMSC-CM and clarify its underlying mechanism(s).

Conflict of Interest

The authors declare that they have no conflict of interest.

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