Original Article

Effects of Mesenchymal Stem Cells Conditioned Medium on Behavioral Aspects of Inflammatory Arthritic Pain Induced by Complete Freund's Adjuvant

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Abstract

Background: Rheumatoid arthritis is a type of inflammatory pain and is an autoimmune and chronic inflammatory disease which can lead to hyperalgesia, edema and decreased motor activity in affected area. Mesenchymal stem cells conditioned medium (MSC-CM) has anti-inflammatory mediators which can regulate the immune responses, alleviate inflammatory symptoms and has a paracrine effects too. The aim of this study was to evaluate the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory arthritic pain which induced by Complete Freund's adjuvant (CFA).

Materials and Methods: Complete Freund's adjuvant-induced arthritis (AA) was caused by single subcutaneous injection of CFA into the rat's hind paw on day zero. MSC-CM was administered daily and intraperitoneal during the 21 days of the study after CFA injection. Hyperalgesia and edema were assessed on days 0, 7, 14 and 21 of the study respectively with radian heat and plethysmometer instrument.

Results: The results of this study indicated the significant roles of MSC-CM in betterment of inflammatory symptoms such as hyperalgesia and edema during different stages of inflammation caused by CFA. The continuing injection of MSC-CM could reduce the inflammatory symptoms.

Conclusion: Long term treatment by MSC-CM can alleviate hyperalgesia and edema and decrease those to the level of the time before induction of inflammation.

Keywords: Inflammation, Pain, Hyperalgesia, Edema, MSC-CM

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Introduction

Inflammatory pain is a type of pathological pain caused by peripheral tissue inflammation and tissue damage which is characterized by an increased sensitivity to stimuli of the affected tissue. Rheumatoid arthritis (RA) is a type of inflammatory pain (1) and is an autoimmune inflammatory disease with unknown etiology which has been lead to pain, swelling, hyperalgesia, dryness and inflammation of the joints and surrounding tissue, cartilage and bone destruction, dysfunction and disability (2). The RA

disease process is variable, often is associated with periods of relapse and sometimes with a reduction in symptoms. Despite the unknown etiology of RA, it seems that pro-inflammatory cytokines elevated during the disease which can cause inflammatory symptoms such as hyperalgesia and edema (3). Inflammatory biochemical factors such as the kinins, prostaglandins, histamine, and serotonin act synergistically to induce hyperalgesia as well as increased vascular permeability and also development of acute pain and edema (4). Hyperalgesia caused due to excessive sensitivity of pain receptors because of decrease of activation threshold as a result of release of some chemicals like histamine, bradykinin and etc. The most important cause of progression of hyperalgesia is activation of pain afferents during inflammation. It seems that the neural mechanism of hyperalgesia is sensitization of the primary afferent nociceptive fibers (5). Following inflammation and injury, an inflammatory response is generated by T-lymphocytes and this is further amplified by producing pro-inflammatory cytokines like TNF-α and IL-1β and maintain the development of pain, hyperalgesia and edema (1). A variety of factors like Complete Freund's Adjuvant (CFA), Formalin, Carrageenan and Capsaicin are used to create an inflammatory pain model and there are differences in underlying mechanisms and clinical symptoms of inflammation induced by these adjuvants. Studies have shown that inflammation induced by CFA is usually more stable than other inflammatory adjuvants. Inflammatory pain model induced by CFA is a biphasic model that in the first phase (inflammatory phase) is associated with increased pain due to the presence of inflammatory cytokines such as TNF-α and IL-1β, while in the second phase (arthritic phase) hyperalgesia due to the elevation of opioid receptors expression dramatically decreased (6). Mesenchymal stem cell (MSC) is a population of pluripotent adult stem cells which have a high capacity for self-renewal and proliferation and represent functional characteristics which have open the way for cell-based therapy for autoimmune disorders (7-9). MSCs are able to secrete a wide range of trophic factors which can demonstrate paracrine effects on other cell types. Hence, mesenchymal stem cell conditioned medium (MSC-CM) have shown potential therapeutic applications in regeneration or in pain alleviation (8). MSC-CM is a supernatant that is achieved from *in vitro* cultured stem cells conditions and can cause effects similar to MSC. In recent years their eventuality to take part in the immune response modulation is discussed (9). The exact mechanisms of immune regulation of MSC-CM are not clear yet, but interactions with other immune regulatory cells, elevation of anti-inflammatory mediators and diminution of pro-inflammatory cytokines may be involved in this process (8, 9). This has led to the expansion of novel applications of MSC-CM for the remedy of inflammatory and degenerative rheumatic diseases such as RA, osteoarthritis (OA) and also bone and cartilage disorders (10). Then, based on the potency of MSC-CM in modulation of immune responses and inflammatory symptoms, in this study, the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory arthritic pain induced by CFA adjuvant was investigated.

Methods

Laboratory animals

In this study, the adult male Wistar rats (n=96) weighing 200–220 gr. were used in all experiments. These animals were housed in polypropylene cages under standard environmental circumstances (22±2ºC, humidity 60–70 %, 12 h light/dark cycle) and allowed free access to food and water. All the experiments were approved by the international association for the study of pain and local ethics committee of the utilization of animals in research for the treatment of animals and the guidelines of the ethical standards for the investigations of experimental pain in animals were followed precisely (11).

In order to determine the effect of MSC-CM on inflammatory pain model and the effectiveness of this treatment, a series of experiments were performed. Rats were randomly divided into different experimental groups, as follows: (a) CFA group, (b) CFA control group, (c) CFA+CM (d) Sham group. According to the study procedure, each group was divided into four subgroups based on different time points of the study (days 0, 7, 14, and 21) and there were 6 rats in each subgroup.

Experimental procedure

Complete Freund's adjuvant (CFA)-induced arthritis was evoked by single subcutaneous injection of (100µL) heat-killed Mycobacterium tuberculosis suspended in sterile mineral oil (10 mg/mL; CFA; Sigma, St Louis, MO, USA) into the rats' right hind paw on day zero (under light anesthesia with methoxyflurane). The CFA control group was received sterile mineral oil once only (100µL) (S.C.). First day after CFA injection, unilateral inflammation was established in the injected hind paw of rat (acute phase), and the next weeks after inflammatory phase were arthritic phase (chronic phase). From the first day after CFA injection, experimental groups received the MSC-CM on a daily basis and were injected intraperitoneal (250µL/rat) (i.p.). The sham group received sterile mineral oil with single subcutaneous (100µL) injection in the rat's right hind paw and also received MSC-CM on a daily basis (250µL/rat) (i.p.). In this study, hyperalgesia and paw edema were assessed on day 0 (immediately before CFA injection), on days 7 (inflammatory phase), 14 and 21 (arthritic phase) (12).

MSC-CM preparation and administration

Bone marrows were obtained from the bone marrow of femurs and tibias of two-month-old male Wistar rats (weighing 200-250 g) under sterile circumstances. The derived tibia and femur bone marrow soaked in cold PBS and eliminated adherent soft tissues. Bone marrow (includes hematopoietic stem cells and stromal cells) were cultured in minimal essential medium alpha (α-MEM, Gibco, Invitrogen, Carlsbad, CA, USA) containing 15% fetal bovine serum (FBS, Gibco, Invitrogen, Carlsbad, CA, USA) and 1% Penicillin/streptomycin (Gibco, Invitrogen, Carlsbad, CA, USA) and incubated at 37°C in the presence of 5% carbon dioxide. The cells reach to passages three were applied for subsequent studies. After 48 hours, the medium of cells were replaced. The medium used in this step is free of FBS. In the next stage, after 48 hours, the medium in flask (MSCs supernatant) were gathered and thereupon filtered by the 0.2 micrometers. Cell supernatant was reserved at -80°C until the injection (13).

Assessment of CFA-induced arthritis and paw edema

To confirm the correct injection of CFA, paw volume was measured in both injected and contralateral paws pre and post-injection during different time points of the study. This measurement was conducted by displacement of an electrolyte solution in a plethysmometer (model 7141; Ugo Basile, Comerio-Varese, Italy). Briefly, the rat hind paw was submerged up to the tibiotarsal joint into the electrolyte-filled Perspex cell of the plethysmometer. The volume of the liquid displacement, which is associated with the paw volume, was illustrated on a digital display. Volume measurements were performed twice for each paw and the average was calculated. The edema was quantified by measuring the differences in the paw volume between the day 0 and other different time points of the study (14, 15).

Behavioral test (Thermal hyperalgesia assessment)

Paw withdrawal latencies (PWL) from noxious heat using the plantar test were assessed in both CFA-injected and control paw on Days 0 (before injection of CFA), 7, 14, and 21. PWL in response to radiant heat by plantar test apparatus was executed in the control and experimental groups (Ugo Basilar, Verse, Italy). 10-15 minutes before the test, rats were placed in a Plexiglas boxes positioned on a glass surface in order to habituate to the test environment. The heat source (infrared light) was positioned under the plantar surface of the affected hind paw and projected focally. The digital timer connected to the heat source recorded the PWL to the nearest 0.1 s. automatically. Heating was terminated at 20 s cut off to prevent tissue damage if an animal failed to withdrawals paw from the stimulus prior to the cut off. Each rat received three trials per hind paw at an interval of 5–10 min. PWL, for each paw at an interval of 5 - 10 min, was done and the results are represented as the difference in the PWL score between injected paw and control paw and statistical comparisons between groups assessed this difference. The value obtained in the negative expressed the hyperalgesia in the inflamed paw (6, 11).

$$H = \frac{(Rt1 + Rt2 + Rt3)}{3} - \frac{(Lt1 + Lt2 + Lt3)}{3}$$

H: The difference between the right and the left response time (Hyperalgesia).

R_t: Right PWL L_t: left PWL

Statistical analysis

Results were represented as mean±standard

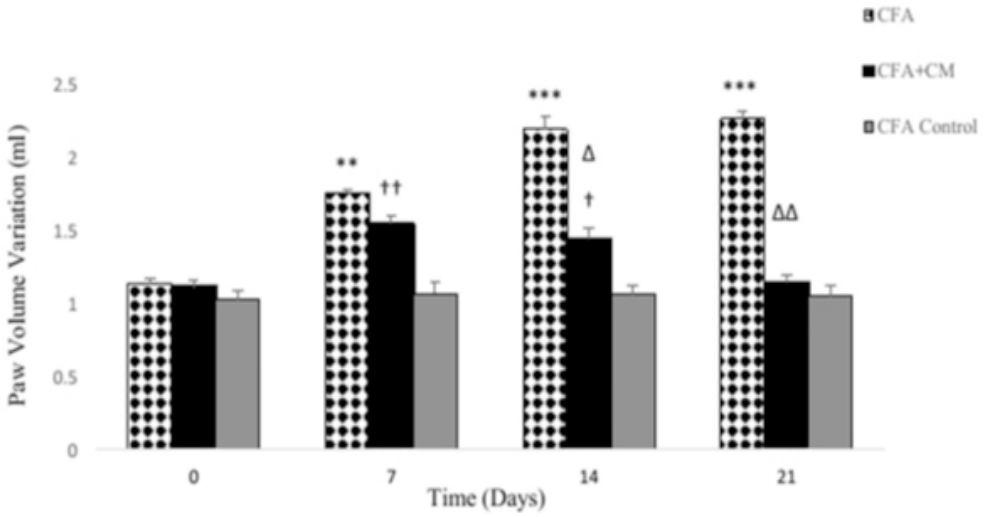

Fig. 1. Paw volume incremented in CFA group in different time points of the study compared to baseline substantially. Long-term injection of MSC-CM downturned paw volume significantly. Results presented as Mean±SEM (n=6/group).
** p≤0.01 and *** p≤0.001 for comparing the paw edema variations between baseline and different days of the study in CFA group. † p≤0.05 and †† p≤0.01 for comparing the paw volume variations between day 0 and different days of the study in CFA+CM group. Δ p≤0.05 and ΔΔ p≤0.01 for illustrating the variations in paw volume at day 14 and day 21 compared to day7 in the CFA+CM group.

error of mean (SEM). To compare the results within the groups (during different time points), repeated measurement ANOVA test (One way ANOVA) and post hoc Tukey multiple range tests were used and unpaired student t- test was applied to identify significant differences of the means on the same days between groups. SPSS software version 21 was used for data analysis and the charts were analyzed by Excel. Statistical significance was accepted at p≤0.05 level.

Results

Paw volume variations during different stages of inflammation

CFA injection in the rats hind paw can induce inflammation and increased ipsilateral paw volume (in the affected paw), which continued up to 21 days after CFA injection. Arthritis due to CFA injection was evaluated by measurements of paw volumes pre- and post-injection (on days 0, 7, 14 and 21). Paw volume significantly increased on days 7, 14, and 21 after CFA injection compared with day 0 and with CFA control group (p≤0.01 for day 7 and p≤0.001 for day 14 and 21). Paw volume on days 7, 14 and 21 compared with day 0 in CFA group showed a considerable increase (p≤0.01 for day 7 and p≤0.001 for day 14 and 21). There were no significant differences in reduction in paw volume following injection of sterile mineral oil to the rat's right hind paw on different days of the study relative to baseline in the CFA control group. Additionally, there were no significant differences in paw volume of rats during 21-days of the study in sham group (Hence, the results of the sham groups are not shown graphically).

Daily administration (i.p.) of MSC-CM in the CFA+CM group could reduce paw volume. MSC-CM injection in CFA+CM group could significantly reduce paw edema throughout this study compared to same days in CFA group (p≤0.05 for day 7, p≤0.01 for day 14 and p≤0.001 for day 21). In comparison between the groups CFA+CM and CFA, the results illustrated that reduction of paw edema following the injection of MSC-CM, at day 21 was higher than day 14 substantially (p≤0.01). Continuing injection of MSC-CM decremented paw volume in CFA+CM group, so that at day 21 of the experiment, no

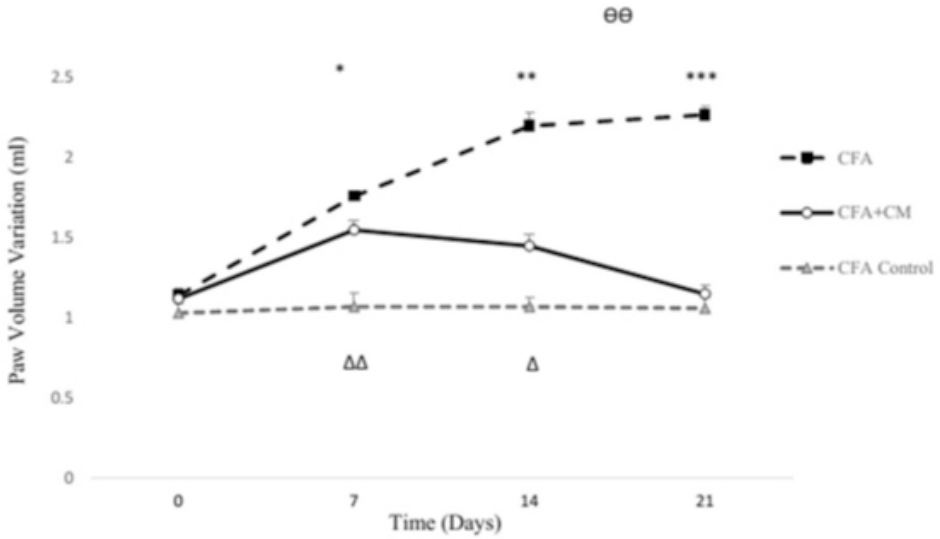

Fig. 2. CFA injection increased paw volume significantly, while MSC-CM administration caused a notable diminution in paw volume compared with CFA group. Results stated as Mean±SEM (n=6/group).
* p≤0.05, ** p≤0.01 and *** p≤0.001 for comparing the paw volume changess between CFA and CFA+CM groups in identical days. Δ p≤0.05 and ΔΔ p≤0.01 for comparing the paw volume variations between CFA+CM and CFA control groups in identical days. ΘΘ p≤0.01 for comparing the differences in paw volume alterations in CFA and CFA+CM groups at day 14 compared to day 21.

significant distinction in paw volume in CFA+CM group compared with CFA control group was perceived.

Variations in thermal hyperalgesia during different stages of inflammation

Intraplantar injection of CFA into the right hind paws of rats caused inflammation and hyperalgesia in the affected paw, which continued up to 21 days after CFA injection. Arthritis due to CFA injection was evaluated by measurements of hyperalgesia pre- and post-injection (on days 0, 7, 14 and 21). Hyperalgesia significantly increased on the 7th day in the CFA group compared with day 0 (p≤0.001), but the persistence of inflammation significantly decreased hyperalgesia on later two days of the study (days 14 and 21) compared to baseline. Howbeit, there was still a significant increase compared to day 0 (p≤0.01). There were no significant differences in reduction of thermal hyperalgesia following injection of sterile mineral oil to the rat's right hind paw on different days of the study relative to baseline in the CFA control group. Considerable difference in the PWL between CFA and CFA control groups on different time points of the study were perceived (p≤0.001). Additionally, there were no significant differences in PWL of rats during 21-days of the study in sham group (Hence, the results of the sham groups are not shown graphically).

Daily administration (i.p.) of MSC-CM in the CFA+CM group declined hyperalgesia, as the continuity of this injection for 21 days also decremented hyperalgesia even more than the baseline (p≤0.05). MSC-CM injection in CFA+CM group significantly declined thermal hyperalgesia throughout this study compared to same days in CFA group (p≤0.05 for day 7 and p≤0.001 for days 14 and 21) and the continued injection for 21 days reduced thermal hyperalgesia even more than CFA control group. In comparison between the CFA and CFA+CM groups, the results illustrated that the rate of reduction of thermal hyperalgesia following the injection of MSC-CM, at day 21th after CFA injection between these two groups was higher than days 7 and 14 substantially (p≤0.001). Continuing injection of MSC-CM decremented PWL in CFA+CM group, so that at day 21 of the experiment, no significant difference in PWL in CFA+CM group compared with

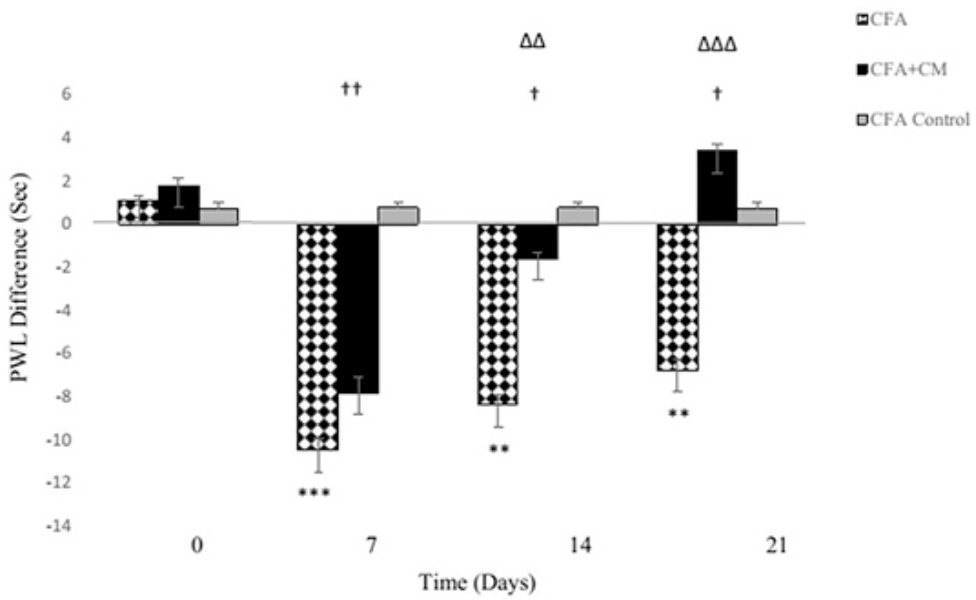

Fig. 3. Thermal hyperalgesia substantially incremented in different time points of the study in CFA group compared to day zero and long-term injection of MSC-CM considerably decremented thermal hyperalgesia. Results presented as Mean ± SEM (n= 6/group).
** p≤0.01 and *** p≤0.001 for comparing the PWL alterations in CFA group between day 0 and different time points of the study. † p≤0.05 and †† p≤0.01 for comparing the PWL variations in CFA+CM group between day 0 and different time points of the study. ΔΔ p≤0.01 and ΔΔΔ p≤0.001for comparing the changes in PWL in the CFA+CM group at day 14 and day 21 compared to day 7.

CFA control group was observed.

Discussion

The main objective of the present study was to evaluate the effects of mesenchymal stem cells conditioned medium on behavioral aspects of inflammatory pain induced by CFA adjuvant. We also assessed hyperalgesia and edema during the long-term administration of MSC-CM.

The results of this study indicated the role of MSC-CM in reducing edema and hyperalgesia during different stages of inflammation caused by CFA adjuvant. The continuing injection of MSC-CM could reduce the inflammatory symptoms to a time before induction of inflammation. Therefore, the CFA+CM group on day 21 of the study did not have significant difference with baseline and the control group.

In this study plantar injection of CFA induced paw inflammation and edema which continued up to day 21 after the CFA injection. Hyperalgesia elevated considerably on day 7 after the CFA injection, but the continuity of inflammation declined hyperalgesia notably on days 14 and 21 of the study compared to day 7. Animal models of inflammatory and neuropathic pain are widely applied to study the mechanisms of acute and chronic pain (16). Arthritis model induced by CFA (containing inactivated Mycobacterium tuberculosis bacteria which is suspended in sterile mineral oil) in rats is an inflammatory model extensively used in etiopathogenic investigational drug and molecular studies due to its similarity to human RA and assay the pathophysiological and pharmacological changes during human RA (11).

Intraplantar injection of inflammatory agents such as CFA causes elevated firing of peripheral afferents in the spinal cord, leading to hyperalgesia (17). Inflammatory pain is specified by an increased sensitivity to mechanical or thermal stimuli of the affected area. Subsequent of tissue injury, an inflammatory response is initiated by local macrophages and reinforced by migrating blood cells (1). Sensitization of the primary afferent nociceptive fiber during inflammation is an important factor in the

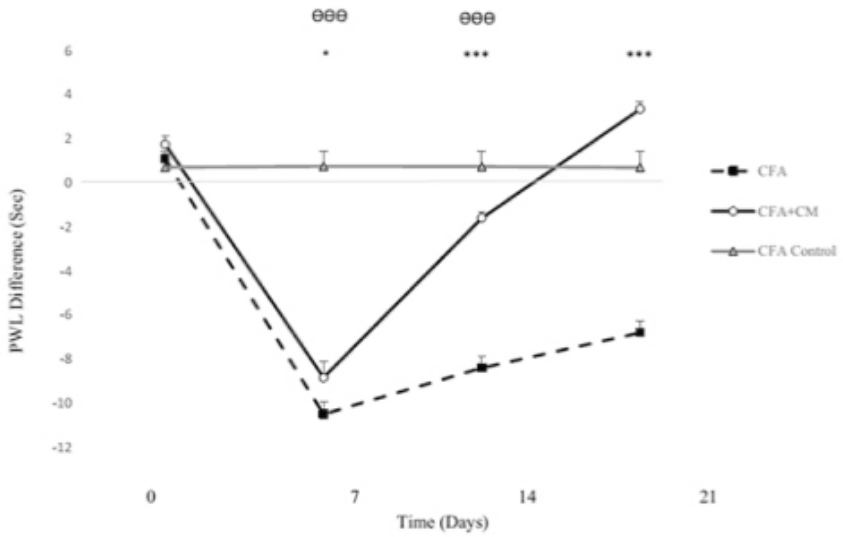

Fig. 4. CFA injection considerably incremented thermal hyperalgesia while MSC-CM administration reduced thermal hyperalgesia compared to the CFA group substantially. Results stated as Mean±SEM (n=6/group). * $p \leq 0.05$ and *** $p \leq 0.001$ for Comparing the PWL variations between CFA and CFA+CM groups in identical days. ΘΘΘ $P \leq 0.001$ for comparing the differences in PWL variations in CFA and CFA+CM groups at days 7 and 14 compared to day 21.

creation and development of hyperalgesia (5, 6).

The behavioral tests as an example hyperalgesia, following inflammatory pain conditions examine the changes in an animal's responses to mechanical or heat stimuli and reflect an alteration of sensory processes. These behavioral tests have been effectual for elucidating the mechanisms of central and peripheral processes which occur in response to inflammation (16).

Pursuant to Hargreaves study, rats due to its inflammation induced by plantar injection of CFA, had withdrawn his affected paw from the thermal stimuli, but some other studies have shown that motor behaviors subsequent of the inflammation are normal and no notable changes in motor activities are observed (18).

A study illustrated that plantar injection of CFA incremented hyperalgesia and edema from 24 hours after the CFA injection and continued up to the first week after the injection (14). However, Cicala *et al,* showed that hyperalgesia existed only between Days 14 and 21 subsequent of arthritis induction due to CFA injection (19). Other studies have indicated that hyperalgesia and edema induced by plantar injection of CFA two hours after the injection are initiated and continued for at least a month. The researchers expressed that injection of inflammatory factors such as CFA to the hind paw through lessen the stimulation threshold of peripheral afferents in the spinal cord lead to hyperalgesia and edema during the first weeks after intervention (6, 11, 20).

Thermal hyperalgesia never happened in the contralateral paw during arthritis induced by CFA adjuvant. Injection of CFA into the one hind paw may have a pivotal role in the induction of hyperalgesia which occurs only in the ipsilateral paw, however the reasons for the absence of hyperalgesia in the contralateral paw should be clarified (6).

The results of this study in line with previous studies have shown that inflammatory model induced by CA injection, is a biphasic model that in the first phase (acute phase) was associated with increase of pain and hyperalgesia due to the presence of pro-inflammatory cytokines such as TNF-α and IL-1β while in the second phase (chronic phase), hyperalgesia substantially declined compared to the previous days due to the presence of opioid receptors (6). Hammond et al., represented that an increase in the potency of opioid agonists alleviates hyperalgesia as an example of inflammatory symptoms through inflammation and arthritis (21). Studies have suggested that MSC-CM containing multiple growth

factors, anti-inflammatory mediators and different chemokines that can alleviate inflammatory symptoms and problems subsequently be involved.

Scientists showed that MSC-CM not only through production of soluble trophic factors can stimulate intracellular mechanisms of damaged cells and inflammatory area but also by inducing secretion of functional active agents via neighboring cells can relieve symptoms of inflammation (8, 22, 23). accordingly, our results not only represent the effectual role of MSC-CM in the betterment of symptoms of acute phase of the inflammation induced by CFA, but also showed that continuing the administration of MSC-CM during inflammation could reduce inflammatory symptoms until day 21 of the study (chronic arthritic phase).

Conclusion

Our study showed that MSC-CM long term treatment significantly reduced hyperalgesia and paw edema during both acute and chronic phases of CFA-induced inflammatory arthritis in male wistar rats. However, further studies are needed to evaluate the effect of MSC-CM on different aspects of inflammation, cytokines production and intracellular signaling pathways activity.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett. 2004;361(1):184-7.
2. Kojima M, Kojima T, Suzuki S, Takahashi N, Funahashi K, Kato D, et al. Alexithymia, depression, inflammation, and pain in patients with rheumatoid arthritis. Arthritis Care Res. 2014;66(5):679-86.
3. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. Pharmacol rev. 2000;52(4):595-638.
4. Troullos ES, Hargreaves KM, Butler DP, Dionne RA. Comparison of nonsteroidal anti-inflammatory drugs, ibuprofen and flurbiprofen, with methylprednisolone and placebo for acute pain, swelling, and trismus. J Oral Maxillofac Surg. 1990;48(9):945-52.
5. Manning DC, Raja SN, Meyer RA, Campbell JN. Pain and hyperalgesia after intradermal injection of bradykinin in humans. Clin Pharmacol Ther. 1991;50(6):721-9.
6. Zaringhalam J, Manaheji H, Mghsoodi N, Farokhi B, Mirzaiee V. Spinal μ-opioid receptor expression and hyperalgesia with dexamethasone in chronic adjuvant-induced arthritis in rats. Clin Exp Pharmacol Physiol. 2008;35(11):1309-15.
7. Fessler E, Dijkgraaf FE, Felipe De Sousa EM, Medema JP. Cancer stem cell dynamics in tumor progression and metastasis: is the microenvironment to blame? Cancer Lett. 2013;341(1):97-104.
8. Platas J, Guillén MI, del Caz MDP, Gomar F, Mirabet V, Alcaraz MJ. Conditioned media from adipose-tissue-derived mesenchymal stem cells downregulate degradative mediators induced by interleukin-1β in osteoarthritic chondrocytes. Mediators Inflamm. 2013;2013.
9. Ivanova-Todorova E, Bochev I, Dimitrov R, Belemezova K, Mourdjeva M, Kyurkchiev S, et al. Conditioned Medium from Adipose Tissue-Derived Mesenchymal Stem Cells Induces CD4. Biomed Res Int. 2012;2012.
10. Jorgensen C, Noel D. Mesenchymal stem cells in osteoarticular diseases: an update. Int J Mo1 Cell Med Winter. 2012;1(1):2.
11. Zaringhalam J, Akhtari Z, Eidi A, Ruhani AH, Tekieh E. Relationship between serum IL10 level and p38MAPK enzyme activity on behavioral and cellular aspects of variation of hyperalgesia during different stages of arthritis in rats. Inflammopharmacology. 2014;22(1):37-44.
12. Zaringhalam J, Hormozi A, Tekieh E, Razavi J, Khanmohammad R, Golabi S. Serum IL-10 involved in morphine tolerance development during adjuvant-induced arthritis. J Physiol Biochem. 2014;70(2):497-507.
13. Aali E, Mirzamohammadi S, Ghaznavi H, Madjd Z, Larijani B, Rayegan S, et al. A comparative study of mesenchymal stem cell transplantation with its paracrine effect on control of hyperglycemia in type 1 diabetic rats. J Diabetes Metab Disord. 2014;13(1):1.
14. Rezazadeh S, Zaringhalam J, Manaheji H, Kebryaeezadeh A. Anti-inflammatory and anti–hyperalgesic activities of Stachys athorecalyx extracts on CFA-induced inflammation. J Med Plant Res. 2009;3(5):368-76.
15. Zaringhalam J, Tekieh E, Manaheji H, Akhtari Z. Cellular events during arthritis-induced hyperalgesia are mediated by Interleukin-6 and p38 MAPK and their effects on the expression of spinal mu-opioid receptors. Rheumatol Int. 2013;33(9):2291-9.
16. LaBuda CJ, Fuchs PN. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp Neurol. 2000;163(2):490-4.
17. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain. 1988;32(1):77-88.
18. Iadarola MJ, Brady LS, Draisci G, Dubner R. Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: stimulus specificity, behavioral parameters and opioid receptor binding. Pain. 1988;35(3):313-26.
19. Cicala C, Ianaro A, Fiorucci S, Calignano A, Bucci M, Gerli R, et al. NO-naproxen modulates inflammation, nociception and

downregulates T cell response in rat Freund's adjuvant arthritis. Br J Pharmacol. 2000;130(6):1399-405.
20. Nagakura Y, Okada M, Kohara A, Kiso T, Toya T, Iwai A, et al. Allodynia and hyperalgesia in adjuvant-induced arthritic rats: time course of progression and efficacy of analgesics. J Pharmacol Exp Ther. 2003;306(2):490-7.
21. Hammond D. Persistent inflammatory nociception and hyperalgesia: implications for opioid actions in the brainstem and spinal cord. Hyperalgesia: Molecular Mechanisms and Clinical Implications (Brune K and Handwerker HO eds) pp. 2004:291-309.
22. Yew T-L, Hung Y-T, Li H-Y, Chen H-W, Chen L-L, Tsai K-S, et al. Enhancement of wound healing by human multipotent stromal cell conditioned medium: the paracrine factors and p38 MAPK activation. Cell Transplant. 2011;20(5):693-706.
23. Maumus M, Jorgensen C, Noël D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: role of secretome and exosomes. Biochimie. 2013;95(12):2229-34.