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CONDITIONED MEDIUM OF MESENCHYMAL STEM CELLS: DUAL ANTI-INFLAMMATORY AND ANALGESIC EFFECTS IN RHEUMATOID ARTHRITIS MODEL

Fedir HLADKYKH[1,2]✉, Volodymyr STUDENT[2], Tetiana LIADOVA[2], Halyna LYKHATSKA[3], Roman KOMOROVSKY[3], Svitlana SMIYAN[3]

[1] Radiation Pathology and Palliative Medicine Group, Radiology Department, Grigoriev Institute for Medical Radiology and Oncology, Kharkiv, Ukraine
[2] Department of General Surgery, Anesthesiology and Palliative Medicine, School of Medicine, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine
[3] Department of Internal Medicine II, Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

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Abstract

Background. Rheumatoid arthritis (RA) is a common autoimmune disease. Current therapies, including anti-inflammatory drugs, corticosteroids, disease-modifying antirheumatic drugs and biologic agents, have limitations such as adverse effects and loss of efficacy. The conditioned medium of mesenchymal stem cells (CM-MSC) may offer an alternative due to its regenerative and immunomodulatory properties.
The objective of the study was to characterize the anti-inflammatory and analgesic activity of the CM-MSC in an adjuvant arthritis (AA) model in rats.
Materials and methods. CM-MSC was obtained by culturing umbilical mesenchymal stem cells (MSCs) in serum-free medium, filtering, and standardizing for galectin-1 content (6.0 pg/mL). It was administered intramuscularly to rats at a dose of 0.6 ml/kg body weight. AA was induced by injecting complete Freund's adjuvant into the right hind limb. Treatments

Résumé

Milieu conditionné de cellules souches mésenchymateuses: double effet anti-inflammatoire et analgésique dans un modèle de polyarthrite rhumatoïde

Introduction. La polyarthrite rhumatoïde est une maladie auto-immune fréquente. Les traitements actuels, incluant les médicaments anti-inflammatoires, les corticoïdes, les antirhumatismaux modificateurs de la maladie et les agents biologiques, présentent des limites telles que des effets indésirables et une perte d'efficacité. Le milieu conditionné de cellules souches mésenchymateuses (CM-CSM) pourrait offrir une alternative grâce à ses propriétés régénératives et immunomodulatrices.
L'objectif de l'étude a été la caractérisation de l'activité anti-inflammatoire et analgésique du CM-CSM dans un modèle d'arthrite adjuvante induite chez le rat.

✉ Address for correspondence: Fedir HLADKYKH
Department of General Surgery, Anesthesiology and Palliative Medicine,
School of Medicine, V. N. Karazin Kharkiv National University
Address: 4, Svobody Sq, Kharkiv 61022, Ukraine
E-mail: fedir.hladkykh@gmail.com; Phone: +380997827872

were given from day 14 to 28 post-induction. The anti-inflammatory activity was measured by changes in limb volume, while analgesic activity was assessed via mechanical compression. Rats were divided into four groups: control, diclofenac sodium, CM-MSC, and saline.

Results. CM-MSC reduced paw edema by 22.0% ($p = 0.01$) and increased the pain threshold by 42.7% ($p < 0.01$) in a rat model of AA. Diclofenac sodium produced more pronounced effects; however, CM-MSC still demonstrated statistically significant anti-inflammatory and analgesic activity.

Conclusions. These findings suggest that CM-MSC holds promise as a cell-free therapeutic approach for managing chronic inflammation and pain in rheumatoid arthritis. Further studies are needed to elucidate the underlying mechanisms and optimize treatment protocols.

Keywords: mesenchymal stem cells, rheumatoid arthritis, adjuvant arthritis, autoimmune diseases, stem cell therapy.

List of abbreviations:
RA – rheumatoid arthritis
AD – autoimmune diseases
MSCs – mesenchymal stem cells
CM-MSC – conditioned medium of mesenchymal stem cells
AA – adjuvant arthritis
NSAIDs – non-steroidal anti-inflammatory drugs
DS – diclofenac sodium
CFA – complete Freund's adjuvant

Matériels et méthodes. Le CM-CSM a été obtenu par culture de cellules souches mésenchymateuses issues du cordon ombilical dans un milieu sans sérum, suivie d'une filtration et d'une standardisation basée sur la teneur en galectine-1 (6,0 pg/ml). Il a été administré par voie intramusculaire aux rats à la dose de 0,6 ml/kg de poids corporel. L'arthrite adjuvante a été induite par injection d'adjuvant complet de Freund dans le membre postérieur droit. Les traitements ont été administrés du 14e au 28e jour suivant l'induction. L'activité anti-inflammatoire a été évaluée par les variations de volume du membre, et l'activité analgésique, à l'aide de tests de compression mécanique. Les animaux ont été répartis en quatre groupes: témoin, diclofénac sodique, CM-CSM et solution saline.

Résultats. Le CM-CSM a permis une réduction de l'œdème de la patte de 22,0% ($p = 0,01$) et une augmentation du seuil de douleur de 42,7% ($p < 0,01$) dans un modèle d'arthrite adjuvante chez le rat. Le diclofénac sodique a induit des effets plus marqués; toutefois, le CM-CSM a montré une activité anti-inflammatoire et analgésique statistiquement significative.

Conclusions. Ces résultats suggèrent que le CM-CSM constitue une approche thérapeutique acellulaire prometteuse pour la prise en charge de l'inflammation chronique et de la douleur dans la polyarthrite rhumatoïde. Des études complémentaires sont nécessaires afin d'élucider les mécanismes sous-jacents et d'optimiser les protocoles thérapeutiques.

Mots-clés: cellules souches mésenchymateuses, polyarthrite rhumatoïde, arthrite adjuvante, maladies auto-immunes, thérapie cellulaire.

Introduction

In recent years, particular attention has been paid to the increasing incidence of autoimmune diseases (AD), as viral infections are frequently identified as key environmental factors in their development[1]. Traditionally, viral infections have had a complex relationship with various ADs, including rheumatoid arthritis (RA)[2-4]. The mechanisms underlying the link between viruses and autoimmunity remain poorly understood. Among the proposed mechanisms are cross-reactive T-cell recognition, known as molecular mimicry, and activation of T-cells by a foreign agent, leading to epitope spreading. These are considered the primary mechanisms through which infections can trigger a T-cell-mediated autoimmune response[2]. Similar to infections, vaccination has also been associated with the development of autoimmunity[5].

By comparing the absolute figures of 704 million COVID-19 cases and 696 million AD cases, and considering the data on the frequency of inflammatory rheumatic diseases following SARS-CoV-2 infection, which amounts to 12.5%, we can hypothesize the potential emergence of 88 million new cases of rheumatic diseases[6,7]. This would lead to a potential increase in the number of AD cases by 12.6% (an increase of 88 million to 696 million existing cases). The projected rise in global AD incidence clearly imposes the search for new, innovative approaches to treating patients with these diseases.

RA is the most widespread autoimmune inflammatory arthritis, affecting 0.5–1.0% of the population in the northern part of the globe, with an annual incidence of 24–45 cases per 100,000 people[8]. It is classified as a joint disease of unknown origin, with advanced stages potentially causing severe pain and immobility due to synovial inflammation (synovitis) that disrupts bone metabolism. This leads to the replacement of cartilage with mineralized bone, causing degeneration of bone tissue. Eventually, RA

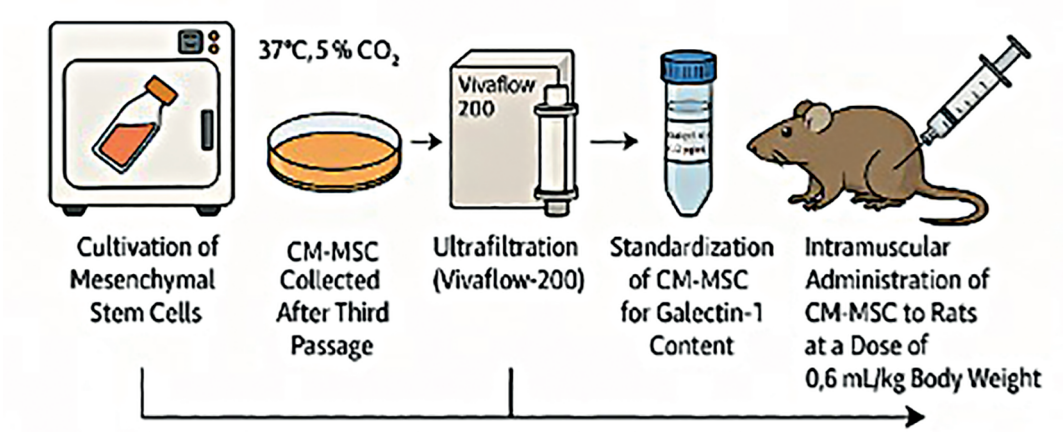

Figure 1. Schematic representation of the preparation and administration protocol of CM-MSC in a rat model of AA.

can severely damage cartilage and bone, resulting in disability, joint deformities, and limited mobility[9,10]. A comprehensive review revealed that within 2–3 years of RA onset about one-third of the patients leave their jobs, and after 10–15 years, nearly two-thirds (50–60%) may become unable to work[11].

In clinical practice, the standard treatment options for RA include steroid medications, anti-rheumatic drugs, and biological therapies. However, prolonged use of these treatments is often associated with side effects, and some RA patients may eventually become resistant to them. This has led to growing interest in alternative therapies, such as those utilizing mesenchymal stem cells (MSCs) and their acellular derivatives, including exosomes, secretomes, and conditioned media. These approaches are increasingly viewed as promising strategies for RA treatment[12].

MSCs have gained significant attention due to their remarkable regenerative properties, including tissue repair, self-renewal, and potent immunosuppressive effects. These properties enable MSCs to modulate the immune system by inhibiting pro-inflammatory cell activation in both innate and adaptive responses. Studies have shown that MSCs suppress natural killer (NK) cell activation, hinder dendritic cell maturation, reduce T- and B-cell proliferation, and promote regulatory T cell (Treg) generation[13].

Recent research has highlighted that the therapeutic benefits of MSCs in immune-mediated diseases are primarily attributed to the secretion of bioactive molecules, rather than their function as progenitor cells. To overcome the challenges associated with stem cell transplantation, secretomes derived from MSCs are being actively explored as a promising acellular therapeutic component[14,15].

The objective of the study was to characterize the anti-inflammatory and analgesic activity of the conditioned medium of mesenchymal stem cells (CM-MSC) in an adjuvant arthritis (AA) model in rats.

Materials and methods

The CM-MSC was obtained during the cultivation of native umbilical MSC cultures in a gas incubator (37°C, 5% CO_2) in serum-free Igla nutrient medium modified by Dulbecco's (*Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 – DMEM/F12*). CM was collected after the third passage when the cell growth entered the stationary phase. The stationary growth phase of the stable MSC line, which is when CM maturation occurs, was evaluated by the formation of a confluent cell layer using an inverted microscope. CM-MSC underwent ultrafiltration using the Vivaflow-200 system (*Sartorius, Germany*) with membranes (*Millipore, Germany*). CM-MSC was aliquoted and frozen for storage at –20°C. CM-MSC was standardized for galectin-1 content (6.0 pg/mL), which was determined by an immunoenzymatic method and adjusted with phosphate-buffered saline. The CM-MSC preparation with galectin-1 content of 6.0 pg/ml was administered intramuscularly (i.m.) to rats at a dosage of 0.6 mL/kg body weight (Figure 1)[16-18].

The experimental model of RA – AA in rats – replicates the morphological and functional characteristics of RA, including a typical immune response mainly driven by T-cell immunity. AA in rats was induced through a subplantar injection of Freund's Complete Adjuvant (*Thermo Fisher Scientific, USA*) into the right hind limb, with a dose of 0.1 mL per rat[19]. The acute phase of the inflammatory autoimmune process lasts up to day 14. Following this, generalized (or manifest) AA develops, characterized by swelling extending to multiple joints, including those of the contralateral paw, and affecting various

organs and systems, accompanied by clear morphological and functional changes. By days 28–30, the inflammation progresses to a chronic stage. The day of adjuvant administration was considered day "0" of the experiment. The peak of the local inflammatory response, marked by significant limb swelling, occurred on days 12–14, after which the inflammatory activity gradually subsided[19]. Treatment of AA was conducted from day 14 to day 28. CM-MSC were administered intramuscularly at 2-day intervals (five injections total) on days 14, 17, 20, 23, and 26. As a reference drug, sodium diclofenac (DS, *Biolik, Ukraine*) was administered intramuscularly at a dose corresponding to its ED50 for anti-inflammatory activity (8.0 mg/kg)[20]. This dose is equivalent to a human dose of 88 mg (1.25 mg/kg), consistent with clinical recommendations for long-term use of 75–100 mg/day[20]. On days 0 (baseline), 14, and 28, oncometric studies and pain sensitivity assessments were performed using electrical and mechanical stimulation methods, including the Randall-Selitto test.

The efficacy of CM-MSC in AA was evaluated in 42 male rats with a body weight of 200–220 g, randomly assigned into 4 groups based on individual pain sensitivity, which was measured using the tail withdrawal test in response to thermal stimuli:

1. Group I (negative control) – intact rats (n=7) who received intramuscular injections of normal saline (1.0 mL/kg body weight) on days 14, 17, 20, 23, and 26.
2. Group II – rats with induced AA (n=7) who received no treatment (control group) but were given intramuscular injections of normal saline (1.0 mL/kg) on the same days.
3. Group III – rats with induced AA (n=7), who received the reference drug DS (8.0 mg/kg) intramuscularly on days 14, 17, 20, 23, and 26[20].
4. Group IV – rats with induced AA (n=7), who received CM-MSC (0.6 mL/kg) intramuscularly on the same days[17,18].

On day 28, the animals were euthanized by cervical dislocation while under narcosis and samples of blood were obtained.

Oncometric assessment of the anti-inflammatory activity

The progression of the inflammatory response was evaluated by tracking the changes in limb volume (in mL) over time, measured using electronic scales (Radwag WLC 0.2/C/1, Poland) and a container filled with liquid. The limb volume was determined by the amount of liquid displaced by the limb upon immersion[21]. Water was chosen as the liquid. Considering that at a temperature of 14–17°C, the density of water is 0.998–0.999 g/mL, the volume measurements of the submerged limb were assumed to be equivalent to the mass of the displaced water: 1 g = 1 mL.

Mechanical stimulation according to Randall-Selitto

The study was conducted using a mechanical paw pressure analgesia meter "Paw Pressure Analgesia Meter DAQ 37215" (*Ugo Basile, Italy*). The pain threshold was determined by the maximum mechanical pressure (in grams) applied to the dorsal aspect of the hind limb during an increasing compression force at a rate of 39.2 g/s, triggering a reflexive withdrawal of the limb. The maximum pressure values achievable by the device are 375 g (25 cm). The pain threshold was measured three times, with the arithmetic mean calculated for each animal[22,23].

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of variable distributions within each group. For normally distributed independent variables, comparisons were made using Student's t-test and ANOVA. Normally distributed data are presented as the mean and standard error of the mean (M ± SE) or as the mean with a 95% confidence interval (95% CI).

This study received approval from the Committee on Bioethics at the V. N. Karazin Kharkiv National University of the Ministry of Education and Science of Ukraine (Protocol nr. 4/3, December 11, 2024).

Results

Table 1 presents the dynamics of paw edema volume (in mL, M ± m (95% CI)) in rats with AA over the experimental period. The study compared four groups: Group I (healthy rats without AA), Group II (AA-induced rats without treatment), Group III (AA-induced rats treated with sodium diclofenac, DS), and Group IV (AA-induced rats treated with conditioned medium of mesenchymal stem cells, CM-MSC). Measurements were taken on days 0 (baseline), 14, and 28.

At baseline (day 0), no significant differences in paw volume were observed among the groups ($p > 0.05$), indicating comparable starting conditions. By day 14, a marked increase in paw edema was observed in the untreated AA group (Group II), with the mean volume nearly doubling compared to baseline ($p = 0.009$, +124.5%), reflecting the peak of the acute inflammatory response. Both treatment groups (III and IV) also showed significant increases in edema volume by day 14 compared to baseline, although to a slightly lesser extent (+117.1% and +104.6% respectively).

By day 28, in the chronic phase of inflammation, Group II still exhibited markedly elevated paw

Table 1. Effect of CM-MSC and DS on paw edema volume over time in rats with AA (mean ± SEM, 95% confidence interval; n = 42; mL)

Time point	*Group I (1) Healthy Rats*	*Group II (2) Control (AA without treatment)*	*Group III (3) AA + DS*	*Group IV (4) AA + CM-MSC*	*Statistical significance [%]p_{2-1}*
Day 0	1.51±0.06 (95% CI: 1.40–1.63)	1.46±0.05 (95% CI: 1.36–1.55)	1.50±0.11 (95% CI: 1.28–1.72)	1.56±0.06 (95% CI: 1.45–1.67)	0.5 [3.8%]
Day 14	1.57±0.06 (95% CI: 1.45–1.70) p_d0 = 0.1 [3.8%]_d0	3.27±0.08 (95% CI: 3.12–3.42) p_d0 = 0.009 [124.5%]_d0	3.26±0.08 (95% CI: 3.10–3.42) p_d0 = 0.009 [117.1%]_d0	3.19±0.11 (95% CI: 2.97–3.40) p_d0 = 0.009 [104.6%]_d0	<0.001 [108.2%]
Day 28	1.60±0.06 (95% CI: 1.49–1.71) p_d0 = 0.07 [5.7%]_d0 p_d14 = 0.09 [1.8%]_d14	3.01±0.06 (95% CI: 2.90–3.13) p_d0 < 0.01 [106.9%]_d0 p_d14 = 0.01 [7.9%]_d14	2.07±0.19 (95% CI: 1.70–2.44) p_d0 = 0.01 [38.1%]_d0 p_d14 = 0.01 [36.4%]_d14	2.49±0.08 (95% CI: 2.32–2.65) p_d0 = 0.01 [59.6%]_d0 p_d14 = 0.01 [22.0%]_d14	<0.001 [88.4%]

Notes:
1. p_{2-1} – statistical significance of differences between groups;
2. [%] – percentage difference in parameters;
3. Indices 1, 2, 3, 4 indicate the group numbers between which comparisons were made;
4. Indices d0, d14 indicate time points in the dynamics (compared with Day 0 or 14).

Table 2. Effect of CM-MSC and DS on pain threshold in rats with AA over time, as measured by the Randall–Selitto test (mean ± SEM, 95% confidence interval; n = 42; arbitrary units*)

Time point	*Group I (1) Healthy Rats*	*Group II (2) Control (AA without treatment)*	*Group III (3) AA + DS*	*Group IV (4) AA + CM-MSC*	*Statistical significance [%]p_{2-1}*
Day 0	311±7 (95% CI: 298–325)	309±10 (95% CI: 290–327)	303±11 (95% CI: 280–325)	317±9 (95% CI: 299–336)	0.8 [0.9%]
Day 14	313±8 (95% CI: 298–328) p_d0 = 0.5 [0.5%]_d0	149±7 (95% CI: 135–162) p_d0 < 0.009 [51.9%]_d0	144±11 (95% CI: 123–166) p_d0 < 0.009 [52.4%]_d0	147±9 (95% CI: 130–164) p_d0 < 0.009 [53.6%]_d0	<0.001 [52.5%]
Day 28	306±8 (95% CI: 291–320) p_d0 = 0.17 [1.8%]_d0 p_d14 = 0.34 [2.3%]_d14	189±8 (95% CI: 172–205) p_d0 < 0.01 [38.9%]_d0 p_d14 = 0.01 [26.9%]_d14	231±11 (95% CI: 210–253) p_d0 = 0.01 [23.6%]_d0 p_d14 < 0.01 [60.4%]_d14	210±7 (95% CI: 196–224) p_d0 = 0 [33.8%]_d0 p_d14 < 0.01 [42.7%]_d14	<0.001 [38.3%]

Notes: *Values expressed in arbitrary units based on device output (Randall–Selitto analgesimeter).
1. p_{2-1} – statistical significance of differences between groups;
2. [%] – percentage difference in parameters;
3. Indices 1, 2, 3, 4 indicate the group numbers between which comparisons were made;
4. Indices d0, d14 indicate time points in the dynamics (compared with Day 0 or 14).

volumes, though a slight decrease from day 14 was noted (p = 0.01, –7.9%). In contrast, treatment with DS (Group III) resulted in a substantial reduction in paw volume compared to day 14 (p = 0.01, –36.4%), with an overall edema increase of only +38.1% from baseline. CM-MSC treatment (Group IV) also reduced edema compared to day 14 (p = 0.01, –22.0%), though to a lesser extent than DS, resulting in a +59.6% increase from baseline.

Notably, both DS and CM-MSC treatments demonstrated significant anti-inflammatory effects compared to the untreated AA group at day 28 (p < 0.001), with edema reductions of 88.4% and 88.4%, respectively. However, DS was more effective in reducing edema volume than CM-MSC over the treatment period.

These findings indicate that both DS and CM-MSC attenuate the progression of paw edema in rats with AA, though DS demonstrates a more pronounced effect. CM-MSC, while less potent in absolute terms, still exhibits a significant anti-inflammatory effect, supporting its therapeutic potential in managing chronic inflammation in RA.

The Randall-Selitto test was used to evaluate pain sensitivity in rats with AA over the course of 28 days. The results, presented in Table 2, highlight the impact of CM-MSC and DS on mechanical hyperalgesia development and progression in AA.

At baseline (Day 0), no significant differences in pain thresholds were observed across the study groups. The average thresholds ranged from 303 to

317 arbitrary units (a.u.), confirming the comparability of the groups prior to AA induction or treatment initiation (p_{2-1} = 0.8, 0.9% difference).

By day 14, a sharp and statistically significant reduction in pain threshold was observed in untreated AA rats (Group II), decreasing by approximately 51.9% from baseline ($p < 0.009$). This indicates the development of pronounced mechanical hyperalgesia characteristic of the acute phase of inflammation in the AA model. Similarly, both treatment groups – AA + DS (Group III) and AA + CM-MSC (Group IV) – also exhibited significant pain threshold reductions at this time point (by 52.4% and 53.6%, respectively), reflecting the peak of hyperalgesia associated with systemic inflammation. Notably, no statistically significant differences were observed between the treated and untreated AA groups at Day 14 ($p_{2-1} < 0.001$, 52.5% difference), suggesting that the full analgesic effect of the treatments had not yet emerged during the acute phase.

By day 28, a dynamic shift in pain thresholds was evident. Untreated AA rats (Group II) showed a modest recovery, with a 26.9% improvement from Day 14, but their thresholds remained significantly lower than baseline (–38.9%, $p < 0.01$), reflecting ongoing chronic pain. In contrast, rats treated with DS demonstrated a significant increase in pain threshold compared to both baseline (–23.6%) and Day 14 (+60.4%, $p < 0.01$), indicating a strong analgesic effect of DS. Similarly, CM-MSC-treated rats exhibited a significant recovery in pain threshold compared to Day 14 (+42.7%, $p < 0.01$), although the magnitude of this effect was slightly less than that of DS. When compared to healthy controls, however, pain thresholds in all AA groups (treated and untreated) remained lower by 26–38%, underscoring the persistent, though partially mitigated, hyperalgesia in the chronic phase of AA.

Overall, these findings indicate that both DS and CM-MSC treatments effectively attenuate mechanical hyperalgesia in AA rats during the chronic inflammatory phase, with DS demonstrating a somewhat greater effect in restoring pain thresholds. Importantly, the ability of CM-MSC to alleviate hyperalgesia, albeit to a lesser extent than DS, highlights its potential as a promising alternative or adjunctive therapy for managing chronic pain in RA and other autoimmune conditions.

Discussion

This study demonstrates the anti-inflammatory and analgesic effects of CM-MSC and DS in a rat model of AA, which replicates the key pathological features of RA, including chronic synovitis, joint swelling, and mechanical hyperalgesia.

The progression of paw edema, as an indicator of inflammatory response, showed a significant increase in the untreated AA group by day 14, corresponding to the peak phase of inflammation. The edema volume in these animals nearly doubled compared to baseline, reflecting the activation of systemic inflammatory processes. Treatment with both DS and CM-MSC from day 14 resulted in a reduction of edema volume by day 28, indicating attenuation of the inflammatory response in the chronic phase. DS demonstrated a stronger anti-inflammatory effect, as reflected by a 36.4% decrease in paw volume from day 14 to day 28 and a final increase of only 38.1% compared to baseline. CM-MSC also reduced paw edema, albeit to a lesser extent, with a 22.0% reduction from day 14 and a 59.6% increase from baseline. These findings align with the established anti-inflammatory effects of DS as a NSAID through cyclooxygenase inhibition and suppression of prostaglandin synthesis, which are key mediators of inflammation in RA.

The analgesic effects observed in the Randall–Selitto test further substantiate the therapeutic potential of CM-MSC and DS in AA. By day 14, all AA groups exhibited a marked decrease in pain threshold, indicating the development of mechanical hyperalgesia characteristic of inflammatory arthritis. This aligns with the known pathogenesis of RA, where persistent synovial inflammation and the release of pro-inflammatory cytokines sensitize peripheral nociceptors, leading to chronic pain. By day 28, DS-treated animals showed a significant recovery in pain threshold compared to both baseline and day 14, confirming the established analgesic properties of DS in arthritis models. CM-MSC treatment also led to a significant increase in pain threshold from day 14, although the effect was less pronounced than DS. Notably, despite improvements, pain thresholds in all AA groups remained lower than in healthy controls, suggesting the persistence of some degree of hyperalgesia, which is consistent with the chronic nature of RA.

The observed effects of CM-MSC are consistent with the current understanding of the immunomodulatory properties of MSC-derived secretome, which exerts anti-inflammatory and anti-nociceptive actions through the release of bioactive molecules, including cytokines, growth factors, and extracellular vesicles. CM-MSC is known to modulate immune responses by inhibiting T cell proliferation, promoting regulatory T cells, suppressing pro-inflammatory cytokine production (such as IL-1β, IL-6, and TNF-α), and inducing a shift toward an anti-inflammatory cytokine profile. The reduction in edema and partial restoration of pain threshold in the CM-MSC group supports the

hypothesis that secretome-based therapies may offer a cell-free alternative for managing chronic inflammatory and pain-related components of RA.

Importantly, while DS demonstrated a more robust anti-inflammatory and analgesic effect, CM-MSC treatment still produced significant improvements, highlighting its potential as an adjunctive or alternative therapy, particularly in cases where NSAID use is limited due to side effects or contraindications.

Overall, these findings provide further evidence for the therapeutic relevance of MSC-derived secretome in the management of RA-like pathologies and support its potential translation into clinical applications. However, additional studies are necessary to fully elucidate the mechanisms of action of CM-MSC, optimize dosing regimens, and evaluate long-term safety and efficacy.

These results also resonate with a growing body of experimental and translational research emphasizing the therapeutic potential of cell-free biologics in autoimmune and inflammatory conditions. Several recent works have shown that cryopreserved extracts and conditioned media can reduce systemic inflammation, oxidative stress, and immune dysregulation across a variety of experimental models, including arthritis, myocarditis, thyroiditis, and hepatitis[25-27]. The convergence of these data suggests that the effects observed in our study are not isolated phenomena but represent a broader, reproducible pattern of action for MSC-derived cell-free products. In this context, the ability of CM-MSC to achieve a measurable decrease in paw edema and a partial recovery of pain thresholds supports the growing consensus that secretome-based interventions can provide dual anti-inflammatory and analgesic benefits[28].

The underlying mechanisms appear to involve a complex interplay of bioactive molecules, including cytokines, chemokines, growth factors, and extracellular vesicles, which collectively exert immunomodulatory and tissue-protective effects[29]. Evidence suggests that CM-MSC can inhibit T-cell proliferation, reduce the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and promote the expansion of regulatory T-cell populations[29]. This multifaceted immunoregulation is critical, since rheumatoid arthritis and its experimental analogues are driven by a combination of aberrant immune activation and chronic tissue damage. In this regard, the observed effects of CM-MSC provide direct functional evidence for what has been hypothesized in mechanistic reviews: that the MSC secretome can reorient the immune response toward resolution and repair[30,31]. Importantly, the therapeutic activity of CM-MSC in our study mirrors that described in other models of autoimmune disease, underscoring the versatility of this approach beyond the joints[27,28].

From a translational perspective, the feasibility of CM-MSC production represents an additional advantage. While extracellular vesicles isolated from MSCs have received attention as potent immunoregulatory agents, their large-scale manufacturing and quality control remain technically demanding[29]. In contrast, CM can be generated in a more straightforward and cost-efficient manner, facilitating standardization and scalability[30]. Several comprehensive reviews emphasize that conditioned media may represent an undervalued but highly promising tool for regenerative and immunomodulatory medicine, particularly in chronic degenerative and autoimmune diseases[31,32,33]. Our results, which demonstrate simultaneous anti-edematous and analgesic effects, are aligned with these assessments and contribute further experimental validation.

The clinical relevance of these findings is supported by the work of Kay and colleagues, who showed that MSC-conditioned medium not only reduced clinical severity but also modulated immune responses in experimental arthritis[34]. Their observations echo our conclusion that cell-free secretome therapies are capable of modifying disease progression, thereby moving beyond symptomatic control. This is consistent with the broader paradigm shift from symptomatic to disease-modifying interventions in rheumatology, where targeted modulation of immune mechanisms is increasingly prioritized[32,33]. Moreover, Vizoso and co-authors have suggested that MSC secretome may contribute to systemic homeostasis and functional recovery across a spectrum of inflammatory conditions, thereby reinforcing its potential as a multi-system therapeutic agent[35].

Taken together, the present study provides an important addition to the evidence base supporting CM-MSC as a disease-modifying and cell-free therapeutic strategy. While diclofenac sodium remains a benchmark anti-inflammatory and analgesic agent, its toxicity profile and limited disease-modifying capacity underscore the need for complementary or alternative options. CM-MSC, by contrast, offers the potential to reduce both inflammation and pain through immunomodulation rather than enzyme inhibition, making it suitable for longer-term strategies and patient populations with contraindications to NSAIDs[27-31]. Furthermore, the reproducibility of beneficial effects across multiple models of autoimmune disease[25,28,34,35] suggests that CM-MSC may eventually be applied not only in rheumatoid arthritis but also in a broader range of immune-mediated disorders.

Future research should prioritize rigorous mechanistic studies to delineate the relative contributions of

soluble mediators versus extracellular vesicles in the observed outcomes. Additionally, dose-response relationships, optimal timing of administration, and combination strategies with standard therapies warrant systematic exploration[29,30,31]. Long-term studies addressing safety, durability of effects, and potential systemic benefits will be essential to justify translation into clinical trials. In this regard, the current findings serve as both proof-of-concept and a call for expanded investigations, reinforcing the notion that MSC-derived secretome holds genuine promise as a next-generation therapeutic platform in autoimmune pathology[32,33,35].

Conclusions

CM-MSC significantly reduced paw edema volume in AA rats by 22.0% ($p = 0.01$) compared to day 14, with a 59.6% increase from baseline. Pain threshold improved by 42.7% ($p < 0.01$) compared to day 14. These results demonstrate the anti-inflammatory and analgesic properties of CM-MSC, supporting its potential as a therapeutic approach for chronic inflammatory conditions such as RA.

While DS showed stronger effects, CM-MSC still provided significant reductions in paw edema and improvements in pain threshold. CM-MSC offers a promising, cell-free alternative for RA treatment, with fewer risks than long-term NSAID therapy. Further studies are needed to clarify the mechanisms of action and evaluate clinical applications of CM-MSC.

Author contributions:

Conceptualization, methodology, design of the work F.H., wrote the manuscript, analysis F.H., T.L., project administration, draft preparation V.S., R.K., H.L., S.S., interpretation of data for the work, editing, validation V.S., T.L., H.L., S.S. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. The research reported in this paper was carried out in accordance with recommendations for laboratory research involving animals (American Physiological Society) and in line with the ARRIVE guidelines."

"All institutional and national guidelines for the care and use of laboratory animals were followed"

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Data and Materials Statement:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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