IJMS
Vol 49, No 2, February 2024

Original Article

Adipose-Derived Stem Cells' Secretome Attenuates Lesion Size and Parasite Loading in Leishmaniasis Caused by *Leishmania Major* in Mice

Iman Khodkar[1], MSc; Jasem Saki[1,2], PhD; Reza Arjmand[1], PhD; Ghasem Saki[3,4], PhD; Layasadat Khorsandi[3,4], PhD

[1]Department of Medical Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;
[2]Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;
[3]Department of Anatomical Sciences, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;
[4]Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Correspondence:
Jasem Saki, PhD;
Second Floor, Department of Medical Parasitology, School of Medicine, Jundishapur University of Medical Sciences, Golestan Blvd., Postal code: 393-61155, Ahvaz, Iran
Tel: +98 61 33335296
Email: jasem.saki@gmail.com
Received: 14 August 2022
Revised: 20 November 2022
Accepted: 25 December 2022

Abstract

Background: Stem cell-derived secretome (SE) released into the extracellular space contributes to tissue repair. The present study aimed to investigate the impact of isolated secretome (SE) from adipose-derived mesenchymal stem cells (ASCs) on *Leishmania major* (*L. major*) lesions in BALB/c mice.

Methods: This experimental study was conducted at Ahvaz University of Medical Sciences (Ahvaz, Iran) in 2021. Forty female BALB/c mice were infected with stationary phase promastigotes through intradermal injection in the bottom of their tail and randomly divided into four groups (n=10 per group). The mice were given SE (20 mg/mL), either alone or in combination with Glucantime (GC, 20 mg/mL/Kg), meglumine antimoniate (20 mg/mL/Kg) for the GC group, and phosphate-buffered saline (PBS) for the control group. After eight weeks, the lesion size, histopathology, the levels of Interleukin 10 (IL-10), and Interleukin 12 (IL-12) were assessed. For the comparison of values between groups, the parametric one-way ANOVA was used to assess statistical significance.

Results: At the end of the experiment, the mice that received SE had smaller lesions (4.56±0.83 mm versus 3.62±0.59 mm, P=0.092), lower levels of IL-10 (66.5±9.7 pg/mL versus 285.4±25.2 pg/mL, P<0.001), and higher levels of IL-12 (152.2±14.2 pg/mL versus 24.2±4.4 pg/mL, P<0.001) than the control. Histopathology findings revealed that mice treated with SE had a lower parasite burden in lesions and spleen than the control group.

Conclusion: The current study demonstrated that ADSC-derived SE could protect mice infected with *L. major* against leishmaniasis.

Please cite this article as: Khodkar I, Saki J, Arjmand R, Saki G, Khorsandi L. Adipose-Derived Stem Cells' Secretome Attenuates Lesion Size and Parasite Loading in Leishmaniasis Caused by *Leishmania Major* in Mice. Iran J Med Sci. 2024;49(2):121-129. doi: 10.30476/IJMS.2023.96413.2795.

Keywords ● Adipose-derived mesenchymal stem cells ● Secretome ● Leishmania ● Wound ● Cytokines ● Mice

What's Known

- Adipose-derived stem cells can improve wound healing caused by the *leishmania* parasite by increasing the levels of cytokines and growth factors. Furthermore, it is known that secretomes of stem cells exert more sophisticated functions than their cellular provenance, which can lead to a better outcome in the treatment of *leishmania* wounds.

What's New

- We found that the administration of adipose-derived stem cell secretomes can clean the wound and spleen of the *leishmania* parasite, comparatively through the immune system and cytokines regulation.

Introduction

Cutaneous leishmaniasis (CL) is a prevalent vector-borne parasitic disease that primarily affects under-developing countries in South and Central America, Central Asia, the Middle East, and the Mediterranean Basin.[1] In Iran, CL is endemic, but in recent

years considerable changes in the distribution of the disease have emerged, and nearly 25000 cases were detected annually.[2]

In the ancient world, *Leishmania major* (*L. major*), which is transmitted through the blood-feeding of a female sand fly, is the primary agent that causes zoonotic and anthroponotic cutaneous leishmaniasis (ZCL and ACL).[3] Clinical infections caused by *L. major* prominently manifest as ulcer-crusted lesions on the extremities that rapidly necrotize and produce wet sores. Following infection, more than half of the ZCL patients recover within 2-8 months.[4]

One of the remarkable features of successful CL treatment is a reduction in wound size. Successful parasite removal from lymphoid organs such as the spleen is another intriguing feature of leishmaniasis treatment.[5]

A wide range of treatment approaches, including antibiotics, antifungal medicines, and several anti-tumor compounds have been evaluated or repurposed for CL. Meglumine antimoniate is a manufactured medicine, known as Glucantime (GC), which is the gold standard therapy for leishmaniasis.[5] However, patients begin to experience its side effects such as joint pain, headache, nausea, heart dysfunction, and pancreatitis. Thus, to combat CL, novel and safe medicines must be developed.[6]

Recently, stem cell therapy is widely regarded as a key treatment for conditions such as osteoarthritis, heart failure, neurological diseases, and even wound healing.[7] Adipose-derived stem cells (ASCs) promoted wound healing by decreasing inflammation, inducing angiogenesis, stimulating the proliferation of fibroblasts, and generating an extracellular matrix.[8] ASCs can be quickly acquired using liposuction from autologous subcutaneous adipose tissue with high cellular activity and without any ethical concerns. The secretory substances (Secretome) of ASCs (ASCs-SE) have many compounds, including growth factors, cytokines, chemokine, and extracellular vesicles, that are involved in cell signaling.[9] There is convincing evidence that SE exerts more complex functions than their cellular provenance. SE is additionally a suitable candidate for treating various diseases, because they can be easily synthesized at a low cost in laboratories with limited facility settings.[7] Moreover, SE regulates the immune system[10] and may increase the secretion of some critical cytokines throughout the CL healing process.

To regulate inflammation, the ACSs transform the macrophage from pro-inflammatory (M1) to anti-inflammatory (M2), which has beneficial effects on skin wounds in individuals with vascular dysfunction, diabetes, radiation, and burns history.[11]

L. major secretes Interleukin 10 (IL-10) to suppress pro-inflammatory cytokine secretion and increase parasite survival.[12] Decreasing Interleukin 12 (IL-12) and increasing IL-10 levels lead to changes in the macrophage phenotype and inhibit parasite clearance.[13] *L. major* impairs IL-12 production of macrophages *in vitro*, even in the presence of interferon-gamma (IFN-γ).[14, 15]

Since a definitive treatment of the wound and complications caused by *L. major* has yet to be discovered, and the role of cytokines IL-10 and IL-12 in the pathogenesis of *L.major* is known, this study aimed to evaluate the effect of the ASC-SE, which has various growth factors and cytokines, on the lesions caused by *L. major* in BALB/c mice.

Materials and Methods

Study Design

Forty female BALB/c mice (age=four weeks) were purchased from Royan Research Center (Tehran, Iran). The rats were housed in a standard vivarium, fed a standard diet, and water *ad libitum*, and kept under standard conditions (12-hour light:dark cycle, temperature 20-25 ºC, and humidity 25-35%). Experimental protocols were according to the Guide for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee of Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethical Approval No. IR.AJUMS.ABHC.REC.1398.054).

Establishing Infected Animals

The standard *L. major* (code: MRHO/IR/75/ER) promastigotes of the second-round passage were obtained from Iran's Pasteur Institute. The promastigotes were cultured in Falcon tubes containing RPMI 1640 (Roswell Park Memorial Institute 1640, Gibco, NM, USA) supplemented with 10% fetal calf serum (FCS, Gibco, USA) and incubated at 25 °C. Subsequently, the stationary phase parasites were centrifuged at 1,200 rpm for three min, and the supernatant was discarded. Finally, a suspension of 10^6 parasites in 0.2 mL of sterile PBS was intradermally injected into the mice at the base of their tail. The mice were examined every day from the inoculation moment until the emergence of lesions (4-6 weeks).

Stem Cell Extraction from Adipose Tissue

Four young inbred male BALB/c mice were euthanized using ketamine (100 mg/Kg; Alfasan, Netherlands) and xylazine (10 mg/Kg; Alfasan, Netherlands) to obtain ASCs. The epididymal

fats were then isolated and washed using sterile PBS containing penicillin-streptomycin (Sigma-Aldrich, USA) antibiotics. After mesh-based slicing of fat tissues (1 mm pieces), they were placed into a Falcon tube containing 9 mL DMEM medium (Gibco, USA), and 1 mL collagenase (Gibco, USA), and were shaken for 30-40 min. The resultant suspension was centrifuged at 1,200 rpm for 10 min, and the cellular pellet was cultured in 5 mL DMEM supplemented with 15% FCS and placed in a standard cell culture incubator. The culture medium was replaced with fresh DMEM containing 10% serum and antibiotics every three days. Osteogenic and adipogenic potentials, at passage 3, were evaluated using Alizarin-Red (Sigma-Aldrich, USA) and Oil-red O (Sigma-Aldrich, USA) staining, respectively.

For detecting specific surface markers, ASCs (1,000,000 Cells), at passage 3, were exposed to fluorescein isothiocyanate (FITC)-conjugated antibody CD90 (ab11155), CD44 (ab30405), CD73 (ab239246), and CD34 (ab18227) diluted in PBS for 40 min on ice. After washing with PBS, the fluorescence intensity of ASCs was measured using a flow cytometry instrument (Becton Dickinson, USA), and the data were analyzed using Flow-Jo software.

Secretome Extraction

The culture medium was replaced with 5 mL serum-free DMEM at passage 3. This medium was collected after 48 hours in a special Falcon tube (Amicon® Ultra-10k, Ireland) and concentrated for 18 hours using a high-speed centrifugation at 5,000 g. The amount of the secreted protein was quantified and kept at -70 °C until use.

Animal Groups

The mice were randomly divided into four groups, and each group received 0.2 mL of treatment compound every seven days:

Control: received only phosphate buffered saline (PBS).

GC: treated with meglumine antimoniate (GC) (20 mg/mL/Kg)

SE: received secretome (20 mg/mL/Kg)

SE+GC: received SE and GC (20 mg/mL/Kg)

All injections were administered intralesionally. The length and width of the lesions were measured with a caliper, and then the lesion size (mm^2) was determined using the following formula: D+d/2, where “D” is the length of the lesion, and “d” is its width.

Measuring Interleukin-10 and 12

At the end of the experiment, the mice were euthanized using ketamine (100 mg/Kg; Alfasan, Netherlands) and xylazine (10 mg/Kg; Alfasan, Netherlands), and blood samples were collected from their hearts. The serum was separated and stored at -70 °C. The protein levels of the IL-10 and IL-12 were measured using ELISA kits (R&D Systems, USA).

Histopathological Examination

To prepare slides (5 µm) for histopathological examinations of the parasite burden, the spleen samples were fixed in formalin 10%. Then, the slides were stained with hematoxylin and eosin (H&E) and examined using light microscopy.

Statistical Analysis

The data were analyzed using SPSS software (version 21; IBM, USA). All data were examined for normal distribution by the Shapiro-Wilk test. The parametric one-way ANOVA was used to determine statistical significance, followed by Tukey’s multiple comparison *post hoc* test. The results were expressed as mean±SD. $P<0.05$ was considered to be statistically significant.

Results

Characterization of ASCs

Flow cytometry method revealed that ASCs had highly expressed mesenchymal stem cell surface markers including CD44, CD73, and CD90, but low expression of hematopoietic stem cell surface markers, i.e., CD34 (figure 1). The Alizarin Red (A) and Oil Red O (B) staining indicated the osteogenic and adipogenic differentiation potential of ASCs (figure 2).

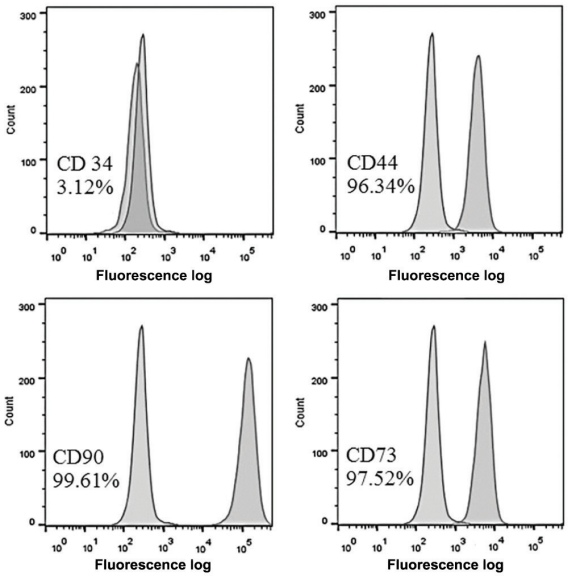

Figure 1: Characterization of ASCs is shown by flow cytometry. CD44, CD73, CD90, and mesenchymal stem cell surface markers were highly expressed. CD34, hematopoietic stem cell surface markers indicated low expression.

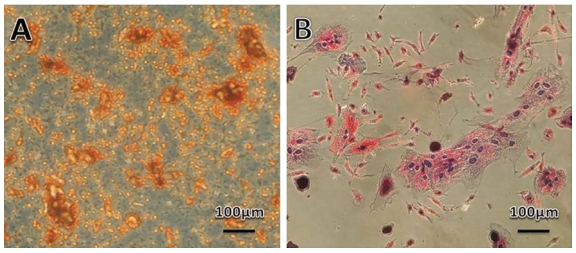

Figure 2: Characterization of ASCs is illustrated using histochemistry. The Alizarin Red (A) and Oil Red O (B) staining indicated the osteogenic and adipogenicity differentiation potential of ASCs.

Lesion Size

The results of Tukey's *post hoc* test, on the seventh day, showed no statistically significant difference between the average of the control group and all the experimental groups.

On day 28, the mean of the control group (10.24 mm) was higher than the mean of the GC group (6.13 mm), which indicated a significant difference between the two groups (P=0.034). The average wound size in the SE group was significantly smaller (7.26 mm; P=0.019) than the control group (10.24 mm). The average wound size in the SE+GC group (4.54 mm) was significantly smaller than the control (10.24 mm; P=0.038).

On the 56th day, there was a significant difference between the mean of GC and the control group (P=0.004); the control group mean (12.79 mm) was higher than the GC mean (3.62 mm). The average wound size in the control group (12.79 mm) was significantly larger than the SE (4.56 mm; P=0.002). The average wound size in the SE+GC group (4.35 mm) was significantly smaller than the control group (12.79 mm; P=0.003). These findings are presented in table 1, figures 3 and 4.

Histopathological Examination

In the SE and MA groups, parasites disappeared from the spleens. *L. major* was found in the spleens of the control group. Only one mouse of the SE+CG group had parasites in its spleen (figure 5).

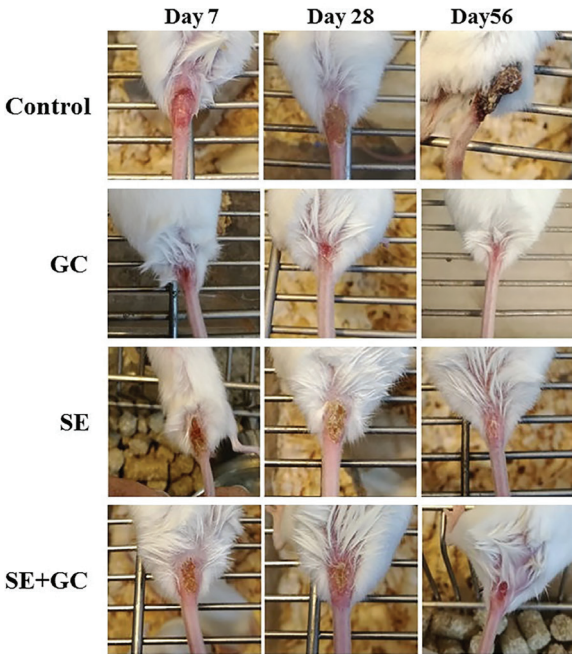

Figure 3: Treatment of cutaneous leishmaniasis wounds with Glucantime (GC), Secretome (SE), and Secretome+Glucantime (SE+GC) and comparing with the control group on days 7, 28, and 56 days after treatment. On day 7, the size of the wounds in the control and treatment groups did not show significant changes. On days 28 and 56, the size of the wounds in the experimental groups was smaller than the control group.

Cytokine Assay

Cytokine levels were examined using a one-way ANOVA followed by Tukey's *post hoc* test. The control group and other experimental groups had significantly different means for IL-10 and IL-12. The mean of the IL-10 level in the control group (258.4 pg/mL) was significantly higher than the mean of GC (48.15 pg/mL; P=0.001).

Table 1: Wound size analysis results in different groups of mice (n=40)

Groups		Mean±SD (mm)	P value
Day 7	Control	5.43±1.12	-
	GC	5.51±1.23	0.093
	SE	5.62±0.98	0.103
	GC+SE	5.46±0.87	0.085
Day 28	Control	10.2±1.72	-
	GC	6.13±1.41*	0.034
	SE	7.26±1.53*	0.019
	GC+SE	4.54±0.67*	0.038
Day 56	Control	12.7±1.39	-
	GC	3.62±0.59**	0.004
	SE	4.56±0.83**	0.002
	GC+SE	4.35±0.54**	0.003

Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. All the comparisons were made between the experimental groups and the control group. GC: Glucantime; SE: Secretome; SE+GC: Secretome+Glucantime; *P<0.05; **P<0.01

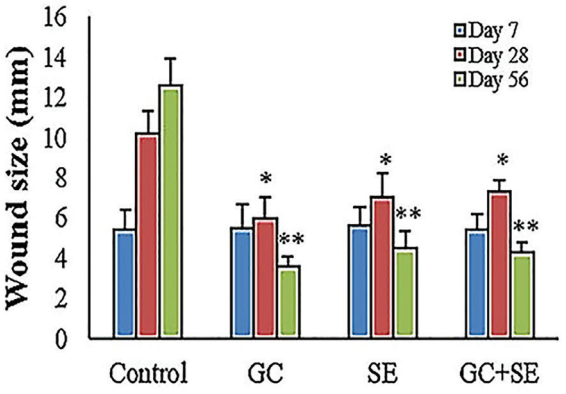

Figure 4: Average sizes of CL in different groups (mean±SD). GC: Glucantime; SE: Secretome; SE+GC: Secretome+Glucantime; *Compared with the control group (P<0.05); **P<0.01

Furthermore, the average IL-10 level in the control group (258.4 pg/mL) was significantly higher than the SE (66.57 pg/mL) and SE+GC (54.29 pg/mL) groups (P<0.001). The mean of the IL-12 level in the control group (24.2 pg/mL) was significantly lower than the mean of the GC group (95.01 pg/mL; P=0.004).

Additionally, the average IL-12 level in the control group (24.2 pg/mL) was significantly lower than the SE (152.2 pg/mL) and SE+GC (140.5 pg/mL) groups (P<0.001). The protein levels of IL-10 and IL-12 in the SE (152.2 pg/mL) and SE+GC (140.5 pg/mL) groups were significantly higher than the GC group (95.01 pg/mL; P<0.001). Table 2 and figure 6 show the results of the pair-wise comparison of serum cytokine levels.

Discussion

In this study, ASC-SE effectively improved CL wound healing in a BALB/c mice model. In line with our findings, intradermal injection of ASC-SE resulted in a significant decrease in wound size in diabetic ulcer subjects.[16] Another study found that intradermal injection of ASCs enhanced skin wound healing in mice.[17]

In the present study, there was a significant

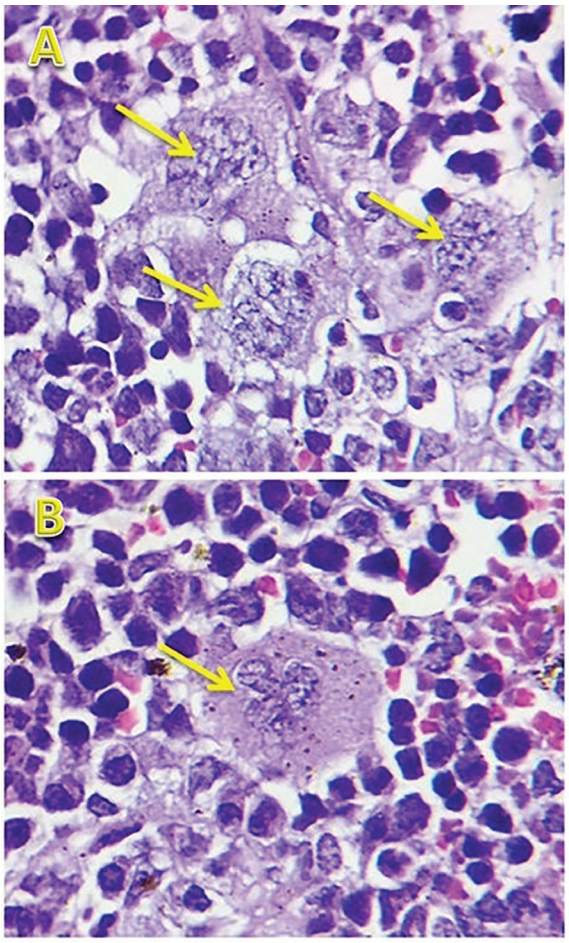

Figure 5: Parasites (arrows) were observed in the spleen of the mice of the control group (A) and GC+SE (B) groups. Magnifications: ×400; GC: Glucantime; SE: Secretome

decrease in the wound size and parasite burden in the spleens of the experimental groups after 28 days. Similarly, Navard and others found that at the end of the MSC therapy (day 30), the size of leishmanial lesions in the mice was decreased, and the parasite burden in histological examinations was significantly diminished.[18] Tadeu and colleagues indicated that ASCs in combination with GC significantly improved CL.[19] According to Bahrami and others, ASCs reduced the size of CL in a manner that was comparable to that of GC.[20] In the present

Table 2: Serum levels of the IL-10 and IL-12 analysis in the different groups

Groups		Mean±SD (pg/mL)	P value
IL-10	Control	258.4±25.2	-
	GC	48.1±8.9***	0.0001
	SE	66.5±9.7***	0.0001
	GC+SE	54.2±11.1***	0.0001
IL-12	Control	24.2±4.4	-
	GC	95±12.1**	0.004
	SE	152±14.2***	0.0001
	GC+SE	140.5±13.3***	0.0001

Data were analyzed using a one-way ANOVA followed by Tukey's *post hoc* test. All comparisons were made between the experimental groups and the control group. GC: Glucantime; SE: Secretome; SE+GC: Secretome+Glucantime; **P<0.01; ***P<0.001

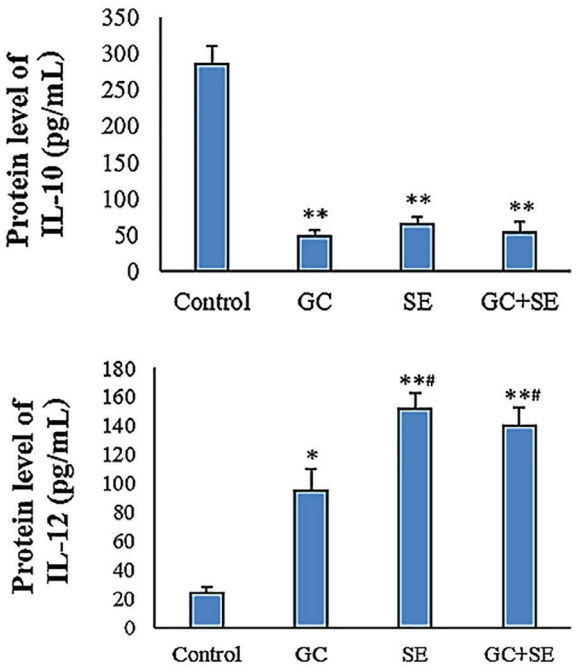

Figure 6: Serum concentrations of IL-10 and IL-12 in different groups analyzed with the Mann-Whitney U test (mean±SD). GC: Glucantime; SE: Secretome; SE+GC: Secretom+Glucantime; *P<0.01; **P<0.001; #P<0.05; * and # show comparison to the control and GC groups

study, the wound size was slightly larger than in the GC-treated group, which indicated the efficacy of SE on CL healing.

This study did not reveal the exact mechanism through which the CL was improved by the ASCs-SE. ASCs-SE may improve wound healing by boosting the levels of cytokines and growth factors involved in angiogenesis and epithelial cell proliferation.[21] Ma and others reported that injection of ASCs-SE into the wound of mice enhanced wound healing and increases vascular epithelial growth factor (VEGF). VEGF promotes the proliferation and migration of endothelial cells.[22]

SE, as mentioned in the results, could clean the parasites from the spleen and wound lesions. Macrophages are the prominent resident and effector cells for the *Leishmania* parasites. These cells play a crucial role in controlling and eliminating parasites by secreting critical cytokines and chemokines that activate T cells.[23] Similarly, Zanganeh and others reported that the intravascular administration of ASCs significantly reduced the parasite burden in the spleen and decreased T regulatory cells in BALB/c mice.[24]

In the present investigation, parasite clearance from the spleen was followed by an increase in IL-12 and a decrease in IL-10 levels in the SE group. In line with our findings, intravenous injection of ASCs reduced IL-10 levels and eliminated the *leishmania* parasite from the spleen of BALB/c mice.[24] *The in vivo* co-culture of BALB/c-derived macrophages and ASCs could improve macrophage's phagocytic activity to engulf parasites.[24] According to the findings of recent studies, *L. major* stimulates macrophages to release IL-10 for suppressing inflammatory responses, which is necessary for *Leishmania* wound healing by inhibiting Natural Killer cells and reducing the proliferation of T helper lymphocytes.[25, 26] The IL-10 levels were elevated in the wild-type C57BL/6 mice infected with the *L. donovani* parasite (visceral leishmania).[27] Another study found that IL-10 defective BALB/c and C57BL/6 mouse models of visceral leishmaniasis were resistant to *L. donovani* infection.[28] Therefore, SE might improve wound healing and parasite burden by suppressing IL-10 secretion from the macrophages.

In a previous study, bone marrow-MSCs injection into *P. berghei*-infected mice reduced the ratio of IL-10 to IL-12 and the number of regulatory T cells. IL-12 has a crucial role in the ability of treated visceral leishmaniasis patients to generate IFN-γ, whereas IL-10 inhibits this response.[29] Li and colleagues investigated the effect of IL-12 on collagen structure and cellular metabolic activity during skin wound healing. Their findings revealed that IL-12 increased cellular metabolic response in the skin wound of the radiation-exposed mice.[30]

One of the limitations of the present study was that we could not measure the amount of IL-12 in the wound-injected SE. The increased plasma level of IL-12 in the infected mice might be due to the presence of IL-12 in the injected SE or stimulating macrophages to release IL-12 by SE. Besides, unknown SE compounds might stimulate other immune cells to produce IL-12, which in turn stimulates macrophages to eliminate the parasites.

Conclusion

The present study indicated that administration of ADSC-SE into the CL accelerated wound healing by enhancing plasma levels of IL-12 in mice. It is also noteworthy that the SE may inhibit the migration of *L. major* into the spleen by regulating the immune system. The high efficacy of the SE against CL could be attributed to macromolecules, such as IL-12, involved in wound healing. However, further investigations need to determine the precise mechanism of SE on the CL produced by *L. major*.

Acknowledgment

We would like to thank all those involved in this

research project. This study was extracted from a PhD thesis by Iman Khodkar, a PhD student of Medical Parasitology, granted by the Ahvaz Jundishapur University of Medical Sciences (Grant number: OG- 9822).

Authors' Contribution

I.KH: Contributed to data acquisition and drafting the manuscript; J.S: Contributed to the initial plan and drafting of the manuscript; R.A: Contributed to the initial plan and critical revision; Gh.S: Contributed to initial plan and data acquisition and writing; L, S, KH: Contributed to data acquisition and drafting and critical revision of the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

References

1 Firooz A, Mortazavi H, Khamesipour A, Ghiasi M, Abedini R, Balighi K, et al. Old world cutaneous leishmaniasis in Iran: clinical variants and treatments. J Dermatolog Treat. 2021;32:673-83. doi: 10.1080/09546634.2019.1704214. PubMed PMID: 31869258.

2 Sabzevari S, Teshnizi SH, Shokri A, Bahrami F, Kouhestani F. Cutaneous leishmaniasis in Iran: A systematic review and meta-analysis. Microb Pathog. 2021;152:104721. doi: 10.1016/j.micpath.2020.104721. PubMed PMID: 33539962.

3 Hajjaran H, Saberi R, Borjian A, Fakhar M, Hosseini SA, Ghodrati S, et al. The Geographical Distribution of Human Cutaneous and Visceral Leishmania Species Identified by Molecular Methods in Iran: A Systematic Review With Meta-Analysis. Front Public Health. 2021;9:661674. doi: 10.3389/fpubh.2021.661674. PubMed PMID: 34249836; PubMed Central PMCID: PMCPMC8267797.

4 Akhoundi M, Hajjaran H, Baghaei A, Mohebali M. Geographical distribution of leishmania species of human cutaneous leishmaniasis in fars province, southern iran. Iran J Parasitol. 2013;8:85-91. PubMed PMID: 23682265; PubMed Central PMCID: PMCPMC3655245.

5 Mcgwire BS, Satoskar A. Sir William Osler Medicine Master Class Leishmaniasis: Clinical Syndromes and Treatment. 2013.

6 Alves F, Bilbe G, Blesson S, Goyal V, Monnerat S, Mowbray C, et al. Recent Development of Visceral Leishmaniasis Treatments: Successes, Pitfalls, and Perspectives. Clin Microbiol Rev. 2018;31. doi: 10.1128/CMR.00048-18. PubMed PMID: 30158301; PubMed Central PMCID: PMCPMC6148188.

7 Zhao L, Johnson T, Liu D. Therapeutic angiogenesis of adipose-derived stem cells for ischemic diseases. Stem Cell Res Ther. 2017;8:125. doi: 10.1186/s13287-017-0578-2. PubMed PMID: 28583178; PubMed Central PMCID: PMCPMC5460534.

8 Zeng N, Chen H, Wu Y, Liu Z. Adipose Stem Cell-Based Treatments for Wound Healing. Front Cell Dev Biol. 2021;9:821652. doi: 10.3389/fcell.2021.821652. PubMed PMID: 35087840; PubMed Central PMCID: PMCPMC8787271.

9 Trzyna A, Pikuła B, Ludwin A, Kocan B, Banaś-Ząbczyk A. The influence of an electromagnetic field on adipose-derived stem/stromal cells' growth factor secretion: Modulation of FGF-2 production by in vitro exposure. Archives of Biological Sciences. 2020;72:339-47. doi: 10.2298/ABS200321028T.

10 Serejo TRT, Silva-Carvalho AE, Braga L, Neves FAR, Pereira RW, Carvalho JL, et al. Assessment of the Immunosuppressive Potential of INF-gamma Licensed Adipose Mesenchymal Stem Cells, Their Secretome and Extracellular Vesicles. Cells. 2019;8. doi: 10.3390/cells8010022. PubMed PMID: 30621275; PubMed Central PMCID: PMCPMC6356584.

11 Eleuteri S, Fierabracci A. Insights into the Secretome of Mesenchymal Stem Cells and Its Potential Applications. Int J Mol Sci. 2019;20. doi: 10.3390/ijms20184597. PubMed PMID: 31533317; PubMed Central PMCID: PMCPMC6770239.

12 Ma X, Yan W, Zheng H, Du Q, Zhang L, Ban Y, et al. Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells. F1000Res. 2015;4. doi: 10.12688/f1000research.7010.1. PubMed PMID: 26918147; PubMed Central PMCID: PMCPMC4754024.

13 Khandibharad S, Singh S. Computational System Level Approaches for Discerning Reciprocal Regulation of IL10 and IL12 in Leishmaniasis. Front Genet. 2021;12:784664. doi: 10.3389/fgene.2021.784664. PubMed PMID: 35126456; PubMed Central PMCID: PMCPMC8807686.

14 Carrera L, Gazzinelli RT, Badolato R, Hieny

S, Muller W, Kuhn R, et al. Leishmania promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. J Exp Med. 1996;183:515-26. doi: 10.1084/jem.183.2.515. PubMed PMID: 8627163; PubMed Central PMCID: PMCPMC2192469.
15 Chandrakar P, Parmar N, Descoteaux A, Kar S. Differential Induction of SOCS Isoforms by Leishmania donovani Impairs Macrophage-T Cell Cross-Talk and Host Defense. J Immunol. 2020;204:596-610. doi: 10.4049/jimmunol.1900412. PubMed PMID: 31882519.
16 Holm JS, Toyserkani NM, Sorensen JA. Adipose-derived stem cells for treatment of chronic ulcers: current status. Stem Cell Res Ther. 2018;9:142. doi: 10.1186/s13287-018-0887-0. PubMed PMID: 29764508; PubMed Central PMCID: PMCPMC5952370.
17 Rodriguez J, Boucher F, Lequeux C, Josset-Lamaugarny A, Rouyer O, Ardisson O, et al. Intradermal injection of human adipose-derived stem cells accelerates skin wound healing in nude mice. Stem Cell Res Ther. 2015;6:241. doi: 10.1186/s13287-015-0238-3. PubMed PMID: 26645735; PubMed Central PMCID: PMCPMC4672563.
18 Navard SH, Rezvan H, Haddad MHF, Ali SA, Nourian A, Eslaminejad MB, et al. Therapeutic effects of mesenchymal stem cells on cutaneous leishmaniasis lesions caused by Leishmania major. J Glob Antimicrob Resist. 2020;23:243-50. doi: 10.1016/j.jgar.2020.09.005. PubMed PMID: 32977079.
19 Ramos TD, Silva JD, da Fonseca-Martins AM, da Silveira Pratti JE, Firmino-Cruz L, Maciel-Oliveira D, et al. Combined therapy with adipose tissue-derived mesenchymal stromal cells and meglumine antimoniate controls lesion development and parasite load in murine cutaneous leishmaniasis caused by Leishmania amazonensis. Stem Cell Res Ther. 2020;11:374. doi: 10.1186/s13287-020-01889-z. PubMed PMID: 32867857; PubMed Central PMCID: PMCPMC7457509.
20 Bahrami S, Safari M, Razi Jalali MH, Ghorbanpoor M, Tabandeh MR, Rezaie A. The potential therapeutic effect of adipose-derived mesenchymal stem cells in the treatment of cutaneous leishmaniasis caused by L. major in BALB/c mice. Exp Parasitol. 2021;222:108063. doi: 10.1016/j.exppara.2020.108063. PubMed PMID: 33412170.
21 L PK, Kandoi S, Misra R, S V, K R, Verma RS. The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. Cytokine Growth Factor Rev. 2019;46:1-9. doi: 10.1016/j.cytogfr.2019.04.002. PubMed PMID: 30954374.
22 Ma H, Lam PK, Siu WS, Tong CSW, Lo KKY, Koon CM, et al. Adipose Tissue-Derived Mesenchymal Stem Cells (ADMSCs) and ADMSC-Derived Secretome Expedited Wound Healing in a Rodent Model - A Preliminary Study. Clin Cosmet Investig Dermatol. 2021;14:753-64. doi: 10.2147/CCID.S298105. PubMed PMID: 34234501; PubMed Central PMCID: PMCPMC8255652.
23 Hosein S, Blake DP, Solano-Gallego L. Insights on adaptive and innate immunity in canine leishmaniosis. Parasitology. 2017;144:95-115. doi: 10.1017/S003118201600055X. PubMed PMID: 27094260; PubMed Central PMCID: PMCPMC5264656.
24 Zanganeh E, Soudi S, Zavaran Hosseini A. Intralesional Injection of Mouse Mesenchymal Stem Cells Reduces IL-10 Production and Parasite Burden in L. major Infected BALB/c Mice. Cell J. 2020;22:11-8. doi: 10.22074/cellj.2020.6838. PubMed PMID: 32779429; PubMed Central PMCID: PMCPMC7481897.
25 Ahangar P, Mills SJ, Cowin AJ. Mesenchymal Stem Cell Secretome as an Emerging Cell-Free Alternative for Improving Wound Repair. Int J Mol Sci. 2020;21. doi: 10.3390/ijms21197038. PubMed PMID: 32987830; PubMed Central PMCID: PMCPMC7583030.
26 Li P, Guo X. A review: therapeutic potential of adipose-derived stem cells in cutaneous wound healing and regeneration. Stem Cell Res Ther. 2018;9:302. doi: 10.1186/s13287-018-1044-5. PubMed PMID: 30409218; PubMed Central PMCID: PMCPMC6225584.
27 Mesquita I, Ferreira C, Barbosa AM, Ferreira CM, Moreira D, Carvalho A, et al. The impact of IL-10 dynamic modulation on host immune response against visceral leishmaniasis. Cytokine. 2018;112:16-20. doi: 10.1016/j.cyto.2018.07.001. PubMed PMID: 30017388.
28 Samant M, Sahu U, Pandey SC, Khare P. Role of Cytokines in Experimental and Human Visceral Leishmaniasis. Front Cell Infect Microbiol. 2021;11:624009. doi: 10.3389/fcimb.2021.624009. PubMed PMID: 33680991; PubMed Central PMCID: PMCPMC7930837.
29 Bacellar O, D'Oliveira A, Jr., Jeronimo S, Carvalho EM. IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis. Cytokine. 2000;12:1228-31. doi: 10.1006/

cyto.2000.0694. PubMed PMID: 10930301.
30 Li J, Bower AJ, Vainstein V, Gluzman-Poltorak Z, Chaney EJ, Marjanovic M, et al. Effect of recombinant interleukin-12 on murine skin regeneration and cell dynamics using in vivo multimodal microscopy. Biomed Opt Express. 2015;6:4277-87. doi: 10.1364/BOE.6.004277. PubMed PMID: 26600994; PubMed Central PMCID: PMCPMC4646538.