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Original Article

Role of magnetic Field in the Healing of Cutaneous Leishmaniasis Lesions in Mice

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ABSTRACT

Leishmaniasis is an important tropical cutaneous disease that is endemic in the Middle East, including Iran. There is no consensus on the appropriate therapeutic method, dose, and duration for this disease. The pentavalent antimonial compounds are the first-line treatments of leishmaniasis. With regard to the resistance of this disease against drugs and its treatment failure in some patients, the present study was conducted to investigate the role of alternating magnetic field (AMF) in the treatment of cutaneous leishmaniasis lesions in mice. To this end, 45 male Balb/c mice with the age of 3-4 weeks and weight of 18-20 g were purchased from the Pasteur Institute, Tehran, Iran, to be investigated. The mice were infected with *Leishmania* promastigote (2×10^6) injected in the upper end of the tail. After 3 weeks, the animals were screened for developing lesions. Finally, 15 mice were selected and randomly divided into three equal groups of positive control (treated with the standard drug), negative control (without treatment), and experimental (subjected to AMF at a frequency of 50 kHz for 30 min on a daily basis for 4 weeks). The subjects were followed up for 4 weeks, and the size of their lesions was measured weekly. The data were analyzed by repeated measures test in SPSS software (version 20) at a p-value of < 0.05. There was no significant difference between the experimental and positive control groups ($P>0.05$). However, the negative control group showed a significant difference with the positive control and experimental groups ($P=0.0001$). As the findings indicated, AMF was seemingly able to decrease the size of lesions to the same extent as the standard drug. Consequently, AMF could be suggested as a noninvasive and complementary tool against cutaneous leishmaniasis. However, it is required to perform more studies to address different aspects of this domain.

Keywords: Cutaneous leishmaniasis, Alternating magnetic field, Treatment

Rôle du Champ Magnétique dans la Cicatrisation des Lésions cutanées de Leishmaniose chez les Souris

Résumé: La leishmaniose est une importante maladie cutanée tropicale endémique au Moyen-Orient, y compris en Iran. Il n'y a pas de consensus sur la méthode thérapeutique, la dose et la durée appropriées pour cette maladie. Les composés antimoniaux pentavalents sont les traitements de première ligne de la leishmaniose. Etant donné la résistance de cette maladie aux médicaments et l'échec thérapeutique chez certains patients, la présente étude a été menée pour étudier le rôle du champ magnétique alternatif (CMA) dans le traitement des lésions de leishmaniose cutanée chez les souris. Dans ce but, 45 souris Balb/c mâles âgées de 3 à 4 semaines et pesant de 18 à 20g ont été achetées à l'Institut Pasteur de Téhéran, en Iran, pour le besoin de cette étude. Les souris ont été infectées par *Leishmania* promastigote (2×10^6) injecté dans l'extrémité supérieure de la queue. Après 3 semaines, les animaux ont été examinés pour le possible développement de lésions. Enfin, 15 souris ont été sélectionnées et réparties au hasard en trois groupes égaux de contrôle positif (traités avec le médicament standard), de contrôle négatif (sans traitement), et expérimental (soumis au CMA à une fréquence de 50 kHz pendant 30 min sur une base quotidienne durant 4 semaines). Les sujets ont été suivis durant 4 semaines, et la taille de leurs lésions a été mesurée chaque semaine. Les données ont été analysées par un test de mesures répétées dans le logiciel SPSS (version 20) à une valeur de p <0,05. Il n'y avait pas de différence significative entre les groupes expérimentaux et contrôle positif (P> 0,05). Cependant, le groupe contrôle négatif a montré une différence significative avec le groupe contrôle positif et le groupe expérimental (P = 0,0001). Comme les résultats l'ont indiqué, le CMA était apparemment en mesure de réduire la taille des lésions dans les mêmes proportions que le médicament standard. Par conséquent, le CMA pourrait être suggérée comme un outil non invasif et complémentaire contre la leishmaniose cutanée. Cependant, il est nécessaire d'effectuer plus d'études pour aborder différents aspects de ce traitement.

Mots-clés: Leishmaniose cutanée, Champ magnétique alternatif, Traitement

INTRODUCTION

Leishmaniasis is a global health problem, affecting more than 2 million people worldwide every year. This disease has diverse epidemiology in the world. Cutaneous leishmaniasis (CL) is endemic in 90 countries, mostly in tropical and subtropical regions, as well as in southern Europe. Old World CL has been reported in the Middle East and North Africa. *Leishmania* species as a protozoan has two forms, namely amastigote (inside the body of the vertebrate host) and promastigote (inside the body of the sand fly as a vector) during its life cycle. This species causes three types of leishmaniases, namely visceral, mucocutaneous, and cutaneous, which can be transmitted to humans through the bites of infected female sand flies. The prevalence of leishmaniasis has undergone an increase; moreover, the distribution of this disease has expanded to the new regions of the world. The high rate of immigration, international travel, and military operations in endemic areas has led to the direction of more attention to this disease in developed countries (Shaddel et al., 2018). The incidence of CL has been reported in 20 of 31 provinces of Iran. Shiraz, the capital of Fars province in southwestern Iran, is one of the main endemic areas for this disease in Iran (Parvizi et al., 2017). In addition to humans, different species of rodents have a reservoir role during the life cycle of *Leishmania* in nature. Accordingly, this issue complicates the process of controlling this disease (Karami et al., 2013). There is no collective agreement on the appropriate therapeutic method, dose, and duration for CL. The pentavalent antimonial compounds (*Glucantim*) have been the first-line treatment agents of leishmaniasis. The treatment of this infection by standard drugs is complicated because of the associated side effects, painful process, and drug resistance. Scientists try to achieve an alternative drug that can eliminate the defects of the previous agents (Shaddel et al., 2018). Physical methods, such as microwave, radiofrequency, laser, and ultrasound, increase tissue temperature, leading to hyperthermia. Hyperthermia can be induced by alternating magnetic field (AMF) by producing heat that seems to be beneficial in wound healing (Chen et al., 2016). There are different studies about the biological effects of magnetic fields (MF) (Gmitrov, 2013). Based on a body of evidence, MF has antimicrobial properties (Giladi et al., 2008) and can treat some skin diseases (Athanasiou et al., 2007). As indicated in previous studies, different frequencies might induce various biological effects depending on the intensity of MF and wound dressing (Cheing et al., 2014; Ferroni et al., 2017). The use of a low frequency may be a beneficial measure for the repair of tissue injuries and could be applied for the treatment of some skin injuries (Pesce et al., 2013). The AMF has the potential to improve wound healing. However, there are limited studies about the efficiency of AMF in healing the lesion of *Leishmania* major infection in Iran. With this background in mind, the present study was conducted to investigate the effect of AMF on CL lesions in mice.

MATERIAL AND METHODS

Parasites. For the purpose of the study, MRHO/IR/75/ER strain promastigotes of *Leishmania* major were supplied by the Department of Parasitology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The strains were cultivated in Novy-MacNeal-Nicolle medium and then RPMI medium, supplemental with 10% fetal bovine serum and 2 mM L-glutamine. The stationary phase of the parasites was obtained following a study performed by Shaddel et al. (2018).

Animal. For the purpose of the study, a total of 45 male BALB/C mice aged 3-4 weeks were purchased from the Pasteur Institute, Tehran, Iran. The animals were maintained under a standard condition (i.e., temperature of 22±2°C, with lights on from 6:0 to 18:00) with free access to food and water. After 1 week of acclimatization period in the Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran, the mice were subcutaneously inoculated with approximately 2×10^6 stationary phase of *Leishmania* major (MRHO/IR/75/ER) promastigote in a shaved and disinfected area at the base of the tail and maintained until observing the lesion at the base of the tail.

***In vivo* study.** Only 15 mice developed lesion; therefore, they were subjected to animal experiments. To this end, they were randomly divided into three groups (n=5 for each group). Group 1 (i.e., negative control group) received no treatment, while group 2 (positive control group) were treated with glucantim as a standard drug administered daily for 4 weeks. Additionally, group 3 as the experimental group were treated with AMF. Accordingly, the whole body of the animals in this group was daily exposed to electromagnetic fields for 30 min, at a frequency of 50 kHz, for 4 weeks. The device used for the exposure of mice consisted of two pairs of coaxially arranged Helmholtz coils (diameter of each coil=91.4 cm and distance between the coils in the pairs=50 cm), oriented along the vector of the geomagnetic field (GMF). A hand-held spectrum analyzer (SPECREAN NF-5035, Aaronia AG, Germany) was used to measure the intensity of the applied exposure within the device (Figure 1). The Helmholtz coils were used to produce 50 Hzuniform electromagnetic fields with an intensity of 100 µT. For animal exposure, a mouse was first put into the perforated cage; subsequently, the cage with the mouse was inserted into the exposure chamber of the device. The big (D) and small (d) diameters of the lesions were measured by a caliper at the baseline and every week. The size of the lesions was computed using the following formula (Dalimi et al., 2015): D+d/2 .At the end of the experiments, all mice were sacrificed and buried according to the standard protocols to prevent the spread of the infection.

Statistical Analysis. All data were expressed as Mean±SEM. Data analysis was performed using a two-way repeated measures ANOVA model in SPSS software (version 22, SPSS Inc, Chicago, Illinois, USA). A p-value less than 0.05 was considered statistically significant.

Figure 1. Device used for producing an alternating magnetic field (frequency 50 kHz) for the experimental group

RESULTS

The results revealed no significant difference between the experimental and positive control groups in terms of the mean size of lesions (P>0.05; Table 1 and Figure 2). However, the positive control and experimental groups showed a significant difference with the negative control group in this regard (P=0.0001; Table 1 and Figure 2).

(Shaddel et al., 2018). Accordingly, the present study was targeted toward evaluating the efficacy of AMF in the treatment of CL lesions. Based on the results, the groups subjected to AMF and glucantim showed a significantly higher reduction in the size of lesions than the negative controls (P=0.0001). However, there was no significant difference between the AMF (i.e., experimental group) and glucantim (i.e., positive controls) groups in this regard. These results are in line with those obtained by Cheing et al. (2014), reporting that static MF improved wound healing in diabetic rats and might be a noninvasive therapeutic tool for impaired wound healing in diabetic patients. (De Pedro et al., 2005), investigating the effect of electromagnetic fields on peripheral nerve regeneration, reported that

Table 1. Mean size of lesions (mm) after 1, 2, 3, 4, and 5 weeks of exposure in the three research groups

Group / Time	At the beginning of treatment	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
Experimental					
Mean	8.39	7.66	5.27	4.51	3.20
Std. deviation	3.886	2.895	2.755	2.152	3.054
Positive control					
Mean	8.78	7.44	6.84	5.98	4.24
Std. deviation	0.492	0.385	0.182	0.687	0.371
Negative control					
Mean	6.58	6.76	6.78	6.84	6.86
Std. deviation	0.466	0.537	0.512	0.351	0.270

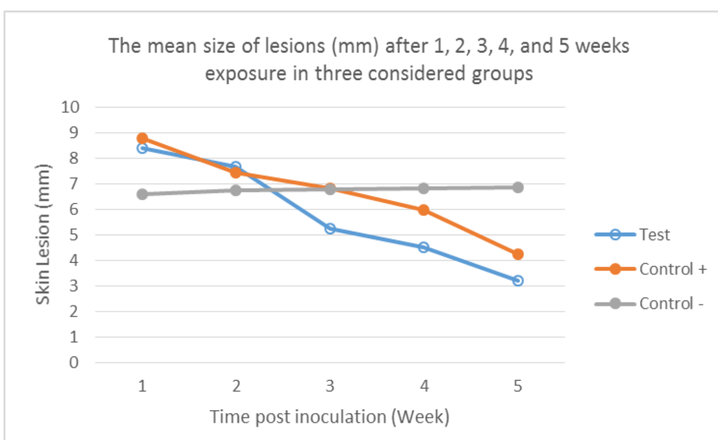

Figure 2. Mean size of lesions (mm) after 1, 2, 3, 4, and 5 weeks of exposure in the three research groups

DISCUSSION

The defects of the current therapeutic approaches for CL have urged the direction of more attention to the identification and development of an alternative method with fewer side effects and higher efficacy pulsed electromagnetic field therapy could accelerate functional recovery after nerve transection. It has been confirmed that pulsed electromagnetic fields can improve peripheral regeneration or crush injury and decrease inflammation (Kanje et al., 1993; Walker et al., 1994; Baptista et al., 2008). Wound healing process can be classified into three different phases, namely inflammatory, proliferative, and remodeling (Rieger et al., 2015). It seems that AMF could affect wound healing (e.g., CL) in several aspects. The AMF might promote the immune response via increasing the anti-inflammatory cytokines (Pesce et al., 2013). The MF increases bloodstream. As mentioned in the study by Gmitrov (2013), AMF decreases the blood pressure by increasing microcirculation in microvascular and heart rate variability. The arterial baroreflex capacity supports nitric oxide (NO)-dependent vasodilation by increasing the sensitivity of the vessels to NO (Gmitrov, 2013). In a study performed by Eskandari et al., it was reported that the elevation of NO and generation of energy had positive effects on the healing of CL lesions (Eskandari et al., 2012). The AMF can also improve endothelial cell proliferation and the formation of epidermal neovascularization (Tepper et al., 2004). Additionally, it can promote the formation of skin collagen and lead to skin regeneration (Choi et al., 2016). Moreover, AMF can produce heat. It is believed that thermotherapy acts against CL by causing severe collateral damage to the parasite and inhibiting parasite growth in a different form (Cardona-Arias et al., 2018). Therefore, AMF as an energy source in the form of heat can inactivate *leishmania* major in cutaneous lesions and improve the healing process of the CL lesions (Chen et al., 2016). In spite of the lack of any significant difference between the groups subjected to AMF and glucantim, in the second week up to the end of the experiment, AMF had a relative preference, and well-being was observed in one case. This highlights the need for performing more investigations in this domain. In order to expose the lesions to AMF, the whole body of the mice was inserted in the device. The efficacy and safety of this method must be verified and compared with the localized method in future studies.

It seems that AMF is able to decrease the size of lesions to the same extent as the standard drug. Based on the findings, AMF can be suggested as a noninvasive and complementary tool against CL. However, it is required to perform further studies to investigate this issue in terms of different aspects using a larger sample size.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contribution

Study concept and design: Dastgheib, M.: Shaddel, M.
Acquisition of data: Shaddel, M.; Saba, V., Homayouni, M. M.; Fereydoni, A.
Analysis and interpretation of data: Dastgheib, M.; Shaddel, M.; Saba, V.; Homayouni, M. M.; Fereydoni, A.
Drafting of the manuscript: Dastgheib, M.; Shaddel, M.
Critical revision of the manuscript for important intellectual content: Dastgheib, M.; Shaddel, M.; Saba, V.; Homayouni, M. M.
Statistical analysis: Dastgheib, M.; Shaddel, M.
Administrative, technical, and material support: Aja University of Medical Sciences; Dastgheib, M.; Shaddel, M.

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References

Athanasiou, A., Karkambounas, S., Batistatou, A., Lykoudis, E., Katsaraki, A., Kartsiouni, T., *et al.*, 2007. The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study. Bioelectromagnetics 28, 362-368.

Baptista, A.F., Gomes, J.R., Oliveira, J.T., Santos, S.M., Vannier-Santos, M.A., Martinez, A.M., 2008. High- and low-frequency transcutaneous electrical nerve stimulation delay sciatic nerve regeneration after crush lesion in the mouse. J Peripher Nerv Syst 13, 71-80.

Cardona-Arias, J.A., Lopez-Carvajal, L., Tamayo-Plata, M.P., Velez, I.D., 2018. Comprehensive economic evaluation of thermotherapy for the treatment of cutaneous leishmaniasis in Colombia. BMC Public Health 18, 185.

Cheing, G.L., Li, X., Huang, L., Kwan, R.L., Cheung, K.K., 2014. Pulsed electromagnetic fields (PEMF) promote early wound healing and myofibroblast proliferation in diabetic rats. Bioelectromagnetics 35, 161-169.

Chen, C., Chen, L., Yi, Y., Chen, C., Wu, L.F., Song, T., 2016. Killing of Staphylococcus aureus via magnetic hyperthermia mediated by magnetotactic bacteria. Appl Environ Microbiol 82, 2219-2226.

Choi, M.C., Cheung, K.K., Li, X., Cheing, G.L., 2016. Pulsed electromagnetic field (PEMF) promotes collagen fibre deposition associated with increased myofibroblast population in the early healing phase of diabetic wound. Arch Dermatol Res 308, 21-29.

Dalimi, A., Delavari, M., Ghaffarifar, F., Sadraei, J., 2015. In vitro and in vivo antileishmanial effects of aloe-emodin on Leishmania major. J Tradit Complement Med 5, 96-99.

De Pedro, J.A., Perez-Caballer, A.J., Dominguez, J., Collia, F., Blanco, J., Salvado, M., 2005. Pulsed electromagnetic fields induce peripheral nerve regeneration and endplate enzymatic changes. Bioelectromagnetics 26, 20-27.

Eskandari, S., Azimzadeh, A., Bahar, M., Naraghi, Z.S., Javadi, A., Khamesipour, A., *et al.*, 2012. Efficacy of Microwave and Infrared Radiation in the Treatment of the Skin Lesions Caused by Leishmania major in an Animal Model. Iran J Public Health 41, 80-83.

Ferroni, L., Bellin, G., Emer, V., Rizzuto, R., Isola, M., Gardin, C., *et al.*, 2017. Treatment by Therapeutic Magnetic Resonance (TMR™) increases fibroblastic activity and keratinocyte differentiation in an in vitro model of 3D artificial skin. J Tissue Eng Regen Med 11, 1332-1342.

Giladi, M., Porat, Y., Blatt, A., Wasserman, Y., Kirson, E.D., Dekel, E., *et al.*, 2008. Microbial growth inhibition by alternating electric fields. Antimicrob Agents Chemother 52, 3517-3522.

Gmitrov, J., 2013. Static magnetic field effect on microcirculation, direct versus baroreflex-mediated approach. Electromagn Biol Med 32, 448-462.

Kanje, M., Rusovan, A., Sisken, B., Lundborg, G., 1993. Pretreatment of rats with pulsed electromagnetic fields enhances regeneration of the sciatic nerve. Bioelectromagnetics 14, 353-359.

Karami, M., Doudi, M., Setorki, M., 2013. Assessing epidemiology of cutaneous leishmaniasis in Isfahan, Iran. J Vector Borne Dis 50, 30-37.

Parvizi, M.M., Handjani, F., Moein, M., Hatam, G., Nimrouzi, M., Hassanzadeh, J., *et al.*, 2017. Efficacy of cryotherapy plus topical Juniperus excelsa M. Bieb cream versus cryotherapy plus placebo in the treatment of Old World cutaneous leishmaniasis: A triple-blind randomized controlled clinical trial. PLoS Negl Trop Dis 11, e0005957.

Pesce, M., Patruno, A., Speranza, L., Reale, M., 2013. Extremely low frequency electromagnetic field and wound healing: implication of cytokines as biological mediators. Eur Cytokine Netw 24, 1-10.

Rieger, S., Zhao, H., Martin, P., Abe, K., Lisse, T.S., 2015. The role of nuclear hormone receptors in cutaneous wound repair. Cell Biochem Funct 33, 1-13.

Shaddel, M., Sharifi, I., Karvar, M., Keyhani, A., Baziar, Z., 2018. Cryotherapy of cutaneous leishmaniasis caused by Leishmania major in BALB/c mice: A comparative experimental study. J Vector Borne Dis 55, 42-46.

Tepper, O.M., Callaghan, M.J., Chang, E.I., Galiano, R.D., Bhatt, K.A., Baharestani, S., *et al.*, 2004. Electromagnetic fields increase in vitro and in vivo angiogenesis through endothelial release of FGF-2. FASEB J 18, 1231-1233.

Walker, J.L., Evans, J.M., Resig, P., Guarnieri, S., Meade, P., Sisken, B.S., 1994. Enhancement of functional recovery following a crush lesion to the rat sciatic nerve by exposure to pulsed electromagnetic fields. Exp Neurol 125, 302-305.