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Original Article

Efficacy of Microwave and Infrared Radiation in the Treatment of the Skin Lesions Caused by *Leishmania major* in an Animal Model

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Abstract

Background: Cutaneous leishmaniasis caused by *L. major* is an important public health problem in endemic areas. The aim of this study was to explore the therapeutic effect of microwave and or infrared radiation in the treatment of lesion induced in BALB/c mice by *L. major* inoculation.

Methods: The footpad lesion was induced in BALB/c mice by inoculation of *L. major* promastigotes subcutaneously. The lesion was treated with 600 watts power, 2.450 GHz frequency and/or infrared device with 150 watts and a wave length of 890 nanometres. The size of the lesion was recorded by footpad swelling measurement every 10 days.

Results: The lesion growth was significantly hampered in treated mice compared with the untreated control group ($P<0.05$). Infrared radiation was more effective than microwave in inhibiting ulcer enlargement.

Conclusion: Infrared radiation and microwave significantly hampered *L. major* lesion growth in BALB/c mice. This therapeutic effect was more in infrared radiation treated mice than microwave treated mice.

Keywords: Microwave, Infrared, *Leishmania major*, Mouse

Introduction

Leishmaniasis is a vector-borne parasitic disease caused by 20 different species of *Leishmania* and transmitted by the bite of various species of sand fly vectors. Leishmaniasis reported from 98 countries and regions mostly developing ones. Clinical manifestations of the disease include cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), post kala azar leishmaniasis (PKDL) and visceral leishmaniasis (VL) (1-5). The most common clinical form of leishmaniasis is CL which is a major health problem in Iran. Both Anthroponotic CL (ACL) caused by *L. tropica* and Zoonotic CL (ZCL) caused by *L. major* are endemic in different parts of Iran (3,4).

Although CL lesion is a self limiting disease but healing takes a long time and usually leaves a disfiguring scar (2-4). Most strains of mice are resistant to *L. major* infection which somehow the disease is similar to human CL. Recovery of CL accompanies with full protection against further infection, on the contrary *L. major* infection in BALB/c mice is similar to human Kala azar and diffuse

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cutaneous leishmaniasis (6). Treatment of *L. major* infection in BALB/c mice with standard drug is almost impossible and all mice eventually succumbed to the disease. Treatment of CL is a universal challenging issue, standard WHO recommended treatment is almost solely antimoniate derivatives which are in use for 80 years. Treatment with antimonate needs multiple injections. It is painful and is not always effective (7-9). Various therapeutic strategies are used to treat CL including thermotherapy (10). Photodynamic therapy and electromagnetic radiation was used to accelerate wound healing in rats (11), healing of CL lesion is partly depends on wound healing process.

The aim of this study was to explore the therapeutic effect of microwave and/or infrared radiation in the treatment of lesion induced in BALB/c mice by *L. major* inoculation.

Materials and Methods

In vitro test

To explore the effect of microwave and infrared on promastigote growth, different concentrations of *L. major* promastigotes were exposed to either microwave (2.450 GHz, 600 W) or infrared (890 nm, 150 W) for a period of 0 to 12 minutes.

To check the tolerability of mice to microwave and infrared, a group of mice were exposed to heat and the temperature per minute at the site of exposure was increased.

Device development

A device was designed in such a way that the mouse body was inside water and only the lesion was exposed to infrared or/and microwave (Fig. 1).The mouse was put in the middle of the cylinder surrounded with the water. Several experiments were designed to test the tolerability of the mice. From those test the final instrument was designed.

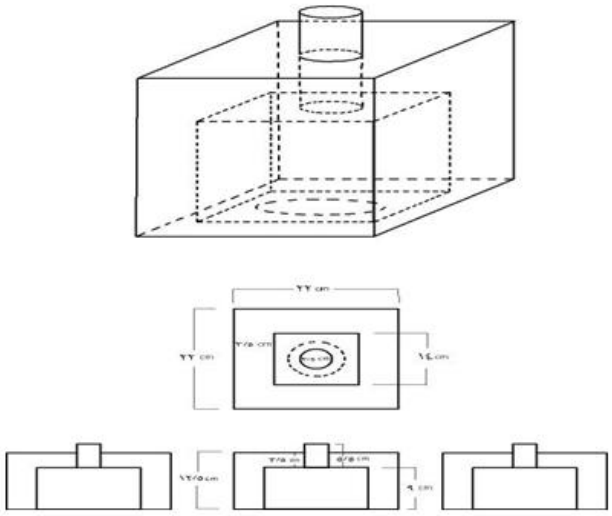

Fig. 1: Instrument for handling of mice for exposing them to infrared or/and microwave

Animals

8-10 weeks old female BALB/c mice were purchased from Pasteur Institute, Karaj, Iran. The mice were maintained in animal house of Center for Research & Training in Skin Diseases & Leprosy, Tehran University of Medical Sciences. The animals were kept in a colony room at 12/12 h light/dark photoperiod cycle at 21°C with free access to water and food. Animal experiments were carried out according to Tehran University of Medical Sciences ethical Committee Acts.

The virulence of *L. major* strain (MRHO/IR/75/ER) was maintained with passage in BALB/c mice. BALB/c mice were inoculated subcutaneously in the left footpad with 1 X 10^6 *L. major* promastigotes harvested at stationary phase. When the mice developed skin lesion, the lesions were measured using digital calipers and a total of 20 mice with similar lesion size were selected and randomly divided into 4 groups and treated as follow: Group 1 was treated with infrared, 150 watts and wave length of 890 nanometers, group 2 was treated with microwave 600 watts power, 2.450 GHz frequency, group 3 was treated with both microwave and infrared and finally

group 4 considered as control. The footpad lesions were measured and recorded every 10 days.

Results

Vials with different number of *L. major* promastigotes were exposed to either microwave (2.450 GHz, 600 W) or infrared (890 nm, 150 W) for a period of 0 to 12 minutes. The footpad lesions were measured and recorded every 10 days post-treatment. Results showed that the duration of the exposure has direct correlation for parasite killing. There was no significant difference in lesion size of different groups, prior treatment. However, there was a significant different in lesion size at baseline. The lesion size was grown in control group significantly faster than the treatment groups. Lesion growth in group of mice which treated with infrared was significantly slower than the other groups (Fig. 2).

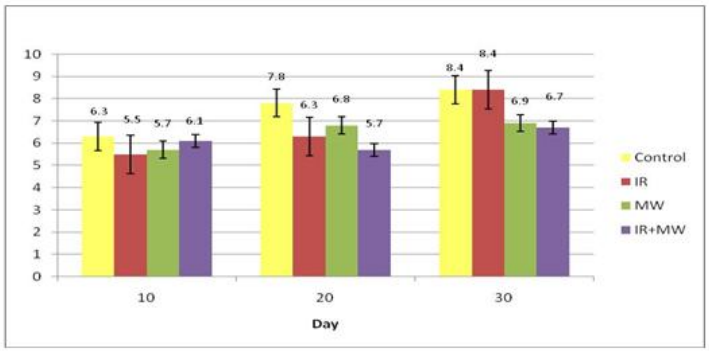

Fig.2: Ulcer size (mm) based on group and days

Discussion

Treatment of CL is a major problem for the patients and health authorities, current available treatment is solely antimonite derivatives which needs multiple injections with poor compliance which results in low efficacy (7-9). In this study the therapeutic effect of microwave and/or infrared radiation in the treatment of lesion induced in BALB/c mice by *L. major* inoculation was studied. It was shown that infrared beam with wave length of 890 nanometer caused recovery of skin lesions with increase in NO production (12). In another study microwave affects white blood cells in rabbits which cause faster healing than control group (13). Two studies showed that 30 minutes exposure to infrared/microwave induces significantly faster healing of non-infectious lesions than control groups, the healing was faster when the animals were exposed to both infrared and microwave (14)

The results of the current study showed that infrared and microwave inhibit *L. major* promastigotes growth *in vitro*. *In vivo* results showed that microwave and infrared as well as the combination of microwave and infrared significantly hampered lesion growth. Mixture tune of lesion growth resulted from *L. major* in BALB/c mice are reduced. Microwave and infrared were used to treat various disorders (15-19).

Physical basis using electromagnetic beams in medicine from interaction with the material is caused if an electromagnetic wave is collided directly with an atom, energy is generated and when the energy is enough one or a few electrons are evoked. The evoked electrons during return to the original state dispatched electromagnetic. Electromagnetic microwave beam and ultraviolet were used to treat lesions induced by *L. major* infection in BALB/c mice. Wave lengths of these radiations were between 10 μm and 10 centimeters (corresponding with frequencies 100 terahertz and 10 giga hertz). One of the most important features of microwave is ease concentration on the main goal (19). Microwave waves on some molecules like OH group have the most effect and cause production of vibration and friction. Due to this phenomenon, the energy of microwave radiation is absorbed in molecules and change to heat energy. In most cases, treatment due to microwave took time and does not have instantaneous effects.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submis-

sion, redundancy, etc) have been completely observed by the authors.

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