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A pilot study – acute exposure to a low-intensity, low-frequency oscillating magnetic field: effects on carrageenan-induced paw edema in mice

Estudo piloto – exposição aguda a campo magnético oscilante de baixa intensidade e baixa frequência: efeitos sobre edema de pata induzido por carragenina, em camundongos

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Abstract

The purpose of this paper was to evaluate the effects of an oscillating magnetic field (MF) on edema evolution in an animal model. Paw edema was induced in 32 female Swiss mice by injecting 50 µL of 1.0% carrageenan, diluted in saline solution in the left hind footpad. Animals were randomly assigned into four Experimental (exposed to different field frequencies) and two Control Groups. Groups 1 (0 Hz), 2 (3 Hz), 3 (9 Hz) and 4 (15 Hz) were exposed for 60 seconds to an oscillating MF (300 mT) in the first, second, and third hour after the injection. Control Groups (CGN and DCL) were not exposed to the MF and diclofenac was administered to DCL Group one hour after the edema induction. Paw volumes were determined every hour using a water plethysmometer. The results were graphed against time and, to evaluate the edema, the area under the curve (AUC) was measured. All groups receiving some form of intervention (1, 2, 3, 4 and DCL) revealed AUC values that were substantially lower than those of the CGN Group. DCL had the lowest reduction percentage (25.0±6.1%) and Group 3, the highest (46.9±4.0%). Compared to the results of DCL, only Groups 2 and 3 showed significantly lower AUC values. Also, with statistical relevance, Group 3 showed lower AUC values than Groups 1 and 4. According to this experiment, acute exposure to oscillating MF yields positive results in the regression of carrageenan-induced edema in mice, with indications that such effect depends on the field frequency.

Keywords: magnetic field, edema, carrageenan, inflammation, mice.

Resumo

O objetivo desse trabalho foi avaliar os efeitos da exposição aguda a um campo magnético (CM) oscilante sobre a evolução do edema em um modelo animal. O edema foi induzido através de injeção subcutânea de 50 µL de carragenina (diluída a 1,0 % em solução salina) na região subplantar da pata traseira esquerda de 32 camundongos fêmeas da linhagem Swiss. Os animais foram distribuídos de forma aleatória em 4 grupos experimentais (expostos a diferentes frequências deCM) e 2 grupos controle. Os Grupos 1 (0 Hz), 2 (3 Hz), 3 (9 Hz) e 4 (15 Hz) foram expostos a CM oscilante (300 mT) durante 60s na primeira, segunda e terceira horas após a injeção de carragenina. Os Grupos Controle (CGN e DCL) não foram expostos ao CM, e o Grupo DCL recebeu diclofenaco sódico 1h após a indução do edema. O volume das patas foi determinado a cada hora com auxílio de pletismógrafo digital. Os resultados foram dispostos em gráfico contra o tempo, e foi calculada a área sob a curva (AUC) para avaliação do edema. Todos s grupos que receberam algum tipo de intervenção (1, 2, 3, 4 e DCL) apresentaram valores de AUC significativamente menores do que CGN. DCL apresentou a menor porcentagem de redução (25,0±6,1%) e o grupo 3, a maior (46,9±4,0%). Em relação ao DCL, somente os grupos 2 e 3 apresentaram valores de AUC menores e significativos. O grupo 3 apresentou, com relevância estatística, valores de AUC menores do que os grupos 1 e 4. De acordo com esse experimento, a exposição aguda a CM oscilante promove efeitos positivos na regressão de edema induzido por carragenina em camundongos, com indicações de que tais efeitos são dependentes da frequência do CM.

Palavras-chave: campo magnético, edema, carragenina, inflamação, camundongos.

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Introduction

In 1979, the Food and Drug Administration (FDA, USA) approved, for the first time, the use of pulsed electromagnetic field (PEMF) for the treatment of non-united fractures and failed arthrodesis[1]. Since then, several studies have been conducted to demonstrate the benefits of electromagnetic fields (EMFs) for the treatment of various conditions, such as psoriasis[2], edema[3-5], osteoarthritis[6], and difficult-to-heal wounds[7-9]. The positive effects of EMFs in the modulation of hemodynamics[10], and pain relief caused by carpal tunnel syndrome[11], besides the established use for bone formation, were also reported. The mechanisms that explain the occurrence of these observed effects, however, are yet to be completely understood.

Considering the principles that govern the electromagnetic phenomena, it is possible to infer that, in biological systems and living organisms, EMFs are ubiquitous and constantly created by physiological processes (cell movements, ionic fluxes, fluids flow in the circulatory systems, mitochondrial electron transport chain, action potentials, and so on). Therefore, biological effects can result from the interaction of these fields with exogenous EMFs.

A recent review[12] points out how electrical oscillations may play important physiological roles in the living organism. Previous studies have reported that right after the occurrence of a wound, an electric field is created at the injured tissue, and this may be a primary stimulus perceived by epithelial cells to start the repair process of the tissue. This "electrical disturbance" caused by the injury may remain for several hours, and it only ceases when the tissue is re-epithelialized. These observations indicate that the cells are sensitive to electrical changes, being able to respond specifically to them.

The major purposes of this paper were to evaluate the effects of acute exposure to a low-intensity, low-frequency oscillating magnetic field on paw edema induced by carrageenan in mice, and to verify if these effects depend on the magnetic field (MF) frequency. It is also our objective to compare the possible effects of the MF exposure to those promoted by a renowned anti-inflammatory drug (sodium diclofenac).

Materials and methods

Animals

All experiments were conducted in accordance with the rules and regulations for animal care and use set forth by the Institute of Biomedical Sciences (University of São Paulo). Since this pilot study was based on classically established protocols, submission to the Institute's Ethics Committee was not obligatory.

Thirty-two female Swiss mice, 44 days-old, weighting between 21 and 28 g, kept under ideal temperature conditions, with water and food *ad libitum*, in 12 hours/ 12 hours dark/light cycles, were used for this study. At the day of the experiment, animals were randomly allocated in groups of five or six inside PVC cages and were kept conscious throughout the experimental procedure.

Paw edema induction

To induce the paw edema, the experiment followed the classical model proposed by Winter et al., in 1962[13], 50 μL of 1.0% carrageenan (Sigma Chemical Co., St. Louis, MO, USA) diluted in a 0.9% saline solution were subcutaneously injected in the left hind footpad of all mice. Carrageenan, a sulfated polysaccharide that turns into gel in room temperature, is widely applied in the promotion of acute inflammatory response in animal models, as it induces the release of inflammatory mediators, such as histamine, bradykinin and prostaglandins, among others.

Edema evaluation

The volume of each animal's left hind paw (submersed until the knee articulation) was determined using a water plethysmometer (Plethysmometer 7150 – Ugo Basile®, Italy, 0.01 mL precision). Evaluations were made immediately before (T_0) and one, two, three and four hours (T_1, T_2, T_3 and T_4) after the carrageenan injections. Each measurement was repeated three times for every animal; therefore, the paws' volumes are expressed as averages.

In order to evaluate the edema evolution, relative volumes (%) of the animal's paws were calculated in relation to their basal volumes, for each of the four hours in the experiment, using the formula:

$$\text{Relative Volume} = \frac{(VF_m - VB_m)}{VB_m \times 100}$$

Where,

VB_m refers to the average basal volume at T_0;

VF_m is the average final volume at T_1, T_2, T_3 and T_4.

With the values obtained for each animal, the averages of their relative volumes were calculated for each group. The relative volume averages were graphed against time, and the area under the curve (AUC) was measured. With the AUC values, the percentages of edema reduction for each group in relation to the CGN group were calculated.

Exposure to MF

The equipment (NVL70, Linfospin, Brazil) used for the present study induces an oscillating MF, with adjustable frequencies (0 to 24 Hz). Each variation cycle induces a polar inversion, which switches back and forth between the North-South and South-North orientation. The field's intensity, measured with a gaussmeter (TMAG-1T, Globalmag, Brazil, 1 mT precision), was of 3,000 G (300 mT) on the surface of the equipment.

Animals were placed in the orthostatic position on the equipment, with the edema-inflicted paw immobilized and directly touching its surface. Even though there was a full body exposure to the MF, the injured paws were exposed to the highest field intensity. During the exposure (with 60 seconds of duration), the mice were immobilized through the torso-cervical region.

Experimental design

Experimental and Control Groups are described in Table 1.

Groups 1, 2, 3 and 4 were exposed to MF of the same intensity, during the same time period, with variations only regarding the field's frequency (0, 3, 9 and 15 Hz, respectively). The exposures were carried out during the first, second, and third hours, starting from the carrageenan injection.

Two Control Groups with edemas induced through the same protocol were included, though without exposure to the MF: CRG and DCL – the animals in this last group were administered an intramuscular injection of sodium diclofenac (1 mL/ kg) one hour after the carrageenan injection.

Statistical analysis

A Z-test was conducted to compare the AUC values obtained from different groups throughout the experiment, with p≤0.05 values being considered statistically relevant.

Results

The relative volume averages for each group, along with the AUC values, can be found in Figures 1 and 2, respectively. The edema reduction percentages are detailed in Table 2.

All groups receiving some form of intervention (1, 2, 3, 4 and DCL) revealed AUC values that were substantially lower than the ones of CGN Group (p≤0.05). DCL had the lowest reduction percentage (25.0±6.1%) and Group 3, the highest (46.9±4.0 %). Compared to the results of DCL, only Groups 2 (3 Hz) and 3 (9 Hz) showed significantly lower AUC values (p≤0.05). Also, with statistical relevance (p≤0.05), Group 3 (9 Hz) showed lower AUC values than Groups 1 (0 Hz) and 4 (15 Hz).

Conclusion

Acute exposure to a 300 mT oscillating MF yields positive results in the regression of carrageenan-induced edema in mice, with indications that such effect depends on the field frequency.

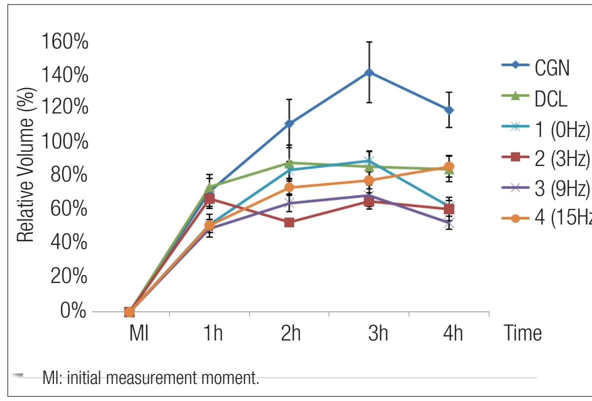

Figure 1. Relative volume averages (%) and their respective standard errors (groups DCL and 3 (9 Hz), n=6; other groups, n=5).

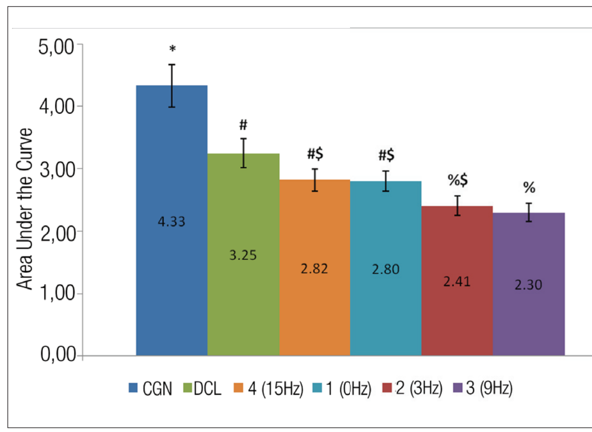

Figure 2. AUC values and their respective standard errors calculated throughout the four hours of experiment (groups DCL and 3 (9 Hz), n=6; other groups, n=5). Different symbols represent statistical difference between the respective groups (p≤0.05).

Table 2. Edema percentage reduction (reduction%) and the respective standard errors (±SE% reduction) calculated in relation to the CGN Group (Groups DCL and 3 (9 Hz), n=6; other groups, n=5).

Groups	Reduction %	±SE% reduction
CGN		
DCL	25,0	6,1
1 (0 Hz)	35,3	4,6
2 (3 Hz)	44,5	4,2
3 (9 Hz)	46,9	4,0
4 (15 Hz)	34,9	4,9

Table 1. Experimental and Control groups.

Groups	Number of subjects	Average weight (g)	Frequency (Hz)	Intensity (mT)	Exposure duration (seconds)	Exposure moment
1	5	24,6	0	300	60	T_1, T_2 & T_3
2	5	25,0	3	300	60	T_1, T_2 & T_3
3	6	24,8	9	300	60	T_1, T_2 & T_3
4	5	24,2	15	300	60	T_1, T_2 & T_3
CGN	5	25,0	Animals with induced paw edema not exposed to the MF			
DCL	6	24,5	1 hour after the carrageenan injection, the animals received a high anti-inflammatory dose (sodium diclofenac 1 mL/ kg) of intramuscular injection			

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