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In vitro Trypanosoma cruzi growth inhibition by extremely low-frequency electromagnetic fields

Short communication

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Abstract. The influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on microorganisms has been a subject of experimental investigations with promising results. In the present study, it was demonstrated that 2.0 mT 60Hz ELF-EMFs inhibited *Trypanosoma cruzi* epimastigotes growth. In addition, no synergistic or antagonistic parasite growth and viability effects were observed after cultures were co-exposed to magnetic fields and Nifurtimox, a well-known anti-trypanosome drug.

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Moreover, the current study represents the first report regarding direct magnetic fields effects on Trypanosomas.

Key words: *Trypanosoma cruzi*, antiprotozoal activity, 60-Hz sinusoidal magnetic fields, anti-trypanosome.

INTRODUCTION

Trypanosoma cruzi, the etiologic agent of Chagas disease, considered a public health problem in Latin America, is a kinetoplastid parasite that has been reported as a highly resistant organism to energetic radiation (Regis-da-Silva *et al.* 2006; Vieira *et al*. 2014). Efforts have been made to control species of this hemoflagellate protozoan parasite with drugs (Bern 2011) and plant extract agents (Molina-Garza *et al*. 2014). In regard to parasite growth inhibition using unconventional procedures, the effect of 60 Hz magnetic fields at 1.5 and 2.0 mT on *Entamoeba invadens* trophozoites growth inhibition and encystation *in-vitro* has been previously demonstrated (Rodríguez-De la Fuente *et al*. 2008). Furthermore, it was reported that by transferring electronic-metronidazole information to water samples, *Entamoeba histolytica* and *Trichomonas vaginalis* growth was significantly inhibited (Heredia-Rojas *et al*. 2011).

There are no recent reports relating ELF-EMFs and *Trypanosomes*, however, about four decades ago, French researchers reported anti-trypanosome effect of magnetic fields on rabbits (Pautrizel *et al*. 1970) and mice (Pautrizel *et al*. 1975); they observed that the control of the infection was not due to a direct action of the radiation on trypanosomes, but to a very strong stimulation of the immune system. Moreover, Bellosi (1983) found no-effect of a static magnetic field of 400 mT on mice infected with *T. equiperdum*; in addition, there was no difference between the length of survival of the animals exposed to magnetic fields, as compared with non-exposed mice. The

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present study was undertaken to evaluate the potential of ELF-EMFs to inhibit *Trypanosoma cruzi* epimastigote growth.

MATERIALS AND METHODS

Bioassays were developed using *T. cruzi* CL Brener strain cultured at 27ºC in liver infusion triptose medium, supplemented with 10% fetal bovine serum and harvested during the exponential growth phase (cell density of about 4×10^6 epimastigotes/mL) (Molina-Garza *et al*. 2014). Experiments were performed in triplicate in 96-well microtiter plates containing 200 μL/well of the parasite suspension at a density of 1.0×10^6 epimastigotes/mL. The following treatment and control groups were used: (*a*) cells exposed to ELF-EMFs at 60 Hz and 2.0 mT, (*b*) untreated cells, used as controls, (*c*) cells treated with Nifurtimox at 0.066 and 0.132 μM/mL (Bern 2011), used as positive controls, and (*d*) cells co-exposed to ELF-EMFs and Nifurtimox. Nine replicate determinations of three independent experiments, providing a total of 27 cultures analyzed per group were performed. When cultures reached the end of logarithmic phase, they were exposed to ELF-EMFs for 24 h. Magnetic field exposure was developed by using a standardized device, as we previously reported (Rodríguez-De la Fuente *et al*. 2008). In summary, this device comprised a coil that was built by winding 552 turns of 1.3 mm diameter enamel insulated copper wire to form a cylindrical solenoid with a radius of 13.5 cm and a length of 71 cm. The solenoid was connected to a step-down transformer and to a variable transformer that was plugged in to a 110 V AC source. The samples were allocated in pre-determined zones where de magnetic field was homogeneous. The magnetic flux density (rms) was evaluated by an axial Hall-effect probe (Bell FW 6010 Gaussmeter, Orlando, Fl, USA), coupling an oscilloscope (BK-Precision model 2120) to monitor the resulting field; a 2.0 mT 60 Hz

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alternating sinusoidal electromagnetic field was then generated, obtaining a nearly pure 60 Hz field frequency (<3% total harmonic distortion). On the other hand, to test the combination of ELF-EMF treatment and Nifurtimox (Sigma-Aldrich, St. Louis, MO, USA) on epimastigote growth and viability, a co-exposure experiment was included. Nifurtimox solutions at 0.066 and 0.132 μM/mL were prepared in 1% dimethyl sulfoxide (DMSO). Cultures were co-exposed at the same time for 24 h. For determining the cytotoxic effects, epimastigotes density was blindly evaluated and counted three times in a Neubauer chamber; parasite viability was determined by a colorimetric technique using the tetrazolium salt 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma-Aldrich) (Molina-Garza *et al*. 2014). Statistical differences among groups were calculated by using analysis of variance for normal distributions, followed by a parametric Tukey test to establish individual differences ($p<0.01$).

RESULTS AND DISCUSSION

A significant ($p<0.01$) decrease in epimastigote growth was observed in cultures treated with magnetic fields (64% growth inhibition), as compared with controls (Fig. 1). Moreover, magnetic field-treated cultures showed significant ($p<0.01$) lower cell counts when compared with 0.066 μM/mL of Nifurtimox (25% growth inhibition, as compared with control), but higher counts when compared with 0.132 μM/mL Nifurtimox (87% growth inhibition, as compared with control) (Fig. 1). On the other hand, cultures co-exposed to ELF-EMFs and Nifurtimox showed no significant differences when compared with cultures treated with Nifurtimox alone. Cultures treated with Nifurtimox showed lower cell density as compared with negative controls, as expected ($p<0.01$). In regard to cell viability, magnetic field exposure caused significant ($p<0.01$) reduction of epimastigote cultures viability, as compared with

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untreated control; in addition, no synergistic or antagonistic effects were observed after co-exposing cultures to ELF-EMFs and Nifurtimox, as compared with Nifurtimox alone (Fig. 2). As expected, cultures treated with Nifurtimox showed significant (p<0.01) lower percentages of viability, as compared with controls (Fig. 2).

These results agreed with our previous report on *Entamoeba invadens* trophozoites; growth inhibition after exposing them to 2.0 mT (Rodríguez-De la Fuente *et al*. 2008). Additionally, a diminution of *Entamoeba histolytica* and *Entamoeba dispar* trophozoite gowth was observed in axenic cultures after exposure to 900 MHz electromagnetic fields (Aksoy *et al*. 2006). To date, there are no reports involving ELF-EMFs effects on *T. cruzi*. However, related studies have been reported in protozoans. Amaroli *et al*. (2006) observed a decreased cellular fission rate and a delay in the early phase of the differentiation cycle in single-cell amoebae of *Dictyostelium discoideum* exposed to 50 Hz, ELF-EMFs at 200 µT. Our findings suggested that lower cell counts were related to ELF-EMF exposure, which may involve alterations in cell cycle progression. In addition, it has been suggested by others that electroporation or electro-permeabilization induced by electric and 50-60 Hz oscillating magnetic fields destroys cell membranes and acts as an antimicrobial procedure (Zimmermann 1998).

Exposure to ELF-EMFs results in a number of important biological changes. With regard to the present study, one possibility is that the parasites reacted to magnetic fields in a similar way to that observed under cellular stress. This is supported by the observation that there is an increase in heat shock gene transcripts (e.g., hsp70) following exposure to ELF-EMFs, where no increases in temperature were measured or expected due to the design of the exposure system with similar characteristics as the one used in the present study. Evidence for this possibility was proposed by Goodman and

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Blank (1998), and later by our research group after exposing human cells to 60 Hz sinusoidal magnetic fields at 8 and 80 μT (Heredia-Rojas *et al*. 2010).

In addition, significant reduction in cell viability was observed after *T. cruzi* cultures were exposed to magnetic fields, suggesting a direct anti-parasite effect. In the existing literature, there are no reports indicating a direct effect of magnetic fields on *T. cruzi* viability. Furthermore, our results showed no synergistic or antagonistic growth and viability effects of ELF-EMFs exposure and Nifurtimox combination. There are reports attempting to investigate synergistic or antagonistic effects by co-exposure conditions for a variety of radiations, chemicals, and cytological endpoints. We have previously observed an antagonistic effect between magnetic field exposure and mitomycin-C, a well-known cytotoxic agent, on human lymphocytes proliferation (Heredia-Rojas *et al*. 2001), and for chromosomal aberrations and sperm morphology in germ cells of mice (Heredia-Rojas *et al*. 2004). In this concern, Yoon *et al*. (2014) found that a 2.0 mT ELF-EMF exposure potentiated the expression of y-H2AX and y-H2A foci production when combined with ionizing radiation, but not when combined with hydrogen peroxide in non-tumorigenic human cell lines.

In conclusion, the present study suggested that 60 Hz magnetic fields induced a significant decrease in *T. cruzi* epimastigotes growth and viability after 24 h exposure to 2.0 mT. However, with the results presented here, we are not supporting any therapeutic technique nor recommending electromagnetic approaches, rather we showed evidence for a significant and measurable biological effect induced by magnetic fields on *T. cruzi*.

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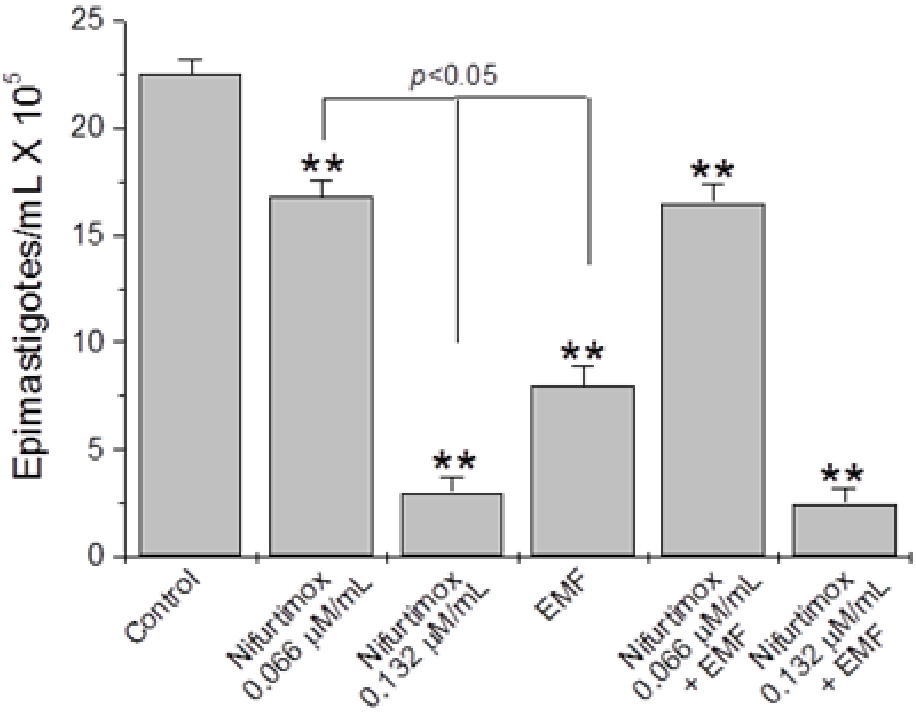

Figure 1. Effect of 60 Hz sinusoidal magnetic fields at 2.0 mT, and 24 h exposure on cell growth of *T. cruzi* epimastigote cultures. Bars represent arithmetical grouped means ± standard deviations.

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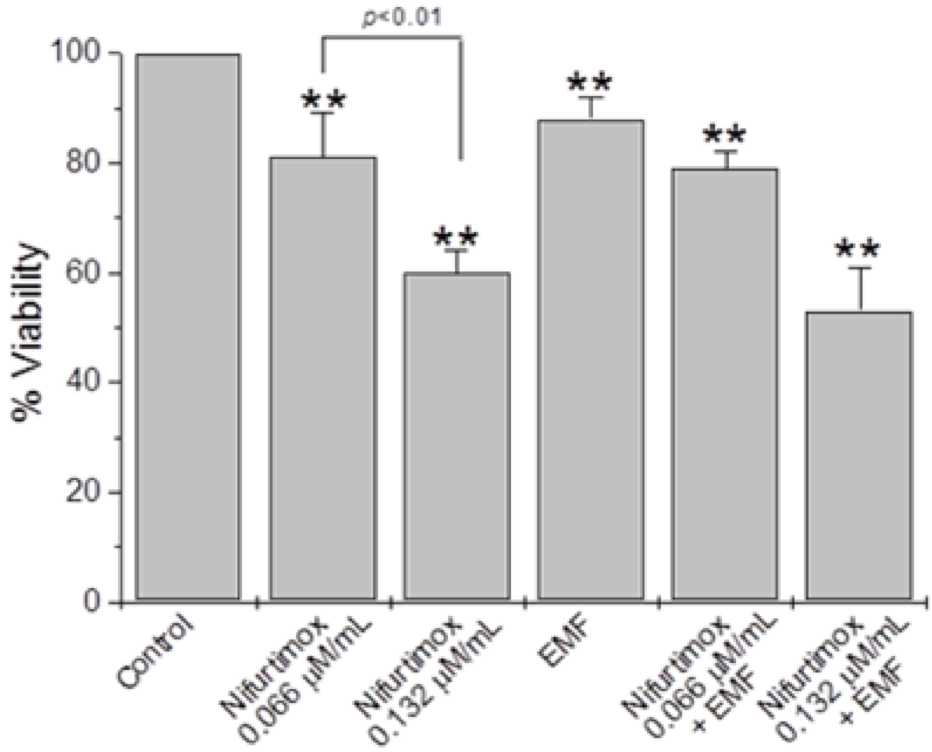

Figure 2. Effect of 60 Hz sinusoidal magnetic fields at 2.0 mT and 24 h exposure on cell viability of *T. cruzi* epimastigote cultures. Bars represent arithmetical grouped means ± standard deviations.