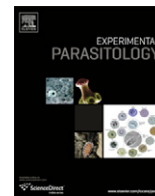




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## *Entamoeba histolytica* and *Trichomonas vaginalis*: Trophozoite growth inhibition by metronidazole electro-transferred water

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### ABSTRACT

The influence of low-frequency electromagnetic (LF-EM) waves on microorganisms has been a subject of experimental investigations for more than two decades and the results are promising. In parallel, an interesting procedure known as biophysical-information-therapy or bioresonance therapy (BRT) which in principle is based on LF-EM stimulation, has emerged. BRT was discovered in the late 1980's but it is still poorly studied. This paper demonstrates that by transferring metronidazole information to water samples by an electronic amplifier (BRT device), the growth of axenically cultured trophozoites of *Entamoeba histolytica* and *Trichomonas vaginalis* is significantly inhibited, compared with those cultures treated with non and sham electro-transferred water samples. A positive control of metronidazole, a well-known cytotoxic drug against parasites, was used as a reference.

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### 1. Introduction

There is a number of alternative and complementary therapeutics that are unproven or not properly tested. For instance, the use of electric and magnetic fields, homeopathy, bioresonance therapy, and many others. For the last decade, the transferring of bioenergetic information has been recognized as a novel scientific approach that may contribute to the therapy of diverse diseases (Kreisli, 1998).

More recently, results of experimental work on the interaction of water with resonant circuits were presented in an attempt to have a physical justification for the changes that are produced in water after this treatment, suggesting permanent modifications

in the physico-chemical properties of water molecules (Cardella et al., 2001). For instance, it is known that water possesses several paradoxical physical characteristics in comparison with other substances and it is extremely sensitive to the impact of physical factors such as temperature, magnetic and electric fields, and even mechanical effects; however, the influence of very weak electromagnetic energy on water has not yet been investigated, but it is the subject of intense biophysical research, which concludes that one of the most peculiar features of water is its ability to achieve structural changes and preserve information from specific signals (Smirnov et al., 2005).

There exist a lot of procedures which attempt to inhibit microbial and parasitic growth, including the conventional (orthodox medicine) use of drugs (Mahajan et al., 1974; Muller, 1983; Samuelson, 1999) or by using unconventional approaches such as electric and magnetic fields. For instance, an early contribution from Rowley et al. (1974) showed an inhibition of infecting microorganisms in human wounds by exposure to alternated elec-

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tric fields. Furthermore, Qjn et al. (1996) reported that processing liquid foods with high-intensity pulsed electric fields inactivated microorganisms with the advantage of only a small increase in food temperature.

Regarding parasite growth inhibition using unconventional procedures, we recently demonstrated an inhibitory growth effect of 60 Hz sinusoidal magnetic fields at 1.5 and 2.0 mT on *Entamoeba invadens* trophozoite cultures. In addition, we found that magnetic field exposure inhibited the encystation process of this eukaryotic protozoan (Rodríguez-De la Fuente et al., 2008).

On the other hand, results from Thomas et al. (2000) reported the activation of human neutrophils by electronically transmitted phorbol-myristate acetate (PMA), suggesting that PMA molecules emit signals that can be transferred to neutrophils by artificial physical means, in a manner that seems specific to the source molecules. Earlier, Kreisl (1998) observed that by transferring acetic acid information to inorganic salt solutions by means of an electronic amplifier, the pH of the inorganic solutions decreases slightly but significantly. This finding demonstrates that chemical signals can be transferred via an electronic amplifier device. Moreover, it has been reported that this kind of information appears to be coded as a frequency of alternating magnetic vector potential. Imprinting a frequency into water may be performed by applying the field of a permanent magnet or an alternating magnetic field at a specific frequency (Smith, 2004).

In view of this interesting issue involving unorthodox antimicrobial strategies and the possibility to transfer drug information to water molecules by means of resonant circuits, we have undertaken the present study to further evaluate the cytotoxic effect of water samples transferred with electronic information of metronidazole, a well-known cytotoxic drug against parasites, by using a bioresonance device on *Entamoeba histolytica* and *Trichomonas vaginalis* trophozoite growth.

## 2. Materials and methods

### 2.1. Parasites

*E. histolytica* strain HM1-IMSS was maintained axenically in PEHPS medium (Said-Fernández et al., 1988). Briefly, culture tubes containing 11.0 mL of PEHPS were inoculated with  $1 \times 10^4$  trophozoites/mL, incubated at 37 °C and sub-cultured every 3 days.

*T. vaginalis* GT-13 reference strain was initially isolated by Dr. Fernando Anaya-Velázquez, Universidad de Guanajuato, México and originally maintained in TYI-S-33 medium with 15.5% bovine serum plus the 10 vitamin mixture (Castro-Garza et al., 1996). Cells were then sub-cultured in 13 × 100 mm screw-capped borosilicated culture tubes containing 5.5 mL of PEHPS. The strain was incubated at 36.5 °C and re-inoculated every 48 h with  $1 \times 10^2$  trophozoites/mL.

### 2.2. Experimental design

Two independent experiments using *E. histolytica* or *T. vaginalis* trophozoites were carried out. In each one, the following treatment regimen and controls were considered: (a) cells treated with electronically-transferred metronidazole water samples, as explained below, (b) cells treated with sham electro-transferred water samples, this is transferring the information from pure water to water, (c) cells treated with non-transferred water, as a negative control and, (d) cells treated with 0.124 µg/mL of metronidazole (IC<sub>50</sub>) as a positive control. In the case of *T. vaginalis* bioassay, an IC<sub>100</sub> dosage of metronidazole (0.150 µg/mL) was used. Nine cultures were included for each treatment regimen and controls for a total of 36 culture tubes for each type of parasite.

All chemicals were supplied by Sigma–Aldrich (St. Louis, MO, USA).

### 2.3. Transmission apparatus

The equipment used for electronic transmission comprised a bioresonance therapy device, Bicom version 4.4 by Regumed (Regulative Medicine Technik GmbH, Germany) serial No. 202057299.

### 2.4. Metronidazole transmission to water

The source flask containing 0.124 µg/mL or 0.150 µg/mL of metronidazole, in a total volume of 10.0 mL of bi-distilled water was placed inside the input coil coupled to a bioresonance amplifier, while in the output coil, a flask containing pure bi-distilled and sterile water was allocated at room temperature. Metronidazole solutions and bi-distilled water were sterilized by filtration. The oscillator was then turned on for the 15 min transmission period. During this procedure, the various parameters such as power, voltage, capacitance and impedance remained constant. Thus, the nature of the source tube (metronidazole versus vehicle) was the only variable. According to the bioresonance device manufacturer, a specific program labelled as #196 was used for electronic transmission substance to substance. At the end of the transmission period, the flasks were kept away from light and stored at room temperature 1.0 h before being used in bioassays. About 1.0 mL of transferred-water was added to each culture tube for treatment and controls.

### 2.5. Bioassays

In order to test the effect of electronically-transferred metronidazole, the density of trophozoites was determined in a blind way when both types of parasite cultures reached the end of logarithmic phase, they were chilled in ice-cold water for 10 min and trophozoites were subsequently harvested by centrifugation at 450g and 4 °C, and then washed three times with fresh medium. The resultant pellet was re-suspended in fresh medium and counted in triplicate with a hemocytometer Neubauer chamber.

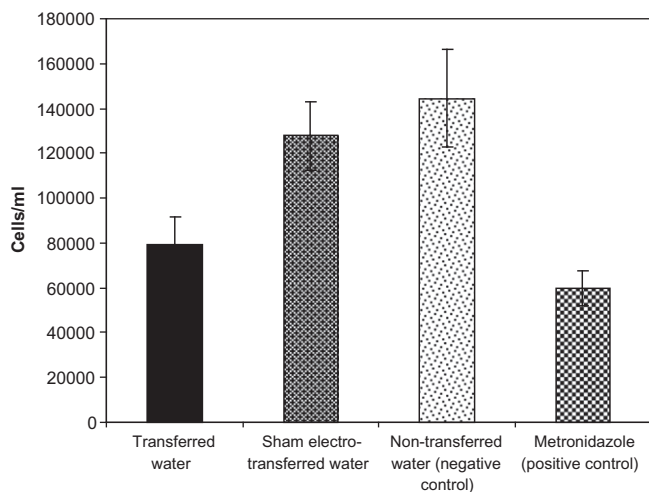
### 2.6. Statistical analysis

The statistical differences were calculated among groups for trophozoite growth by using analysis of variance for normal distributions. The normality of the data was estimated by means of Kolmogorov–Smirnov test ( $p < 0.05$ ). All analyses were done using the SPSS package version 10.0. Differences were considered to be significant when the probability values were lower than 0.05.

## 3. Results

This study evaluated the effect of electronically transmitted metronidazole to water samples on *in vitro* *E. histolytica* and *T. vaginalis* trophozoite growth. Fig. 1 shows the cell density grouped mean (for a total of 27 cell counts per group) scored in trophozoites of *E. histolytica* after being treated with water samples previously transferred with metronidazole and controls. A statistically significant decrease in trophozoite growth was observed in cultures treated with electronically-transferred metronidazole water samples compared with non and sham electro-transferred water samples. In trophozoites directly exposed to metronidazole (positive control), a decrease of about 50% in cell density was found, as expected.

Also, the growth of *T. vaginalis* trophozoites was altered when exposed to metronidazole electro-transferred water samples. Fig. 2 shows the arithmetical grouped means of cell densities of



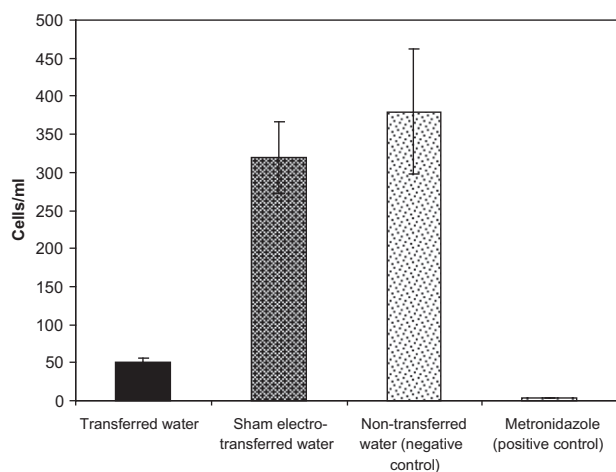
**Fig. 1.** Effect of metronidazole electro-transferred water samples on growth of *Entamoeba histolytica* trophozoites (HM1-IMSS strain). The amoebas were cultured in PEHPS medium, inoculated with  $1 \times 10^4$  trophozoites/mL and incubated at 37 °C. Nine tubes were used for each treatment regimen and controls, giving a total of 36 cultures. The cell density was determined in triplicate and in a blind way by using a hemocytometer Neubauer chamber.

those treatments and controls. The cultures treated with electro-transferred metronidazole showed a lower cell density when compared with sham and non electro-transferred water samples ( $p < 0.05$ ). Related to the positive control cultures, a significant decreased trophozoite growth was observed in cells exposed to  $IC_{100}$  of metronidazole, as expected.

In addition, in both bioassays, a slight but statistically significant difference was found in trophozoite growth when comparing the grouped mean values between non-transferred (negative controls) and sham electro-transferred (water to water) water samples, indicating some induced effect of the bioresonance device on water.

#### 4. Discussion

There is a trend toward the use of alternative and complementary therapeutics, mainly in controlling microbial illnesses. Consid-



**Fig. 2.** Effect of metronidazole electro-transferred water samples on growth of *Trichomonas vaginalis* trophozoites (GT-13 strain). The parasites were cultured in PEHPS medium, inoculated with  $1 \times 10^2$  trophozoites/mL and incubated at 36.5 °C. Nine tubes were used for each treatment regimen and controls for a total of 36 cultures. The cell density was determined in a hemocytometer in triplicate and in a blind way.

ering this trend and the lack on consensus on the effectiveness of these therapies, it is of considerable interest to examine if there is a significant and measurable biological effect of such unconventional procedures. The question has been raised as to whether these unorthodox biomedical techniques can really modify the microbial or parasitic growth.

In the present study, we have observed a statistically significant reduction of both *E. histolytica* and *T. vaginalis* trophozoite growth after axenic cultures were treated with water samples processed in a bioresonance device by using a specific procedure called “substance to substance transference”.

These results agreed with previous reports indicating that molecular information can be scanned and transferred by a bioresonance instrument. Thus, Endler et al. (1995) demonstrated that the metamorphosis of tadpoles could be greatly slowed down by transferring information from a toxic solution of the hormone thyroxin to the aquarium water in a number or parallel blind trials. It has also been reported the transfer of the activity of 4-phorbol-12-B-myristate acetate by electronic means on the activation of human neutrophils (Thomas et al., 2000). On the contrary, Jonas et al. (2006) found no effects from digital signals on the inhibition of thrombin/fibrinogen coagulation by a digital signal used instead of the original molecule.

A full explanation about the possibility of water capacity for storing electronic information is not yet clear, but it is accepted that electromagnetic waves interact with water. One mechanism that can explain the effect of electromagnetic fields on water is related to the existence of “defects” in its molecular structure. These stable structural changes were detected in experiments by the UV luminescence spectrophotometer and they have been related to different water structural defects that include specific centers of luminescence; the nuclear proton spins were considered to be a primary target of external magnetic fields, since proton lattice of water molecules is unstable and asymmetric (Binhi, 1998). In this regard, Wong and Lo (1998) have suggested that the “anomalous” states of water are peculiar and unexpected. Their existence may be connected to the occurrence of meta-stable polymorphic states of water at room temperature; it is therefore important to confirm or to refute the observations of these anomalous states by independent experimental investigations.

In addition, it has been proposed that the orientation of nuclear proton spins may influence biochemical processes in biological systems, as a result of associations and disintegrations of above mentioned structural defects of water, since ionic structural such defects are chemically active (Smirnov et al., 2005).

On the other hand, experiments from Cardella et al. (2001) have proven that exposing water to resonant circuits can permanently alter some of its physico-chemical properties. Moreover, they assure that water samples after exposure acquire “biological-like” behavior that lasts for a significant period of time. Based on these findings, it is assumed that such behavior will somehow affect any chemical and/or biochemical reactions in which the exposed water may become involved.

The basis for an understanding of the antimicrobial effects of bioresonance techniques is the assumption that the alternating electromagnetic fields, which are detectably emitted by living organisms and molecules and are characterized by intensity (amplitude) and frequency, contain biologically significant information that is used for transmitting a number of signals between cells, tissues, and even molecules (Likhoded et al., 2007).

Moreover, in the existing literature there are no original articles published on *E. histolytica* nor *T. vaginalis* or related parasite growth inhibition by transferring antiparasitic drugs to water samples. On the other hand, while bioresonance procedures are denigrated by some, research trials conducted primarily in Russia, Germany, and Eastern Europe, indicate that electromagnetic waves

do, indeed, affect biology in single-cell models that can include microorganisms (Islamov et al., 2002).

In the present study, we also observed that sham electro-transferred water samples showed a behavior different from those non-transferred water samples or pure water as considered negative controls. It is possible that despite water exhibits no magnetic properties, the water clusters could be altered in some way due to the action of electromagnetic waves, because sham electro-transferred water samples are under electromagnetic influence of the bioresonance apparatus. Actually, it is known that water clusters are extremely sensitive to the influence of physical factors such as magnetic and electric fields, even low and ultra-low fields (Liu et al., 1996). This water capacity to acquire paradoxical configurations induced by low and extremely low intensity electromagnetic fields, should be considered when trying to explain resonant intermolecular transfer of electromagnetic energy in liquid water samples (Woutersen and Bakker, 1999).

In conclusion, our *in vitro* study suggests that water samples that are electronically-transferred with vibrational information of metronidazole are capable of inhibiting trophozoite growth of axenically cultured *E. histolytica* and *T. vaginalis*. However, with the results presented here, we are not supporting any therapeutic procedure nor recommending bioresonance therapy; rather we showed evidence for a significant and measurable biological effect induced by electro-transferred water samples that appear to mimic an antiparasitic drug against protozoans. Furthermore, an advantage of using a single-cell model for evaluating the effectiveness of bioresonance techniques is that the placebo effect, claimed by those who refuse any alternative or complementary therapeutics, is absent.

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