CONTROL OF SALMONELLA ACTIVITY IN RATS BY PULSED ELF MAGNETIC FIELD (IN VIVO STUDY)

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Abstract

In this work, the frequency of the magnetic impulses that interfere with the bioelectric signals generated during salmonella typhimurium (STm) cellular division is investigated.

The experiment was expanded to in vivo study for the obtained data in which rats were infected with STm and then whole body were exposed to square magnetic pulses (SMP) that caused inhibition to the microbial cellular growth. Another group of animals was infected by previously inhibited bacteria with SMP then the histological and molecular structures of the liver were investigated for all the animal groups. Dielectric relaxation studies for the liver in the frequency range 100 KHz – 4.5 MHz was used to determine molecular structure changes.

The results indicated a highly significant inhibition of cellular growth for STm in addition to pronounced changes in the cellular morphology after the exposure of the micro-organism to the resonance frequency of 0.8 Hz SMP for 75 minutes. From the Histological and dielectric relaxation measurements and results, it was indicated that the liver for animals infected by STm and then exposed to SMP showed significant improvement in their health state as compared with infected and non exposed group. Moreover, the liver for the animals infected with previously treated bacteria with SMP showed highly significant decrease in cellular damage as compared with untreated bacteria.

It was concluded that treatment of salmonella by 0.8 Hz SMP acts on the structure and biological activity of the bacteria and it is a promising methodology to control salmonella activity in vivo and in vitro applications.

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Introduction

Typhoid is a serious disease difficult to be treated with conventional drugs. Infection of Salmonella Typhi (ST) leads to the development of enteric fever which is characterized by general ill-feeling, abdominal pain, a high (over 103 degrees F) fever, severe diarrhea, rose spots on the belly and chest, agitation and bloody stools[1].

Long-term treatment of typhoid fever with antibiotics has its drawbacks on human health especially for pregnant women because of the association of the antibiotics treatment with birth defects[2]. In addition, indiscriminate use of antibiotics has led to the emergence of multidrug-resistant strains of ST[3,4]. Therefore, considerable efforts have been made towards the development of alternative method for the treatment of bacterial infections.

Over the last few years, efforts had been devoted to control bacterial growth through

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exposure to electromagnetic fields[5-9]. However, the medical applicability of this technique is limited due to the need of very high field strengths of several KV/cm and very high temperatures. In recent work, carried by our group, efforts were devoted to control cellular activities by using electromagnetic waves of very low field intensity and frequencies which resonates with bioelectric signals generated during a particular metabolic activity. These trials succeeded to control the growth of Ehrlich tumors in mice[10,11] and fungi[12]. In a more recent work[13], it was possible to control ST cellular division and cause changes in the structure of the DNA after the exposure of the micro-organism to 0.8 Hz square amplitude modulated waves (QAMW) for 75 min.

ST though a major cause of morbidity and mortality in humans is a virulent in animals, including mice. But Infection of mice with STm causes a disease similar to the one caused by ST in humans[14]. STm infection in mice is focused on the spleen and liver and prolonged infection can lead to sepsis and death. In the present work, a trial was made to find out the resonance frequency of SMP that can inhibit the activity of STm and to investigate the changes that may occur at the molecular and cellular levels. Moreover, to carry some structural and histological studies for the liver of whole body exposed infected rats by STm to SMP at resonance frequency.

Materials and methods

Microorganism growth conditions

The STm strain (ATCC 14028) were purchased from the Holding Company for Biological Products and Vaccines (VACSERA) in Egypt and used in this study.

A broth subculture was prepared by inoculating a test tube containing 5ml of sterile nutrient broth of pH 7.1 (Biolife, Milan, Italy) with two single colonies of bacteria from nutrient agar plate, followed by incubation at 37°C for 18 h. With this subculture, 500 ml screw-capped flasks containing 150 ml of sterile nutrient broth were inoculated to reach a final concentration of 10^7cfu/ml.

The cultures were then incubated at 37°C, but the incubation was interrupted each one hour, as sample was taken for absorbance measurement (using sterile broth medium as reference) at wavelength 600 nm using a spectrophotometer model (6405 UV/Vis) manufactured by Jenway in UK. Each experiment was made in triplicates and the average was considered. A standard count-absorbance calibration curve was plotted between the absorbance of the samples at 600 nm and the concentration of the cells (cfu/ ml) as determined by plate counting technique[15].

An overnight grown culture of STm was mixed with clean broth medium in a proportion of 1:40. After mixing (time T=0 h) the new culture was divided into eleven groups, one control, the others were exposed to SMP for different frequencies in the range 0.1 to 1 Hz in steps of 0.1 Hz for 75 min in order to determine the resonance frequency of growth inhibition.

The morphological changes of control group and group exposed to 0.8 Hz SMP have been determined using transmission Electron Microscope **(**TEM). The bacterial samples were prepared for imaging by the TEM through some processing [16]**.** TEM investigation was done in TEM lab FARP, Faculty of Agriculture, Research Park-Cairo University.

SMP Exposure System

The SMP was generated inside a locally made hollow cylindrical copper solenoid of 56 cm in diameter and 80 cm in length, manufactured at the Electronic workshop in the Faculty of Science, Cairo University.

The solenoid was constructed from thirty two electrically insulated copper wire coils of 1 mm thickness wound around the outer surface of an electrically insulated copper cylinder. The terminals of the coils were connected in parallel to a direct current power supply through an electronic switching device (locally manufactured by the faculty of science, Cairo university workshop) to produce a square pulsed current with varying frequencies. The homogeneity of the magnetic field inside the solenoid was measured at different locations by using a Gauss/Tesla meter (Model 4048 with probe T-4048.001 by Bell Technologies, Inc., USA) of accuracy ±2%.

The SMP was displayed using the Linear Hall-effect IC sensor on the oscilloscope. The copper cylinder has a copper jacket with inlet and an outlet for the flow of tap water to keep the temperature inside the chamber always constant similar to room (25^o C). The current passing in the coils to produce a magnetic field of 0.5 Gauss was only one Ampere. This current did not show any

measurable changes in temperature inside the solenoid.

Experimental animals

50 adult albino male rats, 45 days age and average weight 150 ± 5 g., purchased from the Faculty of Veterinary Medicine, Cairo University, were used. The animals were housed in plastic cages and feed with constant balanced diet and tap water. After death or being sacrificed the animals were excluded through coordination with the Holding Company for Biological Products and Vaccines (VACSERA) in Egypt.

The animals were classified into five groups namely G1, G2, G3, G4 and G5. G1 group was of five animals used as control, which did not receive any treatment. G2 was of 5.0 animals omit and exposed to 0.8 Hz SMP only for 75 min. G3; of 15 animals; was infected orally by 10^8 cfu/ml of STm. G4 of 10 animals; was infected orally by 10^8cfu/ml of STm, and after 5 days of infection was treated with 0.8 Hz SMP for 75 min. G5; of 15 animals; was infected orally by 0.8 Hz SMP treated bacteria for 75 min. At day 15 post infection of G3, G4 and G5 animals were sacrificed then livers were removed and prepared for histo-pathological and dielectric relaxation studies.

All international ethics for treatment of animals were strictly followed; even there are no official restrictions in Egypt for the treatment of experimental animals.

Dielectric relaxation studies for the liver

Animals were sacrificed then the liver was immediately excised and placed between a pair of 1 cm diameter black platinum circular electrodes for dielectric measurements.

The sample between the electrodes was maintained at a constant pressure, and the distance between the electrodes was measured through the use of a micrometer, while the liver sample was filling the whole volume between the electrodes. Dielectric measurements were made in the frequency (f) range from 0.1 MHz to 4.5 MHz using a loss factor meter type Hioki, 3532, LCR Hi TESTER, 1999, Japan.

During measurements, the sample between the electrodes was kept at a constant temperature of 24 ± 0.1°C. The capacitance (C) of the tissue was measured at each frequency and the resistance (R) was recorded. Each run was repeated three times. The relative permittivity of the sample was calculated for each frequency using the relation:

$$\acute{\varepsilon} = Cd/\varepsilon_o A \quad (1)$$

Where, ε_o permittivity of free space, d is the inter-electrode distance in meter, A area of electrode in m^2 measured from the cell used.

The loss tangent (tanδ), the dielectric loss ε″, the AC conductivity σ were calculated from the relations:

$$\tan\delta = 1/2\pi fRC = \varepsilon''/\acute{\varepsilon} \quad (2)$$

$$\sigma = 2\pi f \varepsilon''\varepsilon_o \quad (3)$$

The value of the dielectric constant έ falls from high value $\acute{\varepsilon}_s$ to $\acute{\varepsilon}_\infty$ as the frequency increases through the dispersion region where έ is the real part of the complex permittivity. The dielectric dispersion (Δέ) was calculated by applying the relation:

$$\Delta\acute{\varepsilon} = \acute{\varepsilon}_s - \acute{\varepsilon}_\infty \quad (4)$$

The relaxation time (**T**) was calculated from the equation:

$$T = 1/2\pi f_c \quad (5)$$

Where f_c is the critical frequency corresponding to the midpoint of the dispersion curve. All the measurements had been done for animals of all groups.

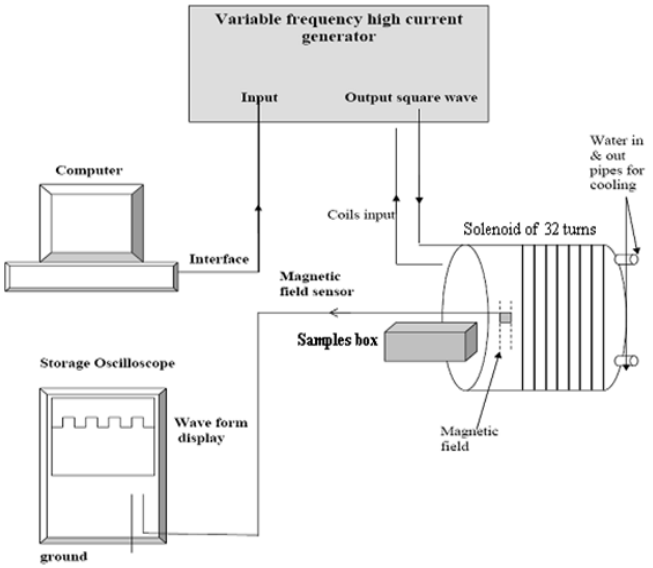

Figure 1: Sketch Diagram of the magnetic field exposure system.

Histopathological investigations

Specimens of liver tissues were taken from all groups and prepared for the histological and histopathological sections following Banchroft and Stevens work, 2006 [17] and examined by light microscope.

Statistical Analysis

Data from bacterial growth studies were compared for statistical significance using Student t-test and ANOVA analyses, the level of significance was set at P<0.05.

Results

Figure (2a) illustrates the growth curve characteristics for control and treated STm by 0.8 Hz SMP. The difference from control was significant (P < 0.05). Figure (2b) shows the change in cellular growth (relative to control) of the micro-organism, measured in the saturation region, as a function of the applied SMP frequency. The results indicate a resonance frequency of the SMP that cause maximum inhibition of the growth of STm strains at 0.8 Hz after exposure for 75 min.

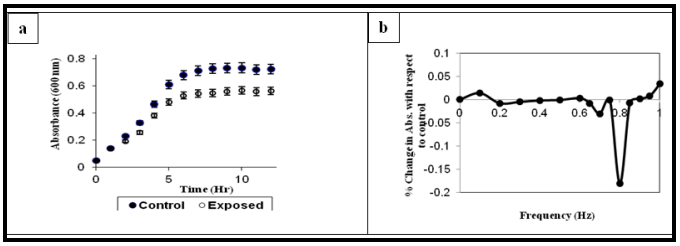

Figure 2. (a): growth curve characteristics for control (closed circle) and treated (opened circle) STm by 0.8 Hz SMP for 75 min. (b): the change in cellular growth (relative to control) of the micro-organism, measured in the saturation region, as a function of the applied SMP frequency.

Morphological changes

Figures 4 (A, B) illustrate TEM images for *STm* for control and treated cells with 0.8 Hz for 75 min. SMP respectively. Figures (A1&A2) show similar morphological form as previously published [18]. In figures (B1&B2) degradation, deformation, disruption and disintegration of cell wall in addition of retraction of cytoplasm membrane and also *STm* ghost cell.

Effects on the molecular structure

a) Dielectric properties of the liver

Figures 3 (a, b, c, d and e) illustrate the dielectric relaxation curves for the livers from groups G1, G2, G3, G4 and G5 respectively, as measured in the range of (100 KHz-4.5 MHz). The results indicate a dielectric dispersion in the frequency range demonstrated.

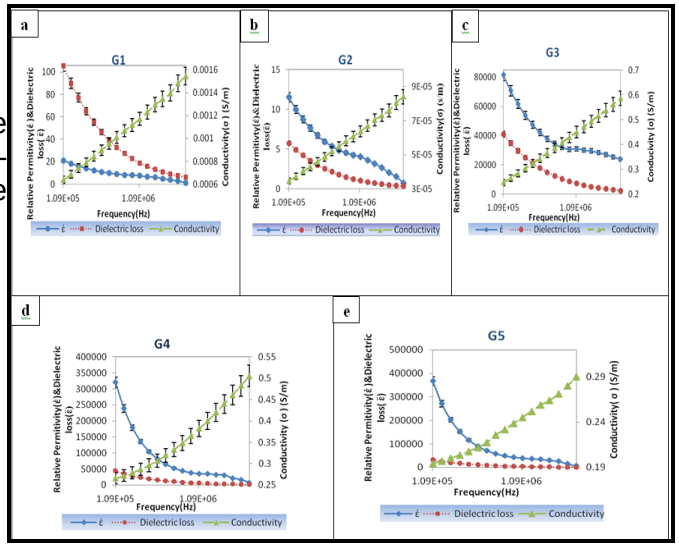

Figure 3. (a, b, c, d and e): illustrate the variation of the relative permittivity ɛ́ (solid line), the dielectric loss ɛ̋ (dot line) and the electric conductivity σ (dash line) as a function of the frequency for the livers from groups: Control (G1),exposed to SMP (G2), infected orally by 10^8 cfu/ml of STm (G3), infected and then treated by SMP (G4) and infected with treated bacteria (G5) respectively. (Note: The frequency is represented on log scale).

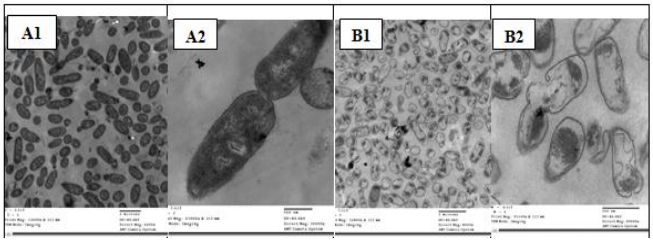

Figure 4: (A1, A2): TEM images for control STm cells, showing normal structure of STm cells; (Magnification: 6000x for image A1, 30000x for image A2). **(B1, B2):** TEM images for treated STm cells with 0.8 Hz for 75 min. SMP, showing degradation, deformation, disruption and disintegration of cell wall in addition of retraction of cytoplasm membrane and *STm* ghost cell.(Magnification: 8000x for image B1, and 40000x for image B2).

The data also indicated that any decrease of the dielectric loss for the sample is associated with an increase of the electrical conductivity; this

yields a consistency test for the data as reported by Kramers-Kronig relations[19]. The results indicate a dielectric dispersion for the liver for all the animals from all groups. It can also be noticed that the formation of two dispersion regions in this frequency range for control animals and those infected with the bacteria.

The second dispersion started at frequencies higher than one megahertz. The values of **T**, and σ were calculated from the curves for all the livers from all groups as given in table (1). The results in the table indicate high significant increase ($P<0.0001$) in the value of σ for animals infected by the bacteria.

Sample	Relaxation time τ (μsec)	Dielectric increment $\Delta\varepsilon = \varepsilon_o - \varepsilon_\infty$	Conductivity σ (s/m), at 4.5 MHz
G1	$7.9 * 10^{-5}$	4.82	$1.54*10^{-3}$
G2	$7.1*10^{-5}$	2.62	$8.40*10^{-5}$
G3	$7.1*10^{-5}$	$2.8*10^{4}$	$5.86*10^{-1}$
G4	$1.35*10^{-4}$	$2.55*10^{4}$	$5.05*10^{-1}$
G5	$6.29*10^{-5}$	$2.77*10^{4}$	$2.90*10^{-1}$

Table 1. Dielectric parameters for of the samples: exposed to 0.8 Hz, 75 min SMP (G2), infected orally (G3), infected then treated (G4) and infected with treated bacteria (G5) as compared with control (G1).

b) Histopathological Investigations

On microscopic level; Histological observations showed normal architectures of liver tissues Figure (5a). Figure (5b), showed approximately normal structure for the liver of the rats at 15 days post exposure to SMP only.

Figure (5c), showed enlargement in the nuclei, thickening in some cell membranes may be due to fibrosis, enlargement in the cytoplasm of the hepatocytes and depletion in their chromatin material for the liver tissues of the rats after 15 days of infection.

The rats infected then exposed to SMP showed recovery in most cells after 15 day of infection Fig. (5d). Rats from G5 which were infected with treated microbe for 15 days showed nearly normal except some enlarged nuclei with fragmented chromatin material Fig. (5e).

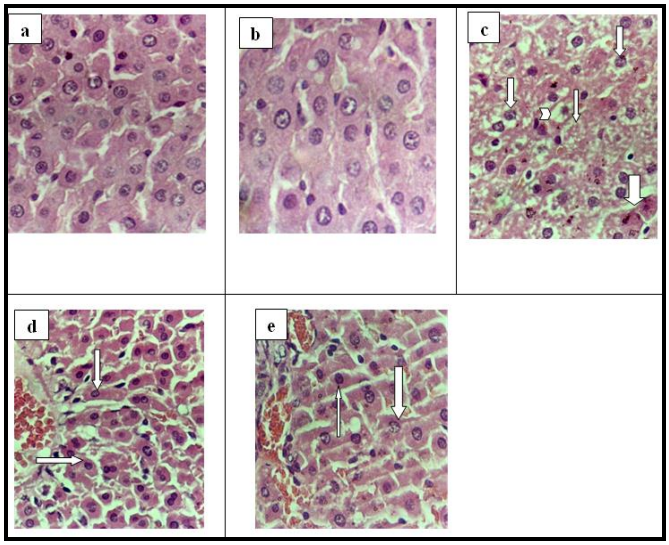

Figure 5. Photomicrographs of liver sections for: Control rat, **(G1),** showing normal architectures of liver tissues (a). Rats exposed to 0.8 Hz for 75 min. SMP, **(G2)**, showing approximately normal structure for the liver at 15 days post exposure to SMP only (b). Rats infected orally by 10^8 cfu/ml of STm, **(G3),** showing enlargement in the nuclei (arrows), thickening in some cell membrane (arrow head), enlargement in the cytoplasm of the hepatocytes (thick arrow) and depletion in their chromatin material (thin arrow) (c). Rats infected orally by 10^8cfu/ml of STm, and after 5 days of infection was treated with 0.8 Hz SMP for 75 min, **(G4),** showing nearly recovery in the hepatocytes after 15 day of infections (arrows) (d). Rats infected orally by 0.8 Hz SMP treated bacteria for 75 min, **(G5),** showing nearly normal hepatocytes (arrow); some enlargment in the nuclei with fragmented chromatin material (thick arrow) (e). (H & E 400 X).

Discussion

In this work; the resonance frequency of SMP that inhibits cellular division was determined. The experiment was then expanded to in vivo study where the microbe was used to infect rats and the injuries in the liver resulting from the toxins secreted were evaluated for animals did not receive any further treatment and those whole bodies exposed to the inhibiting magnetic pulses and animals infected with previously irradiated bacteria.

It is well known that living biological systems run their metabolic mechanisms through ionic motion. The rate of movement of these ionic charges forms ionic currents which result in ionic

potentials. The wave form and frequency of these bioelectric potentials represent the running physiological process which can be understood as the finger print of the organ. These bioelectric currents generate loops of bio magnetic fields which perturb the metabolic functions of the neighboring cells. From the basic understanding of physical concepts, energy can be only transferred from one oscillating system to another when both systems are at resonance i.e. they have the same frequency.

Based on the Metabolic Bio-magnetic Resonance Model (BMRM), suggested by Fadel (1998), an external applied electromagnetic signal can interfere with a bioelectric signal when they are at resonance. The resultant of the interference is the algebraic summation of the two waves which may be instructive or destructive, i.e., enhancement or inhibition, respectively, for the running process. Based on these bases the present work was planned.

The damage in liver due to infecting the animals with STm indicated as noticed in the histological sections is due to the toxins secreted by the microorganism which caused the observed damage. Moreover, there was noticed some damage to liver following the whole body exposure of healthy animals to SMP which were repaired after 15 days post exposure. The damage thus occurred can be analyzed depending on the interaction mechanism of magnetic fields with biological systems.

From the basic understanding and Wright hand rule, when an external magnetic field is applied on moving ions, a magnetic force is generated on the ions perpendicular to their flow direction and the applied magnetic field. This magnetic force will shift the direction of the moving ions to circular pathway. The shift of these ions from their targeted direction will deteriorate the running physiological process.

Recent studies [20, 21] reported that exposure to ELF magnetic fields affects the packing properties of the phospholipids macromolecules forming the cellular membrane and can cause changes in the Vander Val forces binding these molecules together to form the membrane. These changes in the membrane mechanical properties cause changes in the membrane permeability which can be a reason for the noticed destruction of the microbial cellular membrane noticed in the TEM images for the treated microbe by SMP. The improvement of the health state of the animals infected with salmonella and treated with SMP, as can be noticed from the histological sections of the liver, is a marker for the success of this method for the treatments of infections with Salmonella.

Conclusion

It may be concluded from the present findings that the advantage of this technique over the running medication technique for typhoid is of being non destructive, non expensive, safe and fast, where only 75 min are needed for the exposure of the infected region to stop the microbial activity and to avoid secondary harms caused by prolonged treatments with antibiotics and health complications that follow treatments.

Moreover, the noticed fast recovery of the animals infected with previously treated microorganism with the SMP opens a new promising field for the preparation of new vaccine against infections with salmonella.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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