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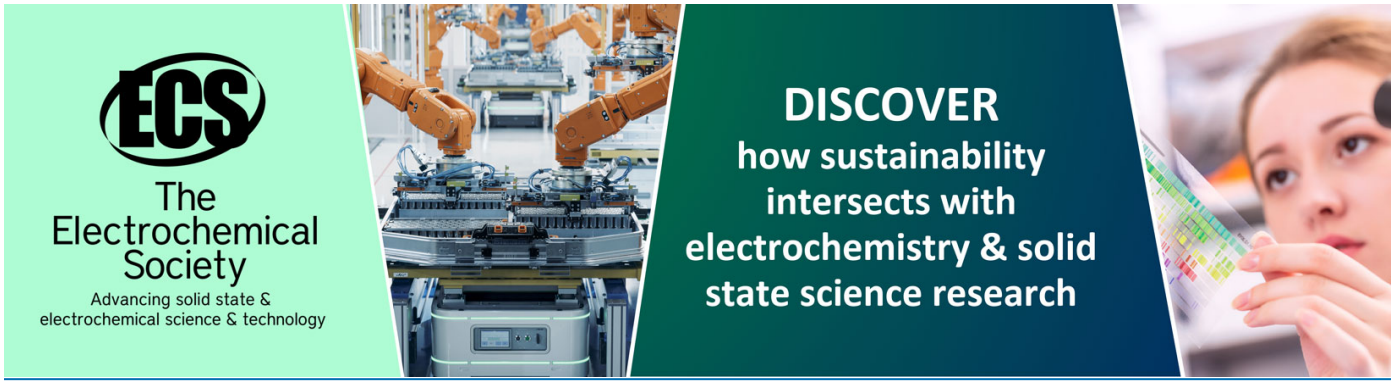

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Medical Applications of Electromagnetic Fields

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Abstract. In this article, we describe two possible applications of low-intensity non-ionizing electromagnetic fields (EMF) for the treatment of malaria and cancer, respectively. In malaria treatment, a low-intensity extremely-low frequency magnetic field can be used to induce vibration of hemozoin, a super-paramagnetic polymer particle, inside malaria parasites. This disturbance could cause free radical and mechanical damages leading to the death of the parasite. This concept has been tested in vitro on malaria parasites and found to be effective. This may provide a low cost effective treatment for malaria infection in humans. The rationale for cancer treatment using low-intensity EMF is based on two concepts that have been well established in the literature: (1) low-intensity non-thermal EMF enhances cytotoxic free radicals via the iron-mediated Fenton reaction; and (2) cancer cells have higher amounts of free iron, thus are more susceptible to the cytotoxic effects of EMF. Since normal cells contain minimal amount of free iron, the effect would be selectively targeting cancer cells. Thus, no adverse side effect would be expected as in traditional chemotherapy and radiation therapy. This concept has also been tested on human cancer cell and normal cells in vitro and proved to be feasible.

1. Introduction

Non-ionizing electromagnetic fields (EMF), from extremely-low frequency to radiofrequency, have been shown to cause biological effects even at low intensity. Some of these effects may be applied for medical treatments. One example is their effect on the physiology of bone cells that led to the use of these fields for treatment of bone fracture and to improve and facilitate bone healing. In this article, we describe two areas of research on medical applications of electromagnetic fields that we have been engaging in, namely, treatments of malaria infection and cancer. Even through preliminary data support the feasibility of these two applications, mechanisms of effect are still speculative and further research is needed to fully develop them for human treatment.

2. Treatment of Malaria

Malaria affects and kills millions of people in the world, particularly in developing countries. Resistance to anti-malarial drugs is widespread and has been a major problem in malaria treatment and eradication. The need novel approaches for malaria treatment is enormous. We have been investigating such a novel approach for the treatment of malaria using an alternating magnetic field.

The basis of this approach is that malaria parasites generate a super-paramagnetic particle known as hemozoin during their process of feeding. The parasite feeds on hemoglobin in erythrocytes in the

blood of the host. A hemoglobin molecule consists of a protein-moiety globin and heme, an iron-containing porphyrin molecule. The globin portion of the hemoglobin molecule is broken down inside the food vacuole of the parasite to amino acids which are used by the parasite for protein synthesis and reproduction [1, 2]. The heme portion, ferriprotoporphyrin IX, is left intact, since the parasite lacks the enzyme to break it down. Free heme is highly toxic to the malaria parasite, because it is an inhibitor of an enzyme (Na^+/K^+-ATPase) that is involved in metabolic energy production [3]. Free heme can also cause a chain reaction of free radical-induced oxidation of unsaturated fatty acids, leading to membrane damage [4]. To eliminate their toxic effects, heme molecules in the food vacuoles of the parasite are packed by an enzymatic process into a polymer called hemozoin. In hemozoin, the heme polymer is formed by covalent bonding of the iron of one heme molecule with the oxygen of the carboxylate group in one of the side chains of another heme molecule [5, 6]. This molecular arrangement makes hemozoin super-paramagnetic [7] and it behaves like a small bar magnet in a magnetic field. We hypothesized that exposure of the parasites to an alternating magnetic field would shake hemozoin molecules inside the parasite. If the energy exerted on the molecules by the magnetic field is large enough, the rate of enzymatic formation of hemozoin would be decreased. Accumulation of free heme in the parasite would then produce toxic effects.

We have tested the effects of alternating magnetic fields on growth of malaria parasites cultured in erythrocytes. Our data show that exposure of malaria parasites cultured in erythrocytes to a low-frequency, low-intensity alternating magnetic field (5 Hz with a duty cycle of 1 second on, 1.5 seconds off and peak-to-peak intensity of 1.5 millitesla) significantly decreased the number of parasites and synthesis of macromolecules (incorporation of [^{3}H]-hypoxanthine into DNA and RNA) by the parasite. Details of the experiment and the results have been published [8]. The 5 Hz frequency was chosen arbitrarily in this preliminary experiment. It is possible that other frequencies may work better.

We propose two mechanisms by which an alternating magnetic field may be effective in causing a deleterious effect on malaria parasites. First, if the field is applied during the erythrocytic stage of the parasite lifecycle in which free heme molecules are being actively polymerized into hemozoin, it may inhibit polymerization of heme into hemozoin. Excess free heme leads to oxidative molecular damages and, in turn, growth retardation and death of the parasite. The second mechanism is that an oscillating magnetic field would cause oscillation of hemozoin, leading to mechanical damage to the organelles of the parasite. Mechanical stress is known to cause cell death via apoptosis [9].

An important advantage of using alternating magnetic fields for the treatment of malaria is that development of resistance would be unlikely. The alternating magnetic field would act directly on hemozoin rather than on an enzyme or other gene product, thus producing no biological selection. Another possibility is that there may be an additive effect, in which alternating magnetic fields would increase the sensitivity of parasites to low concentration of anti-malarial drugs and enhance the effectiveness of treatment.

Further research needed to be carried out to identify the optimal magnetic field exposure conditions to disturb the growth of the malarial parasite. Since the magnetic moment of a hemozoin molecule is proportional to its mass, a resonant frequency probably exists that can make a magnetic field most efficient for malaria treatment.

3. Treatment of Cancer

In previous research, we found that acute (2 hr) exposure to a 60-Hz magnetic field caused DNA single and double strand breaks [10], DNA-protein and DNA-DNA crosslinks [11], and increased apoptosis [12] in brain cells of rats. The effects were mediated by free radicals, since they could be blocked by pretreatment with free radical scavengers [12, 13]. Further studies showed that the effects involved iron because pretreating rats before magnetic field exposure with the iron-chelator deferriprone eliminated the effects [13]. We proposed that magnetic fields generate free radicals via

the Fenton reaction (Fig. 1). Other research has also supported the notion that electromagnetic fields in both the extremely-low frequency and radiofrequency ranges, enhance free radical activity/formation in cells and iron could play a role in the process [14].

Iron plays a vital role in cell functions and growth, e.g., in energy metabolism and DNA synthesis. Special molecular mechanisms have evolved for the transport of iron into cells. In vertebrates, an iron transport system involves a specific interaction between the iron-binding protein transferrin in the extracellular fluid and a cell surface transferrin receptors that results in a facilitated transport of iron across cell membrane via receptor-mediated endocytosis [15]. Due to their rapid rate of division, most cancer cells have high rates of iron intake [16] and express a high cell surface concentration of transferrin receptors [17] than normal cells. In general, the aggressiveness of a tumor is positively correlated with cell surface transferrin receptor concentration of its cells. For example, breast cancer cells have 5-15 times of transferrin receptors on their cell surface than normal breast cells [18], and they do take up more iron than normal breast cells [19].

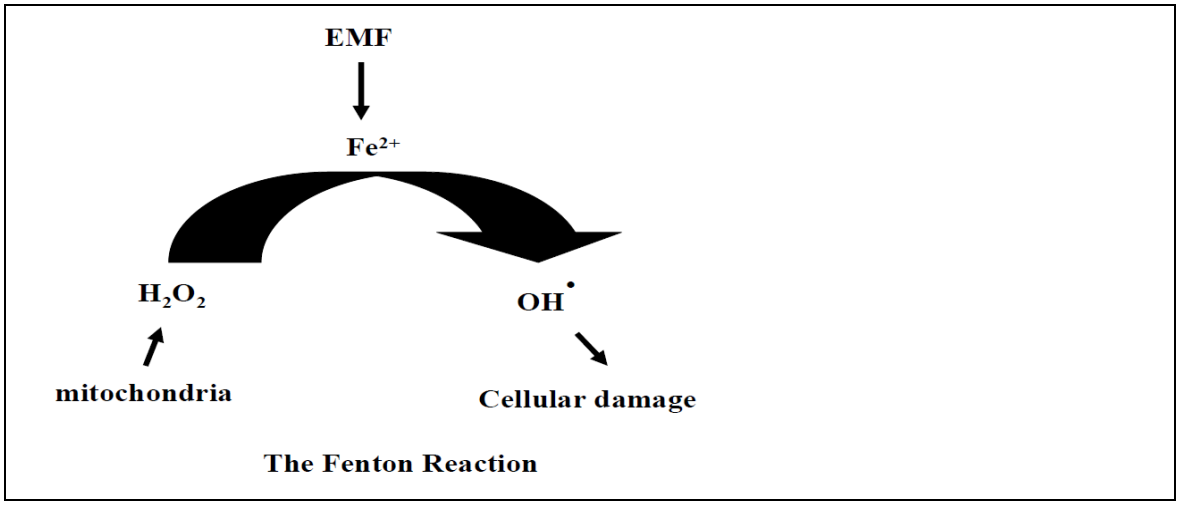

Figure 1. The Fenton reaction: an iron-catalyzed conversion of hydrogen peroxide to a more powerful and damaging hydroxyl free radical that can cause molecular damages and cell death.

Since cellular responses to magnetic fields involve an iron-dependent process, we hypothesize that cancer cells are more responsible to magnetic fields than normal cells. To test this hypothesis, we exposed Molt-4 cells, a human leukaemia cell line, to a 60-Hz magnetic field in the presence and absence of added holotransferrin (i.e., iron loaded transferrin) in the medium. Holotransferrin, as described above, transports iron into cells and increases iron content intracellularly. Molt-4 cells are expected to uptake a large amount of iron when provided with holotransferrin since they have high cell surface concentration of transferrin receptor [20, 21]. An increase in intracellular iron concentration would make these cells more susceptible to the effect of magnetic field. However, since normal lymphocytes do not have high transferrin receptors and do not import a lot of iron via transferrin, they would be less susceptible to the effect of magnetic fields. The following is a description of the experimental procedures.

Molt-4 cells (American Type Culture Collection, Rockville, MD) were grown in 100% humidity at 37^{o}C in 5% CO_2 in air, in RPMI-1640 medium (Life Technologies, Gaithersberg, MD) with 10% fetal bovine serum (Hyclone, Logan, UT). At 24 hrs after splitting into two by adding culture medium, 0.1 ml aliquots were put into microfuge tubes. Half of these samples were incubated with the addition of human holotransferrin (1 mg/ml, Sigma Chemical Co, St. Louis, MO) for 1 hr. Samples from holotransferrin-treated and non-treated cultures were then each further divided into two sets. One of the sets was exposed to a 0.25 mT 60 Hz sinusoidal magnetic field for 2 hrs in an incubator at 37°C. The other was incubated at 37°C without magnetic field exposure. Therefore, there were four

treatment conditions: holotransferrin/ magnetic field, holotransferrin/no magnetic field; no holotransferrin/magnetic field, and no holotransferrin/no magnetic field. Exposure to 60-Hz magnetic field was done in a Helmholtz coil exposure system consisting of two 16 cm diameter coils (250 turns/coil) encased in Perspex. Current input to the coils was adjusted using a variac. The flux density (0.25 mT) within the coils was determined by an Enertech Emdex II magnetic field meter. Similarly, 50 µl of human whole blood obtained from a finger prick was mixed with 1 ml of RPMI-1640 and divided into 0.1 ml samples. These samples were subjected to the same procedures of holotransferrin treatment and magnetic field exposure as described above.

Cell counts were made from each cell sample immediately before and after exposure (2 hrs) and at 22 hrs after exposure. After thoroughly suspending cells, 20 µl of cells were mixed well with 20 µl of 10 µg per ml of acridine orange and 10 µl of this was loaded in a hemocytometer chamber. Acridine orange is a DNA intercalating dye and stains DNA and RNA only. This allowed us to count leukocytes in whole blood without isolating them from the blood. Duplicate cell counts were made from each sample.

Cell count of a sample at a certain time point was converted as a ratio of the count of that sample at time zero (i.e., start of exposure). Four experiments each were run with Molt-4 cells and leukocytes and average responses of the experiments were calculated and plotted. Data were then compared by the Mann-Whitney U test and a difference at $p < .05$ was considered statistically significant.

Cell counts of Molt-4 cells after various times after magnetic field exposure were compared with those of similarly treated normal human leukocytes. Results of the experiment are presented in Figures 2 and 3. Data on Molt-4 cells are presented in Fig. 2. At the end of two hours of exposure, magnetic fields in the presence of holotransferrin significantly decreased the cell count ($p < .014$, compared to control (non-exposed) cells with holotransferrin). Magnetic field alone and holotransferrin alone also showed a slight but significant decrease in cell counts at hour 2. At 22 hrs (hour-24) after exposure, cell counts of control, 'control + transferrin' and magnetic field alone had significantly increased (hour-24 versus hour-2, $p < 0.014$ for all three groups). There was no significant difference among these groups at hour-24. However, cell counts of the 'magnetic field + holotransferrin' treated cells remained low at that time.

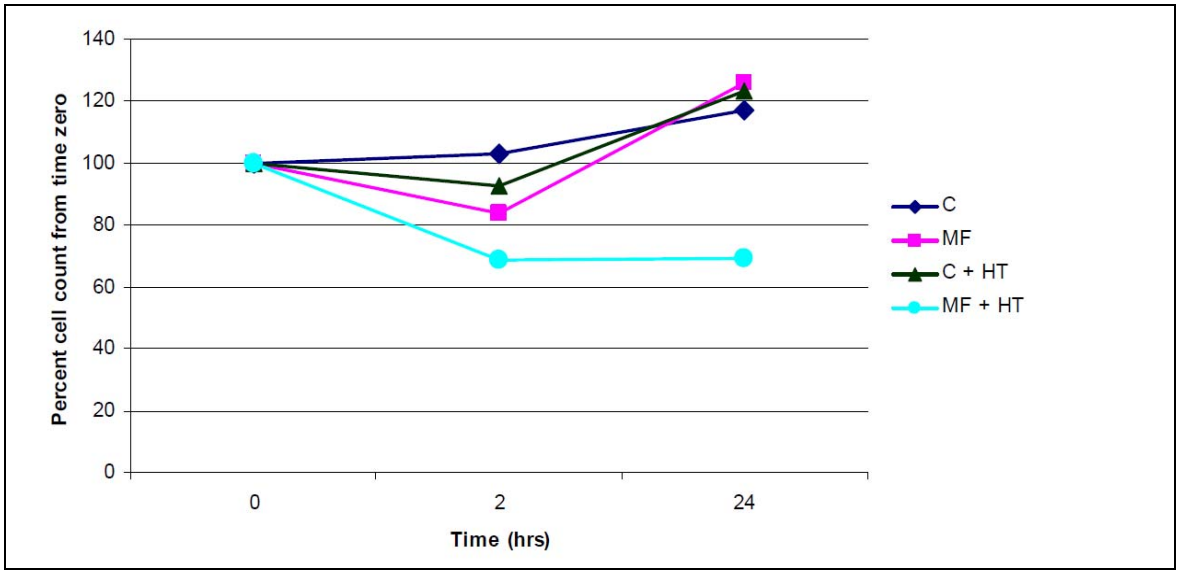

Figure 2. Effects of magnetic field exposure and incubation with holotransferrin on Molt-4 human leukemia cells. Magnetic field exposure started at time zero and lasted for two hours. Each curve represents data from four experiments. C = sham exposed control, MF = magnetic field, HT = holotransferrin.

Data of leukocytes are shown in Fig. 3. At hour-2, there was no significant difference in cell counts among the four treatment groups. Cell counts were significantly decreased at hour-24 (compared to hour-2). This drop in cell count over time is normal for leukocytes in culture. However, the only difference at hour-24 was found between the 'magnetic field + holotransferrin' and 'control' group ($p < .029$). There was no significant difference between 'control + holotransferrin' vs 'control' and 'magnetic field alone' vs 'control'.

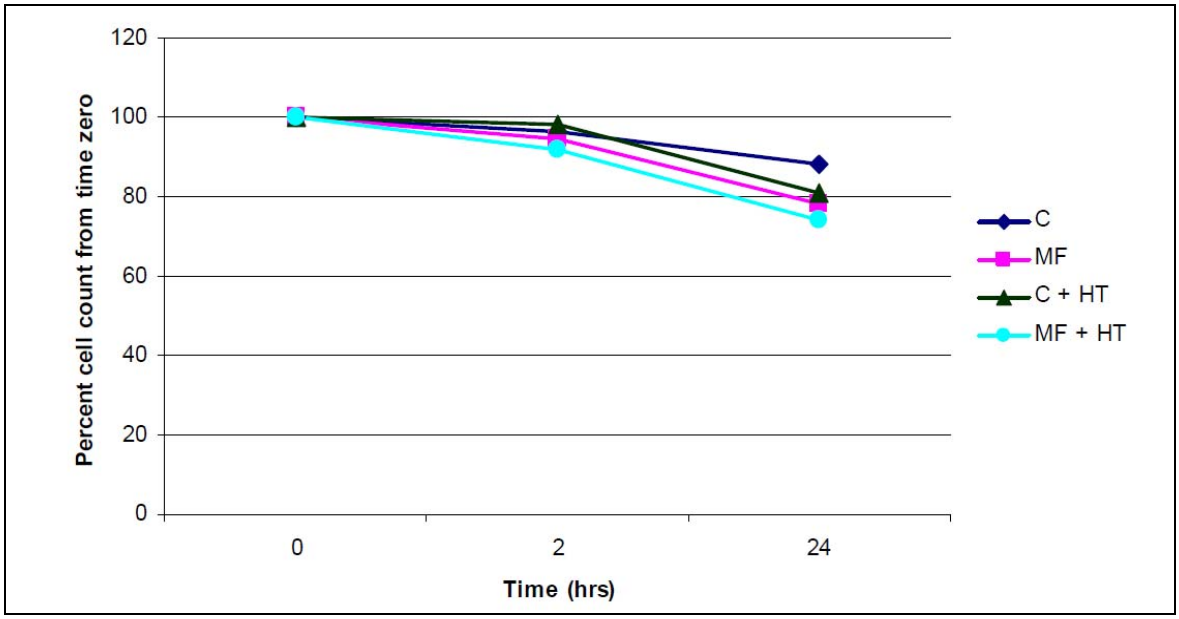

Figure 3. Effects of magnetic field exposure and incubation with holotransferrin on normal human leukocytes. Magnetic field exposure started at time zero and lasted for two hours. Each curve represents data from four experiments. C = sham exposed control, MF = magnetic field, HT = holotransferrin.

Data from this experiment indicate that a 60-Hz magnetic field, in the presence of holotransferrin, has a selective toxic effect on human cancer cells, whereas its effect on normal cells is minimal. This supports our hypothesis that cancer cells are more susceptible to magnetic fields after subjected to a treatment that enhances intracellular iron.

4. Conclusion

The finding that cell counts of Molt-4 cells remained stable at 22 hrs after magnetic field exposure and holotransferrin treatment suggests that the effect of magnetic field is long lasting. The initial decrease in cell count was probably due to cell death as a consequence of DNA and other types of cellular damages triggered by the magnetic field. After that, the remaining cells became stable and stopped proliferating. Free radicals are known to cause cell cycle arrest [22, 23]. A recent study also reports a delay in cell cycling in cells exposed to a 50-Hz magnetic field [24].

Thus, our data indicate that magnetic fields have selective cytotoxic effect on cancer cells, especially under conditions of high iron availability. This can also be applied to in vivo situation. Since transferrin in the circulation is only 30% saturated with iron, it is very easy to increase the availability of iron to cancer cells by simply feeding an animal with a ferrous salt. This will make cancer cells more susceptible to subsequent magnetic field exposure. Oral administration of an iron salt followed by magnetic field exposure can be used for treatment of cancer. Enhanced free radical activity in cells was observed not with 60-Hz magnetic field, similar effects have been reported with

EMF of different frequencies. Thus, EMF of various frequencies could conceivably be used for cancer treatment [25-33].

Thus, EMF may be useful for the treatment of cancer, since cancer cells use high amount of iron and are more susceptible to magnetic fields. Depending on the amount of damage, cell death (apoptosis and necrosis) or cell cycle arrest (e.g., due to DNA strand breaks [34, 35]) can occur. In both cases, the progress of the tumour is retarded.

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