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The role of 217-Hz ELF magnetic fields emitted from GSM mobile phones on electrochemotherapy mechanisms

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ABSTRACT

Electrochemotherapy (ECT), the combination of electric pulses (EPs) and an anticancer drug, is a type of cancer treatment method. We investigated the effect of 217-Hz magnetic fields (MFs) similar to that generated by GSM900 mobile phones, as intervening factors, on proposed mechanisms of ECT including permeability, tumor hypoxia and immune system response. The 4T1 cells were exposed to extremely low-frequency (ELF)-MFs at 93, 120 or 159 µT intensities, generated by Helmholtz coils 10 min, and then put in individual groups, comprising no treatment, chemotherapy, EPs or ECT. The cell viability was evaluated. Then, two treatment protocols were selected for *in vivo* experiments. The mice with 4T1 tumor cells were exposed to ELF-MFs 10 min/day until the day their tumors reached 8 mm in diameter. Then, the tumors were treated to ECT. Tumor hypoxia and immune system response were analyzed through immunohistochemistry assay and enzyme-linked immunosorbent assay technique, respectively. The results *in vitro* indicated a significant decreased ECT efficacy of 60 V/cm, 5 kHz at the flux density of 93 µT. The results *in vivo* showed that pre-exposure to ELF-MFs could increase tumor hypoxia induced by ECT. In addition, exposure to ELF-MFs before ECT caused a significant increase in interferon-γ/interleukin-4 in comparison with ECT alone. More studies, including studies on the effect of ELF-MFs emitted from mobile phones on tumor volume changes induced by ECT, are needed to elucidate how the process of ECT is influenced by the MFs.

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Electrochemotherapy; magnetic fields; tumor hypoxia; immune system

Introduction

Electrochemotherapy (ECT) is an approach to increase the entrance of anticancer drugs into the tumor cells by using electric pulses (EPs) (Domanico et al. 2015; Maglietti et al. 2016). In ECT, the increase in cell membrane permeability by the EPs leads to an increase in the access of chemotherapy drugs into the cytosol (Kunte et al. 2017; Sersa et al. 2008a). In fact, the applied electric fields reach the membrane potential to a critical value, which in turn breaks down the membrane. It thus creates pores to transfer the anticancer drug molecules into the cell (Kotnik et al. 2012). The pores disappear when the pulse is no longer used during a weak electric field (Yao et al. 2017). However, when a strong electric field is used, irreversible damage is made to the cell membrane, so such pores may remain in the membrane (Davalos et al. 2005; Niessen et al. 2013; Pucihar et al. 2011). Although many experimental studies and clinical trials have reported the application of high-voltage ECT (Huang et al. 2018; Miklavcic et al. 2006), treatment using low voltage may have some advantages because of the reduced side effects such as edema and burning (Fujimoto et al. 2005).

In addition to the increased permeability of the membrane of the cancer cells *in vitro*, there are other mechanisms contributing to ECT of the tumors *in vivo*. It was shown in the literature that the EPs affect endothelial cells which in turn leads to a vascular-disrupting effect that modifies tumor blood flow (Cemazar et al. 2001; Sersa et al. 2008a). Several studies in the literature demonstrated that ECT induces a severe reduction in the tumor blood flow, which contributes to the antitumor effect (Gehl et al. 2002; Sersa et al. 1999). Because the tissue oxygenation is related to blood flow in local tissue, tumor cells surviving after ECT are consequently exposed to lack of oxygen and are then pushed into secondary cell death (Sersa et al. 2002).

The involvement of immune response is the other mechanism of action which has already been demonstrated in preclinical reports (Sersa et al. 1997; Seršaa et al. 1997). In fact, ECT can release antigens from the cytosol. Because of the antigen presentation on the cell membrane, it induces immunogenic cell death (Calvet

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et al. 2014). Activation of the immune response may facilitate the recognition and removal of tumor cells at the primary and metastatic tumor sites (Entin et al. 2003). Besides the direct destruction of the primary tumor, triggering the immune response is a major advantage of ECT in comparison with the surgery combined with chemotherapy.

ECT technique is approved for subcutaneous and skin tumors, regardless of their histological origin (Esmaeili and Friebe 2019). Nevertheless, the efficacy of this treatment might be influenced by various factors. The variant stressors can be investigated as intervening factors in cancer treatments. In ECT, electromagnetic fields (EMFs) could be considered as low-dose stressor influencing treatment efficacy. In fact, because of the electric field present in both EMFs and ECT treatment, EMFs might have similar mechanisms to ECT on biological systems that affect treatment outcome. In this way, Mansourian et al. have investigated whether the effect of the 217-Hz MFs similar to those generated by mobile phones is capable of causing a protective response on apoptosis of the cells treated by ECT (Mansourian et al. 2013). Extremely low-frequency (ELF) MFs with a frequency of 217 Hz mainly stem from the current drawn from the battery during transmission. Pulsed radiofrequency signals in GSM900 phones are transmitted with a frame duration of 4.615 ms. It causes the current drawn from the phone battery to be pulsed with the frequency of 217 Hz (Andersen and Pedersen 1997). Because cell death plays a major role in ECT mechanisms, the effect of these ELF-MFs on cell apoptosis induced by ECT may influence all the mechanisms.

Accordingly, in order to understand how these MFs interact with ECT treatment, this study was designed to examine the effect of the 217-Hz MFs similar to those generated by mobile phones on the biological pathways and mechanisms of ECT including cell permeability *in vitro* and tumor hypoxia and immune system response *in vivo*.

In addition, to investigate whether the effect of ELF-MFs on ECT depends on the change of the voltage and frequency of EPs in ECT, we used different protocols of ECT. In other words, we evaluated whether, with the change of ECT protocol, the percentage of efficacy change of ECT induced by ELF-MF is shifted. It helps to evaluate the role of ELF-MF intervention on ECT more precisely and to see whether they can be used to enhance the efficacy of some protocols of ECT.

Materials and methods

In vitro

Cell culture

4T1 mouse mammary tumor cells were grown in RPMI complete medium, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37°C, in a 5% CO_2 humidified incubator. The culture medium of cells was then discarded and 4T1 cells were washed with phosphate-buffered saline (PBS). Trypsin was added to the cells and maintained in the incubator for 2 min. The growth medium was then added to stop trypsinization. The cells were suspended by pipetting up and down several times. They were centrifuged at 500 rpm and washed with RPMI. The number of living cells was estimated by trypan blue exclusion. Then, the cells were diluted to 1×10^5 cells/ml with RPMI starvation medium.

ELF exposure

The signals were generated by the 217-Hz signal generator, connected to a custom-made power signal amplifier. The amplifier output was applied to the Helmholtz pair coils to generate the uniform MF. The coils were composed of 287 turns of 0.78-mm-diameter, insulated copper wire detached by 10 cm. Each coil had a diameter of 20 cm. The inductivity of the coils was 65.995 mH. The duty cycle of the MF was 13%. The pulses were generated in 0.577 ms; every 4.6 ms resulted in 217 Hz pulses (217 Hz = 1/4.6 ms). The waveform of the ELF signal was evaluated by BioLab software (Tehran, Iran) (Figure 1). Flux densities of the MF were measured, using the Tesla Meter (TES-1394; TES Electrical, Electronic Corp, Thedford, Ontario, Canada). The transferred concentration to small-petri-dish was 1×10^5 cell/ml and set between the pair of Helmholtz coil, and pulsed MFs were applied. Cells were exposed to three different MF flux densities of 93, 120 or 159 μT (Moghadam et al. 2009) for 10 min. The measuring current inside the coils was 256, 329 and 432 mA causing flux densities 93, 120 and 159 μT, respectively. The measured field intensity was not different at any petri-dish points. The temperature was measured before and after exposure to the ELF-EMF. No temperature change was observed in the cell culture medium.

Treatments

The experimental groups in our *in vitro* study were as follows:

(A) EMF exposure alone without any treatment

(B) EMF exposure and then treatment with chemotherapy (bleomycin with concentration of 1 μM)

(C) EMF exposure and then treatment with square-wave EPs, 4000 pulses, 100 μs with three following protocols: An amplitude of 70 V/cm and a frequency of 5 kHz, amplitude of 60 V/cm and a frequency of 5 kHz or amplitude of 70 V/cm and frequency of 4 kHz.

(D) EMF exposure and then treatment with ECT (the same EP generation protocol as part C)

In our study, EPs were generated by the pulse generator (ECT-SBDC), in order to produce high-frequency and low-intensity pulses. The uniform electric field was fed to

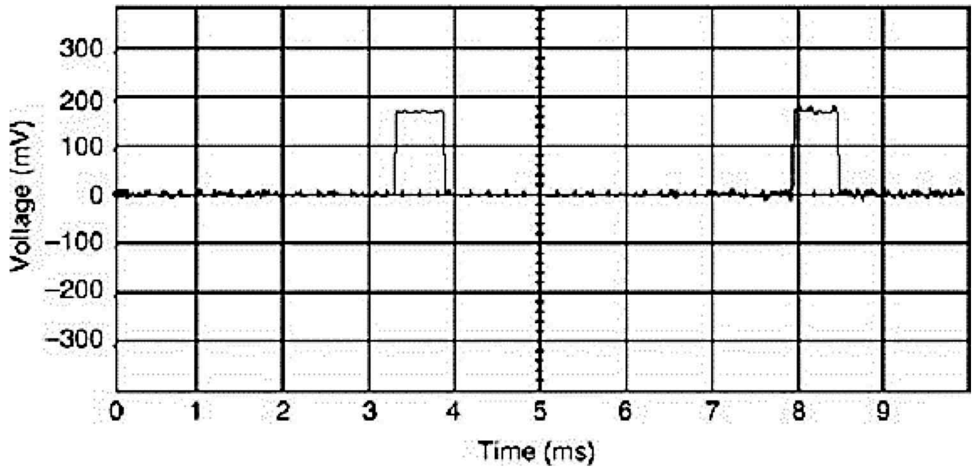

Figure 1. The shape of the pulsed magnetic field (0.576 ms pulse duration, 217 Hz frequency).

the cell suspension, using parallel stainless steel electrodes separated by 1 cm. Three different EP generation protocols (used in experimental groups of C and D) were selected from Shankayi et al. (2014).

Bleomycin with a concentration of 1 μM was used in chemotherapy and ECT treatments. The pulses were applied immediately after adding bleomycin into the cells. After treatments, the cells were put into 96-well plates and incubated for 24 h. All experiments were repeated at least three times.

MTT assay for viability

MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay was performed to measure cell viability. The cell medium was removed. Ten microliters of the MTT solution at appropriate concentrations of 5 mg/ml in PBS and 100 μL RPMI were added to each well. The plates were then incubated for 4 h at 37°C in a 5% CO_2 atmosphere. The supernatant was then carefully discarded, then 100 μL of DMSO was added to each well and the plates were shaken for 10 min to dissolve formazan. Finally, the optical density value of each well was measured by Multiscan MS ELISA reader (LaBsystems Multiskan MS, Basingstoke, UK.), using a 540-nm filter. The cell viability results were illustrated as percentages in comparison with those of the control group.

Statistical analysis

Statistical analysis was performed with a univariate Kruskal–Wallis test in order to evaluate metrics for significant differences, among the four groups (0, 93, 120 or 159 μT) using ELF fields, in different datasets (ECT (70–5), ECT (60–5) or ECT (70–4)). When a significant result was found, Dunn's *post hoc* test with Bonferroni correction (Dunn–Bonferroni) was used for pair-wise comparison, and the adjusted p-values were reported.

In vivo

We chose two protocols of treatments *in vitro* in which ELF MFs had more effect on ECT in comparison to other treatment protocols. One of these groups is the ELF-MF 93 μT before ECT (60 V/cm, 5 kHz). The other group is ELF-MF 120 μT before ECT (70 V/cm, 5 kHz).

Animals

Adult male BALB/c mice ((n = 50) weighting 16–18 g and 5–7 weeks) were obtained from Pasteur Institute of Iran (Tehran, Iran). Animals, under controlled conditions, were housed at 22 ± 2°C in large cages and kept on a 12-h light/dark cycle with *ad libitum* access to fresh tap water and food for 7 days. The animal utilization study committee of Tarbiat Modares University, Tehran, Iran (no. 52D/8436), approved this study complying with Council NR (2010).

Tumor model

Having being supplemented with 10% FBS and 1% penicillin–streptomycin at 37°C, in a 5% CO_2 humidified incubator, 4T1 mouse mammary tumor cells were grown in RPMI 1640 medium. The cells, 1×10^6, were suspended in 100 μL of saline solution and injected subcutaneously into the flank of mice. The mice randomly divided into five groups to be treated as follows:

(1) Control group without any treatment
(2) The group treated to ECT (70 V/cm, 5 kHz)

(3) The group exposed to ELF-MF with flux density 120 μT and then treated to ECT (70 V/cm, 5 kHz)
(4) The group treated to ECT (60 V/cm, 5 kHz)
(5) The group exposed to ELF with flux density 93 μT and then treated to ECT (60 V/cm, 5 kHz)

In groups 3 and 5, the mice were exposed to ELF-MFs 10 min/day until the day their tumors reached 8 mm in diameter. Then, they were treated to ECT. In groups 2 and 4, when tumors reached 8 mm in diameter, the mice were treated to ECT alone.

For experimental group in hypoxia test and cytokine assay, three to five mice were assigned.

ELF exposure

The mice were situated between the pair of Helmholtz coil (similar to *in vitro* experiments), immobilized by using standard Plexiglas restrainers and exposed to ELF-MFs at flux densities of 93 or 120 μT.

ECT protocol

Mice were anesthetized with a mixture of ketamine plus xylazine (100 and 10 mg/kg, respectively) intraperitoneally. Bleomycin (Cell Pharm, Hannover, Germany) was diluted in normal saline (1.5 mg/ml). Two minutes before the application of EPs, 0.16 ml of the diluted bleomycin solution per gram of mouse body weight was injected intratumorally (drug injection intratumorally increases the apoptosis because this drug induces apoptosis (Vernole et al. 1998)). Two parallel, flat and stainless steel electrodes (width 5 mm, length 15 mm) were placed at the opposite margins of the tumor and the EPs were delivered to it. A conductive gel was rubbed on the tumor to ensure good contacts between the skin and the electrodes. We used EPs with 4000 pulses, 100 ms using two following protocols: An amplitude of 70 V/cm and a frequency of 5 kHz or amplitude of 60 V/cm and a frequency of 5 kHz. The EPs were applied to the tumors by an EBCT-EDC (Bioelectromagnetic Laboratory, Tarbiat Modares University, Tehran, Iran).

HIF-1a (Hypoxia-inducible factor 1-alpha) immunohistochemistry (IHC) assay

The excised tumors were removed from the skin of the mice and fixed in 10% buffered formalin and processed through traditional processes in the automatic tissue processor (Sakura VIP, Torrance, CA), embedded in paraffin and cut at 5 μm. The sections were washed in PBS three times. Tissue sections were incubated overnight at 4°C. 2N HCl was applied to sections to antigen retrieval for 30 min followed by borate buffer for neutralization for 5 min. After washing with PBS, 0.3% Triton was added to cell permeabilization for 30 min and then washed again with PBS. Then, 10% goat serum was applied for 30 min to block secondary antibody reaction. The primary mouse monoclonal antibody anti-human HIF-1α was diluted with PBS and applied overnight at 4°C. In the morning, the sections were rinsed in PBS. Then, mouse secondary antibody was added, followed by incubation for 90 min at 37°C. The sections were washed with PBS and stained with DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) solutions. The images were obtained with an Olympus fluorescent microscope (Olympus, Tokyo, Japan).

Separation of splenic mononuclear cells (MNC)

For each experiment, the mice were randomly assigned. The mice were sacrificed by cervical dislocation on the 7th day; spleens were removed and suspended in PBS. The splenic cell suspension was RBC-lysed using a solution of 0.75% NH_4Cl and Tris buffer (0.02%). After washing the cells, their suspension was made in RPMI 1640 containing 10% (FBS) (Gibco, Paisley, UK). Cell densities were determined by hemocytometer counting. The splenocyte viability was above 95%. After washing again with PBS, the suspension was concentrated to 1×10^6 cells per milliliters in RPMI 1640 supplemented with 10% FBS, 100 μg/ml streptomycin and 100 IU/ml penicillin (completed RPMI) and incubated at 4°C.

Splenocyte cytokine production

The isolated spleen MNCs were cultured in 24-well plates at a density of 10^6 cells/well. After 72 h incubation at 37°C and 5% CO_2, the supernatants were gathered and frozen at −70°C in order to perform enzyme-linked immunosorbent assay (ELISA). Interleukin-4 (IL-4) and interferon-gamma (IFN-γ) concentrations were analyzed using R&D DuoSet ELISA Development kit (Minneapolis, MN, USA) according to suggested protocols of the manufacturer. Each sample was examined in triplicates.

Statistical analysis

The entire data were tested for normality. The statistical analysis was performed using the Mann–Whitney non-parametric test for groups related to hypoxia and immune system responses. The significance level was set to $p = .05$.

Results

Cell viability

No significant changes could be detected in cell viability, after exposure to the ELF fields alone, compared with the control group (Figure 2).

In chemotherapy treatments, ELF-MFs could significantly increase the viability ($p < .05$) of the cells exposed at a flux density of 120 µT, but the MFs at the flux densities of 93 and 159 µT cause a slight increase in cell susceptibility to chemotherapy drug (Figure 3a).

In treatment with EPs, the effects of ELF-MFs on various protocols of EPs were different. These fields could give rise to a slight increase in cell susceptibility to EPs of 70 V/cm and 4 kHz, while they slightly reduced the susceptibility of cells to EPs of 70 V/cm and 5 kHz. Although it is insignificant, a protective response to EPs of 60 V/cm and 5 kHz at the flux density of 93 µT of ELF fields was observed, more than the other EP-treated groups (Figure 3b).

In groups relevant to ECT treatments, an insignificant cell viability increase of 15% was seen in cells treated with ECT (70 V/cm, 5 kHz) at 120 µT in comparison to ECT alone (70 V/cm, 5 kHz). Such an increase was also observed in the cells treated with ECT (70 V/cm, 4 kHz) at 120 µT (12%) in comparison with the ECT treatment (70 V/cm, 4 kHz), without any MF exposure.

However, ELF fields significantly increased the percentage of viable cells in ECT (60 V/cm, 5 kHz) at 93 µT by 16% compared with ECT (60 V/cm, 5 kHz) alone ($p < .05$, Figure 3c).

Hypoxia

To investigate the role of mobile phone radiations on hypoxia induced by ECT, we performed IHC assay of HIF-α on tumors of mice. Tumors were excised 2 h after ECT because the maximal reduction in partial oxygen pressure (pO_2) occurred within 2 h after the treatment (Sersa et al. 2002).

The results related to ECT (70 V/cm, 5 kHz) indicated a significant increase in HIF-α level in tumors pre-exposed to ELF-MF at 120 µT in comparison with ECT (70 V/cm, 5 kHz) alone (6%) (Figure 5).

The ELF-MF at 93 µT before ECT (60 V/cm, 5 kHz) could also increase HIF-α level in comparison with ECT (60 V/cm, 5 kHz) alone by 5% (Figure 5).

Immune system response

The results from three independent experiments were also normalized to the control group (healthy mice). The findings showed an insignificant IFN-γ increase and IL-4 decrease in the group treated with ECT (70 V/cm, 5 kHz) at 120 µT in comparison to ECT alone (70 V/cm, 5 kHz). Also, ELF-MF at a flux density of 93 µT before ECT (60 V/cm, 5 kHz) led to an insignificant increase in IFN-γ and decrease in IL-4 level in comparison with ECT (60 V/cm, 5 kHz) alone (Figure 6).

We also considered IFN-γ/IL-4 ratio (Table 1). Because IFN-γ is the indicator of Th1 while IL-4 is the indicator of Th2, the IFN-γ/IL-4 ratio is considered to be a straightforward indicator of Th1/Th2 balance (Wang et al. 2015). Difference in this ratio between group exposed to ELF-MFs at 120 µT before ECT

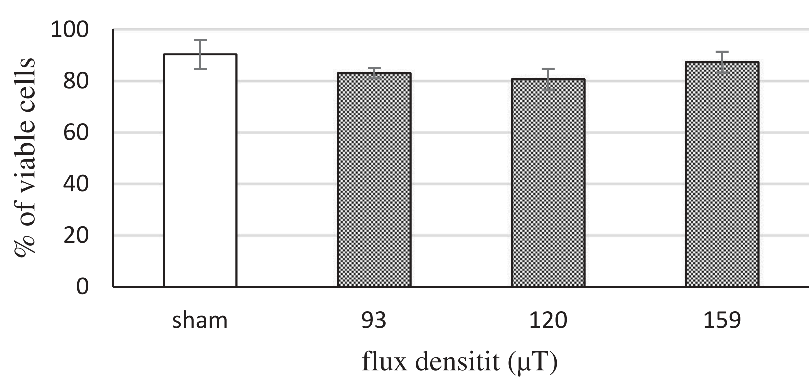

Figure 2. The effect of ELF magnetic fields on cell viability. White column: The percentage of viable cells under experimental conditions, but non-ELF exposure (Sham) normalized to control group; pattern-filled columns: percentage of viable cells in ELF-exposed cells expressed as a percentage of control. Data are shown as a percentage of control mean ± SD of the mean of at least three separate experiments, each performed in triplicate. Statistical significance was determined by the Student t-test analysis as $^{*}p < .05$.

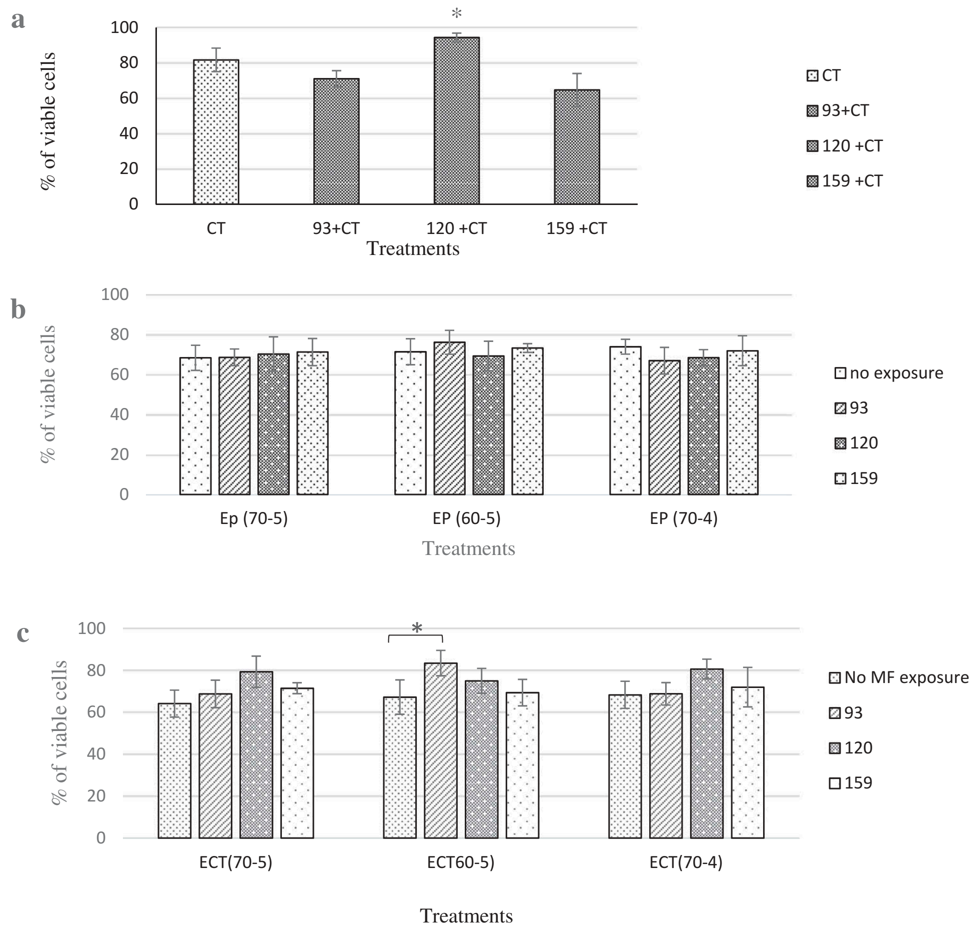

Figure 3. The effect of ELF magnetic fields on the efficacy of three kinds of cancer treatment. (a) The percentage of viable cells for chemotherapy (CT) treatment alone and chemotherapy treatments after exposure to ELF magnetic fields 93, 120 or 159 μT. (b) Percentage of viable cells for treatment by electric pulses alone and treatments (three protocols of electric pulses ((70 V/cm, 5 kHz), (60 V/cm, 5 kHz) and (70 V/cm,4 kHz)) after exposure to ELF magnetic fields 93, 120 or 159 μT. (c) Percentage of viable cells for treatment by electrochemotherapy alone and treatments (three protocols of electrochemotherapy ((70 V/cm, 5 kHz), (60 V/cm, 5 kHz) and (70 V/cm, 4 kHz)) after exposure to ELF magnetic fields 93, 120 or 159 μT. Data are shown as percentage of control mean ± SD of the mean of at least three separate experiments, each performed in triplicate. Statistical significance was determined by the Student *t*-test analysis as $*p < .05$.

(70-5) and ECT (70-5) alone was significant in comparison to difference in IFN-γ/IL-4 ratio between group exposed to ELF-MFs at 93 μT before ECT (60-5) and ECT (60-5) alone.

Discussion

The growth rate of cancer in modern societies has attracted the attention of researchers to improve the treatment techniques. In this regard, the role of intervening factors in cancer treatment efficacy is vital. Because of high exposure to environmental EMFs, the mobile phone waves could be considered as one of the intervening factors in cancer treatments. The current study was designed to ascertain how mobile phone ELF-EMF affects ECT treatment mechanisms.

When assess-ing the mechanism of cell permeability our results, showed a significant increase in the viability of cells treated with chemotherapy pre-exposed to ELF-MF at 120 μT, compared with chemotherapy alone. Also, the same pattern was observed in - ECT pre-exposed to ELF-MF at 93 μT, compared with ECT alone. These findigs suggest that pre-exposure to ELF-MF might result in a protective response. In fact, probably, the exposure of the cells to the ELF-EMF can produce a small "trigger" that does not significantly increase the genetic damage but may stimulate signal transduction pathways resulted in the activation of cell defense mechanisms and consequently provide

Table 1. IFN- γ/IL-4 proportion.

Tumor	ECT (70-5)	ECT (60-5)	120 + ECT (70-5)	93 + ECT (60-5)
1.16 ± 0.3	0.62 ± 0.35	1.18 ± 0.35	1.24 ± 0.55	1.49 ± 0.6

protection from the subsequent genotoxic agent (chemotherapy drug). In addition, ELF-MFs would increase the reactive oxygen species levels (Benassi et al. 2016) which play a role in the activation of cell defenses through signaling pathways (Cao and Tong 2014). The triggered cell defenses could provide the cell the ability to resist high-level damages caused by CT or ECT.

Nonetheless, in spite of being unknown the mechanism of decreased cytotoxicity, caused by ELF MFs, it could be due to an anti-apoptotic effect and decrement of the apoptosis, corresponded with an increment in cell viability fraction. The anti-apoptotic effect of ELF-MF has been demonstrated in many studies (Basile et al. 2011; De Nicola et al. 2006). Such behavior may be determined by different molecular mechanisms and several signaling pathways, reported in the scientific literature. It has been suggested that the protective effects of ELF MFs could be mediated by the expression of heat-shock protein, known to have a key role in cellular defense against the effect of stressors (Garip and Akan 2010). Another influencing mechanism is the Ca^{2+} influx from the extracellular space, enhanced by the ELF-MF (Santini et al. 2005), promoting cell survival. The alterations, including the increase in antioxidant enzyme activity and decrease in DNA damage level in cells, after exposure to the ELF-MFs (Burkart 1989), also may represent a compensatory response to the oxidative stress, induced by a stressor. The other possible suggested mechanisms are associated with inhibiting G1 cell cycle arrest, increasing bcl-2 and decreasing the levels of p21, caspase-3 and p53 (Tian et al. 2002). Furthermore, increase in the levels of BAG3 anti-apoptotic protein, induced by ELF-MF, could contribute to retaining cell survival against the pro-apoptotic effect of EP and chemotherapeutic drugs (Basile et al. 2011).

Interestingly, protective activity, induced by radiations similar to those emitted by mobile phones, occurred at intensity restricted in a specific window could be noted. It shows that the findings confirm the “amplitude windows” hypothesis (Markov 2010). This phenomenon was found for ELF fields similar to the findings of the previous study; thus, the registered effects are not linear over the field intensity spectrum.

The results of the current investigation also showed that the effect of ELF-MFs on various treatments was different at various flux densities. Increase in the viability of cells, pre-exposed to ELF at 120 μT and treated with chemotherapy or ECT, was observed compared with those treated with mentioned treatments alone without ELF-MF exposure. However, at a flux density of 93 μT, the viability in the cells exposed to ELF-MF and treated with chemotherapy decreased compared to chemotherapy alone, while the viability in the cells exposed to ELF-MF and treated with ECT (60 V/cm, 5 kHz) increased compared to ECT alone. In fact, although chemotherapy is a part of ECT, the effect of ELF-MF may be different when chemotherapy is combined with EPs at 93 μT.

After the investigation of the effect of ELF-MFs on the permeability mechanism of ECT, we selected two flux densities of ELF-MFs in which the cell viability had the most percentage and assessed the effect of these two flux densities of mobile phone radiations on two mechanisms of ECT *in vivo*.

The results *in vivo* were different from those *in vitro* . Although ELF-MFs at some flux densities were resulted in an increase in cell viability induced by ECT and a decline in efficacy of permeability mechanism, they caused to improve two other ECT mechanisms *in vivo*.

Because the mobile phone radiations were able to increase the cell viability in the cells treated to ECT, it was expected that the endothelial cell viability increases when exposed to the mobile phone radiations before ECT. In this way, tumor hypoxia induced by ECT could decrease. However, our findings indicated an increase in HIF-α expression due to ELF-MFs similar to those emitted by mobile phone radiations before ECT in comparison with ECT alone (Figure 4). HIF-α is a hallmark of tumor hypoxia expressed in solid tumors (Ren et al. 2016). As a result, tumor hypoxia increased in tumors exposed to mobile phone radiations before ECT. The increase in tumor hypoxia induced by mobile phone radiations could be the result of the anti-angiogenesis property of EMFs. The anti-angiogenesis effect of EMFs was observed by Wiliams *et al.* where the exposure of tumor for 10 min reduced the growth and vascularization of implanted breast cancers in mice (Williams et al. 2001). Cameron *et al.* also showed that the delay of tumor growth accompanied by reduced tumor vascularization could be induced by EMF (Cameron et al. 2014). One of the observations in our study was (not mentioned in the Results section) the retardation in reaching tumor volume (8 mm) for the application of ECT in groups exposed to ELF-MFs in comparison with those without radiation exposure, which showed ELF-MFs similar to those emitted by mobile phones may have an anti-angiogenesis effect. However, the probability of the anti-angiogenesis effect induced by mobile phone radiation is a hypothesis which should be considered in further studies.

The results of the immune system response showed that, although insignificant, ELF-MFs before ECT

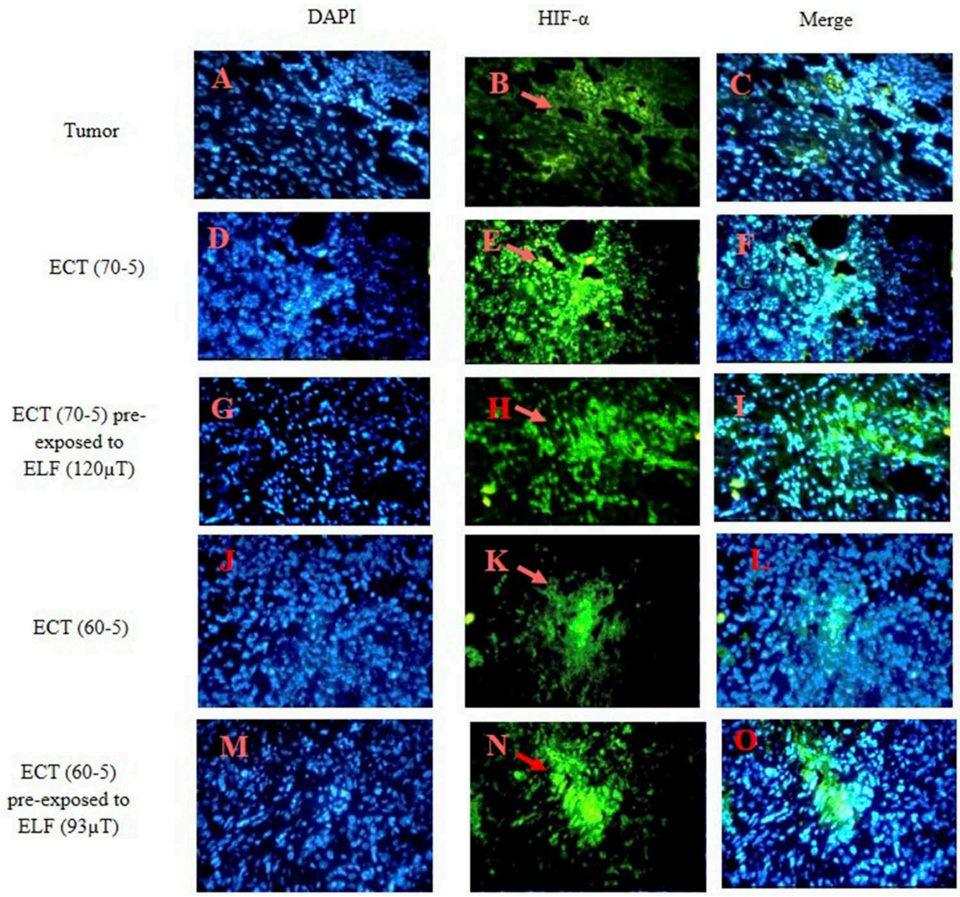

Figure 4. Immunohistochemical analysis of HIF-1α expression. Representative images showing staining for HIF-1α and the nucleus. (a) Nuclei stained by DAPI (blue), primary antibody to HIF-α (green). (a–c) Tumor, (d–f) tumor treated to ECT (70-5), (g–i) tumor treated to ECT (70-5) pre-exposed to ELF (120 μT), (j–l) tumor treated to ECT (60-5), (m–o) tumor treated to ECT (60-5) pre-exposed to ELF (93 μT).

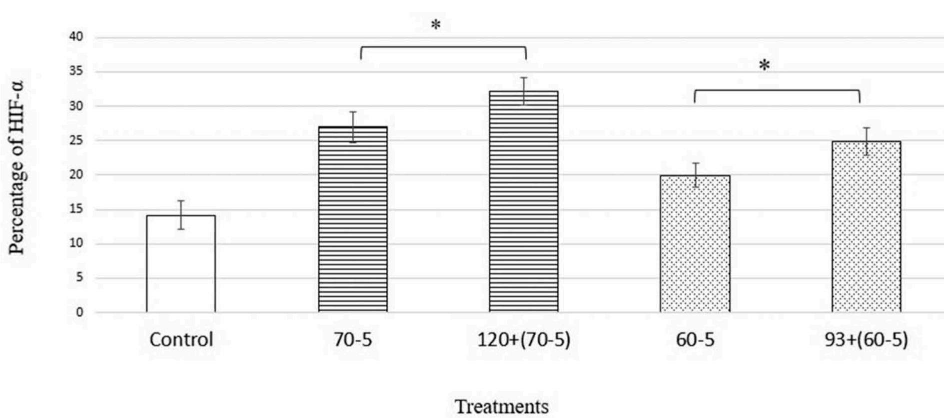

Figure 5. Percentage of HIF-α in treatment groups. Date are shown as mean ± SD. Significantly different at $p < .05$*.

(70 V/cm, 5 kHz) could increase the IFN-γ level in comparison with ECT alone. IFN-γ as a key factor in anti-tumor immunity could suppress the growth of tumor through variant mechanisms (Mojic et al. 2018). Therefore, ELF-MFs might be considered as an assistive factor in treatment.

As observed, despite the negative effect of ELF-MFs *in vitro*, these fields had a positive effect on the ECT

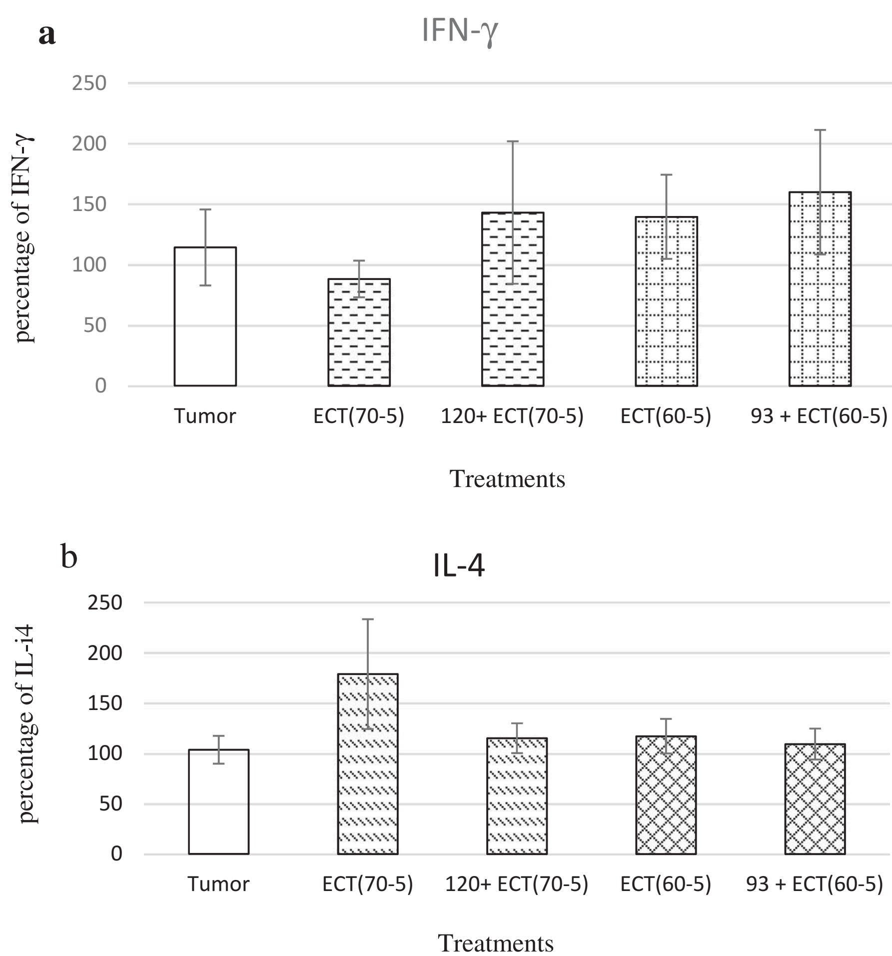

Figure 6. The results of cytokine assay. (a) Effect of exposure to mobile phone radiation on IFN-γ production induced by ECT. Data are represented as the percentage of control IFN-γ production. (b) Effect of exposure to mobile phone radiation on IL-4 production induced by ECT. Data are represented as the percentage of control IL-4 production. The data are represented as mean ± SD of three separate experiments.

mechanism *in vivo*. It could be because of differences in microenvironments. In fact, fibroblast has a significant role in microenvironment. However, further studies are necessary to shed light on many unclear mechanisms underlying this phenomenon.

In conclusion, to the best of our knowledge, this is the first study of its kind to evaluate the effects of ELF-MFs on ECT mechanisms. Our results showed ELF-MFs from mobile phones could enhance ECT efficacy *in vivo*. However, further investigations involving more generalized evaluations are required, including studies that focus on the effect of ELF-MFs emitted by mobile phones on tumor volume changes induced by ECT.

Disclosure of interest

The authors report no conflict of interest.

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References

Andersen, J. B., and G. F. Pedersen. 1997. The technology of mobile telephone systems relevant for risk assessment. *Radiat. Prot. Dosim.* 72:249–57. doi:10.1093/oxfordjournals.rpd.a032097.

Basile, A., R. Zeppa, N. Pasquino, C. Arra, M. Ammirante, M. Festa, A. Barbieri, A. Giudice, M. Pascale, and M. C. Turco. 2011. Exposure to 50 Hz electromagnetic field raises the levels of the anti-apoptotic protein BAG3 in melanoma cells. *J. Cell. Physiol.* 226:2901–07. doi:10.1002/jcp.22641.

Benassi, B., G. Filomeni, C. Montagna, C. Merla, V. Lopresto, R. Pinto, C. Marino, and C. Consales. 2016. Extremely low frequency magnetic field (ELF-MF) exposure sensitizes

SH-SY5Y cells to the pro-Parkinson's disease toxin MPP+. *Mol. Neurobiol.* 53:4247–60. doi:10.1007/s12035-015-9354-4.

Burkart, W. 1989. Effect of 3-aminobenzamide on chromosome damage in human blood lymphocytes adapted to bleomycin. *Mutagenesis* 4:187–89. doi:10.1093/mutage/4.3.187.

Calvet, C. Y., D. Famin, F. M. André, and L. M. Mir. 2014. Electrochemotherapy with bleomycin induces hallmarks of immunogenic cell death in murine colon cancer cells. *Oncoimmunology* 3:e28131. doi:10.4161/onci.28131.

Cameron, I. L., M. S. Markov, and W. E. Hardman. 2014. Optimization of a therapeutic electromagnetic field (EMF) to retard breast cancer tumor growth and vascularity. *Cancer Cell Int.* 14:125. doi:10.1186/s12935-014-0125-5.

Cao, Y., and J. Tong. 2014. Adaptive response in animals exposed to non-ionizing radiofrequency fields: Some underlying mechanisms. *Int. J. Environ. Res. Public Health* 11:4441–48. doi:10.3390/ijerph110404441.

Cemazar, M., C. Parkins, A. Holder, D. Chaplin, G. Tozer, and G. Sersa. 2001. Electroporation of human microvascular endothelial cells: Evidence for an anti-vascular mechanism of electrochemotherapy. *Br. J. Cancer* 84:565. doi:10.1054/bjoc.2000.1625.

Council NR. 2010. *Guide for the care and use of laboratory animals*. Washington (DC): National Academies Press.

Davalos, R. V., L. Mir, and B. Rubinsky. 2005. Tissue ablation with irreversible electroporation. *Ann. Biomed. Eng.* 33:223. doi:10.1007/s10439-005-8981-8.

De Nicola, M., S. Cordisco, C. Cerella, M. C. Albertini, M. D'ALESSIO, A. Accorsi, A. Bergamaschi, A. Magrini, and L. Ghibelli. 2006. Magnetic fields protect from apoptosis via redox alteration. *Ann. N. Y. Acad. Sci.* 1090:59–68. doi:10.1196/annals.1378.006.

Domanico, R., S. Trapasso, M. Santoro, D. Pingitore, and E. Allegra. 2015. Electrochemotherapy in combination with chemoradiotherapy in the treatment of oral carcinomas in advanced stages of disease: Efficacy, safety, and clinical outcomes in a small number of selected cases. *Drug Des. Devel. Ther.* 9:1185.

Entin, I., A. Plotnikov, R. Korenstein, and Y. Keisari. 2003. Tumor growth retardation, cure, and induction of antitumor immunity in B16 melanoma-bearing mice by low electric field-enhanced chemotherapy. *Clini. Cancer Res.* 9:3190–97.

Esmaeili, N., and M. Friebe. 2019. Electrochemotherapy: A review of current status, alternative IGP approaches, and future perspectives. *J. Healthc. Eng.* 2019:11. doi:10.1155/2019/2784516.

Fujimoto, T., H. Maeda, K. Kubo, Y. Sugita, T. Nakashima, E. Sato, Y. Tanaka, M. Madachi, M. Aiba, and Y. Kameyama. 2005. Enhanced anti-tumour effect of cisplatin with low-voltage electrochemotherapy in hamster oral fibrosarcoma. *J. Int. Med. Res.* 33:507–12. doi:10.1177/147323000503300505.

Garip, A., and Z. Akan. 2010. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. *Acta. Biol. Hung.* 61:158–67. doi:10.1556/ABiol.61.2010.2.4.

Gehl, J., T. Skovsgaard, and L. M. Mir. 2002. Vascular reactions to in vivo electroporation: Characterization and consequences for drug and gene delivery. *Biochim. Biophys. Acta. Gen. Subj.* 1569:51–58. doi:10.1016/S0304-4165(01)00233-1.

Huang, K. W., P. C. Yang, U. Pua, M. D. Kim, S. P. Li, Y. D. Qiu, T. Q. Song, and P. C. Liang. 2018. The efficacy of combination of induction chemotherapy and irreversible electroporation ablation for patients with locally advanced pancreatic adenocarcinoma. *J. Surg. Oncol.* 118:31–36. doi:10.1002/jso.25110.

Kotnik, T., P. Kramar, G. Pucihar, D. Miklavcic, and M. Tarek. 2012. Cell membrane electroporation-Part 1: The phenomenon. *IEEE Electr. Insul. Mag.* 28:14–23. doi:10.1109/MEI.2012.6268438.

Kunte, C., V. Letule, J. Gehl, K. Dahlstroem, P. Curatolo, R. Rotunno, T. Muir, A. Occhini, G. Bertino, and B. Powell. 2017. Electrochemotherapy in the treatment of metastatic malignant melanoma: A prospective cohort study by InspECT. *Br. J. Dermatolo.* 176:1475–85. doi:10.1111/bjd.15340.

Maglietti, F., M. Tellado, N. Olaiz, S. Michinski, and G. Marshall. 2016. Electrochemotherapy in non-satisfactory responding tumors in vet patients: Combined administration of bleomycin, systemic and local. In *1st world congress on electroporation and pulsed electric fields in biology, medicine and food & environmental technologies,* pp. 445–448. Springer, Singapore, 2016.

Mansourian, M., S. M. P. Firoozabadi, Z. Shankayi, and Z. Hassan. 2013. Magnetic fields with frequency of 217 Hz can reduce cell apoptosis caused by electrochemotherapy. *Electromagn. Biol. Med.* 32:70–78. doi:10.3109/15368378.2012.708693.

Markov, M. S. 2010. Angiogenesis, magnetic fields and 'window effects'. *Cardiology* 117:54–56. doi:10.1159/000315433.

Miklavcic, D., S. Corovic, G. Pucihar, and N. Pavselj. 2006. Importance of tumour coverage by sufficiently high local electric field for effective electrochemotherapy. *Eur. J. Cancer Suppl.* 4:45–51. doi:10.1016/j.ejcsup.2006.08.006.

Moghadam, M. K., S. M. Firoozabadi, and M. Janahmadi. 2009. Reduction of F1 neuronal excitability by exposure to 217 Hz magnetic fields from GSM 900 mobile phone. *Yakhteh Med. J.* 11:176–83.

Mojic, M., K. Takeda, and Y. Hayakawa. 2018. The dark side of IFN-γ: Its role in promoting cancer immunoevasion. *Int. J. Mol. Sci.* 19:89. doi:10.3390/ijms19010089.

Niessen, C., E. M. Jung, W. A. Wohlgemuth, B. Trabold, M. Haimerl, A. Schreyer, C. Stroszczynski, and P. Wiggermann. 2013. Irreversible electroporation of a hepatocellular carcinoma lesion adjacent to a transjugular intrahepatic portosystemic shunt stent graft. *Korean J. Radiol.* 14:797–800. doi:10.3348/kjr.2013.14.5.797.

Pucihar, G., J. Krmelj, M. Reberšek, T. B. Napotnik, and D. Miklavčič. 2011. Equivalent pulse parameters for electroporation. *IEEE Trans. Biomed. Eng.* 58:3279–88. doi:10.1109/TBME.2011.2167232.

Ren, H. Y., Y. H. Zhang, H. Y. Li, T. Xie, L. L. Sun, T. Zhu, S. D. Wang, and Z. M. Ye. 2016. Prognostic role of hypoxia-inducible factor-1 alpha expression in osteosarcoma: A meta-analysis. *Onco. Targets Ther.* 9:1477–87. doi:10.2147/OTT.S95490.

Santini, M., A. Ferrante, G. Rainaldi, P. Indovina, and P. Indovina. 2005. Extremely low frequency (ELF) magnetic fields and apoptosis: A review. *Int. J. Radiat. Biol.* 81:1–11. doi:10.1080/09553000400029502.

Sersa, C., M. Cemazar, D. Miklavcic, and D. J. Chaplin. 1999. Tumor blood flow modifying effect of electrochemotherapy with bleomycin. *Anticancer Res.* 19:4017–22.

Sersa, G., T. Jarm, T. Kotnik, A. Coer, M. Podkrajsek, M. Sentjurc, D. Miklavcic, M. Kadivec, S. Kranjc, A. Secerov, et al. 2008a. Vascular disrupting action of electroporation and electrochemotherapy with bleomycin in murine sarcoma. *Br. J. Cancer* 98:388–98. doi:10.1038/sj.bjc.6604168.

Sersa, G., M. Krzic, M. Sentjurc, T. Ivanusa, K. Beravs, V. Kotnik, A. Coer, H. Swartz, and M. Cemazar. 2002. Reduced blood flow and oxygenation in SA-1 tumours after electrochemotherapy with cisplatin. *Br. J. Cancer* 87:1047. doi:10.1038/sj.bjc.6600606.

Sersa, G., D. Miklavcˇicˇ, M. Cemazar, J. Belehradek Jr, T. Jarm, and L. M. Mir. 1997. Electrochemotherapy with CDDP on LPB sarcoma: Comparison of the anti-tumor effectiveness in immunocompetent and immunodeficient mice. *Bioelectrochem. Bioenerg.* 43:279–83. doi:10.1016/S0302-4598(96)05194-X.

Sersa, G., D. Miklavcic, M. Cemazar, Z. Rudolf, G. Pucihar, and M. Snoj. 2008b. Electrochemotherapy in treatment of tumours. *Eur. J. Surgical Oncol.* 34:232–40. doi:10.1016/j.ejso.2007.05.016.

Seršaa, G., M. Čemažar, V. Menart, V. Gaberc-Porekar, and D. Miklavčič. 1997. Anti-tumor effectiveness of electrochemotherapy with bleomycin is increased by TNF-α on SA-1 tumors in mice. *Cancer Lett.* 116:85–92. doi:10.1016/S0304-3835(97)00170-5.

Shankayi, Z., S. Firoozabadi, and Z. S. Hassan. 2014. Optimization of electric pulse amplitude and frequency in vitro for low voltage and high frequency electrochemotherapy. *J. Membr. Biol.* 247:147–54. doi:10.1007/s00232-013-9617-9.

Tian, F., T. Nakahara, M. Yoshida, N. Honda, H. Hirose, and J. Miyakoshi. 2002. Exposure to power frequency magnetic fields suppresses X-ray-induced apoptosis transiently in Ku80-deficient xrs5 cells. *Biochem. Biophys. Res. Commun.* 292:355–61. doi:10.1006/bbrc.2002.6661.

Vernole, P., B. Tedeschi, D. Caporossi, M. Maccarrone, G. Melino, and M. Annicchiarico-Petruzzelli. 1998. Induction of apoptosis by bleomycin in resting and cycling human lymphocytes. *Mutagenesis* 13:209–15. doi:10.1093/mutage/13.3.209.

Wang, Y., J. Zhou, S. Fu, C. Wang, and B. Zhou. 2015. A study of association between oral lichen planus and immune balance of Th1/Th2 cells [journal article]. *Inflammation* 38:1874–79. doi:10.1007/s10753-015-0167-4.

Williams, C. D., M. S. Markov, W. E. Hardman, and I. L. Cameron. 2001. Therapeutic electromagnetic field effects on angiogenesis and tumor growth. *Anticancer Res.*;21(6A):3887–91.

Yao, C., Y. Lv, S. Dong, Y. Zhao, and H. Liu. 2017. Irreversible electroporation ablation area enhanced by synergistic high-and low-voltage pulses. *PLoS ONE* 12: e0173181. doi:10.1371/journal.pone.0173181.