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The Effect of ELF Magnetic Field on Tumor Growth after Electrochemotherapy

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Abstract From a fundamental point of view, chemotherapy is the most widely used treatment for cancers despite its side effects on normal cells and tissues. Electrochemotherapy (ECT) is a method for increasing the permeability of cancer cells to drugs and, hence, decreasing their dosage. It apparently creates electropores on the cell membrane using electric pulses. ECT can decrease tumor volume; but this effect is not permanent, and partial regrowth has been reported. The aim of this study was to investigate the potential of magnetic fields in preventing the regrowth of tumors after ECT. Tumoral Balb/c mice were exposed to a magnetic field (15 mT, 50 Hz) for 12 days after treating additionally with 70 V/cm electric pulses and bleomycin at the first day. The magnetic field caused a significant reduction in tumor volumes, while there was no significant difference between the ECT and the electroporation with ECT and magnetic field groups. The exploited magnetic field (15 mT, 50 Hz) could decrease the tumor growth rate significantly, without any effect on ECT efficiency.

Keywords Bleomycin · Electrochemotherapy · ELF magnetic field · Balb/c mice · Breast tumor

Introduction

Twelve percent of women will develop breast cancer at some time in their lives (Neal and Davalos 2009); therefore, determination of therapeutic methods and evaluation of their advantages and disadvantages have long been the focus of attention.

Chemotherapy is one of the most common methods of cancer treatment, being used intensively all over the world. The most important disadvantage of chemotherapeutic drugs is their nonselective absorption by cancer cells (Heller et al. 1999). Thus, to have better antitumor effects, higher doses of these drugs are needed, which cause more intensive side effects and systemic toxicity. In addition, the molecules of some of the chemotherapeutic drugs are too big to pass from the plasma membrane of cells and cannot exert their toxic therapeutic effects to kill cancerous cells (Domenge et al. 1996). To overcome the resistance of the cell membrane in permitting the drugs to pass through, many studies have been carried out to develop more effective drug-delivery systems that require lower doses of the drugs (Heller et al. 1999).

Many selective drug-delivery systems have been described, including local drug application, binding a drug to tumor-specific antibodies, magnetic targeting of the drug, embedding molecules into the liposome or other vesicles and enhancing the plasma membrane permeability to drug molecules by chemical modalities (Lübbe et al. 1996; Deurloo et al. 1991; Steerenberg et al. 1988). Another method for better drug/gene delivery is exposing the tumor region to electric pulses, called "electrochemotherapy" (ECT) or "electrogenetherapy." In this method, electric pulses are expected to increase the permeability of the cell membrane (Pucihar et al. 2002; Miklavcic et al. 2005).

Application of high-voltage electric fields (1 kv/cm) to cells leads to their increased permeability to macromolecules and chemotherapeutic drugs by creating electropores on their membranes.

In spite of its advantages, ECT has some disadvantages, such as inflammation, tissue burning and clinical risk due

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to high voltage. This motivates researchers to use low-intensity electric fields (Akiko et al. 2000; Sersa et al. 1999; Miyazaki et al. 2003; Kitamura 2003; Matsuki et al. 2008).

Low-intensity electric fields can induce apoptosis of cancer cells and allows us to improve drug efficiency by increasing the number of pulse repetitions. Furthermore, due to less pain and fewer contractions, ECT at high frequency is more tolerable than that at low frequency (Pucihar et al. 2002; Miklavcic et al. 2005).

Therefore, considering the importance of low-intensity electric fields with high pulse repetition and frequency, ECT with 4,000 pulse repetitions, 5 kHz frequency and 70 V/cm field intensity has been introduced as the most effective protocol for reduction of tumor volume. However, even with this modality, treated tumors showed regrowth after 12 days of exposure (Shankayi et al. 2010; Shankayi and Firoozabadi 2011).

Tumor regrowth probably also depends on activation of the angiogenesis process, which supports cancer cells in the receipt of needed nutrients. Therefore, control of the angiogenesis process represents a useful modality to prevent tumor growth (Lang and Stoeltzing 2006). According to the results of previous studies, one of these methods is using magnetic fields on tumors.

Various metabolic effects have been reported, based on applied electromagnetic (EM) field parameters and the physiological status of the cells or organisms. In addition, there are many studies about the effects of pulsed and sinusoidal ELF EM fields on tumors and cancer cells. In this regard, tumor volume reduction and survival time enhancement (Yoshiharu et al. 1990; Bellossi and Desplaces 1991; Babincová et al. 2000; Seze et al. 2000; Mikirova et al. 2001; Williams and Markov 2001), decreased apoptosis and increased tumor growth (Jacob et al. 2002) are among the reported results. Meanwhile, a 15-mT magnetic field with 50-Hz frequency was introduced as a tumor necrosis agent by Berg et al. (2010).

Another report on the effect of half–sine wave magnetic field with 120 pulses/s and 15 mT on tumor volume and angiogenesis reduction was presented by William et al. (2001). This modality can be used alone or in combination with other treatments.

Considering tumor regrowth even after 12 days of treatment with low-intensity ECT (Shankayi et al. 2010; Shankayi and Firoozabadi 2011), we postulated that ECT would be more efficient if applied together with an ELF magnetic field. Based on our knowledge, there is no report on the effect of mutual application of a magnetic field and ECT. Therefore, in order to examine the influence of a magnetic field on the efficiency of chemotherapy with and without electric pulses on tumor growth and the permeability of the cell membrane, a magnetic field with 50-Hz frequency for 12 days was applied to breast adenocarcinoma tumors of Balb/c mice after ECT and chemotherapy.

Materials and Methods

Mice and Tumors

Healthy female Balb/C mice, 6–8 weeks old, were purchased from the Pasteur Institute (Tehran, Iran). They were kept at 22 °C with a natural day/night cycle for 10 days for adaptation. Spontaneous mouse mammary tumor (SMMT), i.e., an invasive ductal carcinoma, was transplanted by implanting a 3- to 4-mm fragment into the right flank of anesthetized mice. Approximately 2 weeks after transplantation, when the largest tumor diameter exceeded 8 mm (measured by caliper), the animals were randomly divided into experimental and control groups (10 animals for the control group and for each of the experimental groups).

Tumor volumes (V) were determined with the following formula:

$$V = \left(A \times B^2\right) \times \pi/6$$

where A is the big and B is the small diameter of tumor. The normal volume was obtained by the formula

$$\text{Normal volume on } n\text{th day} = (n\text{th day volume})/(\text{first day volume})$$

where n is the number of days after treatment.

Magnetic Field Exposure System

The experimental apparatus consisted of a vertical rectangular (10 × 12 cm) coil, mounted on a C-shaped magnetic core and powered by an AC generator (Fig. 1). Field intensities were measured by a 3D Hall sensor HI 3550 (Holaday Industries, Eden Prairie, MN), and the magnetic flux density measured in the exposure chamber was 15 mT at 50-Hz frequency. The background magnetic field was considered 400 μT for all of the magnetic field exposure groups.

The exposure chamber was made of Plexiglas and perforated to allow air exchange between the exposure chamber and environment. The temperature change inside the exposure chamber at the end of the magnetic field treatment did not exceed 1 °C, and this change was probably a result of the heat emitted by the mouse body.

ECT Protocol

In this study, bleomycin was diluted in normal saline (1.5 mg/ml), and 0.016 ml of the solution per gram of mouse body weight was injected into the tumors 2 min before the experiments (Shankayi and Firoozabadi 2011). Electric

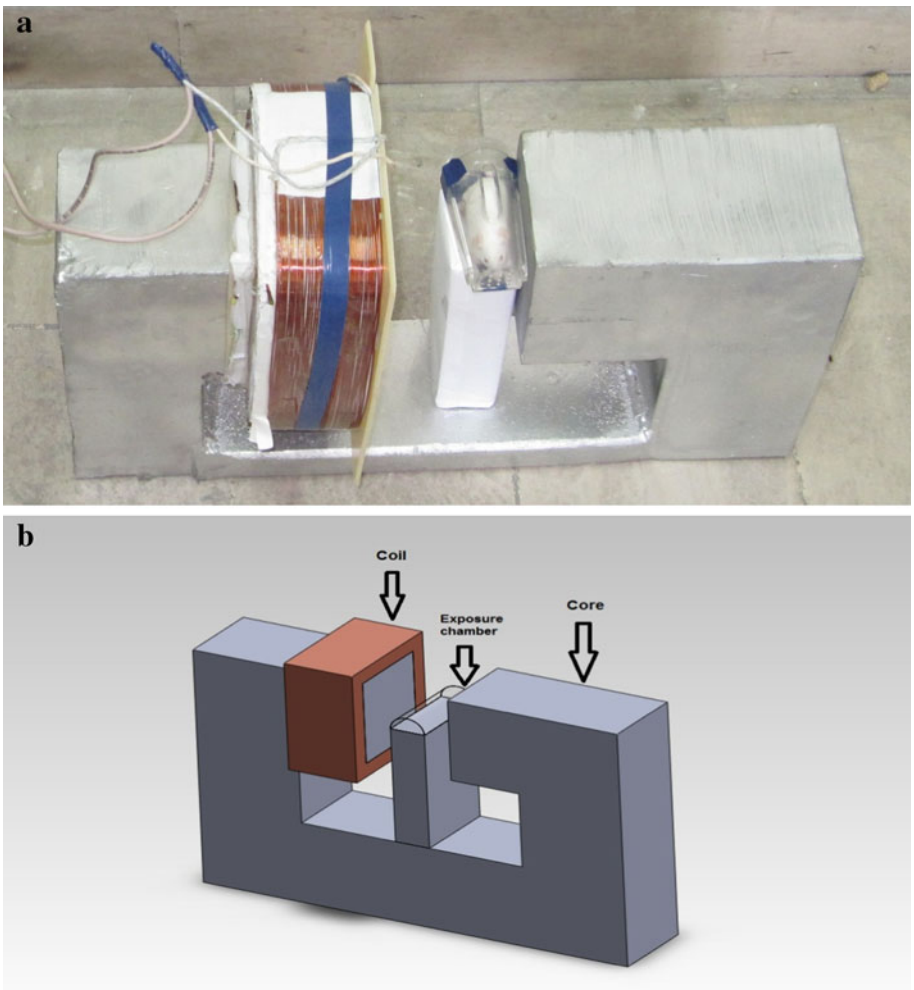

Fig. 1 The magnetic field system and exposure chamber. **a** An actual representation of the laboratory magnetic field system. **b** A schematic representation of the same system

pulses were delivered percutaneously by two flat, parallel, stainless-steel electrodes, which were placed at the opposite margins of the tumor. A conductive gel was used to ensure good contact between electrodes and skin. Four thousand square-wave electric pulses of 70 V/cm at a repetition frequency of 5 kHz were delivered to the mice using an ECT-SBDC (designed and made in the Small Business Development Center and EM Laboratory of the Medical Physics Department of Tarbiat Modares University).

Treatment Groups

The first experimental group was exposed to a magnetic field (50 Hz, 15 mT) for 10 min/day for 12 days. The second group was exposed to a magnetic field just after ECT (70 V/cm, 5 kHz) with bleomycin. The third group was exposed to a magnetic field just after electroporation (EP; 70 V/cm, 5 kHz) without injection of any drug. The fourth treatment group was exposed to a magnetic field with injection of bleomycin at the first day. Other experimental groups were ECT, EP, chemotherapy (bleomycin), and a sham group, which was exposed to a background magnetic field (400 μT) when the apparatus was off. Finally, the control group received no treatment. All of the magnetic field groups were exposed for 12 days, 10 min/day.

Tumor diameters were measured every third day for 30 days, and the normal volumes were calculated.

Statistical Analysis

The statistical significance ($p < 0.05$) of the differences between normal volumes of the tumors in the various groups was assessed by one-way ANOVA. To examine significant differences between two groups, the least significant difference test was used.

Results

Effect of Magnetic Field on Tumor Volume

The results showed that magnetic field exposure for 12 days and 10 min/day can significantly decrease the tumor growth rate compared with control and sham groups

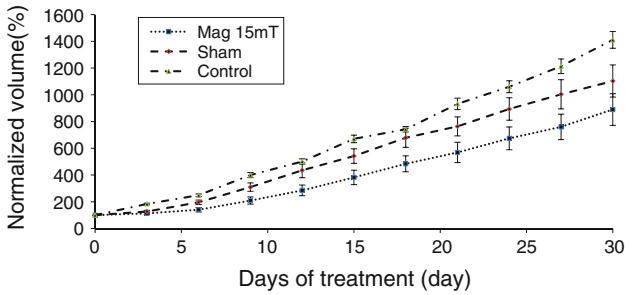

Fig. 2 Effect of magnetic field on tumor growth. *Normalized volume* (%) in the three groups. Sham (static magnetic field with intensity of 400 μT), control group and the Mag15 mT (combined static magnetic field of 400 μT and time-varying magnetic field of 15 mT with frequency of 50 Hz). Results are expressed as mean ± SE

($p < 0.05$) (Fig. 2). There was a significant difference between the normalized volume for the sham and control group on days 3, 9, 15, 21, 24, 27 and 30 with $p < 0.05$.

Effect of Magnetic Field on Bleomycin Toxicity

Comparisons were also performed between the bleomycin group and the group with magnetic field exposure for 12 successive days after bleomycin injection. Consequently, there was a significant difference between the bleomycin and bleo + magnetic field groups and the control group, while no significant difference was observed between normal volumes of the two former groups (Fig. 3).

Effect of Magnetic Field on ECT Persistence

We examined the effect of repetitive ELF magnetic fields (50 Hz, 15 mT) on the persistence of ECT treatment (70 V/cm, 5 kHz).

As shown in Fig. 4, the normal volumes of ECT and EP had a significant difference with the control group. Moreover, a significant difference was found between normal volumes of EP and ECT groups ($p < 0.05$). However, ECT increased bleomycin efficiency when electric pulses 2 min after intratumoral injection of the drug were applied. Nevertheless, there was no significant difference between the EP and chemotherapy groups. Tumors treated by EP had a reduced size during the first 6 days after treatment, and this reduction in tumor size lasted 9 days after treatment for the ECT group.

Considering the effects of magnetic field exposure on the persistence of the treatments, the EP and ECT groups had no significant differences with EP + magnetic field and ECT + magnetic field groups, respectively.

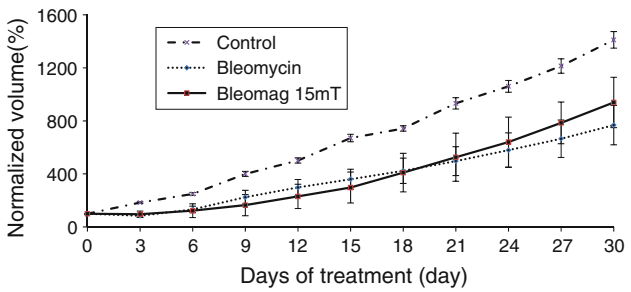

Fig. 3 Effect of magnetic field on bleomycin efficiency as a chemotherapy drug. *Normalized volume* (%) in the three groups. Control group, bleomycin (chemotherapy with bleomycin) and Bleomag15mT (combined magnetic field 15 mT with frequency of 50 Hz and bleomycin injection at the first day). Results are expressed as mean ± SE

Discussion

Effect of Sinusoidal Magnetic Field on Tumor Growth Rate

Magnetic fields (50/60 Hz frequency) are generated in any place that electricity is produced, transported and distributed, such as cables, power lines and any electrical devices (Kim et al. 2010). The intensity of these fields can reach several milliteslas. Some studies show that magnetic fields from power lines increase cancer risk in humans (Lai and Singh 1997; Schmitz et al. 2004; Winker et al. 2005). However, other studies demonstrate that ELF magnetic fields do not induce any toxic effect (McNamee et al. 2002). Anyway, the exact cellular mechanism of these effects has yet to be explained. Effects of magnetic fields on DNA, leading to cell death or increased probability of cancer, impel researchers to do more studies on this subject.

Two mechanisms may influence the impact of magnetic fields. First, metabolically active cells would be more

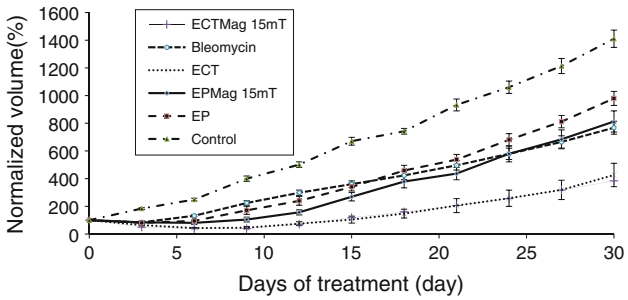

Fig. 4 Effect of magnetic field on EP and ECT treatment duration. *Normalized volume* (%) in the six groups. Control group, bleomycin (chemotherapy with bleomycin), EP (electroporation), ECT (electrochemotherapy with bleomycin), EPMag15mT [combined magnetic field 15 mT with frequency of 50 Hz and electric pulses (electroporation) at the first day] and ECTMag15mT (combined magnetic field 15 mT with frequency of 50 Hz and electrochemotherapy at the first day). Results are expressed as mean ± SE

susceptible to magnetic fields because of the generation of more hydrogen peroxide by the mitochondria of these cells. Second, cells that have a high level of intracellular free iron would be more vulnerable to EM fields. Cancer cells and cells that have abnormal proliferation have shown a higher concentration of free iron due to absorbing more iron (Phillips et al. 2009).

The effectivity of magnetic field exposure on most cancerous cells has been confirmed by other studies (Berg et al. 2010). In the present study, it has been shown that a 15-mT magnetic field with 50-Hz frequency can cause a reduction in tumor growth rate compared to sham and control groups. This is in agreement with the result obtained by William et al. (2001), in which a 15-mT magnetic field with 120 pulses/s led to a reduction of angiogenesis and tumor growth in mice. Likewise, this result confirmed that of Berg et al. (2010) with 15- to 20-mT sinusoidal magnetic fields and MX-1 cancer.

Furthermore, it is probable that the reduction of tumor growth is due to the decline of cancer cell division. On the other hand, it is likely that the enhancement of necrotic cells due to magnetic fields provokes tumor volume reduction (Berg et al. 2010). Based on the effects of repetitive exposure of magnetic fields on DNA breakage and cell death induction, the effect of this magnetic field can be attributed to the repetitive form of exposure (Kim et al. 2010).

However, the reduction of the tumor growth rate contradicts the results of Babincová et al. (2000), in which a magnetic field with 10- to 50-Hz frequency and 10 mT was introduced as an agent for increasing the 2D glioma tumor growth rate. This difference is likely due to the exposure time and type of tumor.

Effect of Sinusoidal 15-mT Magnetic Field on Toxicity of Bleomycin

The results have shown that the 15-mT, 50-Hz magnetic field did not have any significant effect on bleomycin toxicity as a chemotherapeutic agent. In other words, this field did not have a synergistic effect with bleomycin on tumors. This contradicted some other results (e.g., Tofani et al. 2003).

In Tofani et al.'s study (2003), the effects of combined static and sinusoidal magnetic fields were investigated upon cisplatin toxicity on mice B16 melanotic melanoma tumors; and as a result, the magnetic field led to more survival time for exposed mice compared to the cisplatin-only group. The tumors were exposed for 70 min/day for 3 days. In another experiment conducted by Hannan et al. (1994) to investigate magnetic field effects on the therapeutic efficiency of cisplatin, a pulsed magnetic field with average intensity between 0.525 and 0.276 mT was used and the group with magnetic field exposure had the least growth rate among all groups. They proposed that the magnetic field has a synergistic effect with the exploited chemotherapeutic drug.

The evaluation of the combined effect of a 15-mT, 50-Hz magnetic field and bleomycin on MX-1 tumor treatment in vivo showed a synergistic nature in their interaction (Berg et al. 2010). Duration of field exposure was 3 h/day for 8 days.

Considering the contradictory results of these studies, it can be concluded that the intensity of electric currents induced by magnetic fields depends on the conductivity, shape and size of the tumor. Differences in vascular network and structures of intranetwork fluid can change the induced currents. Therefore, if a polar drug is of physiological pH, it will interact with induced current or will be driven by the current. These different results are possibly due to differences between pulse durations and tumor types.

Effect of Sinusoidal 15-mT Magnetic Field on ECT and EP

Our results show that there was no significant difference between the ECT and EP groups with combined magnetic field as well as the ECT with EP groups, while magnetic fields per se could reduce tumor growth.

The reduction of tumor angiogenesis and growth using a 15-mT, 120-pulses/s magnetic field indicates that the magnetic field (15 mT, 50 Hz) with the same exposure durations and protocol may reduce angiogenesis and delay regrowth of treated tumors with also a low-intensity ECT (William et al. 2001).

Ruggiero et al. (2005) used a static magnetic field with an intensity of 200 mT for 3 h on the membrane of chick embryos and demonstrated reduction of angiogenesis. Since the produced magnetic field by a rectified current is quasi-static and the magnetic field used by Williams was rectified (Tofani et al. 2003), it can be concluded that the static property of the field is probably the main cause of its effectiveness on angiogenesis.

In previous studies, the effect of magnetic fields with an intensity on the order of microteslas or teslas was evaluated and survival of the cells was demonstrated. Radiofrequency modulation of a unipolar pulse reduced cell death and cell membrane permeabilization after EP (Jordan et al. 2004). In another study, Mansourian et al. (2013) reported that the cancer cell apoptosis in treatment groups using microtesla magnetic field exposure combined with ECT was lower than that in the ECT group. Also, Chen et al. (2010) demonstrated that EP efficiency can be practicably optimized by inducing pulsed AC magnetic fields using a transcranial magnetic stimulation system.

On the contrary, our results show that the 15-mT magnetic field has no effect on ECT efficiency. Nevertheless, more in vitro and in vivo studies are yet to be carried out. Evaluation of angiogenesis in this combined modality seems to be more informative.

Conclusion

Based on our results, the 50-Hz sinusoidal magnetic field per se can be considered as an effective approach to diminish the tumor growth rate, whereas it did not affect the durability of the EP and ECT treatments. More experiments are needed using a variety of magnetic field parameters in order to develop a better and more effective protocol.

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