ORIGINAL ARTICLE

The Effect of the Shape of Magnetic Field on the Viability of Endothelial Cells

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Abstract

Purpose: Magnetic field is one of the effective and non-invasive modalities on biology and angiogenesis. Studies on the effects of magnetic fields on angiogenesis showed that the shape of the magnetic field could potentially affect angiogenesis. Therefore, this study aimed to control the frequency, intensity, and duration of exposure of magnetic field while investigating the effect of the shape of the magnetic field on the viability of Human Umbilical Vein Endothelial Cells (HUVECs).

Materials and Methods: The HUVECs were exposed to various shapes of 50 and 60 Hz magnetic fields with intensities of 0.5 and 1 mT in acute and chronic exposure regimes. The viability of HUVECs was assessed via MTT assay.

Results: Results showed that the sin type 50 and 60 Hz magnetic fields are more effective in decreasing the viability. The rectified 100 and 120 Hz with 1 and 0.5 mT could increase and decrease the viability compared with 50 and 60 Hz, respectively.

Conclusion: It can be concluded that the shape of the magnetic field can be an effective factor in biology and must be controlled to have a reliable response.

Keywords: Angiogenesis; Endothelial Cell; Magnetic Field; Shapes of Field.

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1. Introduction

As you know angiogenesis is sprouting new capillaries from the old ones caused by some angiogenic factors such as Growth Factors (GFs), hypoxic conditions, and Extracellular Matrix (ECM) components, specifically adhesion molecules [1]. Angiogenesis is an important factor in the tumor growth process and also in many physiological problems like improving the wound healing process [1, 2]. So, finding some ways to control angiogenesis can be an effective step in modifying the clinical conditions. The endothelial cells are the most important and the basic component of the capillary composition. So, if one factor affects endothelial cells viability and proliferation, it can affect the whole process of angiogenesis. Therefore, assessing the endothelial cells' viability is one of the most and common tests for assessing the effect of external chemical or physical agents on angiogenesis.

There are many pharmaceutical methods for increasing and decreasing angiogenesis [3], but nowadays, scientists are finding non-invasive or less-invasive methods to help the patients. According to the results of studies on the effects of magnetic fields on biological processes like accelerating wound healing [2], increasing the local microcirculation [4] and also accelerating the bone fracture healing process [5], increasing single and double-strand DNA breaks [6-10], promoting or preventing tumor growth [11-14], increasing the viability of endothelial cells [15], etc., it can be revealed that various magnetic fields could be a non-invasive modality in medicine.

However different results from different exposure conditions and parameters have been reported. Magnetic fields have some parameters like frequency, intensity, time of exposure, and also the shape of the field like static, sinusoidal, triangle, square, pulsed, and also rectified full-wave and half-wave shapes that can change the results [16]. By the way, some studies reported different biological effects for various Extremely Low Frequency (ELF) magnetic fields (in intensity and frequency, and exposure time) [11, 17-19] but, there is a lack of study on the effectiveness of various shapes of magnetic fields on biological processes and also angiogenesis. Peng et al. reported an angiogenesis-increasing effect of the pulsed magnetic field with 15Hz, 1.5mT, and 30Hz, 3mT for 45min per day on rats. They found that capillary densities were significantly elevated in the 15Hz and 30Hz Pulsed Electromagnetic Field (PEMF)-treated groups as compared to those in the control group [20]. Ashdown et al. reported that a pulsed magnetic field with an intensity of 20 mT in frequencies of 50 and 385 Hz could increase cytotoxicity signal compared to basal protease release by resting control cells at room temperature over the same period (10 min) [21].

Based on the literature, it could be extracted that the shape of the magnetic field could potentially affect angiogenesis [14, 15, 22, 23]. Nevertheless, because of the discrepancy among the parameters and shapes of the applied magnetic fields in various studies, it would be too hard to conclude the effect of the shape of fields on angiogenesis. So, there is a lack of study on the biological effects of the various shapes of magnetic field.

The Human Umbilical Vein Endothelial Cells (HUVECs) could demonstrate stimulation-dependent angiogenesis [24] and they can use for studying hypoxia, inflammation, oxidative stress, response to infection, and both normal and tumor-associated angiogenesis [25].

Therefore, the novelty of this study is control of the frequency, intensity, and duration of the magnetic field while investigating the effect of the shape of the field on the viability of HUVECs to be more conclusive and reliable.

2. Materials and Methods

2.1. Exposure System

Two Leybold Helmholtz coils (320 turns, 13.5 cm coil diameter, 6 Ω resistance) were used for producing the sinusoidal, square, triangle, and rectified sin full-wave with intensities of 0.5 and 1 mT. The power supply was a signal generator connected to a sound amplifier. A diode bridge between the amplifier and the coils was used for converting the sin wave to rectified full-wave. The shapes of fields were controlled by oscilloscope and the intensities of magnetic fields were measured by a Tesla-meter (Axial probe of Leybold Didactic GMBH Teslameter 516-62).

2.2. Cell Culture and Preparation

The HUVECs (prepared from " Stem Cell Research Center " at Tabriz university of medical sciences) were cultured in Dulbecco's. Modified Eagle Medium (DMEM), containing 10% fetal bovine serum, and incubated in 5% CO_2 at the temperature of 37°C. They were seeded in 35 mm Petri Dishes (15000 cells/dish) and incubated for a few hours. Then they were placed on a holder between coils and exposed to magnetic fields (Figure1).

Figure 1. Experimental exposure set-up to study the effect of magnetic field on the viability of HUVECs

2.3. Experimental Groups

The HUVECs in 12 experimental groups were exposed with 1 and 0.5 mT intensity magnetic fields in the frequency of 50 and 60 Hz and sinusoidal, square, and triangle shapes of fields. There were 4 groups with rectified full wave 100 and 120 Hz with 1 and 0.5 mT intensities, too. The exposure protocol was in two phases, acute exposure regime (only one-day exposure on the 5th day after seeding the cells in Petri Dish) and chronic exposure regime (successive five days exposure just after seeding the cells in Petri Dish). The exposure duration of cells was 10 min/day and after the last exposure, they were kept in the incubator for about 72 hours. There were sham groups for all types of exposures.

2.4. Test of Viability of HUVECs

The test of the effects of magnetic fields on the viability of HUVECs was MTT assay. For this test, almost three days (72 hours) after the final exposure of HUVECs, fresh cell culture medium (300 µl/dish) was added to the Petri Dishes. Then, 30 µl of MTT (5 mg/ml) was added to the dishes. After incubating the cells at 37°C for four hours, the residual medium was ejected and 300 µl of dimethyl sulfoxide was added to each dish to solve the violet crystals.

After transferring 200µl of the violet solution of each dish to the 96-well plate, the Optical Density (OD) was measured using a Multimode Microplate Reader (Bio Tek Instruments, Inc., USA) with a 570-nm filter. The final data of the viability of HUVECs were defined as mean values of ODs of each group and reported as a percentage of viable treated HUVECs in comparison with the untreated control cells.

2.5. Statistics

The experiments were done in three-time repetition and the means ± SD were shown as bar graphs. After analyzing the normality of the data by SPSS V.21 (SPSS Inc., Polar Engineering and Consulting), the Analysis Of Variances, (ANOVA) and also t-test were run to compare the differences between the treatment groups. In this study, a P value of less than 0.05 was considered statistically significant.

3. Results

3.1. The Effect of Shapes of the Magnetic Field in 50 and 60 Hz Frequencies on the Viability of HUVECs

The comparison between the effects of different shapes of sin, square, and triangle of 50 and 60 Hz magnetic field on the viability of HUVECs showed that in the intensity of 1 mT, the sin type 50 and 60 Hz magnetic fields are more effective than square and triangle types. The sin 50 and 60 Hz magnetic fields in the acute exposure regime could significantly decrease the viability of endothelial cells ($p<0.05$). There was no significant effect for chronic exposure regimes.

However, when the intensity of magnetic fields was 0.5 mT, none of the chronic and acute exposure regimes of any shapes of magnetic fields (sin, square, and triangle) had significant effects on the viability of endothelial cells (Figure2).

3.2. The Effect of Rectifying the Magnetic Fields of 50 and 60 Hz Sin Waves on the Viability of HUVECs

Moreover, the comparison between the effects of sin 50 Hz and 60 Hz and rectified 100 and 120 Hz magnetic field (respectively) in two intensities of 1 and 0.5 mT

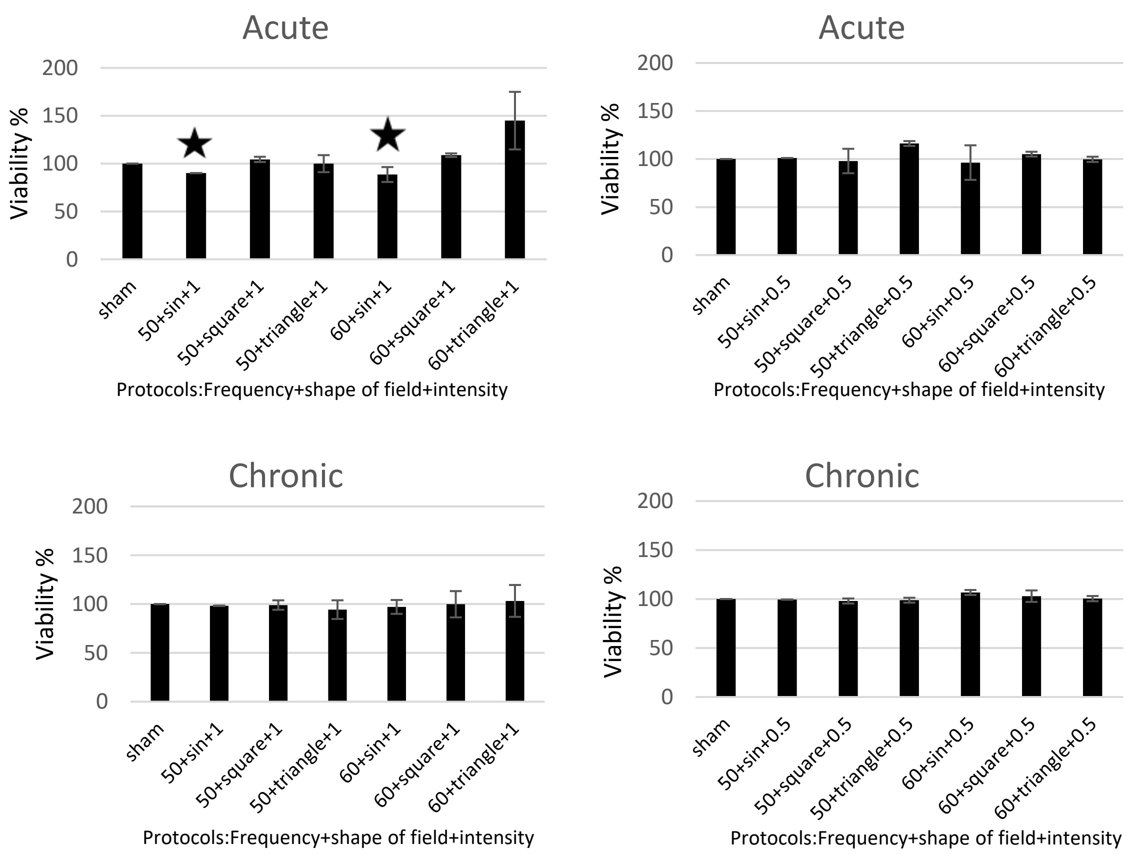

Figure 2. Charts show ths percentage of viable treated HUVECs in comparison with the untreated control cells affected by the 50, 60 Hz magnetic field with 1 and 0.5 mT intensities, respectively, a & b for acute exposure regime and c & d for chronic exposure regime (mean ± SD)

and both of the acute and the chronic exposure regimes showed that the rectified 100 Hz with 1 mT intensity and for acute regime could significantly increase the viability of HUVECs ($p<0.05$). There were not any significant differences between other treatments.

For rectified 120 Hz magnetic field the results have been changed completely. There was a significant decreasing effect on the viability of HUVECs for the group with rectified 120 Hz, 0.5 mT intensity, and chronic exposure regime compared with 60 Hz sin wave magnetic field ($p<0.05$) (Figure3).

4. Discussion

There are many modalities for increasing and decreasing angiogenesis as an important path for growing the tumors

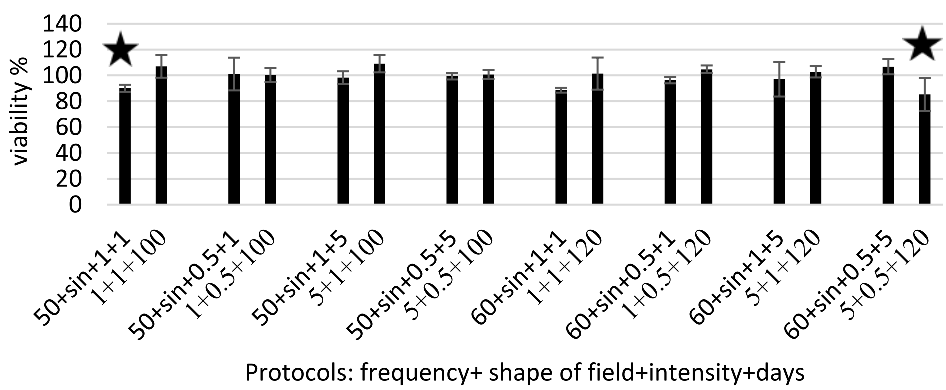

Figure 3. The comparison between the percentage of viable treated HUVECs in comparison with the untreated control cells in sin waves (50, 60 Hz) and rectified waves (100, 120 Hz) of the magnetic field with different intensities 1, 0.5 mT in two acute and chronic exposure regimes (mean ± SD)

and also for improving the wound healing process [3]. Some of these treatments like conventional chemotherapies have toxicity and also drug resistance problems and also some other side effects [3, 26]. Nowadays, there is an interest in assessing external factors like electromagnetic fields on angiogenesis. According to the previous studies done for assessing the effects of magnetic fields on biological processes, it can be revealed that various magnetic fields could be a non-invasive external modality in medicine, but there is no unique rule or instruction for choosing the parameters of fields for each application [7, 11, 22, 27-30]. Biological windows effects of magnetic fields because of their frequency and amplitude make magnetic fields more unpredictable [31, 32]. There is no logic for increasing the effect by increasing the frequency or amplitude of the magnetic field.

The results of this study showed that there is a significant decrease in the viability of HUVECs underexposure of sin 50 and 60 Hz magnetic field in the acute regime and with the intensity of 1 mT. It is in contrast with Mahna et al.'s (2016) study that showed an increase in the viability of HUVECs underexposure of sin 50 Hz magnetic field with an intensity of 46 µT (acute regime and 10 min exposure) [15]. So, one more time we can confirm the existence of the amplitude-window effect of magnetic fields on biology.

Another effective factor of various results of magnetic fields is their shapes. Considering the Fourier series of square, triangle, rectified full sin wave and sin wave reveals that there are different frequency harmonics with different coefficients in them. This reality can be an effective factor in their different manner with HUVECs. The triangle and square waves have odd harmonics of fundamental frequency but the rectified full-wave has both odd and even harmonics (Figure4) [33]. The results showed that 50 and 60 Hz sin waves and also the rectified sin full-wave 120 Hz could decrease the viability of HUVECs. Another significant result was an increase in the viability of HUVECs in the 100 Hz rectified sin full-wave group. But there was no significant effect on the viability by square and triangle exposures. So, it can be revealed that the fundamental frequency and its even harmonics are more effective than odd harmonics.

Controversial results of rectified 100 Hz and 120 Hz magnetic fields, in this study, can have important applications in the treatment of cancer by various modalities. For example, increasing the angiogenesis as an aid to re-oxygenation of the hypoxic areas of the tumor and also

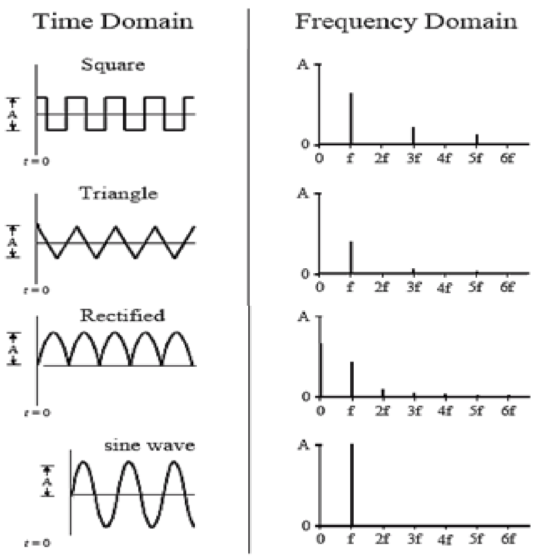

Figure 4. Time-domain and frequency-domain graphs of the square, triangle, rectified, and sin waves

increasing the radio-sensitization through radiotherapy treatment sessions can be an important modality in tumor therapy [34]. For this purpose, it can be likely beneficial treatment to expose the tumor to 100 Hz rectified sin full-wave magnetic field in an acute regime as an effective non-invasive treatment. Furthermore, for decreasing the angiogenesis to prevent tumor growth, probably it can be proposed to expose the tumor to 120 Hz rectified sin full-wave magnetic field in the chronic regime. This latter result is in agreement with Cameron's study in 2001 and 2014 that the 120 Hz rectified magnetic field with an intensity of 15 mT in a chronic regime could decrease angiogenesis and also decrease the tumor size in mice [11, 14].

Also, the result of this study can be in agreement with Henry's study in 2008. A review of the literature reveals that a static magnetic field with an intensity of 23 Gauss (2.3 mT) can improve the rate of wound healing in vivo [29]. Angiogenesis is one the most important factor in the wound healing process [2], so it can be related to the effect of magnetic field on angiogenesis. By the way, the results of this study showed that the 100Hz rectified magnetic field with an intensity of 1mT could increase the viability of the endothelial cells and probably improve the angiogenesis and also the wound healing process. So, replacing the use of the long-term static 2.3 mT magnetic field with 100Hz rectified magnetic field (1 mT) for 10 min in an acute regime for the wound healing process could be a good choice.

5. Conclusion

The results of this study and other similar studies on the effects of magnetic fields on biology showed that the shape of the magnetic field is one of the critical factors in its biological effects. So, we need to do more studies to define the best ones for use in various clinical conditions and have a reliable response.

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