The Effect of 217 Hz Magnetic Field of Cell Phone with Different Intensities on Apoptosis of Normal and Cancerous Cells Treated with Chemotherapy Drug

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Abstract

Background: According to the increasing development of home and business electronic equipment in today's world, the biological effects of ELF magnetic fields have been studied at two molecular-cellular and animal-human levels. Considering the therapeutic viewpoint of this study regarding the effects of low-frequency fields of mobile phone, the effect of acute exposure to this field on chemotherapy will be studied.

Materials and Methods: In this experimental study, based on measurement of the intensity of the magnetic fields from mobile phones in another research, flux densities of magnetic field of 159.44, 93.25 and 120μ tesla with frequency of 217Hz was generated in magnetic field generator system, and the apoptosis level in K562 cancer cells and healthy cells of lymphocytes was assessed after exposure to field using flow cytometry method. This evaluation method was also performed for the cells treated with bleomycin after exposure to this field.

Results: 217 Hz magnetic field exposure significantly increases the rate of apoptosis percentage (p < 0.05) in K562 cancer cells and in two intensities of 120 and 159.44μ tesla compared to the control group, but such effect is not observed in lymphocyte cells. Bleomycin-induced apoptosis percentage following exposure to the mentioned magnetic field shows no significant difference compared to the group of treatment with drug and without field exposure. This lack of significant difference is observed between the groups of drug after field exposure and field alone as well as between groups exposed to field and groups treated with bleomycin.

Conclusion: Study results showed that 217 Hz magnetic field of mobile phone can induce apoptosis on cancer cells, but it has no effect on healthy cells. Thus, in order to use mobile phone as an effective factor in their treatment, some studies should be conducted at animal-human level.

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Introduction

Magnetic fields generated by mobile phones and electrical equipment have effects on biological functions, which has attracted the interest of many scientists to study on this field [1-3]. One of these effects, which can be effective in the treatment of many diseases including cancer, is cell death or acceleration of cell death by these fields during the chemotherapy [4-7].

Among the existing mechanisms for cell death, apoptosis is a very good mechanism of cell death in treatment of cancer, because apoptotic cells are eliminated from the environment by phagocytosis and without causing decay [8]. Several studies show that ELF (extremely low frequency) magnetic fields can cause cell death by apoptosis induction [9]. One of these ELF fields is emitted from mobile phones, which has been considered by so many studies due to the increasing use of this device by users. GSM System (Global System for Mobile) is now one of the most common wireless communications technologies using time-division scheme in which causes the phone battery flow to be established for short consecutive transmissions and changes in flow to generate ELF pulsed magnetic fields whose biological effects have been recently considered [10-11].

The examination of the effects of pulsed magnetic fields shows that these fields can change the efficiency of anticancer drugs, but their interaction mechanism in making this change is unknown [12]. These studies have variously evaluated the absorption level and effect of different drugs at the exposure to fields. For example, the increase of effect of Adriamycin by pulsed magnetic fields in the study of Miyagi et al is of these studies [13]. However, stoppage of deterrent action of tamoxifen in breast tumor cells (MCF-7) by fields with intensity of 1/2μ tesla and frequency of 60 Hz is a result obtained in another study [14].
Bleomycin is an anti-cancer drug which is extensively used in chemotherapy. This drug enters the cell through mechanism of endocytosis and via receptors [15]. Thus, the positive or negative effect of ELF magnetic field radiations of GSM mobile phones in generating apoptosis of cells can be studied using this drug in chemotherapy treatment. Since, on the one hand, some of people who are exposed to mobile radiations are cancer patients and on the other hand, due to the above points, magnetic fields affect cell death and the resistance of tumor cells to anticancer drugs, this study intends to investigate and review the effect of ELF magnetic field of mobile phone on apoptosis of blood cancerous cells K562 (Erythroleukemia) and healthy lymphocyte cells in human peripheral blood. Furthermore, this type of cell death as a result of treatment with anticancer bleomycin following the acute exposure to these fields is researched, so that a proper perspective can be presented in order to provide useful advice to these patients to use their mobile phones as an effective factor in their treatment.

Materials and Methods

This is an experimental study which has been conducted on the cancer cell lines of K562 and normal lymphocyte cells.

Erythroleukemia K562 cells: suspension K562 cells in the medium containing 1640 RPMI and 10% fetal bovine serum (FBS) were cultured in filtered flasks and were kept in humidified incubator of 37°C with 5% CO₂. The medium of cells was replaced with fresh medium every two days.

Extraction and isolation of lymphocytes: To extract lymphocytes, first the required amount of blood was prepared by heparinized syringe and after being diluted by PBS (phosphate buffered saline) it was poured on ficoll (blood/ficoll ratio 2:1). This was done so that the blood would not get mixed with ficoll. After that, it was centrifuged for 20 minutes and the white layer between the two layers of red and yellow, was extracted. The extracted layer was washed by PBS several times to remove ficoll from cell environment and ultimately, lymphocytes cells existing in this layer were tested.

Square pulses were received from a 217-Hz signal generator and were delivered to a 200 watt audio amplifier with output current of 5A. The amplifier output was applied to double Helmholtz coils to create a uniform magnetic field. These coils included a pair of circular coils, which were made of coated copper wire with a diameter of 0.78 mm and 287 rounds. The diameter of each coil was 20 cm and the space between them was equal to the coil radius.

The field shape was studied in each test using Biolab software (made in Iran related to the device). In this pulse shape square, width of each pulse was considered 0.577 milliseconds (interval of two pulses is 4.61 ms) and its number was considered 217 pulses per second. Magnetic flux density in the space between the two coils was also measured with magnetic field meter (TES 1394). Magnetic flux density had three intensities of 159.44, 120 and 93.25 μtesla [16]. After that, cells were exposed to these intensities on the middle axis in the space between the two coils.

Radiation and cell treatment: first, 1.5 × 10⁷ cells per ml were poured in small laboratory petri dishes (35 mm). Then the cells were irradiated in separate groups for 10 minutes (according to Fig. 1) to assess the effects of radiation on apoptosis. In other groups where the effect of magnetic field exposure was evaluated in chemotherapy, the cells were treated with bleomycin of concentration of 0.1 M [17] after exposure to magnetic field for 10 minutes. After that, adding the medium to cells, all cell groups were kept in a humidified incubator of 37°C with 5% CO₂ for 24 hours.

Figure 1. Schematic view of the positioning of cells among the coils producing the magnetic field

Apoptosis measurement: After 24 hours incubation of cells, they were centrifuged (5 min at 1200 rpm) and their medium was discarded. Adding 500 µl of Binding buffer and then annexin and propidium iodide (5 µl of each) the cells were placed in the dark for 5 min, and finally, the number and percentage of apoptosis and their necrosis (Fig. 2) was determined using flow cytometry device (Bicton Dickinson; BD).

All tests were repeated three times on different days and the results obtained in different tests are presented as mean ± standard deviation. Statistical analysis was performed using independent t-test and through SPSS-16 software. Normal nature of data was evaluated using Kolmogorov-Smirnov test and it was shown that data in the different groups were all normally distributed. p<0.05 was regarded as significance level of tests.

Figure 2. An example of flow cytometry graphs and percentages of apoptosis and necrosis
Effect of Magnetic Field on Chemotherapy

Mansourian M et al.

Results

At the beginning of this study, the effect of radiation on cell apoptosis was investigated. Data show that acute exposure of K562 cells to the magnetic field causes significant increase of apoptosis percentage in the intensities of 120 and 159.44 µ tesla compared to the control group (Fig. 3). Apoptosis percentage in this cell line equals 20.54±3.5% in the intensity of 120µ tesla and 18.29±2.9% in the intensity of 159.44µ tesla and apoptosis percentage in the sham group without magnetic field exposure was 5.11±0.91%. However, no significant difference was observed between percentage of apoptosis and sham group in the intensity of 93.25µ tesla. The apoptosis percentage of the lymphocyte cells exposed to magnetic field in no intensity represented the statistically significant difference compared to the control group (Fig. 1).

Further in the present study, the effect of magnetic field exposure was analyzed on apoptosis of cells treated with bleomycin followed by field exposure. The results show that the resistance of K562 cells to Bleomycin drug does not change in any magnetic field intensities. A similar result was obtained for the lymphocytic cells which were treated with Bleomycin after exposure to magnetic fields (Fig. 2). In the groups of drug treatment after field exposure, although the percentage of apoptosis was reduced by increases of magnetic field intensity, this reduction was not statistically significant for each intensity, compared to the other exposure intensities.

Comparison of groups of magnetic field exposure alone and field exposure before treatment with drug showed no significant difference in either cell lines at all intensities. Comparison of apoptosis percent of K562 cells groups and normal lymphocyte cells group, showed that control group and consequently, the other groups in this cell line underwent a greater apoptosis than cancerous cells groups. This difference is caused by the greater sensitivity of lymphocyte cells to the temperature changes, humidity and other physical factors on which the test conditions are inevitably dependent and have influenced the lymphocyte cells.

The comparison between groups of the exposure alone and group of chemotherapy with low dose medication show no significant difference in apoptosis percentage (Fig. 3).
Another finding of this study showed that the effect of ELF fields on cells, suggests that these fields first change the events occurring on cell membrane and then, affect the intracellular performance by transferring consecutive signals [18]. Resistance of lymphocyte cells to fatality caused by ELF field radiation compared to cancer cells is of the results seen also in the other studies. For example, the investigation of difference rate of death in cancer and healthy cells in a study that reviews the effect magnetic field exposure with frequency of 50 Hz shows more apoptosis of cancer cells against longer survival of lymphocyte cells [6]. Another finding confirming this result is the exposure of HL-60 cancer cells and healthy lymphocyte cells to the magnetic field with frequency of 50 Hz and intensity of 45m Tesla, which showed that this radiation for 3.5 hours leads to DNA fragmentation in HL-60 cells, whereas there is no such effect for lymphocyte cells [7]. Thus, the results of this part of study are consistent with the results obtained from other studies.

Resistance of tumor cells to antineoplastic factors is considered a barrier to chemotherapy of cancer cells. The main mechanism of resistance to chemotherapy drugs is multiple drug resistance which reduces the drug level within the cell through P-glycoprotein pump function. This cellular pump takes all drugs out of cell and makes it difficult for cancer chemotherapy [5]. There are several reports indicating that pulsed magnetic fields are able to make changes in the toxicity level of anticancer drugs [19-20]. Results of the second part of this study showed that the effect of bleomycin does not change after exposure to magnetic field. Some studies which have examined the toxicity of medicine after exposure, have achieved the results similar to the results of this study. For example, exposure of MCF-7 cells to the fields with intensity of 1.5 m Tesla and frequency of 25 Hz has not changed the efficiency of methotrexate effect in this cell line and thus, has had no effect [21].

There are also other studies which have had different results. For example, the study of Omote which showed that exposure of cancer cells to pulsed magnetic field immediately after entering methotrexate or mitomycin C, increases anti-tumor activity of these drugs [4]. The increase of effect of daunorubicin on cancer cells through exposure of pulsed magnetic fields (with a maximum intensity of 5.25m Tesla, 250 pulses per second) was the same result obtained in another study [5]. Although in these studies, radiation had a positive effect on the drug effects, Harland and Liburdy who have reviewed the effect of magnetic field with intensity of 1.2µ Tesla and frequency of 60 Hz on the efficiency of tamoxifen effect, observed that this radiation stopped the deterrent action of tamoxifen on breast tumor cells (MCF-7) [14].

There are differences between present study and other studies. For example, In all mentioned studies,
magnetic field radiation has been performed during or after drug entry, while in this study, radiation was performed before drug treatment. They have also used different intensities and frequencies of pulsed magnetic fields. The type of cell and drug are chosen differently. These differences may reflect the fact that factors such as drug type, cell line type, the type and duration of irradiation by field as well as intensity and frequency of the field will be able to affect the impact of pulsed magnetic fields on the toxicity of anticancer drugs. Actually, this study has only considered ELF magnetic fields which modulate mobile phone RF waves; more complete results may be achieved using RF waves modulated by ELF magnetic fields.

In conclusion, the results showed that ELF magnetic fields from mobile phones can lead to cancer cells death, but they have no effect on healthy lymphocyte cells. In addition, these fields make no change in the toxicity level of bleomycin in cancer and healthy cells. Therefore, given that this study is conducted in in-vitro environment and results are obtained at the cellular level. It is necessary to conduct further research in both animal and human levels in order to provide appropriate advice to cancerous patients to use mobile phones as effective factor in their treatment.

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