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ORIGINAL ARTICLE

# Effects of Static Magnetic Fields on Viability and Apoptosis in Normal and Cancerous Cells

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## ABSTRACT

***Background:*** The influence of static magnetic fields (SMFs) on living organisms has been the topic of considerable interest for many years. However, the exact mechanism of SMFs is still unclear. Regarding this, the present study was conducted to evaluate the possible relationship between SMF and cancer treatment and also determine the possible effects of co-treatment with anticancer drugs on normal and cancerous cells.
***Materials and Methods:*** The effects of 10 mT SMF on cell death (sub-G1 and apoptosis/necrosis) were investigated using the flow cytometric methods. The SMF was utilized both in the presence and absence of cisplatin as an anticancer agent in the HeLa cell line and Hu02 as cancerous and normal cell types, respectively.
***Results:*** According to the results, the SMF exposure caused an increase in cell death in Hu02 24 h post-treatment and HeLa cell line both 24 and 48 h after treatment. Moreover, the co-treatment of SMF and cisplatin led to an enhancement in cell death within the first 24 h of treatment via necrosis as well as early and late apoptosis. On the other hand, this co-treatment resulted in the reduction of cell death in the first 48 h post-treatment in both cell types.
***Conclusion:*** Even though 10 mT intensity of SMF was not a high value, it was able to change the cell function and structure, which in turn led to a change in apoptosis rate. The findings of the present study might indicate that the SMF could decrease the cell death. Nevertheless, it is essential to perform more investigations to find the exact related mechanisms.

***Key Words:*** Apoptosis, Cisplatin, Flow cytometery, HeLa cell line, Static magnetic field, Sub-G1

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## INTRODUCTION

The influence of static magnetic field (SMF) on biological system has been the topic of considerable interest for many years [1]. The moderate intensity of SMF (i.e., 1 mT-1 T) is not ionizing and do not exert any thermal effects on the biological systems. This intensity has potentially deep penetration into different tissues. Furthermore, it affects the macromolecule performance and behavior, such as calcium influx, ion transfer, membrane potential differences, radical production, life span, and eventually oxidative stress [1, 2].

The exact mechanism of SMF is still unclear due to differences in the intensity, cell, and time exposure. One of the notable findings of SMF is changing the rate of cell death (apoptosis) [3, 4] and viability. To find out these parameters on cells, the rate of cell death and amount of DNA damage have been evaluated in the presence and absence of SMF and various anticancer drugs [2].

Cisplatin has been used as the first-line therapy for several cancers. This medication can induce apoptosis following DNA damage like many other chemotherapeutic drugs [5]. Anticancer drugs have cytotoxic effects on both normal and cancerous cells. To reduce the associated side effects, a variety of unified treatment is required. Magnetic fields presumably provide a new strategy for the effective treatment of cancers and reduce the side effects of antineoplastic drugs [6].

With this background in mind, the present

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study was conducted to evaluate the possible relationship between SMF and cancer treatment and determine the possible effects of co-treatment of SMF and an anticancer agent on the normal and cancerous cells.

## MATERIALS AND METHODS

### *Chemicals*

For the purpose of the study, normal skin fibroblast (Hu02) and HeLa cell line were obtained from the Iranian Biological and Genetics Reserves Center (2015). Cell culture reagents, Dulbecco's Modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were obtained from Gibco (Great Britain). Furthermore, penicillin strep-tomycin and trypsin-EDTA were acquired from Bioidea (Bioidea Company,Tehran, Iran). The MTT salt, Triton X-100, and propidium iodide (PI) were obtained from Sigma-Aldrich Chemicals. Finally, ribonuclease A and dimethyl-lsulphoxide were acquired from Fermentas-Life Sciences (Fermentas-Life Sciences, Vilnius, Lithuania) and Merck (Darmstadt, Germany), respectively.

### *Cell culture*

The Hu02 and HeLa cell line were grown in DMEM supplemented with 10% FBS, 1% penicillin (100 U/mL), and streptomycin (100 μg/ml). Subsequently, they were incubated at 37±0.5°C in an atmosphere containing 5% $CO_2$ and 100% humidity. In order to cover the 80% surface area of the flask by the cultured cells, the medium was replaced with the fresh one every 48 h before the implementation of any treatment.

### *Magnetic field exposure system*

A locally designed generator was executed for SMF production. This generator was made from two wire coils (3.0 mm in diameter, 1 km in length, and 40 kg in weight) with a heat resistance up to 200°C. These two coils conducted the magnetic field through two iron blades with 1-meter height and a cross section of 10 $cm^2$. The magnetic field was measured by a teslameter (13610.93, PHYWE, Gottingen, Germany). In addition, the presence of any pulsation was tested by a 60 MHz oscilloscope (8040, Leader Electronics Co., Yokohama, Japan).

The electrical power was provided using a 220 V AC power supply equipped with a variable converter and a single-phase full-wave rectifier. The switching power supply could apply a direct current up to 16 V and a potential difference up to 50 V to generate the moderate intensity of magnetic field. The field between the iron blades was measured by a telemeter. For cooling off the system, a gas chiller, consisting of an evaporator, an engine, a condenser, and refrigerant gas, was utilized. This system was equipped with a rectangular cubic (23×20×50 $cm^3$) incubator using three sensors for controlling the temperature, humidity, and $CO_2$ pressure of the air surrounding the flasks. To have a uniform SMF inside the exposure unit, an electronic board was used to stabilize the system.

### *Drug and static magnetic field treatments*

In this study, we employed two cell types. Each cell type was divided into four groups. The first group of cells was control receiving no treatment, and the second group was exposed to the SMF without any interruption. Additionally, the third group was treated with 50% inhibitory concentration (IC50) of cisplatin, and the last one was provided with a combination of both treatments. This study was carried out in two separate times (i.e., 24 and 48 h post-treatment), and three different SMF intensities, the most influential of which was 10 mT.

### *In vitro toxicity*

The standard MTT assay was performed to compare the cytotoxicity of cisplatin with different series of drug concentration to HeLa cell line and Hu02. Multiple doses of cisplatin (with concentrations of 1-100 μl/mL) were utilized. The IC50 values obtained from dose response curve to cisplatin were 3 and 12 μg/mL for HeLa cell line in the first 24 and 48 h of the treatment, respectively. These values were 6 and 22 μg/mL in Hu02 in the first 24 and 48 h of the treatment, respectively.

### *Sub-G1 analysis*

For flow cytometric measurement, a LSR II flow cytometer (Becton Dickinson, Accuri™, US) was employed. The cells were fixed at 4˚C for 24 h prior to the analysis using Win MDI, version 2.8. A typical cell cycle stain (i.e., PI) only staining the dead cells, intercalated into the cellular DNA. The PI fluorescence was collected with a 575/25 nm band pass filter, orange-red fluorescence (FL2) after linear amplification. The flow cytometric data was stored according to a standard format [7].

### *Apoptosis/necrosis measurement*

Apoptosis/necrosis was evaluated by the flow cytometry using Annexin V-FITC Apoptosis Detection Kit (eBioscience, USA). Briefly, the cells were stained with Annexin

V-FITC and PI according to the manufacturer's instruction. Early apoptotic, late apoptotic, necrotic, and normal cells were identified using Annexin+/PI -, Annexin+/PI+, Annexin -/PI+, and Annexin -/PI -, respectively [8]. The flow cytometric measurements were performed using a LSR II flow cytometer (Becton Dickinson).

### *Statistical analysis*

All the experiments were carried out with at least three independent repetitions. Statistical analysis was performed using the Student's t-test with 95% confidence interval. All data were presented as mean and standard deviation. P-value less than 0.05 was statistically significant.

## RESULTS

### *Sub-G1 analysis*

Cisplatin treatment led to a significant decrease in the population of HeLa cells in sub-G1 phase both 24 and 48 h post-treatment. On the other hand, SMF had no significant changes in both cell types (tables 1 and 2). The combination of SMF and cisplatin treatments resulted in the reduction of sub-G1 population in Hu02 (48 h after treatment) and HeLa cells (24 and 48 h post-treatment). However, the results were not significant in this regard.

### *Apoptosis/necrosis analysis*

Tables 3 and 4 present a set of representative results of this measurement in both cell types and the percentage of cells in each phase. The combination of cisplatin and SMF elevated the extent of necrosis and early apoptosis of HeLa cell line in the first 24 h after treatment. Nevertheless, the rate of the late apoptosis reduced within this period of time. However, our data did not show any significant changes in Hu02 in the first 24 h post-treatment.

**Table 1.** Percentage of HeLa cells distributed in Sub-G1 phase

| | 24 h post-treatment | 24 h post-treatment |
|---|---|---|
| **Control** | 1.01±0.44 a | 2.23±0.23 a |
| **MF** | 3.17±0.43 ab | 9.77±0.39 ab |
| **Drug** | 27.06±3.05 c | 29.00±0.95 bc |
| **MF+Drug** | 10.07±1.08 d | 14.74±0.77 bd |

MF: magnetic field, * Different letters vertically show the significant differences in cell cycle phases; P<0.05 is significant, * Errors indicate the standard deviation for four independent experiments

**Table 2.** Percentage of Hu02 cells distributed in Sub-G1 phase

| | 24 h post-treatment | 48 h post-treatment |
|---|---|---|
| **Control** | 0.85±0.11 a | 1.13±0.18 a |
| **MF** | 1.63±0.39 b | 1.35±0.13 ab |
| **Drug** | 3.85±0.26 bc | 4.94±0.20 ac |
| **MF+DRUG** | 6.68±0.43 bd | 3.41±0.21 ad |

MF: magnetic field, * Different letters vertically show the significant differences in cell cycle phases; P < 0.05.
* Errors indicate the standard deviation for four independent experiments

## DISCUSSION

Our data demonstrated that SMF exposure caused an increase in the cell death of HeLa cell line (both 24 and 48 h post-treatment) and Hu02 (24 h after the treatment). The co-treatment of SMF and cisplatin led to the

**Table 3.** Percentage of HeLa cells in different stages of viability

| | Viable | | Necrosis | | Early | | Late | |
|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 |
| **Control** | 93.88±1.03 a | 91.65±0.65 a | 4.18±0.31 a | 6.98±0.31 a | 1.16±0.76 a | 0.62±0.27 a | 0.76±0.34 a | 0.73±0.25 a |
| **MF** | 92.25±1.30 ab | 84.96±2.15 b | 6.64±1.0 b | 13.55±1.72 b | 0.95±0.33 b | 0.46±0.36 ab | 0.32±0.16 b | 1±0.44 b |
| **Drug** | 79.64±1.6 ac | 78.76±3.1 bc | 18.07±1.06 bc | 15.11±1.52 bc | 0.46±0.22 bc | 2.52±0.78 ac | 2.08±0.64 c | 3.25±1.11 c |
| **MF+Drug** | 78.99±1.8 ad | 88.7±2.85 bd | 20.08±1.61 bd | 13.73±2.08 bd | 0.48±0.16 d | 1.12±0.42 ad | 0.44±0.18 bd | 3.44±0.99 cd |

MF: magnetic field, * Different letters vertically show the significant differences in cell cycle phases; *P* < 0.05 is significant * Errors indicate the standard deviation for four independent experiments

**Table 4.** Percentage of Hu02 cells in different stages of viability

| | 24 h post-treatment | 48 h post-treatment | 24 h post-treatment | 48 h post-treatment | 24 h post-treatment | 48 h post-treatment | 24 h post-treatment | 48 h post-treatment |
|---|---|---|---|---|---|---|---|---|
| **Control** | 93.35±0.39 a | 91.15±1.43 a | 4.29±0.29 a | 5.75±0.72 a | 0.76±0.4 a | 1.11±0.28 a | 1.58±0.14 cd | 1.98±0.85 a |
| **MF** | 89.12±1.31 b | 91.46±1.24 ab | 6.24±0.37 ab | 4.17±0.27 bc | 2.66±1.34 b | 1.91±1.16 b | 1.96±0.24 b | 2.44±0.34 b |
| **Drug** | 83.2±0.53 ac | 87.11±0.48 bc | 13.66±0.57 c | 11.63±0.53 ac | 0.92±0.12 c | 0.4±0.14 ac | 2.21±0.26 bc | 0.85±0.29 c |
| **MF+Drug** | 81.49±0.56 ad | 88.41±0.32 cd | 16.76±0.61 cd | 9.47±0.34 cd | 0.7±0.14 cd | 0.59±0.12 ad | 1.03±0.2 bd | 1.51±0.25 d |

MF: magnetic field, * Different letters vertically show the significant differences in cell cycle phases; *P*< 0.05 is significant * Errors indicate the standard deviation for four independent experiments

enhancement of cell death in the first 24 h via necrosis as well as early and late apoptosis. Furthermore, the employment of both interventions resulted in the reduction of cell death 48 h post-treatment in both cell types (tables 3 and 4).

There are some evidence indicating that the moderate intensities of SMFs can influence the living organisms. The previous studies investigating the SMF bioeffects have demonstrated that there is no definition of the exact mechanism of action due to differences in cell types, intensities, exposure periods, etc. [2, 9].

Exposure to SMF and anticancer agents (e.g., cisplatin, taxol, doxorubicin, and cyclophosphamide) collectively lead to the enhancement of anticancer permeability [10].

Qi Hao et al. examined the effect of 12 h exposure to 8.8 mT SMF in combination with Adriamycin on the metabolic activity of K562 cell line. They found a significant inhibition of metabolic activity at the presence of SMF [11]. Sabo et al. described the effect of 8.8 mT SMF on the metabolic activity of human leukemia cell line [12]. These reports suggested that the magnetic field exposure had a synergic effect on anticancer agents. However, decrease in cell death induced by SMF has been also reported in the literature.

Miyakoshi et al. revealed an increase in the mutation rate of the cells following exposure to electromagnetic fields (EMF) [13]. The tumor growth in cancer-susceptible mice strain increased after EMF exposure [14].

Our study is in agreement with the mentioned literatures, especially those reporting decreases in cell death. These bioeffects followed by magnetic field exposure vary depending on cell types, intensities, and exposure periods.

Cell death and viability have been reported to both increase and decrease in the previous studies. Despite the large number of the studies reporting the bioeffects of SMF, none of them have clarified the exact mechanism explaining how SMF modulates cell death/viability. There are many reports in this regard; nonetheless, they have utilized only a single type of cells. We believe that it is essential to evaluate the biological response in the normal and cancerous cells together when different treatments are used in a clinical research.

In order to investigate the bioeffects of 10 mT SMF, HeLa and Hu02 were exposed to magnetic field in the presence and absence of an anticancer agent (i.e., cisplatin). Cisplatin is one of the widespread anticancer drugs for various cancer and solid tumor treatments. This drug exerts cellular toxicity through DNA damage, especially double-strand breaks and reactive oxygen species production, which in turn lead to apoptosis [5, 15].

Our findings are consistent with those reported in the literature, indicating that plasma membrane was a target for SMF [10, 16]. The SMF causes an increase in the intracellular adenosine triphosphate (ATP) content [17], which in turn would affect the ATP-dependent transporters, such as copper transporter. This transporter normally resides in trans-Golgi network, but could be directed to plasma membrane, if necessary [18].

The decrease in cisplatin accumulation could be theoretically due to the increase in cisplatin exported by these copper transporters [18]. These mechanisms would cause changes in cell death following the SMF exposure. The SMF may decrease the concentration of $Ca^{2+}$ ion through influencing either the function of Ca-ATPase or modifying the function of $Ca^{2+}$ binding proteins [9, 19]. These hypotheses might explain all bio-effects of SMF, including the modulation of apoptosis and cell death/viability.

## CONCLUSION

Despite the fact that 10 mT intensity of SMF was not a high value, it was able to change the cell function and structure. As the findings of this study indicated, SMF can decrease the cell death. However, it is essential to perform more investigations to explain the cause of these various functions.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## REFERENCES

1. Fiorani M, Cantoni O, Sestili P, Conti R, Nicolini P, Vetrano F, et al. Electric and/or magnetic field

effects on DNA structure and function in cultured human cells. Mutat Res. 1992; 282(1):25–9.
2. Dini L, Abbro L. Bioeffects of moderate-intensity static magnetic fields on cell cultures. Micron. 2005; 36(3):195–217.
3. Tofani S, Barone D, Cintorino M, de Santi MM, Ferrara A, Orlassino R, et al. Static and ELF magnetic fields induce tumor growth inhibition and apoptosis. Bioelectromagnetics. 2001; 22(6):419–28.
4. Tavasoli Z, Abdolmaleki P, Mowla SJ, Ghanati F, Sarvestani AS. Investigation of the effects of static magnetic field on apoptosis in bone marrow stem cells of rat. Environmentalist. 2009; 29(2):220–4.
5. Basu A, Krishnamurthy S. Cellular responses to Cisplatin-induced DNA damage. J Nucleic Acids. 2010; 2010:1-16.
6. Tofani S, Barone D, Berardelli M, Berno E, Cintorino M, Foglia L, et al. Static and ELF magnetic fields enhance the in vivo anti-tumor efficacy of cis-platin against lewis lung carcinoma, but not of cyclophosphamide against B16 melanotic melanoma. Pharmacol Res. 2003; 48(1):83–90.
7. Sarvestani AS, Abdolmaleki P, Mowla SJ, Ghanati F, Heshmati E, Tavasoli Z, et al. Static magnetic fields aggravate the effects of ionizing radiation on cell cycle progression in bone marrow stem cells. Micron. 2010; 41(2):101–4.
8. Wang X, Chen Y, Huang C, Wang X, Zhao L, Zhang X, et al. Contribution of a 300 kHz alternating magnetic field on magnetic hyperthermia treatment of HepG2 cells. Bioelectromagnetics. 2013; 34(2):95–103.
9. Javani Jouni F, Abdolmaleki P, Movahedin M. Investigation on the effect of static magnetic field up to 15 mT on the viability and proliferation rate of rat bone marrow stem cells. In Vitro Cell Dev Biol Anim. 2013; 49(3):212–9.
10. Liu Y, Qi H, Sun RG, Chen WF. An investigation into the combined effect of static magnetic fields and different anticancer drugs on K562 cell membranes. Tumori. 2011; 97(3):386–92.
11. Hao Q, Wenfang C, Xia A, Qiang W, Ying L, Kun Z, et al. Effects of a moderate-intensity static magnetic field and adriamycin on K562 cells. Bioelectromagnetics. 2011; 32(3):191–9.
12. Sabo J, Mirossay L, Horovcak L, Sarissky M, Mirossay A, Mojzis J. Effects of static magnetic field on human leukemic cell line HL-60. Bioelectrochemistry. 2002; 56(1):227–31.
13. Miyakoshi J, Yamagishi N, Ohtsu S, Mohri K, Takebe H. Increase in hypoxanthine-guanine phosphoribosyl transferase gene mutations by exposure to high-density 50-Hz magnetic fields. Mutat Res. 1996; 349(1):109–14.
14. Morandi MA, Pak CM, Garen RP, Caren LD. Lack of an EMF-induced genotoxic effect in the Ames assay. Life Sci. 1996; 59(3):263–71.
15. Wang Q, Zheng XL, Yang L, Shi F, Gao LB, Zhong YJ, et al. Reactive oxygen species-mediated apoptosis contributes to chemosensitization effect of saikosaponins on cisplatin-induced cytotoxicity in cancer cells. J Exp Clin Cancer Res. 2010; 29(1):159–64.
16. Teodori L, Grabarek J, Smolewski P, Ghibelli L, Bergamaschi A, De Nicola M, et al. Exposure of cells to static magnetic field accelerates loss of integrity of plasma membrane during apoptosis. Cytometry. 2002; 49(3):113–8.
17. Shin J, Yoo CH, Lee J, Cha M. Cell response induced by internalized bacterial magnetic nanoparticles under an external static magnetic field. Biomaterials. 2012; 33(22):5650–7.
18. Borst P, Rottenberg S, Jonkers J. How do real tumors become resistant to cisplatin? Cell Cycle. 2008; 7(10):1353–9.
19. Aldinucci C, Garcia JB, Palmi M, Sgaragli G, Benocci A, Meini A, et al. The effect of exposure to high flux density static and pulsed magnetic fields on lymphocyte function. Bioelectromagnetics. 2003; 24(6):373–9.