

Simultaneous application of cisplatin and static magnetic field enhances oxidative stress in HeLa cell line

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Abstract In this study, we reported the effects of simultaneous application of static magnetic field (SMF) and cisplatin as an anticancer drug on the oxidative stress in human cervical cancer (HeLa) cell line and normal skin fibroblast cells (Hu02). The cells were exposed to different SMF intensities (7, 10, and 15 mT) for 24 and 48 h. IC₅₀ concentrations of cisplatin were obtained by MTT assay. The cytotoxic effects of combined treatment were studied by measuring the intracellular reactive oxygen species content using flow cytometric method and estimation of membrane lipid peroxidation by spectrophotometry. Statistical analysis was assessed using one-way repeated measures analysis of variance (ANOVA) followed by Tukey's test. Based on the obtained results, the highest and lowest death rate, respectively, in HeLa and Hu02 cell lines was observed at the intensity of 10 mT. Also, we found that membrane lipid peroxidation in cancer cells is higher than that of normal counterparts. SMF potently sensitized human cervical cancer cells to cisplatin through reactive oxygen species (ROS) accumulation while it had small effects on normal cells. The combination of both treatments for 48 h led to a marked decrease in the viability percentage of HeLa cells by about 89%

compared to untreated cells. This study suggests that conjugation of both physical and chemical treatments could increase the oxidative stress in HeLa cell line and among three optional intensities of SMF, the intensity of 10 mT led to the higher damage to cancer cells in lower doses of drug.

Keywords Static magnetic field · Lipid peroxidation · Reactive oxygen species · HeLa cell line · Cisplatin · Viability

Introduction

The global cancer burden has changed dramatically over time. The number of new cancer cases is expected to rise by about 70% over the next two decades (WHO|Cancer 2016). Worldwide, cervical cancer is one of the most common causes of cancer and death in women. Chemotherapy is a category of cancer treatment. Cisplatin, a platinum-based chemotherapeutic drug, have been used widespread against different human cancers, including lung, testicular, ovarian, cervical cancers, and many other solid tumors (Gottfried and Ramlau 2008; Türk et al. 2008; El-Bialy and Rageh 2013). The anticancer activity of cisplatin is due to its interactions with chromosomal DNA which inhibit transcription, translation, replication, DNA repair, and such fundamental cellular processes (Suo et al. 1999).

Cancer treatments affect healthy tissues or organs. Cisplatin generates the reactive oxygen species, such as superoxide anion and hydroxyl radicals (Masuda et al. 1994; Wozniak et al. 2004) which is closely associated with an increase in lipid peroxidation in some tissues (Antunes 2000; El-Bialy and Rageh 2013). Consequently, several studies have shown the possible synergism between applying simultaneously magnetic field (MF) and administration of chemotherapeutic drugs to reduce the side effects of antineoplastic drug while keeping its therapeutic efficiency (Tofani 2003; El-Bialy and Rageh 2013).

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Moderate intensity of static magnetic field (ranged from 1 mT–1 T) under certain conditions is non-ionizing. It can influence physiological processes in the organisms and affect different aspects of physiological responses such as calcium signaling (Lindström and Lindström 1995; Fanelli et al. 1999), ion transfer, apoptosis (Fanelli et al. 1999; Buemi et al. 2001; Kamalipooya et al. 2015.), events involving generation of free radicals and their life span and eventually oxidative stress (Roy et al. 1995; Teodori et al. 2002); nevertheless, the modes of action remain unknown (Marędziak and Marycz 2014). It seems that different biological factors are modifiers of the cellular response to magnetic fields (Walleczek and Budinger 1992; Schimmelpfeng et al. 1995; Albuquerque et al. 2016).

Among the possible mechanisms of interaction between the magnetic field and cells, the direct effect of magnetic field on molecules with unpaired electrons (free radicals) was considered as the major mechanism (Tofani 2003). Due to the free radical interaction with the variety of biomolecules, the amount of free radicals dramatically increased (Lacy-Hulbert et al. 1998). There are many types of free radicals, but most of them are coming from oxygen atoms. So the most concern in biological systems is related to derived free radicals from oxygen, which known collectively as reactive oxygen species (ROS). A deleterious condition known as oxidative stress occurs when reactive oxygen species levels exceed the antioxidant capacity of the cells (Lindström and Lindström 1995). Damage of macromolecules (proteins, DNA, and lipids) is the consequence of oxidative stress, which leading to genetic mutation and could modulate the rate of apoptosis/necrosis (Tavasoli et al. 2009). The deregulation of apoptosis would be the mechanism through which exposure to MFs alters the risk of tumors.

To improve the therapeutic approaches for cancer treatment, we have investigated the effects of static magnetic field (SMF) in combination with an antineoplastic drug on the rate of cellular damage in human cervical cancer cell line (HeLa) and fibroblast cells. In this study, we exposed the HeLa cells and Hu02 to the different intensities of static magnetic field (7, 10, and 15 mT) and different exposure times (24 and 48 h) to find out whether the SMF affects the amount of reactive oxygen species and cell death or not.

Material and methods

Cell culture Cervical cancer (HeLa) cell line and normal skin fibroblast (Hu02) (mesenchymal) cells were purchased from Iranian Biological and Genetics Reserves Center (IBRC) and were explanted in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were incubated at $37 \pm 0.5^\circ\text{C}$ in an atmosphere containing 95% humidified air and 5% CO_2 . Cell cultures were split every 2–3 d when 70–80%

confluences of the surface area of the flasks were occupied. Cells in logarithmic phase were used for the more experiments.

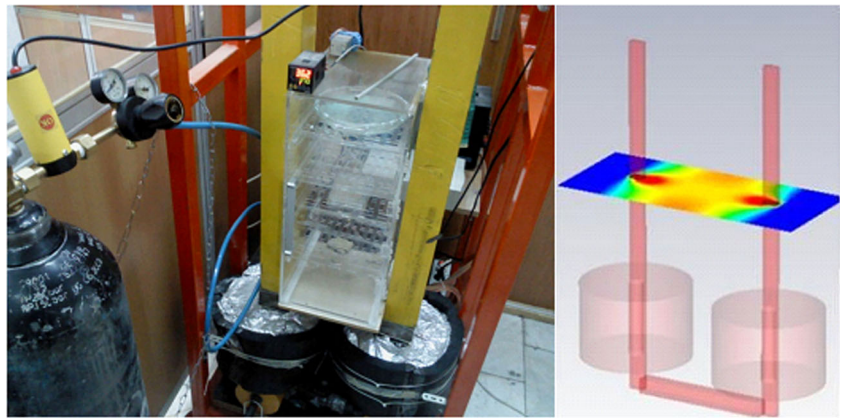
Magnetic field application Exposure to MF was performed using a locally designed SMF generator (Fig. 1). The magnetic field generator consisted of two coils and a DC switching power supply. This generator was made up of two 3.0 mm diameter wire coils with a resistance of 3Ω the inductance of 2H and a heat resistance up to 200°C . Wire length in each coil was about 1 km and each coil weighs approximately 40 kg. These two coils guided the magnetic field through two iron blades with 1 meter height and the cross section of 10 cm^2 .

The electrical power was provided using a 220 V AC power supply equipped with a variable transformer and a single-phase full-wave rectifier. The switching power supply could apply a DC voltage up to 50 V and a current up to 16 A to the coil, for moderate flux density static magnetic field generation as needed. A gas chiller with optimum control of temperature was used to cool off the system. It was consisted of an engine, a condenser, refrigerant gas and an evaporator, covering the outer surface of the coils. The system was equipped with an included rectangular cube ($23 \times 20 \times 50 \text{ cm}^3$) incubator using three different sensors to control the temperature, humidity and CO_2 pressure of the air surrounding the flasks. The field between iron blades was measured by a 13610.93 PHYWE (Gottingen, Germany) teslameter and the presence of any pulsation in the current from rectifier into the SMF generating apparatus, was tested by an oscilloscope (8040, Leader Electronics Co., Yokohama, Japan).

However, the ripple voltage had not been zero, it still was enough small to be neglected and consider the generated magnetic field homogenous. The field produced by the system, was simulated using the CST STUDIO 2011 software. The uniformity of the field is represented with surfaces of the same color. Calibration of the system as well as tests for the accuracy and uniformity of the SMF were performed by a teslameter (13610.93, PHYWE, Gottingen, Germany), and also was calculated with Complete Technology for 3D Simulation CST STUDIO 2011 software (<http://www.CST.com>) for the best site selection of experimental samples within the exposure chamber of SMF generator. The value of the geomagnetic field in our working laboratory was measured $47 \mu\text{T}$ by Tehran Geomagnetic Observatory, Institute of Biophysics, University of Tehran.

Treatment protocols Three independent SMF intensities (7, 10, and 15 mT) were used to find the effective intensity of static magnetic field which causes the maximum effect on cancer cells and the minimum one on fibroblasts. The differences in the viability percent of three intensity of SMF in both cell types for 24 and 48 h were determined. The intensity of 10 mT was selected for the further investigations. The simultaneous effect of 10 mT SMF and cisplatin on Hu02 and HeLa cell line at two different time (24 and 48 h) were investigated. A count of approximately 5×10^5 cells/ cm^2 was seeded in 25 cm^2 flasks and incubated for

Figure 1. The magnetic field generating device. The generator was built in Tarbiat Modares University, Department of Biophysics.



24 h before treatments. Both cell types were divided into four groups; First group was treated with IC_{50} concentration of cisplatin, the second one was exposed to the SMF alone without any interruption, the third group was double-treated (cisplatin + SMF), and the last one was control; all treatments for each cell type were performed at two different time (24 and 48 h).

MTT assay For the assessment of cell viability, cell suspensions containing 1×10^4 viable cells per well were cultivated in 96-well flat-bottomed plates. After 24 h, which is equal to one cell cycle duration of selected cells, cells attached to the flasks and were ready for treatments. Experiments were carried out with at least five repetitions. Plates with or without treatments in a final volume of 100 μ l incubated at 37°C. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide) was prepared at the concentration of 10% (v/v) of 5 mg/ml MTT salt. It was added to each well, and the cultivation was continued for additional 4 h. During this period, the living cells, convert the MTT salt to a blue, insoluble formazan product. The metabolic activity of living cells was correlated with the amount of formazan produced. In order to dissolve the dark blue crystals, 100 μ l of dimethyl sulfoxide (DMSO) was added to each well and mixed thoroughly. After a few minutes, the plates were read on a BIO-TECH MQX200 microplate Elisa reader, using a test wavelength of 540 nm (Tenuzzo and Chionna 2006).

Determination of intracellular ROS In order to detect the amount of intracellular reactive oxygen species (ROS) after each treatment, at least 5×10^5 cells/ml were fully detached by trypsinization and resuspended according to the manufacturer's instruction (DCFDA Cellular ROS Detection Assay Kit, Abcam, UK). Cells were stained with 20 μ M DCFDA (2', 7' - dichlorofluoresceindiacetate) and were incubated for 30 min at 37°C. DCFDA as a fluorogenic dye passively diffused into the cells and was oxidized by ROS into 2', 7' -dichlorofluorescein (DCF), a highly fluorescent compound. Flow cytometric measurement was performed using a LSR II flow cytometer (Becton Dickinson, Franklin Lakes, NJ) for the detection of DCF (excitation/emission 495/529 nm) (Zhao et al. 2011).

Estimation of lipid peroxidation Cell membrane damage was determined by measuring malonyldialdehyde (MDA) as the end product of membrane lipid peroxidation (Vos et al. 1991). In brief, after each treatment, 5×10^5 cells/ml was homogenized in 10% (v/v) trichloroacetic acid (TCA). After sonication on ice, 1 ml of 0.5% (v/v) thiobarbituric acid (TBA) solution was added to 1 mL of the supernatant. The final solution was incubated for 30 min in a 100°C water bath and then transferred to an ice-cold water bath. The absorbance of MDA was read at 532 nm followed by correlation for the nonspecific absorbance at 600 nm. The amount of MDA-TBA

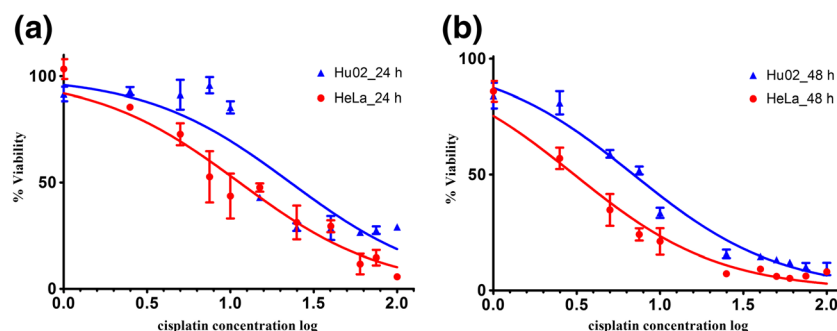


Figure 2. Determination of IC_{50} values. The 50% inhibition concentration (IC_{50}) values of the different concentration of cisplatin on HeLa cell line and Hu02 at 24 h (a) and 48 h (b) were measured by MTT assay. Cisplatin

reduced viability of cells in a dose-dependent manner. IC_{50} values were calculated from the dose response curves by nonlinear regression. Data are means \pm SD, n = at least five independent experiments.

Table 1. The mean viability percent of Hu02 and HeLa cells after exposure to different intensities (7, 10, and 15 mT) at 24 and 48 h

	HeLa			Hu02				
	24 h		48 h	24 h		48 h		
7 mT	104.84 ± 11.32	a*	89.43 ± 6.10	bc	106.90 ± 4.28	a	89.84 ± 4.21	a
10 mT	111.35 ± 4.64	a	101.69 ± 4.79	b	109.08 ± 3.95	a	74.78 ± 3.54	b
15 mT	107.85 ± 0.59	a	89.41 ± 5.89	c	93.46 ± 4.87	b	93.83 ± 1.52	a

Different letters “a, b, c” refer to significant differences according to Tukey’s test ($P < 0.05$). “a” letter means, there was a significant difference between “a” group with “b” group and “c” group, but there was no significant difference between groups with the same letter. “bc” letter means, there was no significant difference between “bc” group with “b” group and “c” group

* Errors indicate the standard deviation (SD) for at least four independent experiments

complex was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Fahimirad 2013).

Statistical analysis All of the experiments were carried out with three independent repetitions, and data were presented as the mean values ± standard deviation (Mean ± SD). Statistical analysis was assessed using one-way repeated measures analysis of variance (ANOVA) followed by Tukey’s test with 95% confidence limits. P value of <0.05 between groups was considered statistically significant by using the GraphPad Prism 6 software.

Results

Determination of the half maximal IC_{50} Inhibitory concentration (IC_{50}) concentration of cisplatin was obtained from the dose response curve by using multiple doses of the drug. According to this procedure, the IC_{50} value of cisplatin was calculated 12 and 3 $\mu\text{g/ml}$ for HeLa cell line in 24 and 48 h, respectively, and 22 and 6 $\mu\text{g/ml}$ for Hu02 cells as the same way (Fig. 2); to investigate the same drug dose effects on both cell types and provide the opportunity of data comparability, the IC_{50} of HeLa cells were used in all treatments. The obtained IC_{50} concentration for Hu02 was greater than that of HeLa cells.

Evaluation of cell viability percent The viability percent of cells in both cell types in different SMF intensities (7, 10, and 15 mT) were determined at both times (24 and 48 h). Table 1

shows the obtained mean viability percent of HeLa cell line and Hu02 after exposure to different intensities of SMF and exposure periods.

The viability of cells had reduced by the increase in cisplatin dose. In the presence of 10 mT SMF exposure and administration of IC_{50} concentration of drug, the viability percent of HeLa cells were $51.44 \pm 8.63\%$ and $11.42 \pm 0.31\%$ at 24 and 48 h, respectively. The viability percent of fibroblast cells were $55.49 \pm 2.08\%$ and $47.49 \pm 4.35\%$ at 24 and 48 h as the same way. Based on the obtained results, the combination of both treatments (SMF and drug) for 48 h led to a marked decrease in the viability percent of HeLa cells by about 89% compared to untreated cells.

Detection of intracellular ROS Similarly, we evaluated the influence of the SMF exposure on intracellular ROS generation in cisplatin + SMF groups compared to cisplatin-treated cells at both times (Table 2). As shown in Fig. 3, our results demonstrated that by applying the static magnetic field, the ROS formation was increased in SMF + cisplatin-treated cells in both cell types. In addition, SMF + cisplatin-treated cells compared with cisplatin-treated group, showed the higher DCF fluorescence in HeLa cell line (Figs. 3 and 4); 47.8 to 81.4 at 24 h and 58 to 78.6 at 48 h, respectively; however, the ROS level of SMF + cisplatin-treated group in Hu02 cells showed a relatively small increase in DCF fluorescence (63.1 to 69.6 at 24 h and 78.2 to 83.1 at 48 h, respectively).

Table 2. The intracellular ROS production in HeLa cell line and Hu02 in treated groups

	HeLa			Hu02				
	24 h		48 h	24 h		48 h		
Control	29.18 ± 3.2	a*	34.96 ± 5.6	a	64.4 ± 4.9	a	68.47 ± 7.3	a
Drug	47.83 ± 1.8	b	58.06 ± 9.2	b	63.14 ± 7.5	a	78.23 ± 4.4	a
MF + drug	81.4 ± 5.8	c	78.62 ± 9.9	c	69.66 ± 8.5	a	83.19 ± 8.04	a

Different letters “a, b, c” refer to significant differences according to Tukey’s test ($P < 0.05$). “a” letter means, there was a significant difference between “a” group with “b” group and “c” group, but there was no significant difference between groups with the same letter

* Data are means ± SD of three independent experiments

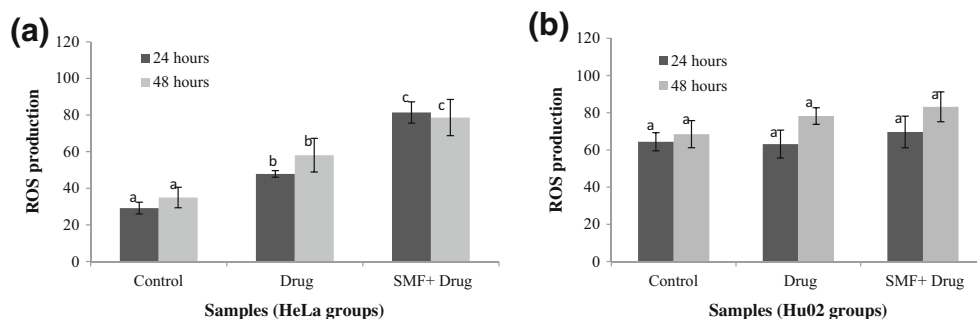


Figure 3. Intracellular ROS production in HeLa cell line (a) and Hu02 (b) in treated groups. Data are means \pm SD of three independent experiments. Different letters “a, b, c” refer to significant differences according to Tukey’s

test ($P < 0.05$). “a” letter means there was a significant difference between “a” group with “b” group and “c” group, but there was no significant difference between groups with the same letter.

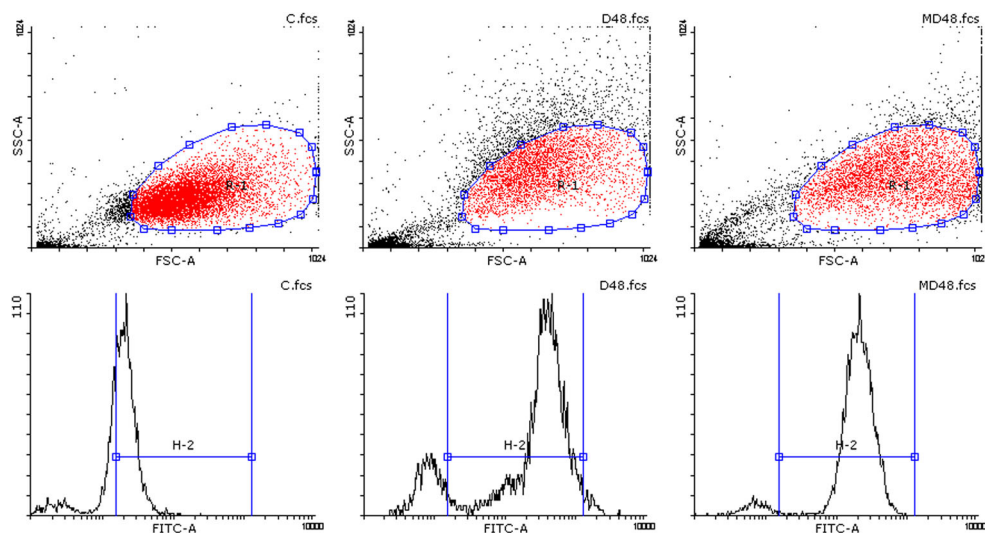
Evaluation of lipid peroxidation The level of peroxidation of membrane lipids was significantly higher in SMF + cisplatin-treated HeLa cells than cisplatin-treated groups at both times (Fig. 5). Interestingly, our results showed that there were obvious increase in cell membrane lipid peroxidation after 48 h exposure; it seems that long time exposure to SMF has more effect on the cell membrane lipid peroxidation compared to short time exposure (Fig. 5). However, there was no significant difference between the levels of membrane lipid peroxidation of Hu02 treated groups compared to the untreated group at both times.

Discussion

In the present study, we report the influence of SMF and cisplatin co-treatment on the oxidative stress and cell death in two kinds of cell type, HeLa cell line as cancer cells and Hu02 as normal cells. For both types of cell, exposure time had a substantial effect. Nonetheless, as shown in Fig. 3, the effect of SMF on cancer cells is markedly greater than fibroblast cells at both exposure times (24 and 48 h). Simultaneous

application of 10 mT SMF and IC_{50} concentration of cisplatin led to an increase in intracellular ROS formation by 34% at 24 h and 20% at 48 h in HeLa cell line; while, combination of both treatments resulted in a slight increase in ROS production (approximately 6%) in normal fibroblast cells. It is well known that DNA is the major target of cisplatin, which forms covalent platinum DNA adducts and also stimulates superoxide radical production. The major presumptive mechanism that could explain the non-thermal effects of MFs on biological systems are the hypothesis in which MF influences the kinetics of chemical reactions with radical pair intermediates which increases in the concentration and/or lifetime of free radicals (Dini and Abbro 2005; Ghodbane et al. 2013). It has been found that the behavior of normal cells in response to static magnetic field exposure are different from cancer cells (Dini and Abbro 2005). Taking into consideration of differences between normal and cancer cells in metabolic reprogramming in mitochondria (Pinton et al. 2008; Gerweck and Seetharaman 1996; Li et al. 2016), transportation of cations (Dini and Abbro 2005), membrane (Kojima 1993), cytoplasm, gene expression (Li et al. 2016), and level of gene products (protein), it is suggested that static magnetic

Figure 4. Representative flow cytometry dot plot and histogram of ROS production by HeLa cells (Hu02 cells are not shown). *C* letter represents control, *D48* represents cisplatin treatment for 48 h, and *MD48* represents cisplatin + MF treatment for 48 h.



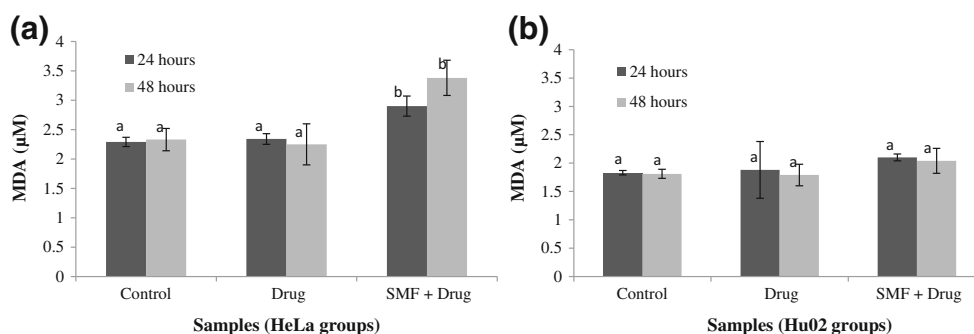


Figure 5. Rate of lipid peroxidation in HeLa cell line (a) and Hu02 (b) in treated groups. Data are means \pm SD of at least three independent experiments. Different letters “a, b” refer to significant differences according to

Tukey’s test ($P < 0.05$). “a” letter means, there was a significant difference between “a” group with “b” group, but there was no significant difference between groups with the same letter.

fields (SMFs) has more potential of altering apoptosis in cancer cells (HeLa) than normal cells. Therefore, static MFs potentially sensitize human cervical cancer cells to cisplatin through ROS accumulation while it has a small effect on background surrounding normal cells.

Lipid peroxidation is a process caused by ROS and leads to severe cell membrane damages. Malonyldialdehyde (MDA) is an important index of lipid peroxidation rate and oxidative stress damages (Uemura and Tominaga 2006; Fahimirad 2013; Shahandashti and Amiri 2013). From the measured MDA level of both cell types during treatments, we found that the MDA amount in cancer cells is higher than their normal counterparts and SMF and cisplatin co-treatment caused a significant increase in MDA levels in HeLa cells. This indicated that the HeLa cell was more sensitive than fibroblast cells; the complexity and sensitivity of cells are a crucial factor in evaluating the cell damage during oxidative stress. Several studies demonstrated that SMF promotes free radical activity in cells (Gray et al. 2000; Cintolesi et al. 2003; Berk et al. 2006; Efimova and Hore 2008), particularly via the catalytic reaction of Fenton (Berk et al. 2006) by which hydrogen peroxide is converted to hydroxyl free radicals that are very potent and cytotoxic molecules (Ghodbane et al. 2013). Free radicals affect cells by damaging macromolecules, such as DNA, protein, and membrane lipids. It was approved that rapidly proliferating cells have a higher metabolism and more sensitive to free radicals than normal cells (Zafari et al. 2015).

Some previous literatures indicated that SMF influences the cell membrane permeability and some holes produced by the action of static MF which is related to the synergic action of SMF and anticancer drugs; these alterations in the cell surface cause the increase in the flow rate of anticancer drug into the cells (Liu et al. 2010). To our knowledge, the current study revealed that SMF sensitizes cancer cells and modulates the redox status. Oxidative stress is caused by an imbalance between ROS production and the biological system’s ability to detoxify the reactive intermediates. Co-administration of cisplatin and SMF exposure could greatly improve the sensitivity of cancer cells to cisplatin, disrupt the integrity of cell

membranes and intact DNA. From a biomolecular point of view, the effects on cell proliferation, viability, and cell death may be linked to the action of free radicals and enhancement of lipid peroxidation. Damage to the lipids in the cellular membrane by exerting SMF increase the cytoplasmic free calcium. Earlier studies provide evidence, depending on the cell type, Ca ion exerts quite different effects on apoptosis and cell death (Teodori et al. 2002). Free radicals, directly and indirectly, participate in the operation of apoptosis and necrosis. Also, the transformation of Ca molecular level could result in apoptosis and cell death and enhance the cytotoxicity of antineoplastic drugs. Previous reports highlighted the alteration of signal pathways through the variation of intracellular Ca concentration (Dini and Abbro 2005). The change in calcium ion concentration led to fluctuation of the apoptotic rate. This hypothesis would explain all of the bioeffects attributed to these fields, including the cell viability (Tatarov et al. 2011; Zafari et al. 2015) and modulation of apoptosis (Tavasoli et al. 2009).

Conclusions

According to the obtained results by cell viability assay for two different times (24 and 48 h) and SMF intensities of 7, 10, and 15 mT, it is believed that intensity of 10 mT had more effects on HeLa cell line and lower effects on fibroblast cells. The viability of cells has been increased by reduction of cisplatin doses. So the combination of SMF with therapeutic approaches may be useful as a therapeutic method for cancer treatment through the potency of SMF for inducing free radical production and eventually apoptosis in cancer cells. The most advantages of such hybrid treatment (both chemical and physical agents) are that it can have a better biological effect with less administration doses of drug. This is a starting point, and there is much more to be done in order to be sure about the protective role of SMFs regarding the normal cells and the concomitant ameliorating role regarding the cancer ones. It can be tested in other cell lines (i.e., TCO-2, JHUCS-3) or even in other types of cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Albuquerque WWC, Costa RMPB, Fernandes T d S, Porto ALF (2016) Evidences of the static magnetic field influence on cellular systems. *Prog Biophys Mol Biol* 121:16–28
- Antunes L (2000) Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. *Pharmacol* 41:405–411
- Berk M, Dodd S, Henry M (2006) Do ambient electromagnetic fields affect behaviour? A demonstration of the relationship between geomagnetic storm activity and suicide. *Bioelectromagnetics* 27:151–155
- Buemi M, Marino D, Di Pasquale G, Floccari F, Senatore M, Aloisi C, Grasso F, Mondio G, Perillo P, Frisina N et al (2001) Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. *Nephron* 87:269–273
- Cintolesi F, Ritz T, Kay C, Timmel C, Hore P (2003) Anisotropic recombination of an immobilized photoinduced radical pair in a 50- μ T magnetic field: a model avian photomagnetoceptor. *Chem Phys* 294:385–399
- Dini L, Abbro L (2005) Bioeffects of moderate-intensity static magnetic fields on cell cultures. *Micron* 36:195–217. doi:10.1016/j.micron.2004.12.009
- Efimova O, Hore P (2008) Role of exchange and dipolar interactions in the radical pair model of the avian magnetic compass. *Biophys J* 94:1565–1574
- El-Bialy NS, Rageh MM (2013) Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. *Biomed Res Int* 2013:189352. doi:10.1155/2013/189352
- Fahimirad S (2013) Cold-induced changes of antioxidant enzymes activity and lipid peroxidation in two canola (*Brassica napus* L.) cultivars. *J Plant Physiol Breed* 3:1–11
- Fanelli C, Coppola S, Barone R (1999) Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca²⁺ influx. *FASEB J* 13:95–102
- Gerweck LE, Seetharaman K (1996) Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer Res* 56:1194–1198
- Ghodbane S, Lahbib A, Sakly M, Abdelmelek H (2013) Bioeffects of static magnetic fields: oxidative stress, genotoxic effects, and cancer studies. *Biomed Res Int* 12:1. doi:10.1155/2013/602987
- Gottfried M, Ramlau R (2008) Cisplatin-based three drugs combination (NIP) as induction and adjuvant treatment in locally advanced non-small cell lung cancer: final results. *J Thorac* 3:152–157
- Gray JR, Frith CH, Parker JD (2000) In vivo enhancement of chemotherapy with static electric or magnetic fields. *Bioelectromagnetics* 21:575–583
- Kamalipooya S, Soleimani H, Abdolmaleki P, Sabet A, Hajipour B, Jouni FJ (2015) The effects of static magnetic fields on viability and apoptosis in normal and cancerous cells. *Stress* 1:2
- Kojima K (1993) Molecular aspects of the plasma membrane in tumor cells. *Nagoya J Med Sci* 56:1–18
- Lacy-Hulbert A, Metcalfe J, Hesketh R (1998) Biological responses to electromagnetic fields. *FASEB J* 12:395–420
- Li C, Zhang G, Zhao L, Ma Z, Chen H (2016) Metabolic reprogramming in cancer cells: glycolysis, glutaminolysis, and Bcl-2 proteins as novel therapeutic targets for cancer. *World journal of surgical oncology* 14:15
- Lindström E, Lindström P (1995) Intracellular calcium oscillations in a T-cell line after exposure to extremely-low-frequency magnetic fields with variable frequencies and flux densities. *Bioelectromagnetics* 16:41–47
- Liu Y, Qi H, Sun RG, Chen WF (2010) An investigation into the combined effect of static magnetic fields and different anticancer drugs on K562 cell membranes. *Tumori* 97:386–392
- Maređziak M, Marycz K (2014) The influence of static magnetic fields on canine and equine mesenchymal stem cells derived from adipose tissue. *Vitr Cell Dev Biol-Anim* 50:562–571
- Masuda H, Tanaka T, Takahama U (1994) Cisplatin generates superoxide anion by interaction with DNA in a cell-free system. *Biochem Biophys Res Commun* 203:1175–1180
- Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R (2008) Calcium and apoptosis: ER-mitochondria Ca²⁺ transfer in the control of apoptosis. *Oncogene* 27:6407–6418
- Roy S, Noda Y, Eckert V, Traber M, Mori A (1995) The phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils is increased by a 0.1 mT (60 Hz) magnetic field. *FEBS Lett* 376:164–166
- Schimmelpfeng J, Stein J, Dertinger H (1995) Action of 50 Hz magnetic fields on cyclic AMP and intercellular communication in monolayers and spheroids of mammalian cells. *Bioelectromagnetics* 16:381–386
- Shahandashti S, Amiri R (2013) Change in membrane fatty acid compositions and cold-induced responses in chickpea. *Mol Biol* 40:893–903
- Suo Z, Lippard S, Johnson K (1999) Single d (GpG)/cis-diammineplatinum (II) adduct-induced inhibition of DNA polymerization. *Biochemistry (Mosc)* 38:715–726
- Tatarov I, Panda A, Petkov D, Kolappaswamy K, Thompson K, Kavirayani A, Lipsky MM, Elson E, Davis CC, Martin SS, Detolla LJ (2011) Effect of magnetic fields on tumor growth and viability comparative. *Medicine* 4:339–345
- Tavasoli Z, Abdolmaleki P, Mowla SJ, Ghanati F, Sarvestani AS (2009) Investigation of the effects of static magnetic field on apoptosis in bone marrow stem cells of rat. *Environmentalist* 29:220–224. doi:10.1007/s10669-008-9210-4
- Tenuzzo B, Chionna A (2006) Biological effects of 6 mT static magnetic fields: a comparative study in different cell types. *Bioelectromagnetics* 27:560–577
- Teodori L, Grabarek J, Smolewski P, Ghibelli L, Bergamaschi A, De Nicola M, Darzynkiewicz Z (2002) Exposure of cells to static magnetic field accelerates loss of integrity of plasma membrane during apoptosis. *Cytometry* 49:113–118. doi:10.1002/cyto.10160
- Tofani S (2003) 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* 48:83–90. doi:10.1016/S1043-6618(03)00062-8
- Türk G, Ateşşahin A, Sönmez M, Çeribaşı A, Yüce A (2008) Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. *Fertil Steril* 89:1474–1481
- Uemura M, Tominaga Y (2006) Responses of the plasma membrane to low temperatures. *Physiol Plant* 126:81–89
- Vos CHR, Schat H, Waal MAM, Vooijs R, Ernst WHO (1991) Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiol Plant* 82:523–528

- Walleczek J, Budinger T (1992) Pulsed magnetic field effects on calcium signaling in lymphocytes: dependence on cell status and field intensity. *FEBS Lett* 314:351–355
- WHO | Cancer (2016) Online database: [WWW Document] WHO. URL <http://www.who.int/cancer/Cited> 12 Sept 2016
- Wozniak K, Czechowska A, Blasiak J (2004) Cisplatin-evoked DNA fragmentation in normal and cancer cells and its modulation by free radical scavengers and the tyrosine kinase inhibitor STI571. *Chem Biol Interact* 147:309–318
- Zafari J, Javani Jouni F, Abdolmaleki P, Jalali A, Khodayar MJ (2015) Investigation on the effect of static magnetic field up to 30 mT on viability percent, proliferation rate and IC50 of HeLa and fibroblast cells. *Electromagn Biol Med* 34: 216–220. doi: [10.3109/15368378.2015.1076452](https://doi.org/10.3109/15368378.2015.1076452)
- Zhao G, Chen S, Wang L, Zhao Y, Wang J, Wang X, Zhang W, Wu R, Wu L, Wu Y, Xu A (2011) Cellular ATP content was decreased by a homogeneous 8.5 T static magnetic field exposure: role of reactive oxygen species. *Bioelectromagnetics* 32:94–101. doi:[10.1002/bem.20617](https://doi.org/10.1002/bem.20617)