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Selective destabilization of tumor cells with pulsed electric and magnetic sequences: a preliminary report

S. Crocetti[1,2], F. Piantelli[2] & C. Leonzio[1]

[1]*Department of Environmental Science, University of Siena, Siena, Italy, and* [2]*I.M.O. Centre, University of Siena, Siena, Italy*

Background: Various studies *in vitro* suggest that low electric and magnetic fields may modify cancer cell growth and recent studies *in vivo* have revealed anti-tumoral effects. After screening different tumor cell lines, we identified specific sequences of localized magnetic and electric fields (MESQ) that reduce cancer cell survival *in vitro*. This finding led us to design an experiment to determine the actual efficacy of above sequences in selectively destabilizing tumor cells and their effect on healthy cells.
Materials and Methods: We exposed the MCF7 cancer cell line and normal fibroblasts to MESQ for 1, 2, 3 and 6 hours, evaluating cell survival and induction of apoptosis.
Results: Exposure to MESQ reduced MCF7 survival, inducing apoptosis in a timedependent way, whereas fibroblasts were completely unaffected.
Conclusion: These results have promising implications for the treatment of cancer and warrant further research.

Keywords Cancer cell, electric and magnetic field, apoptosis, proliferation, mitosis

INTRODUCTION

The literature abounds in studies aimed at defining the effects of electric and magnetic fields on humans, but the results have often been contradictory. This is partly because many parameters are involved: field type, frequency and intensity, for example, can invalidate controls, make experiments non reproducible, and obscure the mechanisms of action of electric and magnetic fields (ICNIRP, 2001; Who, 2007). Specifically, research conducted in the field of oncology suggests an association between electromagnetic fields (EMF) and the onset of cancer (Ghabili et al., 2008; Loomis et al., 1994). However, recent evidence demonstrates that EMF do not have genotoxic effects in terms of DNA damage (Fiorani et al., 1992; ICNIRP, 2001; McCann et al., 1998; Who, 2007) and suggests that they do not act as carcinogenic agents by altering oncogene and oncosuppressor expression (Loberg et al., 1999; Morehouse et al., 2000). Moreover, electrical and magnetic fields appropriately modulated in intensity and frequency have been used separately to impair cancer cells *in vitro* (Koh et al., 2008; Santini et al., 2005).

Address correspondence to Sara Crocetti, Department of Environmental Science, University of Siena, 53100 Siena, Italy; E-mail: crocetti2@unisi.it

Very few studies have examined the possible biological effects induced by simultaneous use of sequences of localized magnetic and electric fields (MESQ). The use of localized fields enables better control of field parameters and the possibility of appropriately combining electric and magnetic signals to produce different effects by distinguishing the contribution of the two components (Basu et al., 1989; Borzdov, 2002; McNeil et al., 2010; Mullins et al., 1993). Appropriate modulation of the parameters of magnetic and electric fields is of fundamental importance for manipulating cell functions and behaviour in a predetermined way. This idea has stimulated recent oncological studies aimed at identifying magnetic and electric fields effective for treating neoplasias (Barbault et al., 2009; Bertoni et al., 2009; Besić, 2007; Elson, 2009).

After several years spent screening different tumor cell lines in our institute, we identified localized, high intensity pulsed magnetic fields, combined with a localized electric field, that reduce the survival of cancer cells *in vitro*. This finding led us to design an experiment to determine the actual efficacy of the above sequences in selectively destabilizing tumor cells and their effect on healthy cells. The experiment was conducted applying the magnetic and electric sequences (MESQ) to the MCF7 tumor line and to primary cultured fibroblasts, evaluating the effects produced in terms of cell survival and induction of apoptosis.

MATERIALS AND METHODS

Cell cultures

Mammary adenocarcinoma cell line MCF7 from the American Type Culture Collection (ATCC) was cultured in RPMI 1640 medium (Sigma) supplemented with fetal calf serum (10%), L-glutamine (1%) and penicillin-streptomycin antibiotic (1%). The primary fibroblast culture (PCS-201-012, ATCC) was cultured using Fibroblast Growth Kit - low serum (ATCC). The cells were maintained at 37°C in an atmosphere with 95% humidity and 5% CO_2. For MESQ treatment, the cells were sown in 25 cm^2 flasks at a concentration of $7x10^5$ cellule/ml. After 24 h they were washed with PBS (Sigma), placed in fresh medium and exposed to MESQ for 1, 2, 3, and 6 h to test cell survival. For the apoptosis test, only three exposure times were used (1, 2, and 3 h), on the basis of cytotoxicity test results. The results were compared with those of sham-exposed cells.

MESQ generator and exposure of cell samples

Electric field generator

The electric field was obtained with a high-tension generator connected to a condenser consisting of two copper electrodes 10 cm apart that produced a uniform electric field intensity of 3 kV/m. The generator was driven by a power source having a frequency between 100 Hz and 1 KHz, modulated at low frequency (20 Hz).

Magnetic field generator

The magnetic field was generated by a pair of Helmholtz coils mounted outside the sample compartment consisting in a plexiglass conteiner. They created a magnetic field (intensity 600 Gauss) perpendicular to the direction of the electric field between the two electrodes. The copper coils were connected to a power source that enabled the magnetic field to be regulated in pulsed square-waves at a frequency of 20 Hz.

Sample compartment and temperature control

The cell samples were placed inside the condenser, which was housed in a plexiglass container kept at 37°C by means of a water jacket and thermostat.

Compartment for sham-exposed samples

The thermostat-controlled water jacket also extended to a compartment, completely isolated from magnetic and electric fields, for sham-exposed samples. Cell samples were placed in this compartment to verify the null-hypothesis.

Instruments for monitoring and control

The system was continuously monitored and controlled by an oscillograph and a multimeter equipped with temperature sensors and Hall probes to measure the magnetic and electric fields. The system was coordinated and controlled by computer.

EVALUATION OF CELL SURVIVAL

After MESQ treatment, cell samples were incubated 1:1 with trypan blue (Sigma) for 15 min. The cells were then disposed in a Burker chamber equipped with inverted microscope (Nikon TE 300), where they were observed and counted. Percentage cell survival was evaluated by calculating the ratio of the number of viable cells in exposed and sham-exposed samples.

DETERMINATION OF APOPTOSIS (ANNEXIN V ASSAY)

Induction of apoptosis was evaluated by the Annexin V assay followed by cytofluorimetric analysis and confocal microscopy (Nikon TE 300, Bio-Rad laser system). For cytofluorimetric analysis the cells were collected after a passage in trypsin-EDTA (1X)(Sigma), washed twice with PBS and resuspended in Binding Buffer 1X solution (Bender Medsystems). They were then stained with Annexin V - FITC Apoptosis Detection Kit (Bender Medsystems) (5 μl Annexin V - FITC per 10^5 cells) and with 50 μg/ml propidium iodide solution in PBS buffer 1X (2 μl). The cells were incubated for 15 min at ambient temperature, before cytofluorimetric reading.

For confocal microscopy, the cells were detached with trypsin EDTA 1X (Sigma), washed twice in PBS and transferred to Poly-Prep poly-L-lysine coated slides at a concentration of $1x10^6$ cells/ml. After three washes with Binding Buffer 1X (Sigma) they were stained with Label Staining Solution (AnnCy3) (Sigma), incubated for 10 min in the dark and observed by confocal microscope.

For the two procedures, adhering and floating cells were both collected. The results were normalized to the respective sham-exposed cells, in which the percentage of apoptotic cells was 1–4% for the two types of cells analyzed.

STATISTICAL ANALYSIS

The results were expressed as mean of at least three experiments and had a low coefficient of variation, as shown by the standard deviations indicated in the following graphs.

RESULTS

Cytotoxicity

As shown in Fig. 1, a decrease in survival of MCF7 cells was recorded after only 1 h of exposure (78% ves. 100% sham) and reached 40% after 3 h. By contrast, MESQ exposure was completely innocuous to fibroblasts, where survival remained at 100% throughout the experiment. In order to confirm this observation, we prolonged the duration of exposure to 6 h, finding a continuous

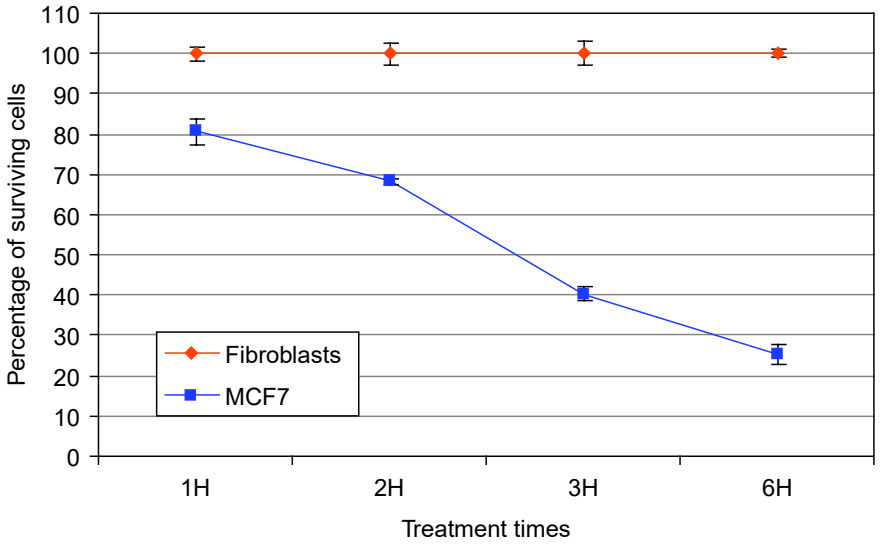

FIGURE 1 Survival of MCF7 cell line and cultured fibroblasts after exposure to MESQ. Bars represents means of viable cell counts of triplicate experiments and error bars represent ± SD.

decrease in the percentage survival of MCF7 cells down to a value of 25%, whereas fibroblasts maintained a survival percentage that did not vary with respect to sham-exposed cells.

Apoptosis

The early apoptotic cells induced by MESQ were examined by flow cytometry analysis of Annexin V staining in fibroblast and MCF7 cells. As shown in Fig. 2, after 1 h of exposure, a small percentage (6%) of apoptotic MCF7 cells were observed. The number of apoptotic cells increased in the course of treatment up to 30% after 3 h of exposure to MESQ. Confocal microscopy analysis of MCF7 apoptotic cells (red fluorescence) show many paired cells (Fig. 3B), suggesting that exposure to MESQ may upset the moment of cell division. Fibroblasts exposed to MESQ did not show any appreciable differences with respect to sham-exposed cells, as shown in Fig. 2.

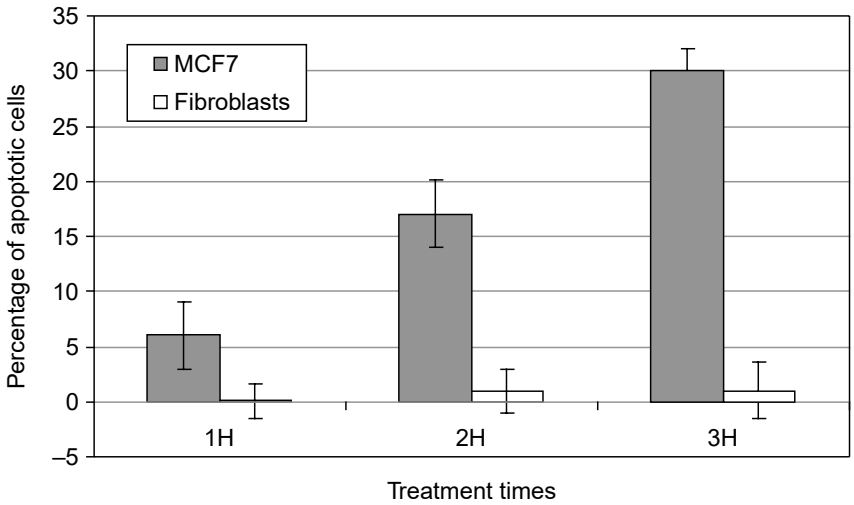

FIGURE 2 Cytofluorimetric analysis of apoptosis during Annexin V – Propidium Iodide assay of MCF7 cells and fibroblasts after MESQ treatment. Columns show means of apoptotic cell percentages of triplicate experiments, bars represent ± SD.

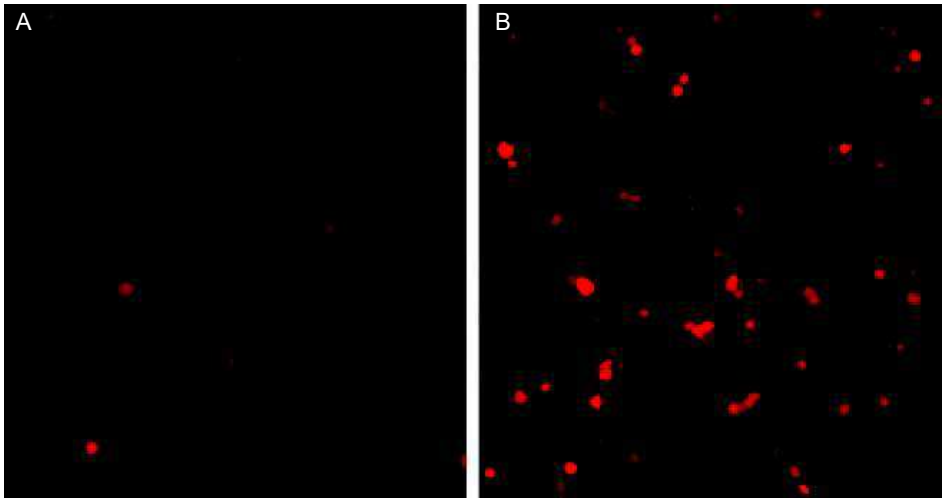

FIGURE 3 Apoptosis of MCF7 cells after 3 h of MESQ treatment. Image obtained by confocal scansion during Annexin V/Cy3 assay. Original magnification, × 200. A: Sham-exposed MCF7 cells; B: MCF7 cells exposed to MESQ.

DISCUSSION

The present results show that pulsed sequences of magnetic (600 G, 20 Hz) and electric (3 KV/m) fields affect cells of the MCF7 tumor line, leading to decreased cell survival by induction of apoptosis. These effects were not observed in normal fibroblasts, the survival of which remained unchanged with respect to sham-exposed fibroblasts (Figs. 1 and 2).

The image of apoptotic cells obtained by confocal microscope (Fig. 3) showed many paired cells, suggesting that the cell population most susceptible to induction of apoptosis by MESQ was that undergoing mitosis. The moment of cell division, when the mitotic spindle forms and chromosomes align at microtubules, is a delicate stage of the cell cycle subject to many mechanisms of regulation. Many articles in the recent literature underline the importance of the spindle assembly check-point and centrosomes in ensuring correct chromosome segregation and as regulators of the whole cell cycle under physiological conditions and stress (Doxsey, 2001; Javerzat, 2010; Watanabe et al., 2009). These articles highlight the importance of electrostatic interactions for two-way anchorage of microtubules and correct chromosome segregation, suggesting that exposure to MESQ may upset the dynamics of these mechanisms.

Death observed in tumor cells but not in normal fibroblasts indicates that destabilization induced by MESQ was completely reversible or innocuous in cells with genomic stability and normal functions, including regulation and repair mechanisms, as already shown in various studies (Ali, 2007; Béghin et al., 2008; Hartwig et al., 2009). On the contrary, cells of the MCF7 tumor line, characterized by genomic instability, aneuploid chromosomes (hypertriploid-hypotetraploid), and aberrant cell function, were completely destabilized by MESQ, as shown by the large population of apoptotic cells after 3 h of exposure (Figs. 2 and 3B). Aneuploidy is known to be associated with faster proliferation and cell-cycle velocity with respect to cells with diploid DNA (Allison et al., 1998; Dowle et al., 1987; Kramer et al., 2004; Lukac et al., 2006; Owainati et al., 1987), preventing aneuploid cells from adapting and responding to stress.

Alhough further research is necessary, these results are in line with the observation that the effects of MESQ on MCF7 cells depend on exposure time (Fig. 1).

Also in line with this hypothesis, MESQ could induce apoptosis progressively in asynchronous cell populations, as cells reach the stage of division. If so, the high rate of proliferation of tumor cells could contribute to selective action of MESQ based on cell type and status.

As already shown in various studies (ICNIRP, 2001; Chiabrera et al., 1985; Who, 2007), the present experiment demonstrates that the nature of the effects caused by electrical and magnetic fields depends on parameters such as intensity, frequency, and waveform, as well as cell type. Different combinations of the variables lead to different biological effects, such as modulation of calcium transport, apoptosis, proliferation and differentiation (Buttiglione et al., 2007; Hall et al., 2007; Sul et al., 2006; White et al., 2004).

Our working hypothesis was based on preliminary studies that showed that exposure only to electric fields (3 KV/m) does not have any effect on cell survival or apoptosis, whereas exposure only to magnetic fields (600 Gauss; 20 Hz) has minor effects compared to those observed when both field components are applied (supplementary data). These observations suggest that electric fields potentiate the effects of magnetic fields. Indeed, common applications of electric fields exploit intensities of the order of 100 KV/m to induce certain effects in cells (Belehradek et al., 1993; Heller et al., 1996; Vernier et al., 2003; Weaver et al., 1999) and Astumian et al. (1995) identified intensity thresholds below which no biological effect is seen. The electric field we used was therefore unlikely to be directly responsible for the effects observed, but may have had a synergic effect with the magnetic field. This action could be due to the capacity of even weak electric fields to influence the distribution of charged molecules (receptors, proteins) in the plasmalemma, as described in other studies (Chiabrera et al., 1985).

Although the present results require further study, they are clearly important for the direct clinical and therapeutic implications of appropriate modulation of electric and magnetic signals.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Supplementary figures available online.