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Enhancement of docetaxel-treated MCF-7 cell death by 900-MHz radiation

Research Article

Marianna Trebuňová[1], Galina Laputková[1,*], Imrich Géci[1], Igor Andrašina[2], Ján Sabo[1]

[1]*Department of Medical and Clinical Biophysics, Faculty of Medicine, P.J. Šafárik University, 040 11 Košice, Slovakia*

[2]*Department of Radiotherapy and Oncology, Faculty of Medicine, P.J. Šafárik University, 041 90 Košice, Slovakia*

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Abstract: The aim of the study was to investigate the effect of high-frequency electromagnetic field of 900 MHz at 8 W input power on metabolic activity of human breast adenocarcinoma MCF-7 cells. With the aid of the colorimetric MTT assay, it was shown that there is significant change in cell culture survival exposed to docetaxel in field-free conditions in comparison with cells treated with docetaxel simultaneously exposed to high-frequency electromagnetic field.

Keywords: *MCF-7 • 900 MHz electromagnetic field • Docetaxel*

1. Introduction

The biological effects of electromagnetic fields, especially in the high-frequency regions of the spectrum ranging from 30 kHz to 300 GHz is a subject that has been under thorough investigation during the last decade. A large number of studies have documented numerous biological effects due to high frequency radiation *in vivo* and/or *in vitro*. Nevertheless, there has been conflicting reports with respect to the potential hazard of high frequency radiation exposure. So far, there are no unambiguous results on the effects of high frequency electromagnetic field especially if the limit values are not exceeded. In several *in vitro* studies, it has been shown that exposure to high frequency electromagnetic fields induce cell death or influence cell proliferation [1-4]. As a biological target, the DNA molecule has received attention with respect to potential high-frequency electromagnetic field damage [5,6], because of its relevance for cell function, proliferation, viability, mutation and carcinogenesis.

High frequency radiation is non-ionizing radiation. The emitted energy required to break chemical bonds and to cause ionizations in atoms and molecules is in the eV order of magnitude. Even though the order of the photon energy of high frequency electromagnetic fields is negligible, the fraction, of the ionization energy or the energy necessary to induce direct chemical changes and break non-covalent bonds directly, indirect radiation-based effects have been discussed [7] and thoroughly reviewed by authors of the study [8].

Regarding electromagnetic fields effects on tumor promotion, some studies show a carcinogenetic effect to the induction of brain tumours [9] and others [10] suggests that high frequency electromagnetic field could not contribute to the initiation stage of tumor formation in mouse C3H10T1/2 cells. However, it may contribute to the promotion stage at the extremely high SAR (≥100 W/kg). A major problem in cytostatic treatment of malignant tumours is the development of the resistance of tumour cells to anticancer drugs. P-glycoprotein, the expression product of the MDR-1 gene is strongly

* *E-mail: galina.laputkova@upjs.sk*

associated with both *de novo* and acquired resistance. The protein functions as a transmembrane drug efflux pump, transporting cytostatic agents. Glutathione and it's dependent enzymes may be involved in resistance to drugs evidenced by cellular protection against free radicals damage [11]. Although the reduction of intracellular drug level due to the P-glycoprotein pump is considered to be the main mechanism in drug resistance [12] and the expression product of the drug-metabolizing enzymes may be responsible for the resistance as well. The overexpression of trans-membrane receptor tyrosine kinase HER2 is associated with increased tumor aggressiveness and chemoresistance to several anti-cancer drugs including docetaxel and paclitaxel [13,14].

In a series of publications, it has been shown that static, low or high frequency electromagnetic fields, pulsed and/or unpulsed can produce alterations in antineoplastic drug cytotoxicity [15]. Microtubule inhibitor docetaxel, which is semi-synthetically derived from a natural compound extracted from the pacific yew tree Taxus, belongs to the most active cytotoxic drug in patients with metastatic breast cancer. However, most of breast cancer patients relapse upon or after treatment due to either intrinsic or acquired drug resistance [13]. Systemic toxicity can seriously decrease the effectiveness of the drug as well, that's why a lower dose must be administrated to avoid drug toxicity. Therefore, attempts to enhance antitumor efficacy of cytostatics by combination with the effect of the electromagnetic field was made. The exposure to low-frequency pulsed electromagnetic field on experimental mouse T-cell lymphoma EL4 slowed down the growth of tumor mass and prolonged the survival of experimental animals [16]. The effect was synergistic with suboptimal dose of synthetic HPMA copolymer-based doxorubicin. Mechanochemically activated doxorubicin in combination with 40 MHz frequency irradiation was demonstrated to improve drug delivery to A-549 human lung carcinoma and greater cell kill than does conventional doxorubicin [17]. In several studies, docetaxel was combined with electromagnetic hyperthermia [16], The *in vivo* study of amplitude-modulated electromagnetic fields of tumor-specific frequencies in conjunction with chemotherapy seems to be promising for the treatment of cancer too [18]. However, studies of docetaxel and preferentially the effect of high frequency electromagnetic fields are still controversial and unsatisfactory. To contribute to the advancement of anti-tumor strategies, the possible additive effect of 900 MHz electromagnetic field and docetaxel on the metabolic activity of MCF-7 cells was the aim of this study.

2. Experimental Procedures

2.1 MCF-7 culturing

The cell line MCF-7 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-7 mammary adenocarcinoma cells used as a standard *in vitro* model retain characteristics of differentiated mammary epithelium including ability to process estradiol *via* cytoplasmic oestrogen receptors. Cells were grown as monolayer culture in 10% Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, California, USA) containing foetal bovine serum (FBS, Invitrogen), mixture of 5000 units of penicillin (base) and 5000 µg of streptomycin (base)/ml in 0.85% saline (PenStrep) (Invitrogen). They were kept in the Heracell 150i incubator (Thermo Scientific, USA) at 37°C in 5% CO_2 and 95% humidity. All solutions used were of cell culture grade quality. The equipment employed was commercially pre-sterilized and disposable.

2.2 Metabolic assay

For the measurement of a cell population's response to external factors, the reduction of the yellow compound (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) was used in all experiments. Briefly, the cell suspension in a final volume of 100 µl containing 6×10^3 cells per well was cultivated in 96 well tissue plates.

The optical density of each well was measured spectrophotometrically at 480 nm in the MRX Dynatech ELISA reader (UK). After 24 h of field-free incubation of the plates at 37°C, the experiment was started by adding docetaxel (generous gift from Dr. Andrašina). Cell samples were exposed to cytostatics for 6 hours with consequent replacement of the medium or keeping them in the medium with cytostatics for 72 hours depending upon the experimental conditions. Docetaxel was added to the Dulbecco's Modified Eagle Medium so that the concentration of cytostatics in medium was consequently: C1: 1 µmol l^{-1}, C2: 3 µmol l^{-1}, C3: 5 µmol l^{-1}, C4: 50 µmol l^{-1}, C5: 100 µmol l^{-1}, C6 250 µmol l^{-1}. The microplates were then transferred to another incubator Heraeus, one plate was placed in the Transversal Electromagnetic Mode (TEM) cell and the other control plate distant from it. Subsequently, proliferation was allowed to continue for another 24 h in field-free conditions. The cytotoxic effect of docetaxel and metabolic activity of cells after exposure to the ultra-high frequency electromagnetic fields were evaluated on the eighth day of the cell growth, e.g. all experiments were performed with exponentially growing cells [19] or immediately after 6 hours exposure to docetaxel. The viability of drug treated cells and cells after the exposure

to the ultra-high frequency electromagnetic fields was expressed as a percentage of metabolic activity in comparison with controls taken as 100%.

2.3 Statistical analysis.

The results for the viability of cells evaluated by the MTT test were expressed as the mean from four independent experiments ± standard deviations (SD). Multiple comparisons between groups were assessed using one-way analysis of variance (ANOVA). Probability values $P<0.05$ were accepted as statistically significant.

2.4 Electromagnetic field exposure system

Cells were irradiated with a high frequency electromagnetic field generated within the certified gigahertz TEM cell manufactured by Fischer Custom Communications (FCC-TEM-JM3, CA, FCC, USA) providing uniform incident plane wave exposure conditions. A schematic diagram of the experimental setup is shown in Figure 1.

Signal generator was used to produce electromagnetic field frequency of 900 MHz (MG 3642A, Anritsu, Japan). The cell cultures, growing in sterile 96-well microtiter plate (Sarstedt) and residing as a thin monolayer at the flat bottom of the flat well, were placed so that the H-field vector was parallel to the plane of the culture medium. For the supplied plane wave signal 8 W input power was required to achieve a 465 V m^{-1} electric field:

$$E \quad \frac{V}{b} \quad \frac{\sqrt{P\,Z_0}}{b}$$

where

V - rms voltage on the septum (centre conductor),

b - separation distance between the septum and lower or upper walls: 0.043 m,

P - net power flow to the cell, W,

Z_0 - real part of the cell's characteristic impedance ≅50 Ω.

Negative control cells were kept in the same experimental conditions, but were not exposed to ultra high electromagnetic radiation.

During the experiments, the microplates were exposed to 900 MHz for 72 hours at 37±0.1°C. The system's temperature was kept 37±0.1°C for the entire duration of the experiment. The temperature difference between the control and the exposed plates with cell cultures did not exceed 0.1°C. The temperature change was checked inside the incubator with the aid of the temperature probe placed in the vicinity of the TEM cell.

3. Results

Docetaxel cytotoxicity was assayed by exposure to MCF-7 cells at different doses in the range of 1-250 μmol l^{-1} of the drug in field-free conditions. Surviving cell fractions at each dose were expressed as the optical density and were used to make dose–response curves (Figure 2) in order to determine the appropriate experimental conditions for the exposure of the cell culture to the electromagnetic field of ultra-high frequency. Concerning the effect of docetaxel,

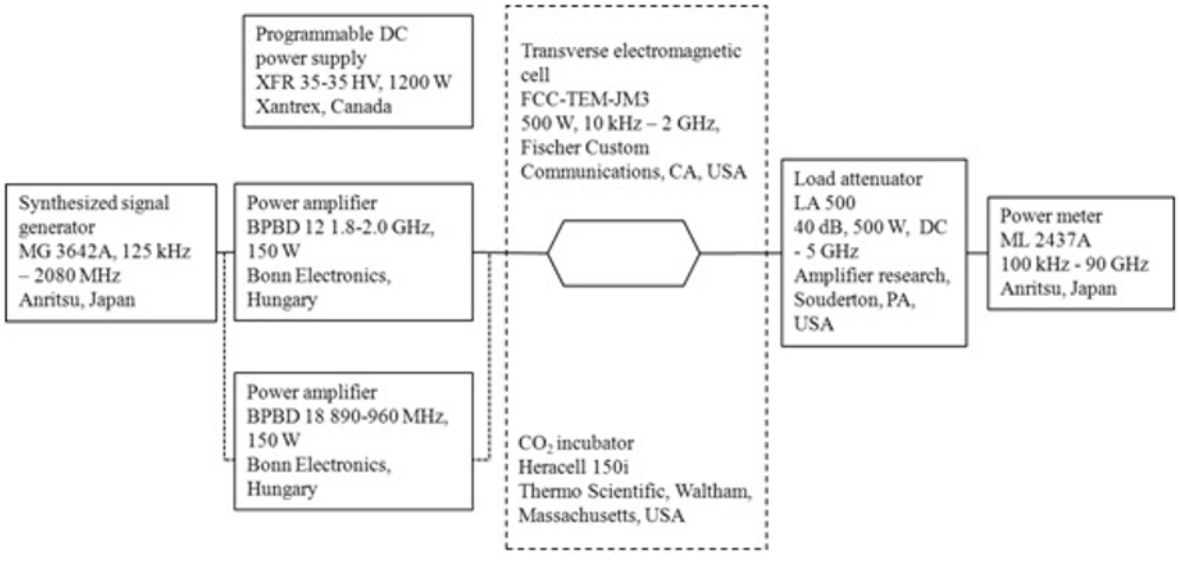

Figure 1. Schematic diagram of experimental set-up.

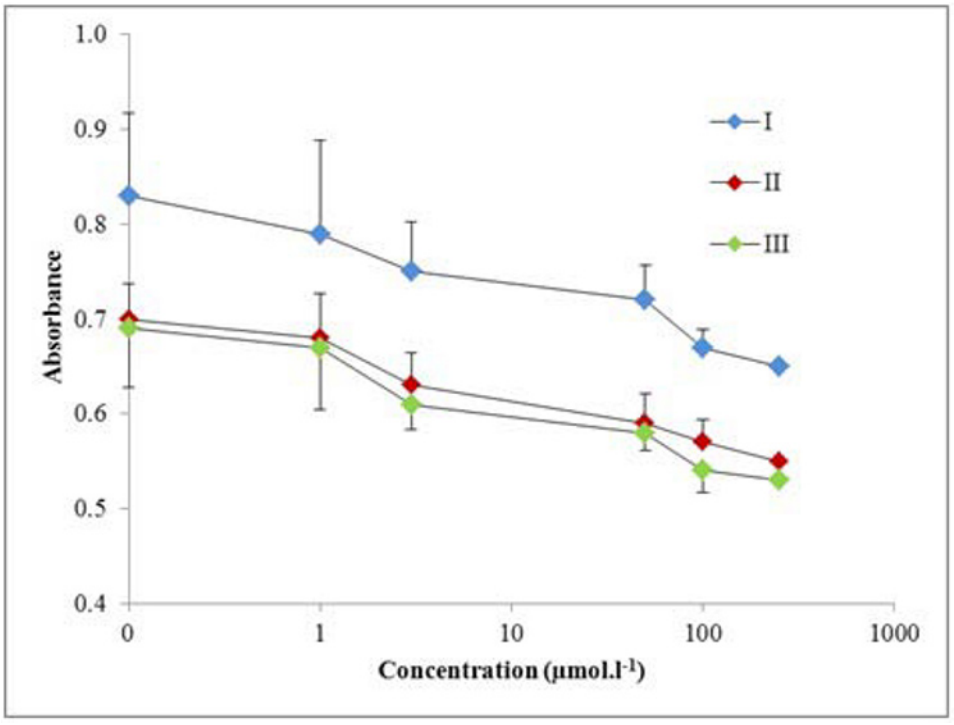

Figure 2. Docetaxel cytotoxicity curves obtained for MCF-7 breast cancer cells: I - 6 hour treatment with docetaxel, II - 6 hour treatment with docetaxel, replacement of the culture medium, 72 hour growth; III - 72 hour growth in the medium with cytostatics.

three subsequent patterns of the treatment of MCF-7 cells were chosen: I - MCF-7 cells were treated with docetaxel for 6 hours with subsequent colorimetric evaluation of the surviving cells, II – MCF-7 cells were treated with docetaxel for 6 hours followed by the replacement of the fresh culture medium; after that the cells were allowed to grow in cytostatic free medium up to 72 hours succeeded by the MTT test or III. The cells were exposed to cytostatics for the entire period of the growth (72 hours) and assessed by a colorimetric test.

The results of all experiments show that docetaxel inhibited the metabolic activity. The higher dose of docetaxel obtained from the dose–response curves, the lower metabolic activity was observed. The comparison of pattern II and III revealed that there was no significant change in suppression of metabolic activity of MCF-7 cells. To study the influence of 900 MHz electromagnetic field on the anti-proliferative effect of docetaxel in MCF-7 cells, 6 hour treatment with docetaxel was followed by replacement of the culture medium for the cytostatics-free medium for the remaining time of the culture growth.

As shown in Figure 3. the exposure of MCF-7 in docetaxel-free culture medium to 900 MHz electromagnetic field induced changes in the metabolic activity of cells. The presence of the electromagnetic field produced a decrement of 27.5% with a cell viability corresponding to a *P* value of *<0.05.*

The docetaxel response of different concentrations in either a configuration without electromagnetic field and or using a sinusoidal electromagnetic field of 900 MHz, the effect of these conditions on MCF-7 cells is presented in Figure 4. MCF-7 cells showed increased susceptibility to docetaxel when exposed to ultra-high electromagnetic field. Additive effect of docetaxel and electromagnetic field on the metabolic activity was observed at all concentrations of the cytostatic drug. When combining 1 µmol l^{-1} docetaxel with exposure to the electromagnetic field, the metabolic activity of MCF-7 cells decreased to the level that is even lower than the effect of the highest concentration of docetaxel used (250 µmol l^{-1}).

The statistical analysis confirmed that the suppression of metabolic activity by ultra-high radiation was significant for all concentrations of docetaxel if comparing untreated cytostatics cells in field-free conditions. A comparison of the proliferation of MCF-7 cells as % of control I (MCF-7 cells untreated with docetaxel) to the metabolic activity of MCF-7 cells as % of control II (MCF-7 cells untreated with docetaxel, exposed to the electromagnetic field) showed that the significant difference of the data was limited to the concentration of 3 µmol l^{-1} of docetaxel.

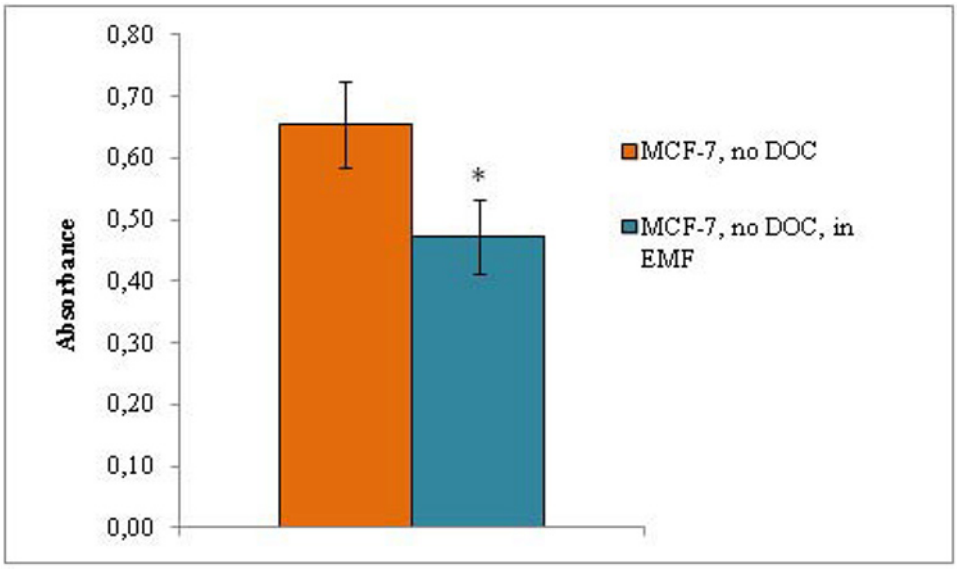

Figure 3. Effect of electromagnetic field (EMF) on metabolic activity of MCF-7 cells. Bars represent the mean±SD of four experiments. The error margins represent SD. *Significantly different from the control in field-free conditions at *P<0.05*.

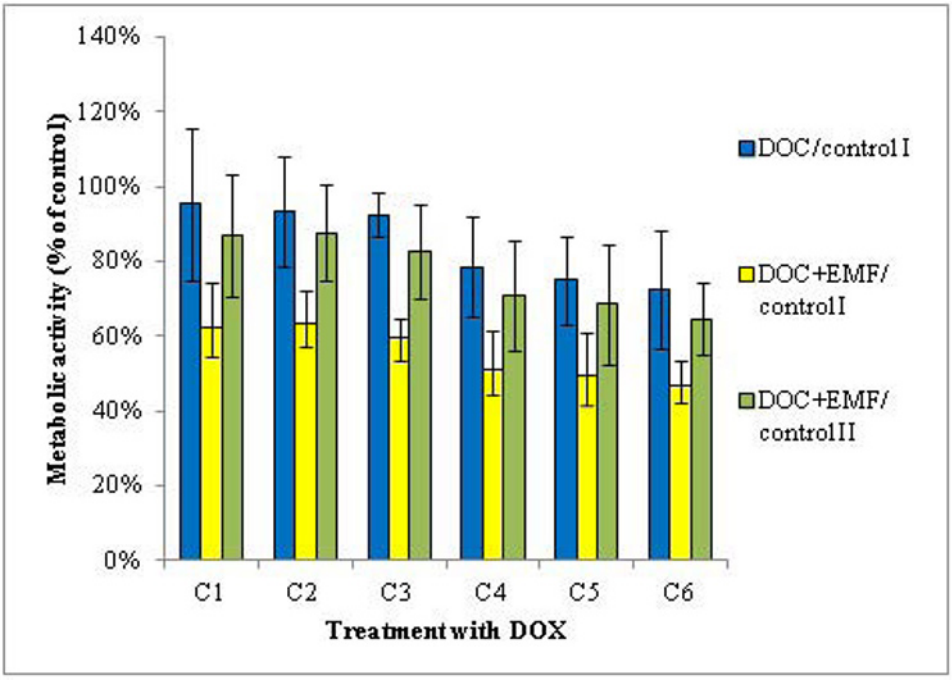

Figure 4. Effect of EMF on metabolic activity of MCF-7 cells treated with docetaxel C1: DOC=1 μmol l^{-1} (P<0.05 *versus* control I, P>0.05 *versus* control II), C2: DOC=3 μmol l^{-1} (P<0.01 *versus* control I, P<0.01 *versus* control II), C3: DOC=5 μmol l^{-1} (P<0.05 *versus* control I, P>0.05 *versus* control II), C4: DOC=50 μmol l^{-1} (P<0.01 *versus* control I, P>0.05 *versus* control II), C5: DOC=100 μmol l^{-1} (P<0.01 *versus* control I, P>0.05 *versus* control II), C6: DOC=250 μmol l^{-1} (P<0.01 *versus* control I, P>0.05 *versus* control II). Bars represent the mean±SD of four independent experiments (n=4). Control I: MCF-7 cells – no treatment with docetaxel, no exposure to EMF; Control II: MCF-7 cells – no treatment with docetaxel, exposure to EMF.

4. Discussion

Toxicity of conventional chemotherapeutic agents and chemotherapy resistance remains a major drawback in the tumor treatment. Further research into development of more selective drugs and treatment protocols that target cancer-specific defects is needed in order to maximise the benefit and at the same time minimise toxicity of anticancer drugs. Cancer cells are under firm control between viability/proliferation and death.

Any external influence upon each of these processes may lead to uncontrolled cell growth or cell death. Understanding the pathways associated with drug sensitivity and the ways in which tumor cells modulate these processes to promote their death or survival may contribute to the treatment selection.

The aim of the current study was to determine whether an additive response would be observed when human adenocarcinoma MCF-7 cells used as a model system for therapeutic studies were exposed to electromagnetic field of 900 MHz at 8 W input power to achieve a 465 V m^{-1} for 3 days whilst simultaneously treated with the chemotherapeutic drug docetaxel (1-250 μmol l^{-1}). A viability of cells related to toxicity of docetaxel and combination of the drug and electromagnetic field exposure was examined.

The effects of emf on cell population can be the consequence of the primary interaction of the physical agent either with the plasmatic membrane or with subcellular, intracellular and molecular structures. The ability to avoid apoptosis and uncontrolled proliferation are the characteristic properties of cancer cells. Apoptosis is characterized by numerous cytological changes, including DNA fragmentation, condensation of chromatin and activation of cysteinyl aspartate-specific proteinases: the caspases. The pathways leading to apoptosis may be dependent on or independent of caspases. Caspase-dependent apoptosis was described and studied first, but caspase-independent apoptosis is also a commonly accepted phenomenon [20]. Considering the fact that the original MCF-7 breast cancer line from the American Tissue Type Culture Collection according to author of the study [21] does not express caspase-3 and we decided not to analyse caspase content.

The observations reported in several studies suggested that Ca^{2+} levels belong to the main intercellular communication systems observed among biological cells playing an important role in triggering of apoptosis in response to a variety of external and internal stimuli. An increase in cytosolic concentration of Ca^{2+} through the involvement of the secondary messenger molecule (*i.e.*, IP3 or adenosine-5'-triphosphate (ATP)) resulting from a stimulus is produced by an excited cell [22]. Two different pathways, the signal among adjacent cells propagates through, were distinguished: signalling through gap junctions of IP3 molecules or free diffusion of ATP molecules. It has been experimentally acknowledged that both frequency and amplitude components of intercellular calcium current strongly depend on the morphological or phenotypical cell forms. It is assumed by authors of the study [23] that the oscillations of calcium concentration are responsible for the signal transmission preferably to the stationary calcium concentrations. The effect of calcium oscillations is observed even in nonexcitable cells not possessing an obvious oscillatory biological function. Sometimes it has been discussed temporal encoding of Ca^{2+} oscillations. The precise pattern of spikes of intercellular calcium waves seems to be more important for the signal transmission than the overall frequency of oscillations.

It was shown [24] that the altered Ca^{2+} dynamics and increase in the cytosolic Ca^{2+} in stem cell-derived neuronal cells can be influenced by nonthermal radiofrequency radiation exposure from 700 to 1100 MHz. The increase in the Ca^{2+} spiking activities was dependent on the frequency but not on the SAR between 0.5 to 5 W kg^{-1}.

It has been shown [25] that the membrane and, in particular, the membrane permeability is affected by EMF. Authors found that cell viability and Ca^{2+} influx in brain and dorsal root ganglion neuron of rats exposed to 2.45 GHz electromagnetic radiation were increased compared with controls. Investigating oxidative stress and Ca^{2+} homeostasis in the neuron cells of rat exposed to electromagnetic radiation concluded that melatonin supplementation have protective effects on the 2.45 GHz-induced increase Ca^{2+} influx where EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca^{2+} channels. In spite of the different experimental set up of the study and different SAR and intensities of EMF, it can be speculated that altered cytosolic Ca^{2+} levels trigger the observed change in viability of MCF-7 cells exposed to 900 MHz emf in our experiments as well. However, it is also necessary to take into account the relatively high intensity of the electromagnetic field used during our experiments. The application of pulsatile or longer duration electromagnetic fields to cells leads to transient or permanent permeabilization of the membrane. Holes on cell membrane can influence physiological responses of cells, including the change of ion channels and intracellular calcium flux, which may result in irreversible changes of cell function including the apoptosis of cells.

To electroporate the membrane, the voltage applied to the cell has to be chosen so that the threshold value of the transmembrane potential is exceeded. Electric field strength around 1000 V cm^{-1} is supposed to be high enough to evoke irreversible electroporation in the pulsatile mode. From our results, it can be seen that viability of MCF-7 cells can be also influenced by applying higher field intensity (465 V m^{-1}) at 900 MHz of continuous wave instead of using single pulses or small numbers of subsequent pulses. The mechanism of the membrane break up remains controversial. The theory of

the formation of hydrophilic pores in the lipid bilayer that are stable long enough to allow the passage of molecules belongs to the most discussed. No less attention is paid to the idea that the electric field facilitates the entrance of water molecules into the defects in the lipid bilayer making the membrane more permeable to hydrophilic molecules. However, it deserves mentioning that these pores have never been observed experimentally [26]. Indirect indication of relevancy of the theory attributed the effect of EMF to the entrance of water molecules in the defects of the membranes can be correlated to the hydrophilic analogue of docetaxel showing increased inhibition of cell proliferation with cell cycle delay at G2/M phases along with induction of apoptosis compared to the original docetaxel in MCF-7 cells [27].

EMF could also affect apoptosis-related genes and genes related to signal transduction. There are several genes that are related to apoptosis pathways like p53. The p53 tumor suppressor gene is thought to play a critical role in the cellular response like DNA damage, cell cycle arrest and apoptosis. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through apoptosis. Although no significant changes in the expression and activation of the p53 protein were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells [28], it would be useful to repeat this study at higher field intensities used in our experimental set-up.

In the long term the mechanism underling the cytotoxic effect of docetaxel has been thoroughly studied from all aspects. Generally, it is supposed that docetaxel inhibits cell growth and proliferation primarily through its effect on the cell cycle and induction of apoptosis and necrosis. The apoptosis and necrosis effect was found to be concentration and time dependent [29]. The mode of BT-20 and MDA-MB-231 cell death was found to be mainly by necrosis at low drug equivalent concentration and by apoptosis at high drug equivalent concentration. Analogous to our results, it was shown that at 72 hours exposure to the cytostatic, the percent apoptotic cell fragments were significantly higher for BT-20 and MDA-MB-231 cells.

The combined application of docetaxel and EMF in the sequence docetaxel→EMF showed that there is statistically a significant reduction of cell viability in the case of combination treatment in comparison with docetaxel alone: e.g. 1 μmol l^{-1} of docetaxel in combination with EMF has the same effect as 250 μmol l^{-1} of the drug alone. In contrast, it was found [30] that in the sequence of the events, during EMF exposure to cytostatic drug, the EMF pre-exposure can 'protect' cells from the toxicity induced by subsequent cytostatics treatment. The results obtained in our study cannot be compared completely with the observations in that report because of the obvious differences in exposure set up and the parameters used for the assessment of EMF exposure. Besides, our study included treatment with docetaxel followed by exposure of MCF-7 cells to EMF. Nonetheless, it would be useful to repeat this study at a more appropriate field intensity and SAR level.

Based on our observations we can conclude that the combination of docetaxel with high-frequency EMF in the sequence is more effective than the drug alone to change the viability of human breast carcinoma cells *in vitro*. However, further investigations are required to clarify the mechanism of action of the applied EMF exposure. Authors believe that HRM analysis of melting profile of genomic DNA extracted from the control and EMF-affected MCF-7 cells using MDR1 primer pairs could be useful in that task.

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References

[1] Panagopoulos D.J., Chavdoula E.D., Nezis I.P., Margaritis L.H, Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation, Mutat. Res., 2007, 626, 69-78

[2] Velizarov S., Kwee S., Raskmark P., The effects of radiofrequency fields on cell proliferation are non-thermal, Bioelectrochem. Bioenerg., 1999, 48, 177-180

[3] Lossi L., Alasia S., Salio Ch., Merighi A., Cell death and proliferation in acute slices and organotypic cultures of mammalian CNS, Prog. Neurobiol., 2009, 88, 221-245

[4] Chavdoula E.D., Panagopoulos D.J., Margaritis L.H., Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation, Detection of apoptotic

cell-death features, Mutat. Res. – Genet. Tox. En., 2010, 700, 51-61

[5] Franzellitti S., Valbonesi P., Ciancaglini N., Biondi C., Contin A., Bersani F., et al., Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay, Mutat. Res., 2010, 683, 35-42

[6] Luukkonen J., Hakulinen P., Mäki-Paakkanen J., Juutilainen J., Naarala J., Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872 MHz radiofrequency radiation, Mutat. Res. – Fund. Mol. M., 2009, 62, 54-58

[7] Sheppard A.R., Swicord M.L., Balzano Q., Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes, Health Phys., 2008, 95, 365-396

[8] Funk R.H.W., Monsees T., Nurdan Ö., Electromagnetic effects – From cell biology to medicine, Prog. Histochem. Cytoc., 2009, 43, 177-264

[9] Miyakoshi Y., Kajihara Ch., Shimizu H., Yanagisawa H., Tempol suppresses micronuclei formation in astrocytes of newborn rats exposed to 50-Hz, 10-mT electromagnetic fields under bleomycin administration, Mutat. Res., 2012, 747, 138-141

[10] Wang J., Sakurai T., Koyama S., Komatubara Y., Suzuki Y., Taki M., et al., Effects of 2450 MHz electromagnetic fields with a wide range of SARs on methylcholanthrene-induced transformation in C3H10T1/2 cells, J. Radiat. Res., 2005, 46, 351-361

[11] Orel V., Romanov A., The effect of spatially inhomogeneous electromagnetic field and local inductive hyperthermia on nonlinear dynamics of the growth for transplanted animal tumors, In: Todd Evans (Ed.), Nonlinear Dynamics, INTECH, Croatia, 2010

[12] Gottesman M.M., Ling V., The molecular basis of multidrug resistance in cancer: The early years of P-glycoprotein research, FEBS Lett., 2006, 580, 998-1009

[13] de Hoon J.P.J., Veeck J., Vriens B.E.P.J., Calon T.G.A., van Engeland M., Tjan-Heijnen V.C.G., Taxane resistance in breast cancer: A closed HER2 circuit?, Biochim. Biophys. Acta, 2012, 1825, 197–206

[14] Gavelová M., Hladíková J., Vildová L., Novotná R., Vondráček J., Krčmář P., et al., Reduction of doxorubicin and oracin and induction of carbonyl reductase in human breast carcinoma MCF-7 cells, Chem.-Biol. Interact., 2008, 176, 9–18

[15] Zhijian Ch., Xiaoxue L., Yezhen L., Shijie Ch., Lifen J., Jianlin L., et al., Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells, Mutat. Res., 2010, 695, 16–21

[16] Říhová B., Etrych T., Šírová M., Tomala J., Ulbrich K., Kovář M., Synergistic effect of EMF–BEMER-type pulsed weak electromagnetic field and HPMA-bound doxorubicin on mouse EL4 T-cell lymphoma, J. Drug. Target, 2011, 19, 890-899

[17] Orel V.E., Kudryavets Yu.I., Satz S., Bezdenezhnih N.A., Danko M.L., Khranovskaya N.N., et al., Mechanochemically activated doxorubicin nanoparticles in combination with 40 MHz frequency irradiation on A-549 lung carcinoma cells, Drug Deliv., 2005, 12, 171-178

[18] Barbault A., Costa F.P., Bottger B., Munden R.F., Bomholt F., Kuster N., et al., Amplitude-modulated electromagnetic fields for the treatment of cancer: Discovery of tumor-specific frequencies and assessment of a novel therapeutic approach, J. Exp. Clin. Canc. Res., 2009, 28, 51, DOI: 10.1186/1756-9966-28-51

[19] Trebunova M., Laputkova G., Slaba E., Lacjakova K., Verebova A., Effects of docetaxel, doxorubicin and cyclophosphamide on human breast cancer cell line MCF-7, Anticancer Res., 2012, 32, 2849-2854

[20] Mathiasen I.S., Jaattela M., Triggering caspase-independent cell death to combat cancer, Trends Mol. Med., 2002, 8, 212-220

[21] Janicke R.U, MCF-7 breast carcinoma cells do not express caspase-3, Breast Cancer Res. Treat., 2009, 117, 219-221

[22] Kuran M.S., Tugcu T., Edis B.Ö., Calcium signaling: Overview and research directions of a molecular communication paradigm, IEEE Wirel Commun, 2012, 19, 20-27

[23] Schuster S., Marhl M., Höfer T., Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling, Eur. J. Biochem., 2002, 269, 1333-1355

[24] Rao V.S., Titushkin I.A., Moros E.G., Pickard W.F., Thatte H.S., Cho M.R., Nonthermal effects of radiofrequency-field exposure on calcium dynamics in stem cell-derived neuronal cells: Elucidation of calcium pathways, Radiat. Res., 2008, 169, 319-329

[25] Nazıroğlu M., Çelik Ö., Özgül C., Çiğ B., Doğan S., Bal R., et al., Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca2+ channels in brain and dorsal

root ganglion in rat, Physiol. Behav., 2012, 105, 683-692

[26] Breton M., Mir L.M., Microsecond and nanosecond electric pulses in cancer treatments, Bioelectromagnetics, 2012, 33, 106-123

[27] Fauzee N.J., Wang Y.L., Dong Z., Li Q.G., Wang T., Mandarry M.T., et al., Novel hydrophilic docetaxel (CQMU-0519) analogue inhibits proliferation and induces apoptosis in human A549 lung, SKVO3 ovarian and MCF7 breast carcinoma cell lines, Cell Prolif., 2012, 45, 352-364

[28] Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C., Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells, Bioelectromagnetics, 2013, 34, 52-60

[29] Naik S., Patel D., Chuttani K., Mishra A.K., Misra A., In vitro mechanistic study of cell death and in vivo performance evaluation of RGD grafted PEGylated docetaxel liposomes in breast cancer, Nanomed-Nanotechnol., 2012, 8, 951-962

[30] Jin Z., Zong C., Jiang B., Zhou Z., Tong J., Cao Y., The effect of combined exposure of 900 MHz radiofrequency fields and doxorubicin in HL-60 cells, PLoS ONE, 2012, 9, e46102