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The effect of superposition of 900 MHz and incoherent noise electromagnetic fields on the induction of reactive oxygen species in SP2/0 cell line

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► ***Short report***

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ABSTRACT

Background: Induction of cellular response after exposure to electromagnetic fields is limited to coherent fields. An incoherent noise field is supposed to suppress the bioeffects of regular RF electromagnetic fields. The purpose of this study was to investigate the effect of GSM mobile phone-induced radiofrequency (RF) on the induction of oxidative stress in SP2/0 cell line. ***Materials and Methods:*** This study was also an attempt to assess whether these RF-induced effects can be blocked by superposing the RF radiation and an incoherent magnetic noise. Three groups of cultured cells were used in this study. The cells in the first group were only exposed to RF radiation emitted from a mobile phone simulator. The second group was only exposed to an incoherent noise field and the third group was simultaneously exposed to RF radiation and incoherent noise field. The exposure duration in all groups was 2 hours. The level of ROS production in the cells was quantified by the CM-H2DCFDA fluorescence probe, using flow cytometry technique. ***Results:*** Although our results showed increased ROS production after exposure to 900 MHz RF radiation, superposition of 900 MHz RF and the incoherent noise fields did not lead to increased levels of ROS in any experiment. However, the differences between RF exposure group and superposition of RF and noise exposure group were not statistically significant. ***Conclusion:*** Altogether our results cannot support the neutralizing effect of noise theory but may confirm the concept that just the coherent fields can be bioeffective while the incoherent noise fields cannot cause any biological effects.

Keywords: *Electromagnetic fields (EMF), superposition, noise, reactive oxygen species (ROS).*

INTRODUCTION

Some studies have been conducted to prove this theory and they improved while the constant or coherent field induced a cellular response; simultaneous exposure of cells to coherent and incoherent EMFs do not cause any response [1-12]. Some recent studies showed that

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radiofrequency radiation causes oxidative stress in living organisms. Also another study proposed a mechanism responsible for the reported effects of RF radiation on cells [13]. It is suggested that ROS generation is followed by stimulation of NADH oxidase in the cellular membrane. This could lead to stimulation of the matrix metalloproteinases and finally signaling cascade is started. Over the past years, our laboratory has focused on studying the health effects of exposure of laboratory animals and humans to some common and/or occupational sources of electromagnetic fields such as mobile phones [14-21] and their base stations [22], mobile phone jammers [23], laptop computers [24], radars [15], dentistry cavitrons [25] and MRI [26]. In this study, we investigated the effect of superposition of an incoherent field and incoherent RF EMF on the induction of reactive oxygen species in SP2/0 cell line (mouse myeloma). It was an attempt to compare this effect with those of exposure of cells to MW (as a coherent) and noise (as an incoherent) fields. The incoherent field was a noise field with an inconstant frequency between 30-90 Hz. 900 MHz electromagnetic field emitted by a simulator was used as a coherent or regular field

MATERIALS AND METHODS

Cell culture

SP2/0 cells (ATCC Ag14 cells, mouse myeloma, B cell, obtained from Diagnostic Labratory Sciences and Technology Research Center, Shiraz University of Paramedical Sciences, Iran) were cultured in RPMI-1640 (Gibco, USA) supplemented with 10% heat-inactivated FBS (FBS) (Gibco, USA) and 100 U/ mL of penicillin, 100 µg/mL of streptomycin maintained with 5% CO2 atmosphere at 37 °C. The cells were seeded in 6-well plates, each well containing 300×10^3 cell, and kept in incubator. Twenty four hours later, they were exposed to the electromagnetic fields. The groups in this study consisted of group I, exposed group in which the cells were exposed to RF EMF (a coherent field), Group II, control group in which the cells were not exposed to any fields, group III, exposed group in which the cells were exposed to RF and noise fields simultaneously (superposition of incoherent and coherent fields), group IV, control group in which the cells were not exposed to any fields, group V, exposed group in which the cells were only exposed to noise field (incoherent).

Exposure system

Noise signal generator

To generate a noise MF, waveguides were wrapped with three rectangular Helmholtz coils made up of copper. The distance between the two coils was calculated to maximize the uniformity of the magnetic field along the axis of the coils. First an array of white Gaussian noise was created. Afterwards, the array was filtered, using a digital bandpass filter. The signal with Gaussian distribution had frequency components in the range of 30 to 90 Hz. The spectrum was white in this range; in other words, overall mean frequency time of the components was independent of frequency. The output current was approximately 10 mA root mean square (RMS) value at maximum device volume. The root mean square (RMS) value for the magnetic flux density at the center of the coil system was 2.4 µT.

Assay of Intracellular ROS

A fresh stock solution of CM-H2DCFDA (10mM) was prepared in DMSO and diluted to a final concentration of 1 µM in 1×PBS (final working concentration adjusted to 2.5µg/ 50 µL). After 2 hours of exposure, the cells were centrifuged (1800 rpm/ 10 min). The dye was added to each group and kept in 37ºC for 30 min. The cells were harvested, washed with 1×PBS; cell-associated mean fluorescent intensity was measured by flow cytometry in FL1 channel Excitation and emission wavelengths were 488 and 525 nm, respectively.

Evaluation of oxidative stress

CM-H2DCFDA passively diffuses into the cells and it is hydrolyzed by intracellular esterase to liberate 2´ -7´ -dichlorofluoressein which is not highly fluorescent but during the reaction with

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ROS, it yields a highly fluorescent compound 2´-7´-dichlorofluorescein (DCF) that is trapped inside the cells. ROS content in the cell is expressed as mean fluorescence intensity of the DCF dye.

Statistical analysis

The results were expressed as the mean value of mean fluorescence intensity ± standard deviation. The statistical differences between means in each group were determined using a two-tailed paired student´s *t*-test. P<0.05 was considered as significantly different from the corresponding control group.

RESULTS

The level of ROS generation (expressed as mean fluorescence intensity) in SP2/0 cell line in the control and exposure groups is shown in figure 1. As shown in the Figure, exposure of the cells to 900 MHz EMF for 2 hours significantly increased the ROS generation compared to non-irradiated cells (P<0.05).

ROS generation level (expressed as mean fluorescence intensity) in SP2/0 cell line in the control and RF+noise exposure groups is shown in figure 2. As shown in the Figure, exposure of the cells to both 900 MHz EMF and noise for 2 hours could not significantly increase the ROS generation compared to non-irradiated cells.

ROS generation level (expressed as mean fluorescence intensity) in SP2/0 cell line in the control and noise exposure groups is shown in figure 2. As shown, exposure of the cells to noise for 2 hours could not significantly increase the ROS generation compared to non-irradiated cells.

Flowcytometry evaluation on ROS generation expressed as mean fluorescence intensity in control samples (A) as well as samples exposed to RF (B), noise field (C), superposition of noise and RF EMF (D) is shown in figure 2.

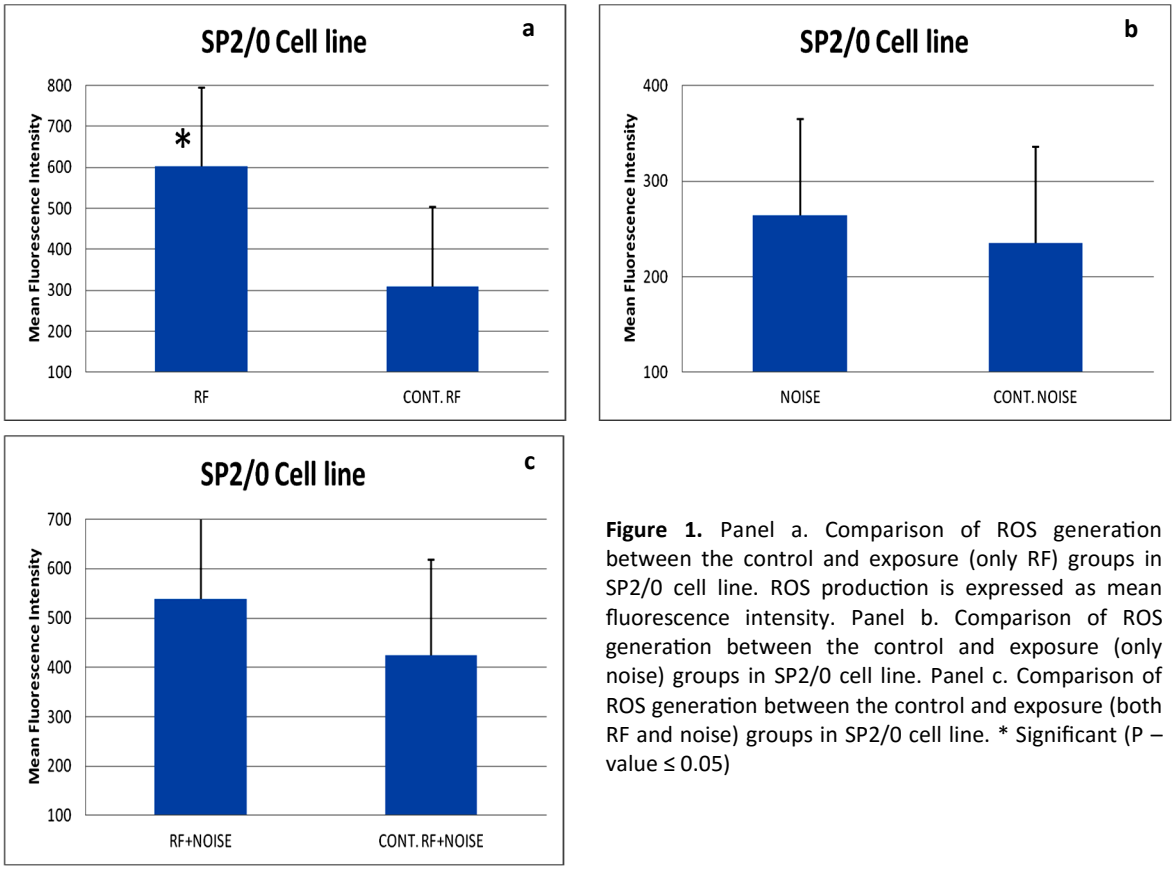


Figure 1. Panel a. Comparison of ROS generation between the control and exposure (only RF) groups in SP2/0 cell line. ROS production is expressed as mean fluorescence intensity. Panel b. Comparison of ROS generation between the control and exposure (only noise) groups in SP2/0 cell line. Panel c. Comparison of ROS generation between the control and exposure (both RF and noise) groups in SP2/0 cell line. * Significant (P – value ≤ 0.05)

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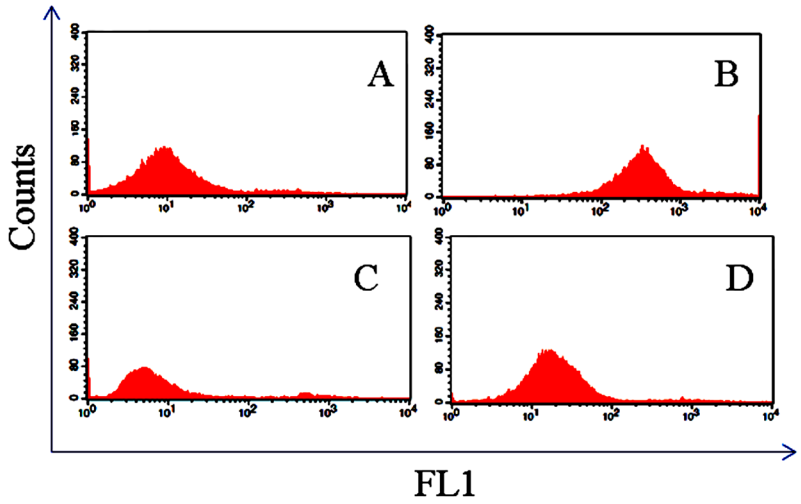


Figure 2. Flowcytometry evaluation on ROS generation expressed as mean fluorescence intensity in the control sample or non-irradiated group (A), sample radiated to RF EMF (B), sample radiated to noise field (C), sample radiated to superposition of noise and RF EMF (D).

DISCUSSION

As shown in figure 1, two hour exposure of SP2/0 cells to 900 MHz EMF caused increased ROS production. However, this was not seen after either exposure to noise field or superposition of 900 MHz and noise fields. Ruediger suggested EMFs induce their genotoxic effects via 3 ways; causing microthermal effect in the cellular system, interference on DNA repair mechanisms, and induction of reactive oxygen species (ROS) and oxidative stress. In our study, first of all, we investigated the effect of MW EMF on the production of ROS. After 2 hour exposure of SP2/0 cells to 900 MHz EMF, the amount of ROS increased in comparison to the control groups. After that, the effect of 2 hour exposure of cells to incoherent noise and superposition of 900 MHz and noise was studied. No induction of ROS was seen in the cells exposed to noise not in the cells exposed to 900 MHz and noise simultaneously. The increased ROS after exposure to 900 MHz was variable; in some samples, this effect was not seen. But the interesting point was that the ROS generation after exposure to an incoherent noise field and a field by superposition of noise and 900 MHz together did not have any difference compared to the control ones, with no exception. Litovitz et al. proposed that the biological effects induced by EMF exclusively belong to coherent fields which have the constant frequency, amplitude and phase for more than 10 sec. They call this kind of field "Temporal Coherent" field. When these fields' vary in less than 10 sec, the cellular receptors do not respond to them. It is called "Temporal Incoherent" field. It is said that cellular response to extracellular EMFs needs a particular degree of coherence. According to temporal sensing theory, cells remember the characters in 10 sec, if they change, the cellular memory resets [27]. They supposed that incoherent noise fields with varying field's characters can suppress the bioeffects of regular EMFs [28]. This happens when cells are exposed to two incoherent noise and coherent fields simultaneously. According to their reports and some studies which were subsequently done later, the suppressing effect of incoherent fields was proved.

Yao *et al.* approve this theory in 2 different works and in both ones reported noise field inhibits the MW radiation induced DNA damage or ROS increase in HLECs [5, 6]. Henry Lai investigated the effect of noise field on single and double DNA strand breaks in rats followed by exposure to MW. In another study, this effect was tested by evaluation the special learning in rats. Suppression effect of noise field was confirmed in both studies [3, 29]. It should be

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emphasized that in this report the incoherent noise field was in the range of extremely low frequency fields which doesn't interfere to the 900 MHz signals.

In conclusion, our results shows that a coherent and regular EMF with a constant frequency could cause oxidative stress in SP2/0 cells. In contrast, an incoherent and irregular EMF with variable frequency could not. In this study, the exposures of cells were done several times. Further research should be performed in the fields such as different endpoints to investigate the superposition of MW and noise EMFs other than oxidative stress.

It can be concluded that based on the results obtained in this study a coherent and regular EMF with a constant frequency could cause oxidative stress in SP2/0 cells whereas an incoherent and irregular EMF with variable frequency could not. However, further research is needed to clarify the biological effects of MW EMFs other than oxidative stress. Furthermore, the effect of intermittent exposures versus continuous exposure and the effect of different types of modulation in MW EMFs should be investigated.

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Conflicts of interest: *none to declare.*

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