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Induction of apoptosis by 900 MHz radiofrequency radiation emitted from a GSM mobile phone simulator in bystander Jurkat cells

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► ***Short report***

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ABSTRACT

Background: Radiation-induced bystander effect is a response which results in damage in non-irradiated cells in response to signals from the irradiated cells. The aim of the present study was to investigate microwave-induced bystander effect from a GSM mobile phone simulator on induction of apoptosis in Jurkat cell line. ***Materials and Methods***: Jurkat cells were divided into three groups of non-irradiated, exposed and bystander (medium transfer from the irradiated cells). The exposed group was subjected to irradiation from GSM mobile simulator for 2h and12h; the medium from irradiated cells was then transferred to the bystander cells. Apoptosis rate was measured12 and 24 hours after treatment by Annex in V 7-AAD kit using flow cytometry. ***Results***: Apoptosis was observed in both exposed and bystander cells of Jurkat cell line. The difference among non-irradiated, exposed and bystander cell groups was significant ($p<0.05$). ***Conclusion***: From the obtained results it can be concluded that microwave radiation exposure in Jurkat cells leads to a significant increase in the apoptosis rate not only in the exposed cells but also in the bystander cells.

Keywords: *Apoptosis, bystander effect, microwave, radiofrequency (RF), Jurkat cell line.*

INTRODUCTION

The levels of electromagnetic radiation in the microwave range are rapidly increasing in our environment [1]. Nowadays, the use of mobile phones has increased exponentially [2, 3]. Electromagnetic radiation, emitted by mobile phones and base stations, has raised the concern of possible health hazards related to exposure to these sources of electromagnetic fields [3, 4]. There are several controversial reports regarding the capability of radiofrequency electromagnetic fields (RF EMFs) to induce apoptosis [5-7]. Investigation of apoptosis rate in the rat's cortical neurons exposed to a 900-MHz global system for mobile communication (GSM) RF field for 24h has shown no statistically significant difference between controls and 24h GSM-exposed neurons [8]. Apoptosis, the programmed cell death, plays a significant role

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in the improvement and regulation of the immune system [9]. Microwave significantly inhibits the metabolic activity, leads to the induction of apoptosis in A549 cells [10], and enhances caspase dependent pathways of apoptosis [5]. Exposure of cells to ionizing radiation results in significant biological effects occurring in both irradiated and non-irradiated cells called bystander effect. Gap junctional intercellular communication and existence of diffusible factors play an important role in this phenomenon but the mechanism is not clearly known [11, 12]. In previous investigations, bystander effect induced by gamma and alpha radiations has been studied in different cell lines [13-15]. In 1992, Nagasawa and Little for the first time investigated the bystander effect of radiation using low doses of alpha particles from a source of plutonium238 in Chinese hamster ovary cells by measuring the frequency of sister chromatid exchange [16]. Early studies have shown that transmission of the medium from irradiated cells can induce biological effects in non-irradiated cells [14]. Cell communication via Gap Junction [11, 15, 17] and the release of soluble factors in medium [14, 18] have been reported as two basic mechanisms of bystander effect. 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 [19-26], laptop computers [27], radar [28] and MRI [29]. All previous studies on bystander effect have been performed using ionizing radiation. The aim of this study was to determine whether 900MHz GSM radiation can induce apoptosis in Jurkat cell line. Also, there was an attempt to investigate the bystander effect of RF as a non-ionizing radiation in Jurkat cells.

MATERIALA AND METHODS

Cell culture conditions

Human T-cell line (Jurkat) purchased from Pastor Institute in Iran was used in this experiment. Jurkat cells were grown in suspension in RPMI-1640 (Sigma) with 10% fetal bovine serum (Gibco) at a density of 10^5cells/mL. Cultures were exposed to MW for 2h at incubator temperature and then suspended in fresh medium at 37^{0}C. Thereafter, analyses were made by a flow cytometry device manufactured by BD Company, USA. Jurkat cells were cultured in laboratory condition (incubator 5% CO_2 at 37° C, RPMI 1640 and 10% FBS and 1% penicillin) in three groups of non-irradiated, exposed and bystander. There were 2×10^5 cells / ml in each well. According to the protocol, the cells were washed twice with cold PBS and then re-suspended in 1X binding buffer at a concentration of 1×10^6 cells/ml. Then, 100µl of the solution was transferred (1×10^5 cells) to a 5 ml culture tube; thereafter, 5µl of PE Annexin V and 5 µl 7-AAD were added to the tube. The cells were gently vortexed and incubated for 15 min at room temperature (25°C) in the dark. 400 µl of 1X binding buffer was added to each tube and analyzed by flow cytometry.

Exposure system

A GSM mobile phone simulator (designed and produced at the school of electrical and computer engineering, Shiraz University in collaboration with the Center for Research in Radiation Sciences (CRRS), Shiraz University of Medical Sciences (SUMS) was used for microwave irradiation. It was used as a coherent EMF source. The frequency was adjustable from 800 to 1800 MHz but in this experiment 900 MHz radiation (a wavelength of about 33.4 cm) that is similar to GSM mobile phone was used. Amplitude was digitally modulated with 217Hz frequency and duty cycle was 0.125 (the signal was switched on for 463 micro seconds periodically with a rate of 217 Hz. The power was adjustable from zero to 3 Watts but we fixed it at 2 W during exposure. The signal bandwidth was 200 kHz (similar to GSM mobile phone channels). The waves were transmitted by a co-axial wire to a waveguide. The cells were in 6 well plates. They were exposed to electromagnetic field of GSM mobile system simulator for 2 hours. After 12 hours, the medium was separated and added to the bystander cell groups. Group A, 12 hours after transferring the medium and Group B 24 hours

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after it were tested. In this study, apoptosis was determined by apoptosis kit Annexin V-PE 7-AAD using flow cytometry method after exposure of the cells to microwave radiation emitted from GSM mobile simulator. Using this method, apoptosis in the early and late stages, as well as necrosis in each group, was determined and analyzed by non-parametric statistical tests, such as Mann-Whitney U and Kruskal Wallis tests. P value less than 0.05 was considered as significant.

RESULTS

The finding of this study showed a statistically significant difference between the non-irradiated and exposed and also between non-irradiated and bystander cells in group A (12h) while the difference between cell groups in group B (24h) was not significant. Figure 1 displays the result of irradiation of Jurkat cells under 2h exposure and 12h after the medium transfer. MW irradiation was found to affect the apoptosis of the Jurkat cells in both exposed and bystander cells in comparison with the non-irradiated cells. The differences found among all the three groups are shown in table 1. (Group A, Means ± SE: 2.72 ± 0.17, 6.84 ± 1.64, 5.75 ± 0.58 respectively). In figure 2, the percentage of apoptosis which was analyzed 24h after the medium transfer is shown in the three groups (Group B, Means ± SE: 1.48 ± 0.15, 2.77 ± 0.56, 1.14 ± 0.35, respectively). No significant differences were seen in the total number of cells in group B with Annexin V 7AAD.

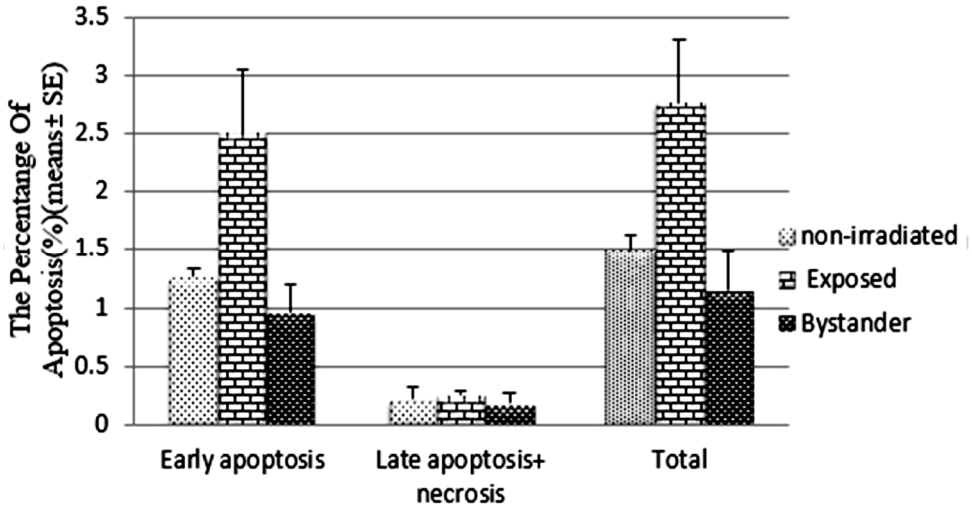

Figure 2. Comparison of apoptosis rate in 3 subgroups of non-irradiated, exposed and bystander T-cell line (Jurkat), (2h exposure to 900 MHz field; 24h later medium transfer was performed and after 12h analysis was conducted by flow cytometry method and Annexin V-AAD kit).

Table 1. Apoptosis rate in different subgroups. The significance level was considered at p<0.05.

Groups	Non-irradiated Cells	Exposed Cells	Bystander Cells	Significance (P-Value)
Group A	2.72 ± 0.17	6.84 ± 1.64		P=0.049
	2.72 ± 0.17		5.75 ± 0.58	P=0.049
		6.84 ± 1.64	5.75 ± 0.58	P=0.827 (NS)
Group B	1.48 ± 0.15	2.77± 0.56		P=0.089 (NS)
	1.48 ± 0.15		1.14 ±0.35	P=0. 42 (NS)
		2.77± 0.56	1.14 ±0.35	P=0.069 (NS)

NS: not significant

*p-value shows the results obtained by performing Mann-Whitney test(a non-parametric test for comparing two means)

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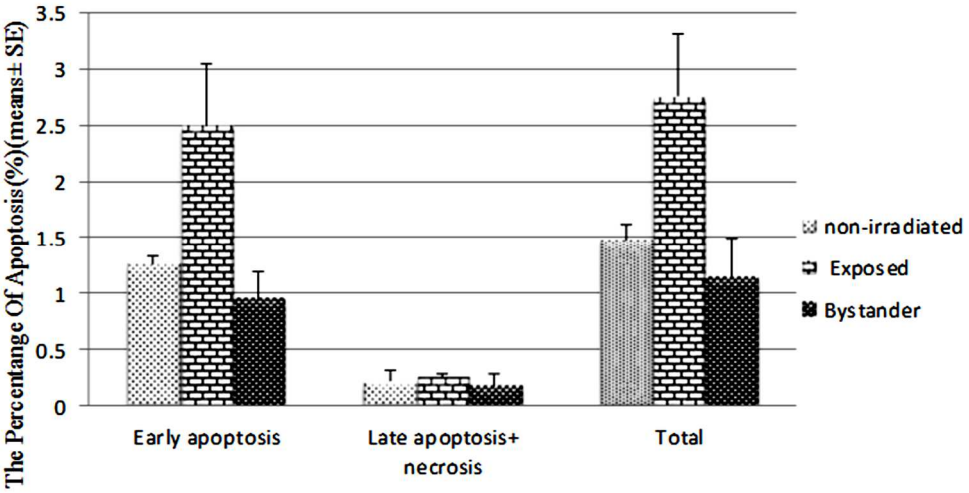

Figure 2. Comparison of apoptosis rate in 3 subgroups of non-irradiated, exposed and bystander T-cell line (Jurkat), (2h exposure to 900 MHz field; 24h later medium transfer was performed and after 12h analysis was conducted by flow cytometry method and Annexin V-AAD kit).

DISCUSSION

Our findings in this study showed that when the cells were exposed to RF radiation for two hours, programmed cell death in group A among non-irradiated, exposed and bystander cells was statistically significant while in group B it was not. To the best of our knowledge, this study is the first investigation on MW-induced bystander effect. Mortazavi *et al.* have previously indicated that laboratory animals pre -irradiated with radiofrequency radiation were less susceptible to subsequent lethal effects of high doses of ionizing radiation [22, 30]. These findings were later confirmed by our subsequent reports [22, 24] as well as the few published reports that investigated the induction of adaptive response after pre-treatment with microwave radiation [31-35].

The use of mobile phones has increased drastically over the past years. This growth has generated concerns regarding the safety of these phones. Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. On the other hand, bystander effect is a radiobiological phenomenon which occurs at low doses of radiation and results in damage to the non-irradiated cells in response to the signals from irradiated cells. There are several controversial reports regarding the ability of RF EMFs to induce apoptosis [5-7]. Previous research showed that with continuous exposure to 2.45-GHz microwave in a Jurkat *T-cell* line, significant non-thermal effects regarding Fas-induced apoptosis occur. On the other hand, our results revealed that under the conditions of the present experiment, RF exposure (GSM-900 MHz) does significantly increase the apoptosis rate in the Jurkat cell line.

Different results have been reported on the effect of RF on the apoptosis. Capri *et al*. had previously indicated that when an apoptotic agent was used in peripheral blood mononuclear cells, radiofrequency at a frequency of 1800 MHz could not induce apoptotic phenomenon in this in-vitro system[4]. Many studies have also shown that microwave radiation can induce apoptosis and cause cell death, but many others could not reveal such an effect. Previous studies reported the use of RF exposure as a therapeutic tool for inducing apoptosis in tumor cells [36].

CONCLUSION

To the best of our knowledge, our study is the first investigation on the MW-induced bystander effect. Based on the finding of this study, it can

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be concluded that microwave radiation exposure in Jurkat cell line leads to a significant increase in the apoptosis rate not only in the exposed cells but also in the bystander cells. This finding may have important implications for radiotherapy.

Conflicts of interest: *none to declare.*

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