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Investigation of the effects of distance from sources on apoptosis, oxidative stress and cytosolic calcium accumulation via TRPV1 channels induced by mobile phones and Wi-Fi in breast cancer cells☆

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ABSTRACT

TRPV1 is a Ca^{2+} permeable channel and gated by noxious heat, oxidative stress and capsaicin (CAP). Some reports have indicated that non-ionized electromagnetic radiation (EMR)-induces heat and oxidative stress effects. We aimed to investigate the effects of distance from sources on calcium signaling, cytosolic ROS production, cell viability, apoptosis, plus caspase-3 and -9 values induced by mobile phones and Wi-Fi in breast cancer cells MCF-7 human breast cancer cell lines were divided into A, B, C and D groups as control, 900, 1800 and 2450 MHz groups, respectively. Cells in Group A were used as control and were kept in cell culture conditions without EMR exposure. Groups B, C and D were exposed to the EMR frequencies at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) for 1 h before CAP stimulation. The cytosolic ROS production, Ca^{2+} concentrations, apoptosis, caspase-3 and caspase-9 values were higher in groups B, C and D than in A group at 0 cm, 1 cm and 5 cm distances although cell viability (MTT) values were increased by the distances. There was no statistically significant difference in the values between control, 20 and 25 cm.

Wi-Fi and mobile phone EMR placed within 10 cm of the cells induced excessive oxidative responses and apoptosis via TRPV1-induced cytosolic Ca^{2+} accumulation in the cancer cells. Using cell phones and Wi-Fi sources which are farther away than 10 cm may provide useful protection against oxidative stress, apoptosis and overload of intracellular Ca^{2+}. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

1. Introduction

Electromagnetic radiation (EMR) produced by mobile phones and their base station antennae is in the range of 900 and 1800 MHz for the Global System for Mobile Communications (GSM). Mobile phones emit radiofrequency EMR which may affect human health based on biological stress responses [5]. Wireless local area network systems (WLAN, 2450 MHz), are an alternative to wired internet access in business centers, homes, and public areas providing means of communication and information exchange [26]. These concerns require further investigation of possible biological effects of exposure to WLAN signals [5]. Cancer, with 10 million new cases of per year, is one of the biggest concerns of humanity [15,16]. A main concern has been the risk of cancer and DNA degeneration among people living near base stations, but the general welfare of all citizens exposed to EMR is becoming a frequent subject of conjecture [5,13,22,25,30]. With regard to this issue, there have been numerous reports of valuable research, but the results are still somewhat inconclusive [5,12,21,29]. Recently, we observed proliferative and tissue injury effects of exposure to 2450 MHz radiation in an HL-60 cancer cell line [22], an MDA-MB-231 breast cancer cell line [14] and in rat tissues through induction of Ca^{2+} influx and oxidative stress [4,13,30].

EMR can alter the energy level and spin orientation of electrons and, as a consequence, increase the activity, concentration and lifetime of ROS [20]. There are various antioxidant mechanisms in cells that neutralize the harmful effects of ROS [22] but exposure to EMR results in increases of ROS due to loss of efficiency of antioxidant mechanisms and alterations in the mitochondrial electron transfer chain [8,12]. However, whether distance affects the induction of oxidative stress and apoptosis in breast cancer cells exposed to 900, 1800 and 2450 MHz EMR is still unknown and deserves further study.

Ca^{2+} homeostasis of the cells is one of the many important functions. The proliferation of cells, to undergo apoptosis, induction of oxidative

Abbreviations: $[Ca^{2+}]_i$, cytosolic free calcium ion; CAP, capsaicin; DMSO, dimethyl sulfoxide; EGTA, ethylene glycol-bis[2-aminoethyl-ether-*N*,*N*,*N*,*N*-tetraacetic acid; EMR, electromagnetic radiation; GSM, Global System for Mobile Communications; HBSS, Hank's buffered salt solution; RF, radiofrequency; ROS, reactive oxygen species; TRP, transient receptor potential; TRPM2, transient receptor potential melastatin 2; TRPV1, transient receptor potential vanilloid 1

☆ This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

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stress and physiological functions such as signal transduction is a part of Ca^{2+} homeostasis [17]. Cytosolic free calcium ion concentration $[Ca^{2+}]_i$, which is dependent on both plasma and intracellular membrane functions, is controlled by many ion channels. The transient receptor potential (TRP) family is one of these channels and they are important non-selective cation channels [23]. TRPV1 is a cation channel and is a member of the subfamily of these channels. It can be activated by capsaicin and it is also heat sensitive (≥ 43 °C) [24,27]. Thermal effects of electromagnetic radiation caused by changes in temperature have been noted [7] and temperature effects of exposure to EMR of 450 MHz and 2450 MHz frequencies have been seen in various tissues and in total blood flow examinations and increase in skin temperature [1]. This issue has been examined in many well performed experimental studies using rats and mice subjected to EMR exposure [19–24].

Since to date there is no report about the mechanism of 900, 1800 and 2450 MHz EMR-induced actions on cellular survival and death, such an investigation may help clarify how free radical formation and apoptosis occur following EMR-induced injury. The present study was designed to determine the effects of 900, 1800 and 2450 MHz EMR exposure on oxidative damage of breast cancer cells, apoptosis and ROS production, as well as the possible protective effects of different distances on the values by analyzing apoptosis, caspase activities, cytosolic ROS production, and accumulation of $[Ca^{2+}]_i$ concentration-induced oxidative stress.

2. Materials and methods

2.1. Cells and reagents

The Michigan Cancer Foundation-7 breast cancer cell line (MCF-7) was used in this study. The cell line was originally obtained from 'The Leibniz Institute-German Collection of Microorganisms and Cell Cultures (DSMZ)' Cell Lines Bank (Braunschweig, Germany). Ethylene glycol-bis(2-aminoethyl-ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) and dimethyl sulfoxide and Roswell Park Memorial Institute (RPMI) 1640 medium were obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA). N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (ACDEVD-AMC), nonidet-P-40 substitute (NP40), 2-(N-morpholino)ethanesulfonic acid hydrate (MES hydrate), PEG, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3-[(3-chomalidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), polyethylene glycol (PEG) and dithiothreitol (DTT) were obtained from Sigma Chemical. Dihydrorhodamine-123 (DHR 123/N-acetyl-Leu-Glu-His-Asp-7-amino-4-methylcoumarin (AC-LEHD-AMC) was purchased from Bachem (Bubendorf, Switzerland. A mitochondrial stain 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was purchased from Santa Cruz (Dallas, TX, USA). All organic solvents were also purchased from Santa Cruz (Dallas, TX, USA). The reagents were equilibrated at room temperature for 30 min before an analysis.

2.2. Cell culture

The MCF-7 cells were cultured in RPMI 1640 medium supplemented with fetal bovine serum in a humidified incubator at 37 °C, 5% CO_2, and 95% air. The cells were counted daily by removing a small volume from the tissue culture flask (filter cap, sterile, 250 ml, 75 cm^2), diluting it with an equal volume of trypan blue (0.4%), and tallying viable cells (trypan blue excluding) with a hemocytometer. Cultures were maintained as a suspension without shaking or stirring at a density of 1×10^6 cells per ml by dilution with fresh media. Cultures were transferred once a week.

2.3. Groups

Cells were seeded in 8–10 flasks at a density of 1×10^6 cells per flask (filter cap, sterile, 250 ml, 75 cm^2) and placed in a circulating water bath (Fig. 1). All cells were cultured in the same culture medium (37 °C) and for an identical time (1 h). The cells were divided into four main groups.

A- Control group: The cells were not exposed to EMR but were kept in falcon tubes containing the same cell culture medium and conditions for 1 h.
B- 900 MHz group: Cells in the group were exposed to 900 MHz EMR at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) for periods of 1 h.
C- 1800 MHz group: Cells in the group were exposed to 1800 MHz EMR at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) for 1 h.
D- 2450 MHz group: Cells in the group were exposed to 2450 MHz EMR at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) for 1 h.

All the exposures at the different distances were repeated 4–6 times. At the end of the 1 h incubation, the control and exposed cells were used for the analyses of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) concentration, apoptosis and caspase.

2.4. Exposure system and design

The exposure system has been described in detail elsewhere [14]. The cells were kept in a circulatory water bath (Fig. 1). The cells were attached to the walls of the flask. The exposure system was performed in a special room that was fitted with plastic furniture such as tables and chairs so as to minimize the possibility of radiation reflection. The walls of the room were covered by chromium–nickel sheets (thickness: 1 mm) for protecting the cells from possible outside electromagnetic interference. The continuous wave of radiofrequency signal (900 MHz with 217 Hz pulses) emitted by the generator was amplified initially and then fed into the cancer cells in the water bath by an antenna

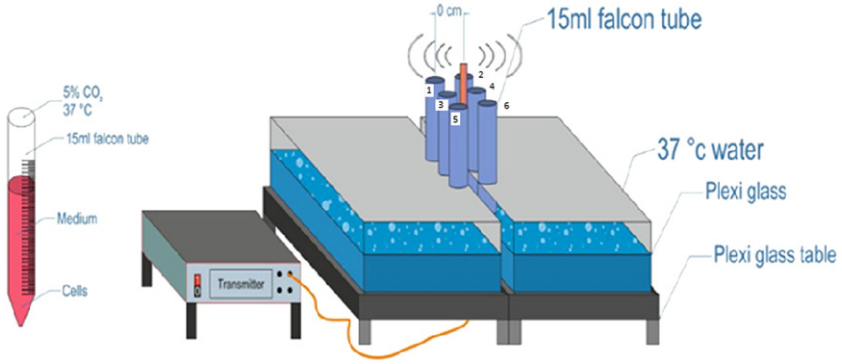

Fig. 1. Schematic diagram of radiofrequency exposure device.

Table 1
Specific absorption rate (SAR) values of falcon tubes (mean ± SD, $n = 6$).

	Tube Numbers					
	1	2	3	4	5	6
SAR (W/kg) (min–max)	0.32 ± 0.05 (0.24–0.39)	0.26 ± 0.08 (0.18–0.40)	0.50 ± 0.06 (0.43–0.58)	0.51 ± 0.06 (0.43–0.58)	0.28 ± 0.08 (0.20–0.40)	0.26 ± 0.04 (0.20–0.31)
SAR (W/kg) mean of 6 tubes	0.36 ± 0.02					

(Biçer Electronic, Sakarya, Turkey). This antenna has a special Falcon holder designed to accommodate the cells for appropriate exposure conditions. The repetition time, frequency, and amplitude of the radio-frequency (RF) energy spectrum were monitored by a satellite level meter (PROMAX, MC-877C, Barcelona, Spain). Radiation reflection and exposure were measured with a Portable RF Survey System (HOLADAY, HI-4417, Eden Prairie, MN, USA) with a standard probe. The EMR dose was calculated from the measured electric field strength (V/m) and digital models based on the FDTD numerical code. Distance was arranged at 0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm between the falcon tubes and probe of the exposure system. Six falcon tubes each containing 1×10^6 cells/ml (5 ml total medium) were placed on a non-conductive plexi glass table at a height of 110 cm at a precise location where the required power density was measured. The RF field inside the special room was probed using a strength meter and the precise positions which provided power densities of 1.2, 12 or 120 μW/cm^2 were determined [14]. The required power density (≤12 μW/cm^2) was continuously recorded every 5 min using a satellite level meter (EXTECH-480836, Extech Instruments, Nashua, NH, USA). The data were saved on a computer. At the top of the flask, the average specific absorption rate (SAR) estimated for 900 MHz exposure at 12 μW/cm^2 power flux density was 0.36 ± 0.02 mW/kg (Table 1). The water bath (Water Bath 601, Jiangsu Zhengji Instruments, Jiangsu, China) installed in the chromium–nickel covered room was maintained at 37 °C (relative

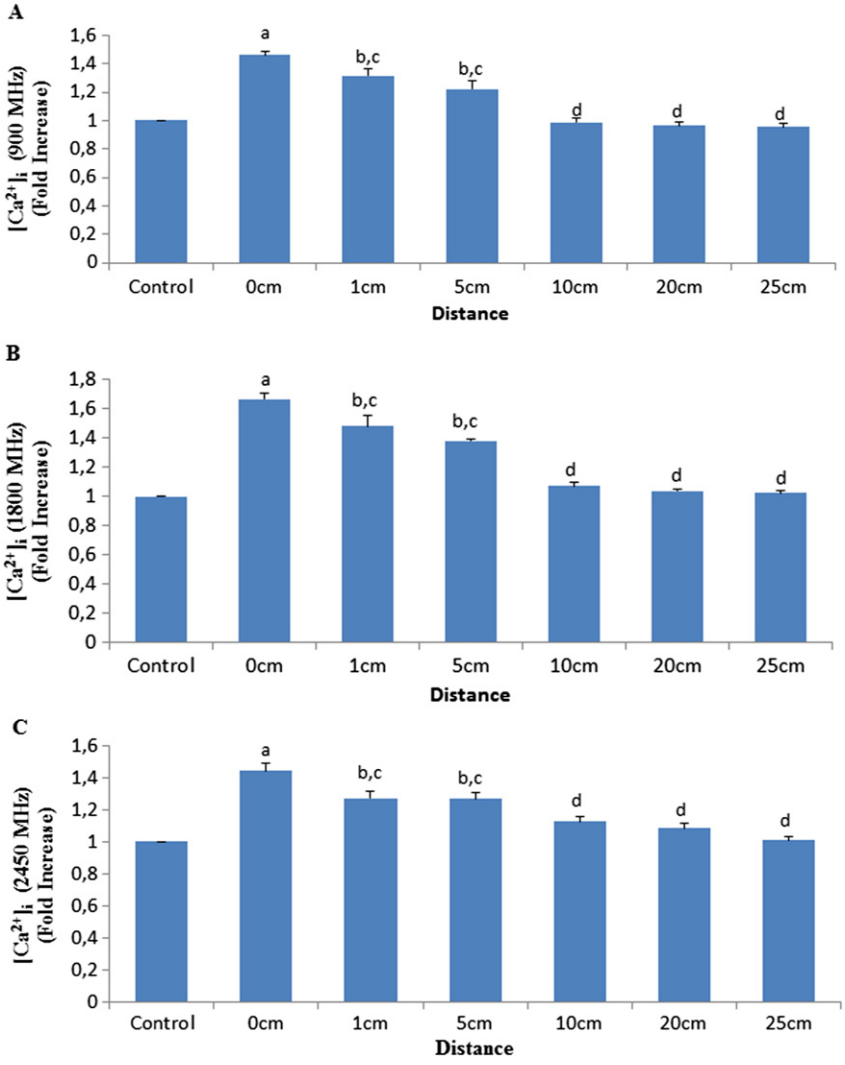

Fig. 2. Effects of EMR (A-900, B-1800 and C-2450 MHz) exposure on cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) concentration in MCF-7 breast cancer cells. The Ca^{+2} entry was estimated as described under Materials and methods sections. Values are presented as mean ± SD of 6 separate experiments and expressed as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.001$ and $^{b}p < 0.01$ versus control. $^{c}p < 0.05$ and $^{d}p < 0.01$ versus 0 cm group.

humidity of 83%) and the inside temperature of the flask was also 37 °C. The SAR values at the input 1.2 μW/cm^2 power flux density were calculated using Burkhardt's formula [3].

2.5. Cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) concentration analysis

The MCF-7 cells were loaded with 4 μM fura-2/AM in loading buffer with 1×10^6 cells per ml for 45 min at 37 °C in the dark, washed twice with phosphate buffer then incubated for an additional 30 min at 37 °C to complete probe de-esterification, and re-suspended in loading buffer at a density of 1×10^6 cells per ml according to a procedure published elsewhere [21]. All groups were immediately exposed to capsaicin (CAP and 0.1 mM) for stimulation of $[Ca^{2+}]_i$ influx. Fluorescence was recorded from 2 ml aliquots of magnetically stirred cellular suspension by using a spectrofluorometer (Cary Eclipsys, Varian Inc, Sydney, Australia) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_i$ were monitored by using the fura-2 340/380 nm fluorescence ratio and they were calibrated according to the method of Grynkiewicz et al. [10]. We performed a total of 6 experiments ($n = 6$) to measure the intracellular calcium concentration. The data were expressed in terms of the fold-increase, relative to the control, after the pretreatment level.

The release of Ca^{2+} was estimated using the integral of the rise in $[Ca^{2+}]_i$ for 150 s after addition of CAP [8,28]. The release is reported as nanomolar concentration (nM) with sampling at 1 s intervals, as previously described [36]. All experiments were carried out at 37 °C.

2.6. Cell Viability (MTT) Assay

To assess EMR adverse effects on cell viability, we evaluated the mitochondrial activity of living cells by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) quantitative colorimetric assay. For these assays, distance differentiated the breast cancer cells were cultured in 96-well culture plates before EMR exposure. After treatments, the cells were washed and incubated with fresh RPMI 1640 medium containing MTT (0.5 mg/ml) at 37 °C for 90 min. Then, the supernatant was discarded and dimethyl sulfoxide was added to dissolve the formazan crystals. Treatments were carried out in duplicate. The optical density in each well was evaluated by measurement of absorbance at 490 and 650 nm using a microplate reader (Infinite Pro200) [14]. We performed a total of 6 experiments ($n = 6$) for the cell viability assay. The data are presented as fold-increase over the pretreatment level (experimental/control).

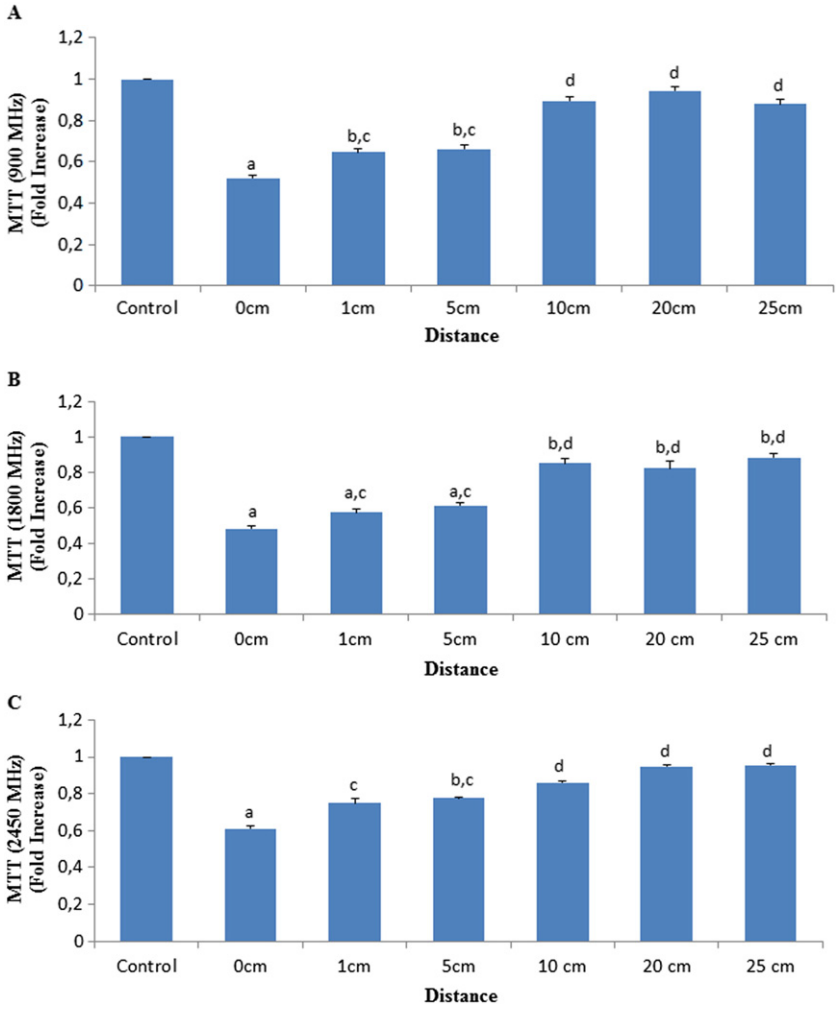

Fig. 3. Effects of EMR (A-900, B-1800 and C-2450 MHz) exposure on cell viability (MTT) levels in MCF-7 breast cancer cells. The MTT level was estimated as described under Materials and methods sections. Values are presented as mean ± SD of 6 separate experiments and expressed as fold increase over the pretreatment level (experimental/control). $^a p < 0.001$ and $^b p < 0.01$ versus control. $^c p < 0.05$ and $^d p < 0.01$ versus group 0 cm.

2.7. Apoptosis assay

The APOPercentage assay (Biocolor, Belfast, Northern Ireland) is a dye-uptake method, which stains only the apoptotic cells with a red dye. When the membrane of apoptotic cell loses its asymmetry, the APOPercentage dye is transported into cells, staining apoptotic cells red, thus allowing detection of apoptosis by spectrophotometry [2]. The apoptosis values were obtained from 6 separate experiments and expressed as fold increase over the pretreatment level (experimental/control).

2.8. Assay for caspase-3 and -9 activities

The determination of caspase-3 and caspase-9 activities was based on a method previously reported [8,23] with minor modifications. Stimulated or resting cells were washed once with PBS. After centrifugation, cells were re-suspended in PBS at a concentration of 10^3 cells/ml. Fifteen microliters of the cell suspension was added to a microplate of a microplate reader and mixed with the appropriate peptide substrate dissolved in a standard reaction buffer that was composed of 100 mM HEPES, pH 7.25, 10% sucrose, 0.1% CHAPS, 5 mM DTT, 0.001% NP40 and 40 μM of caspase-3 substrate (AC-DEVD-AMC) or 0.1 M MES hydrate, pH 6.5, 10% PEG, 0.1% CHAPS, 5 mM DTT, 0.001% NP40, and 0.1 mM of caspase-9 substrate (AC-LEHD-AMC). Substrate cleavage was measured with the microplate reader (Infinite Pro200) with an excitation wavelength of 360 nm and emission at 460 nm. The data were calculated as fluorescence units/mg protein and presented as fold-increase over the pretreatment level (experimental/control). The caspase-3 and caspase-9 assays were performed in 6 separate experiments.

2.9. Intracellular reactive oxygen species (ROS) measurement

DHR 123 is a non-fluorescent, non-charged dye that easily penetrates cell membrane. Once inside the cell, DHR 123 becomes fluorescent upon oxidation to yield rhodamine 123 (Rh 123), the fluorescence being proportional to ROS generation. The fluorescence intensity of Rh123 was measured in an automatic microplate reader (Infinite Pro200). Excitation

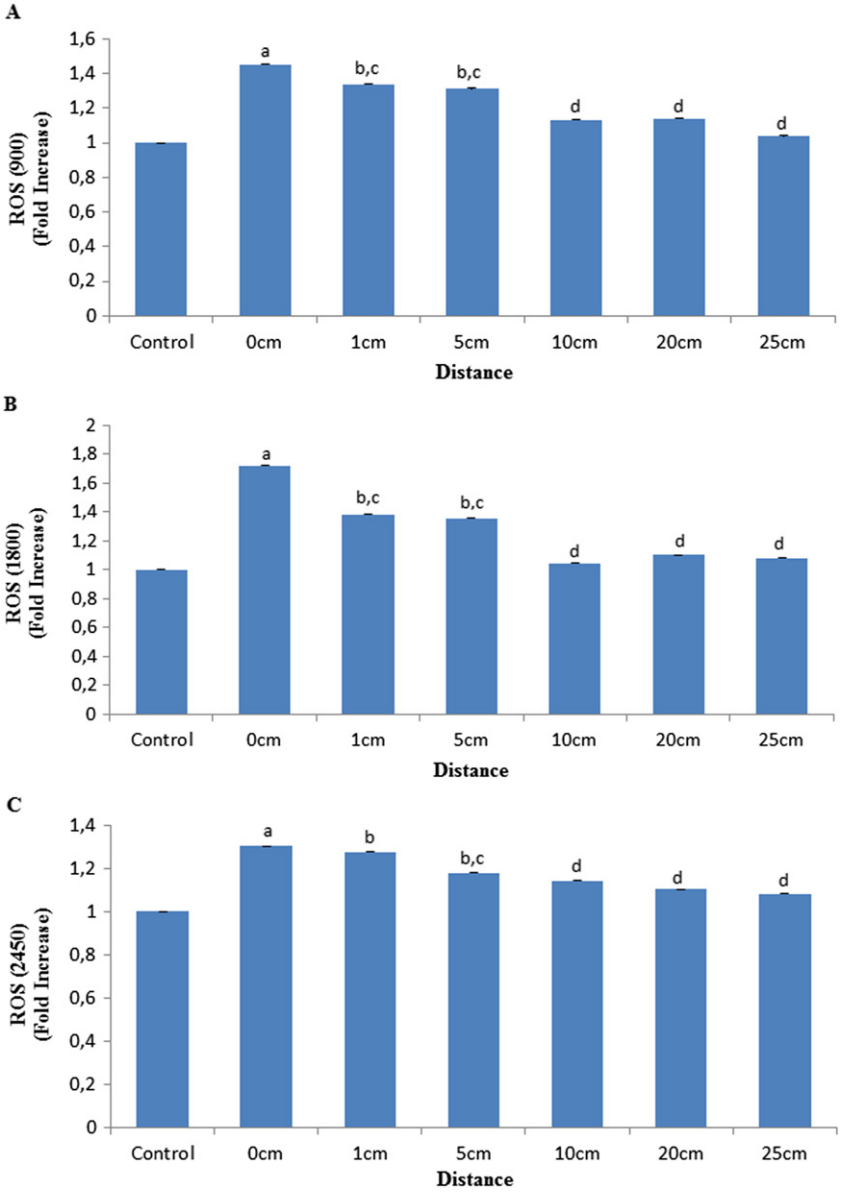

Fig. 4. Effects of EMR (A-900, B-1800 and C-2450 MHz) exposure on intracellular ROS levels in MCF-7 breast cancer cells (mean ± SD and $n = 6$). Values are presented as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.001$ and $^{b}p < 0.01$ versus control. $^{c}p < 0.05$ and $^{d}p < 0.01$ versus group 0 cm.

was set at 488 nm and emission was set at 543 nm [32]. We performed a total of 6 experiments ($n = 6$) for the intracellular ROS assay. The data were presented as fold-increase over the pretreatment level (experimental/control).

2.10. Statistical analyses

Data were analyzed using the SPSS statistical program (version 17.0, software, SPSS. Chicago, IL, USA). All results are expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) and an unpaired Mann–Whitney *U* test were performed; *P*-values of less than 0.01 were regarded as significant. Significant values were assessed with the least significance difference test.

3. Results

3.1. Cytosolic Ca^{2+} Concentration analysis

MCF-7 cells are capsaicin sensitive TRPV1 ion channels and they exhibit activity with EMR exposure and they were became more active with respect to calcium entry to cells compared with the control the EMR exposure. Changes were observed in the cells placed at 0 cm, 1 cm and 5 cm distances from the EMR source. The mean cytosolic Ca^{2+} concentration analysis values in the four groups are shown in Fig. 2 A, B and C. Ca^{2+} values were significantly lower in the 900, 1800 and 2450 MHz EMR groups than in control at 0 cm ($p < 0.001$), 1 cm ($p < 0.01$) and 5 cm ($p < 0.05$). However, there was no difference between any of the 10 cm, 20 cm, 25 cm and control values. Hence, we

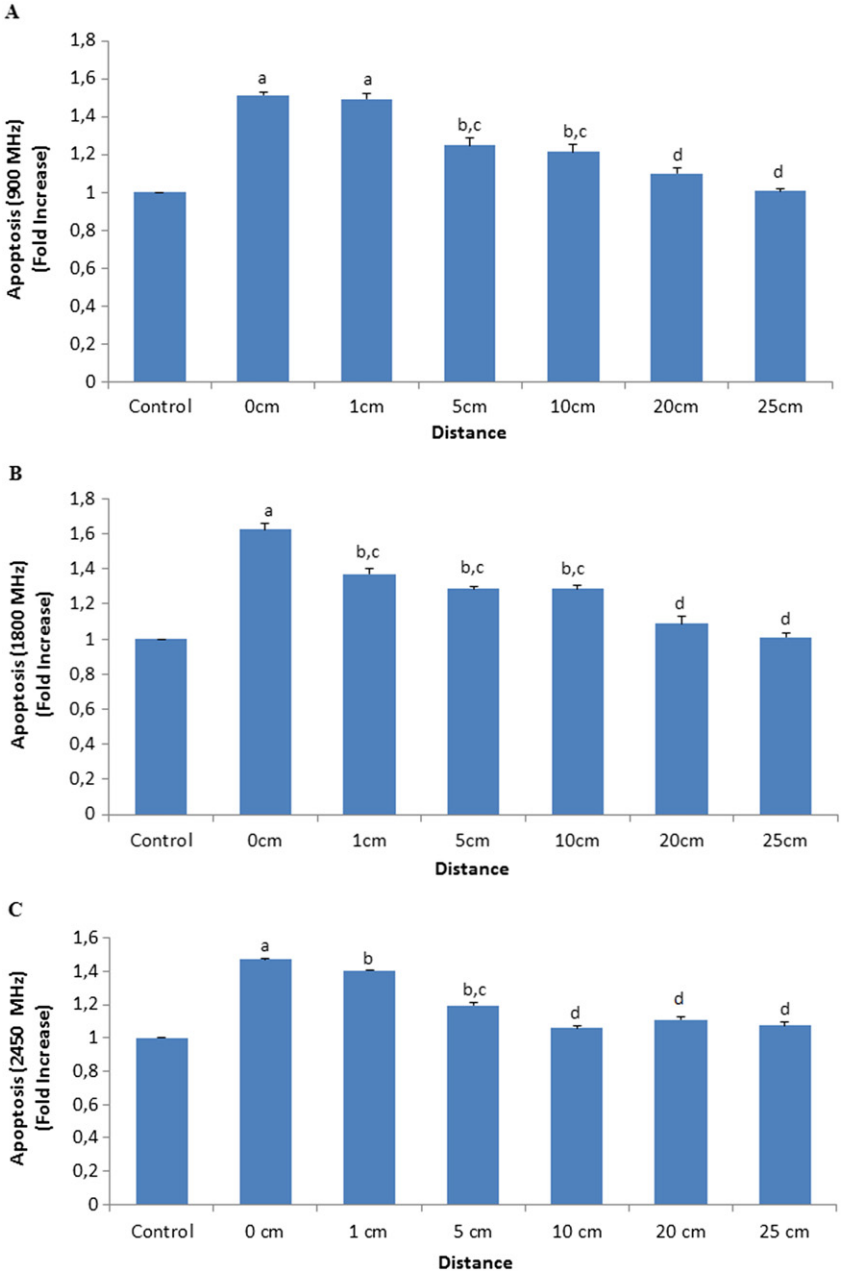

Fig. 5. Effects of EMR (A-900, B-1800 and C-2450 MHz) exposure on apoptosis levels in MCF-7 breast cancer cells. The apoptosis level was estimated as described under Materials and methods sections. Values are presented as mean ± SD of 6 separate experiments and expressed as fold increase over the pretreatment level (experimental/control). [a]$p < 0.001$ and [b]$p < 0.01$ versus control group. [c]$p < 0.05$ and [d]$p < 0.01$ versus group 0 cm.

observed that the cytosolic Ca^{2+} concentration of the cells was decreased if the distance to the EMR probe was within 10 cm.

3.2. Cell viability (MTT) results

The mean MTT values in the four groups are shown in Fig. 3A, B and C. MTT values were significantly lower in the 900, 1800 and 2450 MHz EMR groups than in the control at 0 cm ($p < 0.001$), 1 cm ($p < 0.01$) and 5 cm ($p < 0.05$). However, there was no difference between the 10 cm, 20 cm, 25 cm and control values. Hence, we observed that MTT values of the cells were decreased if they were within 10 cm of the EMR prob.

3.3. Intracellular (ROS) production

The mean cytosolic ROS production levels in the four groups are shown in Fig. 4 A, B and C, respectively. The results showed that the levels of cytosolic ROS production ($p < 0.001$) were significantly higher in the EMR groups than in controls. Exposure to 900 MHz, 1800 MHz and 2450 MHz EMR increased the ROS production at 0 cm, 1 cm and 5 cm distances from EMR prob. However, at distances of 10 cm, 20 cm and 25 cm, the EMR-induced cytosolic ROS production was not statistically different from the control group.

3.4. Effects of 900, 1800 and 2450 MHz EMR on apoptosis and caspase-3 and caspase-9 values

We investigated the effects of 900 MHz, 1800 MHz and 2450 MHz EMR exposure on the rate of programmed cell death as indicated by apoptosis and caspase values in the cancer cells. The results of apoptosis, caspase-3 and caspase-9 values in control, 900 MHz, 1800 MHz and 2450 MHz groups are shown in Figs. 5, 6 and 7, (A, B and C) respectively. The apoptosis ($p < 0.001$) and ($p < 0.005$), caspase-3 ($p < 0.001$) and caspase-9 ($p < 0.001$) values in the EMR group were significantly higher than in the control group. Furthermore, a significant difference

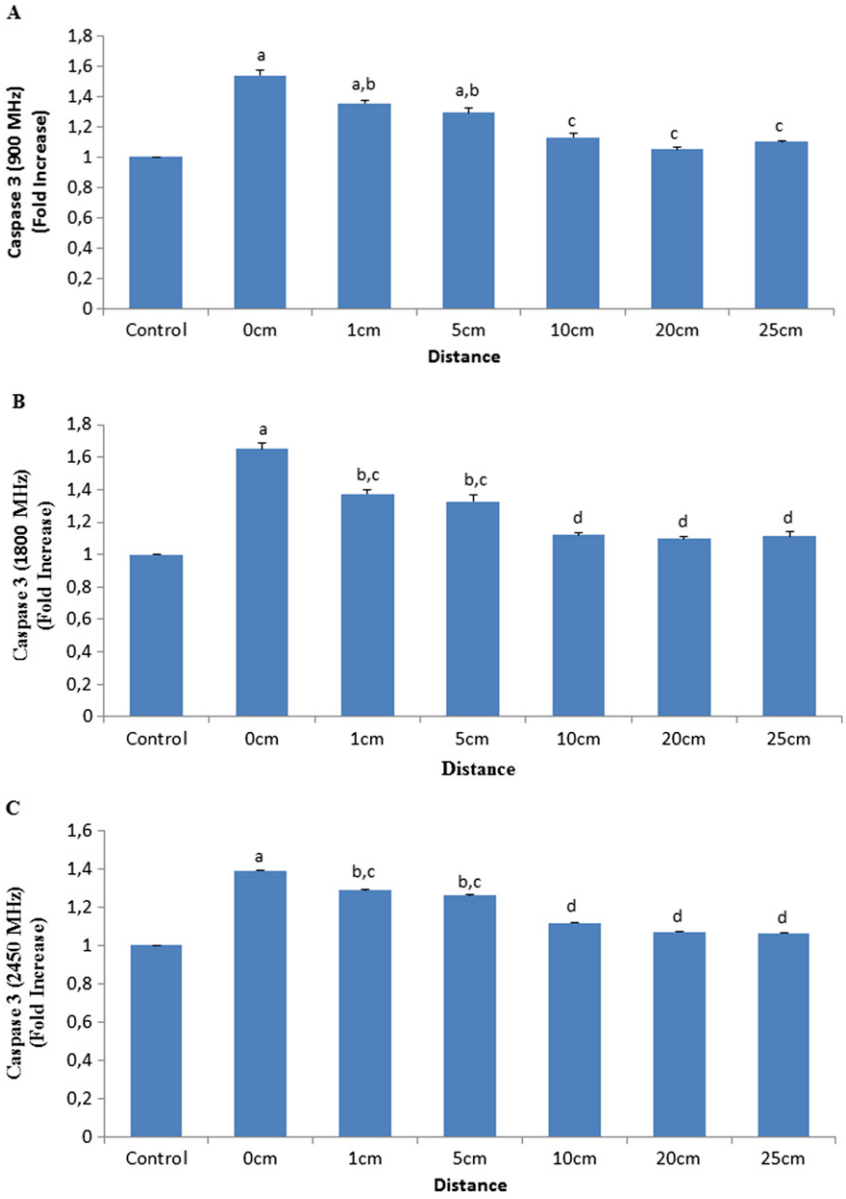

Fig. 6. Effects of EMR (A-900, B-1800 and C-2450 MHz) exposure on caspase-3 activity in MCF-7 breast cancer cells (mean ± SD and $n = 6$). The values expressed as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.001$ and $^{b}p < 0.01$ versus control group. $^{c}p < 0.05$ and $^{d}p < 0.01$ versus group 0 cm.

($p < 0.001$) was observed for 0 cm, 1 cm and 5 cm distances in each group, compared to control. However, no statistical difference was observed at 10 cm and at distances beyond this figure.

4. Discussion

The use of mobile phones and Wi-Fi internet is currently one of the fastest growing technology developments. The likelihood of close proximity of the antenna of such a devices to the breast has raised concern about a possible biological connection between EMR and breast cancer of women [13,27,14]. The exposure to such radiation depends on the length of time and frequency of use, which varies from individual to individual. The most investigated RF waveforms of the GSM-modulated signals are at 900 MHz and 1800 MHz and the Wi-Fi (2450 MHz) signals. The EMR studies have been carried out on different cancer cell types such as HL-60 cancer cell line [22], an MDA-MB-231 breast cancer cell line [14]. Modern cell phone devices and Wi-Fi internet in Turkey and many other countries work at a frequency of 900 MHz, 1800 MHz and 2450 MHz which were consequently selected for the present study.

Cell membranes are a major potential target for absorption of hazardous materials encountered in the environment [27,29]. In the present study, the cytosolic ROS production, apoptosis, caspase-3 and caspase-9 values in the breast cancer cells increased after 900 MHz, 1800 MHz and 2450 MHz exposure. These data are in agreement with reports suggesting that EMR induces oxidative stress and apoptosis of cancer cells by inducing ROS [14,27,31]. We have shown also that the changes induced by EMR are very dependent on the distance from the source in the cancer cells. To our knowledge, it is the first report of the relationship between different distances and 900 MHz, 1800 MHz and 2450 MHz EMR in the MCF-7 breast cancer cells.

The most recent evidence obtained for the effects of ionizing and non-ionizing radiation indicate that they both trigger oxidative stress

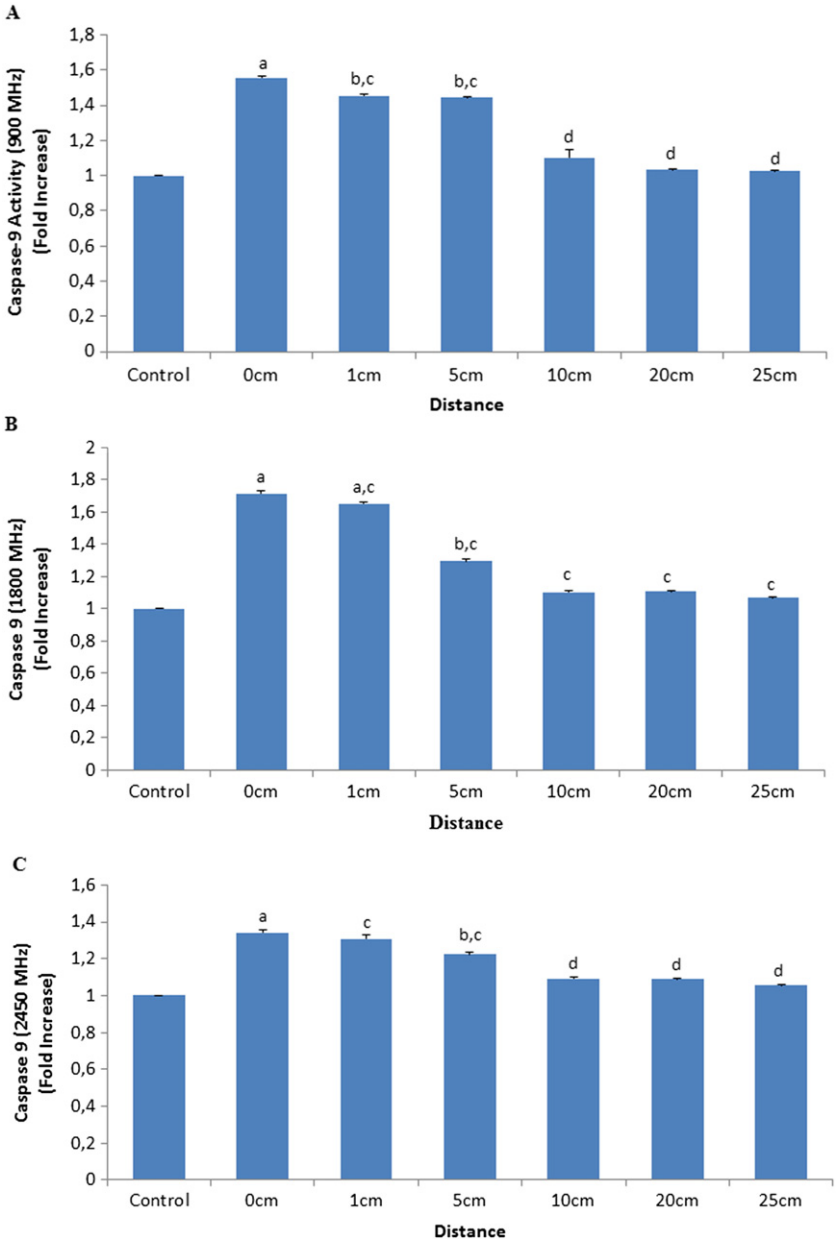

Fig. 7. Effects of EMR (A-900 MHz, B-1800 MHz and C-2450 MHz) exposure on caspase-9 activity in MCF-7 breast cancer cells (mean ± SD and $n = 6$). The values expressed as fold increase over the pretreatment level (experimental/control). $^{a}p < 0.001$ and $^{b}p < 0.01$ versus control group. $^{c}p < 0.05$ and $^{d}p < 0.01$ versus group 0 cm.

in cancer cells and neuronal cells [9,13,14,22] although antioxidants such as selenium and melatonin gave effective protection. [14,21]. In another study it has been reported that 900 MHz EMR causes an increase in temperature of skin and ears [35].Thus 900 MHz, 1800 MHz and 2450 MHz EMR exposure may activate heat-sensitive TRPV1 cation channels and thereby trigger excessive calcium entry into cells. But limited information is available about whether environmental EMR exposure triggers the free radical formation via cytosolic Ca^{2+} accumulation [16].

It is well known that ionized EMR has been used to kill tumor cells in some cancer treatment protocols. Exposure of cells to non-ionized EMR leads to generation of ROS, which is known to disturb the antioxidant defense system and induce oxidative stress [31]. In turn, the radiation-induced increases in ROS cause DNA damage, cell cycle arrest and activation of some transcription and apoptotic factors e.g., the nuclear factor kappa-light-chain-enhancer of activated B cells [18,33]. In the current study, cytosolic ROS production values in the EMR groups were elevated. The results of our experiments confirm that exposure to 900 MHz, 1800 MHz and 2450 MHz caused rises in oxidative stress, cytosolic $[Ca^{2+}]_i$ concentration, ROS production and apoptosis of breast cancer cells. According to the results, ideal distance for protection from oxidative and apoptotic damage of mobile phones and Wi-Fi devices-induced EMR seems 10 cm and up of the devices.

Mitochondrial function is essential for neuronal survival because neurons critically depend on ATP synthesis generated by mitochondrial oxidative phosphorylation [36]. Mitochondrial depolarization activity depends on Ca^{2+} and is fueled by Ca^{2+} entry from the extracellular space via channels such as TRPM2 and TRPV1 when triggered by neuronal activity [2,17]. In return, some cation channels such as TRPM2 and voltage gated calcium channels in cancer and neuronal cells are gated by EMR-induced ROS production [21,22]. In addition the channels are activated by capsaicin, noxious heat (42 °C), low pH and other endogenous ligands. TRPV1 is also activated by extracellular ROS [6,11]. Repeated activation of TRPV1 has previously been shown to result in increased cytosolic free Ca^{2+}, oxidative stress, and apoptotic cell injury [2,11]. TRPV1 activation by capsaicin in cancer cells was also found to increase substantially following mitochondrial oxidative stress [12]. Recently apoptosis and oxidative stress in colorectal cancer cells via activation of capsaicin and TRPV1 channels were reported although the TRPV1 antagonist, capsazepine, potentiated the apoptotic and oxidative stress effects through down-regulation of cell survival proteins and up-regulation of death receptors via the ROS–JNK–CHOP-mediated pathway [35]. Through this mechanism, EMR-induced oxidative stress and temperature increase may modulate TRPV1 responses during cancer cell apoptosis and mitochondrial injury because an overload of cytosolic Ca^{2+} induces depolarization of mitochondria and production of ROS [36]. Until now, no studies dealing with the effects of distance on Wi-Fi and mobile phone frequencies-induced EMR on mitochondrial depolarization, oxidative stress and apoptosis in cancer cells have been published. In the current study, we observed that oxidative stress values as well as cell apoptotic factors were lower in 10 cm, 20 cm and 25 cm distances than in 0 cm, 1 cm and 5 cm distances, all groups compared to control groups. Hence we observed the protective effect of distances exceeding 10 cm on cell apoptosis, caspase-3, caspase-9 in the cells. These results indicate that to some extent, EMR exposure activates TRPV1 channels, consistent with our hypothesis that up-regulation of TRPV1 channels activity by EMR pre-exposure and heat effects should be responsible for the apoptosis and oxidative stress of cancer cells.

Apoptosis is programmed death and it is mediated by specific proteinases namely caspases. There are two major pathways for apoptosis [34]. One involves death receptors and is marked by Fas-mediated caspase-8 activation, and the other is the stress or mitochondrial mediated caspase-9 activation. Both pathways induce caspase-3 activation [8,34]. In the current breast cancer cells, apoptosis, caspase-3 and caspase-9 values were increased by 900 MHz, 1800 MHz and 2450 MHz in cells positioned within 10 cm of the EMR source. It is likely that TRPV1-mediated Ca^{2+} entry in the EMR-exposed breast cancer cell involves accumulation of ROS and opening of mitochondrial membrane pores that consequently leads to mitochondrial dysfunction, substantial swelling of the mitochondria with rupture of the outer membrane and release of apoptosis-inducing factors such as caspase-3 and caspase-9.

In conclusion, the current results demonstrate that 900 MHz, 1800 MHz and 2450 MHz radiations of mobile phones and Wi-Fi internet in breast cancer cells induce apoptosis and ROS through calcium accumulation of activation of TRPV1 channels. However, the increases of apoptosis and oxidative stress are modulated by different distances. We did not detect oxidative and apoptotic damage of breast cancer cells distanced 20 cm and 25 cm from the source of radiation. Using the cell phones and Wi-Fi radiation sources which are far from 10 cm may provide useful distance against oxidative stress, apoptosis and overload Ca^{2+} entry in cancer. In addition, we suggest that use of TRPV1 channel blockers may provide a potential therapeutic approach for the mobile phone and Wi-Fi-induced oxidative stress and apoptosis by calcium accumulation.

Declaration of interest

There is no conflict interest in the study.

Transparency document

The Transparency document associated with this article can be found, in online version.

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References

[1] E.R. Adair, B.L. Cobb, K.S. Mylacraine, S.A. Kelleher, Human exposure at two radio frequencies (450 and 2450 MHz): similarities and differences in physiological response, Bioelectromagnetics (Suppl. 4) (1999) 12–20.
[2] M. Argun, L. Tök, A.C. Uğuz, Ö. Çelik, Ö.Y. Tök, M. Naziroğlu, Melatonin and amfenac modulate calcium entry, apoptosis, and oxidative stress in ARPE-19 cell culture exposed to blue light irradiation (405 nm), Eye (Lond.) 28 (2014) 752–760.
[3] M. Burkhardt, K. Poković, M. Gnos, T. Schmid, N. Kuster, Numerical and experimental dosimetry of Petri dish exposure setups, Bioelectromagnetics 17 (1996) 483–493.
[4] H. Cetin, M. Nazıroğlu, O. Celik, M. Yüksel, N. Pastacı, M.O. Ozkaya, Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring, J. Matern. Fetal Neonatal Med. (2014)http://dx.doi.org/10.3109/14767058.2014.898056 (Epub ahead of print).
[5] E.D. Chavdoula, D.J. Panagopoulos, L.H. Margaritis, Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features, Mutat. Res. 700 (2010) 51–61.
[6] H.H. Chuang, S. Lin, Oxidative challenges sensitize the capsaicin receptor by covalent cysteine modification, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20097–20102.
[7] J.A. D'Andrea, J.M. Ziriax, E.R. Adair, Radio frequency electromagnetic fields: mild hyperthermia and safety standards, Prog. Brain Res. 62 (2007) 107–135.
[8] J. Espino, I. Bejarano, S.D. Paredes, C. Barriga, A.B. Rodríguez, J.A. Pariente, Protective effect of melatonin against human leukocyte apoptosis induced by intracellular calcium overload: relation with its antioxidant actions, J. Pineal Res. 51 (2011) 195–206.
[9] V. Ghazizadeh, M. Nazıroğlu, Electromagnetic radiation (Wi-Fi) and epilepsy induce calcium entry and apoptosis through activation of TRPV1 channel in hippocampus and dorsal root ganglion of rats, Metab. Brain Dis. 29 (2014) 787–799.
[10] C. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (1985) 3440–3450.

[11] S. Hong, L. Agresta, C. Guo, J.W. Wiley, The TRPV1 receptor is associated with preferential stress in large dorsal root ganglion neurons in early diabetic sensory neuropathy, J. Neurochem. 105 (2008) 1212–1222.
[12] S.W. Ip, S.H. Lan, H.F. Lu, A.C. Huang, J.S. Yang, J.P. Lin, H.Y. Huang, J.C. Lien, C.C. Ho, C.F. Chiu, W. Wood, J.G. Chung, Capsaicin mediates apoptosis in human nasopharyngeal carcinoma NPC-TW 039 cells through mitochondrial depolarization and endoplasmic reticulum stress, Hum. Exp. Toxicol. 31 (2012) 539–549.
[13] Z. Jin, C. Zong, B. Jiang, Z. Zhou, J. Tong, Y. Cao, The effect of combined exposure of 900 MHz radiofrequency fields and doxorubicin in HL-60 cells, PLoS One 7 (2012) e46102.
[14] M.C. Kahya, M. Nazıroğlu, B. Ciğ, Selenium reduces mobile phone (900 MHz)-induced oxidative stress, mitochondrial function, and apoptosis in breast cancer cells, Biol. Trace Elem. Res. 160 (2014) 285–293.
[15] M. Koçer, M. Nazıroğlu, G. Koçer, T.T. Sonmez, Effects of bisphosphonate on oxidative stress levels in patients with cancer, Cancer Invest. 32 (2014) 37–42.
[16] M. Koçer, M. Nazıroğlu, Effects of 5-fluorouracil on oxidative stress and calcium levels in the blood of patients with newly diagnosed colorectal cancer, Biol. Trace Elem. Res. 155 (2013) 327–332.
[17] V.S. Kumar, A. Gopalakrishnan, M. Naziroğlu, G.K. Rajanikant, Calcium ion—the key player in cerebral ischemia, Curr. Med. Chem. 21 (2014) 2065–2075.
[18] J.-S. Lee, T.-Q. Huang, T.-H. Kim, J.Y. Kim, H.J. Kim, J.-K. Pack, J.-S. Seo, Radiofrequency radiation does not induce stress response in human T-lymphocytes and rat primary astrocytes, Bioelectromagnetics 27 (2006) 578–588.
[19] M. Mailankot, A.P. Kunnath, H. Jayalekshmi, B. Koduru, R. Valsalan, Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8 GHz) mobile phones induces oxidative stress and reduces sperm motility in rats, Clinics (Sao Paulo) 64 (6) (2009) 561–565.
[20] A.K. Manta, D.J. Stravopodis, I.S. Papassideri, L.H. Margaritis, Reactive oxygen species elevation and recovery in Drosophila bodies and ovaries following short-term and long-term exposure to DECT base EMF, Electromagn. Biol. Med. 33 (2) (2013) 118–131.
[21] M. Nazıroğlu, Ö. Çelik, C. Özgül, S. Doğan, R. Bal, N. Gümral, A.B. Rodríguez, J.A. Pariente, Melatonin modulates wireless devices (2.45 GHz)-induced brain and dorsal root ganglion injury through TRPM2 and voltage gated calcium channels in rat, Physiol. Behav. 105 (2012) 683–692.
[22] M. Nazıroğlu, B. Ciğ, S. Doğan, A.C. Uğuz, S. Dilek, D. Faouzi, 2.45-GHz wireless devices induce oxidative stress and proliferation through cytosolic Ca^{2+} influx in human leukemia cancer cells, Int. J. Radiat. Biol. 88 (2012) 449–456.
[23] M. Nazıroğlu, B. Çiğ, C. Özgül, Neuroprotection induced by N-acetylcysteine against cytosolic glutathione depletion induced-Ca^{2+} influx in dorsal root ganglion neurons of mice: role of TRPV1 channels, Neuroscience 242 (2013) 151–160.
[24] M. Nazıroğlu, D.M. Dikici, S. Dursun, Role of oxidative stress and Ca^{2+} signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels, Neurochem. Res. 37 (2012) 2065–2075.
[25] M. Nazıroğlu, S. Tokat, S. Demirci, Role of melatonin on electromagnetic radiation-induced oxidative stress and Ca^{2+} signaling molecular pathways in breast cancer, J. Recept. Signal Transduct. Res. 32 (2012) 290–297.
[26] M. Nazıroğlu, M. Yüksel, S.A. Köse, M.O. Özkaya, Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signaling pathways in females and males, J. Membr. Biol. 246 (12) (2013) 869–875.
[27] M. Nazıroğlu, Molecular role of catalase on oxidative stress-induced Ca(2+) signaling and TRP cation channel activation in nervous system, J. Recept. Signal Transduct. Res. 32 (2012) 134–141.
[28] M. Nazıroğlu, F.F. Özkan, S.R. Hapil, V. Ghazizadeh, B. Çiğ, Epilepsy but not mobile phone frequency (900 MHz) induces apoptosis and calcium entry in hippocampus of epileptic rat: involvement of TRPV1 channels, J. Membr. Biol. 248 (2015) 83–91.
[29] A. Özorak, M. Nazıroğlu, Ö. Çelik, M. Yüksel, D. Özçelik, M.O. Özkaya, H. Çetin, M.C. Kahya, S.A. Kose, Wi-Fi (2.45 GHz)- and mobile phone (900 and 1800 MHz)-induced risks on oxidative stress and elements in kidney and testis of rats during pregnancy and the development of offspring, Biol. Trace Elem. Res. 156 (2013) 221–229.
[30] D.J. Panagopoulos, L.H. Margaritis, The effect of exposure duration on the biological activity of mobile telephony radiation, Mutat. Res. 699 (2010) 17–22.
[31] S. Reuter, S.C. Gupta, M.M. Chaturvedi, B.B. Aggarwal, Oxidative stress, inflammation, and cancer: how are they linked? Free Radic. Biol. Med. 49 (2010) 1603–16016.
[32] G. Rothe, A. Oser, G. Valet, Dihydrorhodamine 123: a new flow cytometric indicator for respiratory burst activity in neutrophil granulocytes, Naturwissenschaften 75 (1988) 354–355.
[33] R. Schreck, K. Albermann, P.A. Baeuerle, Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review), Free Radic. Res. Commun. 17 (1992) 221–237.
[34] Y. Shi, Apoptosome: the cellular engine for the activation of caspase-9, Structure 10 (2002) 285–288.
[35] B. Sung, S. Prasad, J. Ravindran, V.R. Yadav, B.B. Aggarwal, Capsazepine, a TRPV1 antagonist, sensitizes colorectal cancer cells to apoptosis by TRAIL through ROS-JNK-CHOP-mediated upregulation of death receptors, Free Radic. Biol. Med. 53 (2012) 1977–1987.
[36] A.C. Uğuz, M. Nazıroğlu, J. Espino, I. Bejarano, D. González, A.B. Rodríguez, J.A. Pariente, Selenium modulates oxidative stress-induced cell apoptosis in human myeloid HL-60 cells via regulation of caspase-3,-9 and calcium influx, J. Membr. Biol. 232 (2009) 15–23.