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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

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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

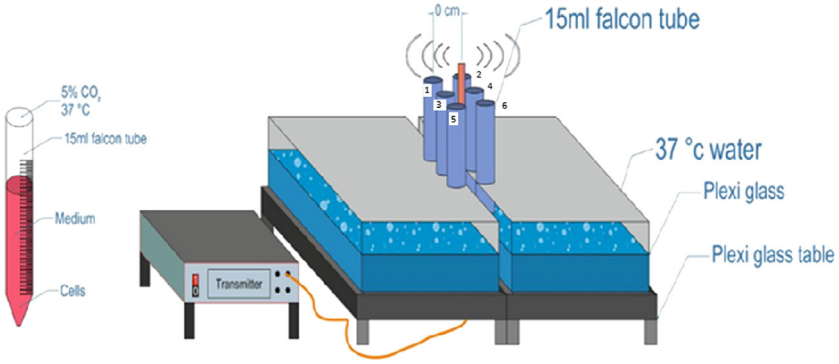

Fig. 1. Schematic diagram of radiofrequency exposure device.

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