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Post-exposure Effects of PEMF on ROS levels in H_2O_2-treated Glioblastoma Cell Line

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Abstract - Pulsed electromagnetic fields (PEMF) of extremely low frequency are non-ionizing and use a series of magnetic pulses of electrical energy into exposed biological tissue to accelerate tissue repair without induced thermal effects. Till date, there is no report showing the post-exposure effects of PEMF on ROS production in human glioblastoma cells treated with hydrogen peroxide (H_2O_2). Therefore, the present work aims to investigate possible post-exposure effects of 75 Hz PEMF on basal ROS levels in U87-MG cells treated with H_2O_2 at different time intervals. Cells are grouped as sham-control (group-I), cells treated with H_2O_2 for 30 min, 24 h, and 48 h (group-II), and cells post-exposed to PEMF following H_2O_2 treatment with the same duration as group II (group-III). After cell viability measurement, basal ROS levels in each group are determined spectrophotometrically. Statistical analysis is carried out by SPSS v.23. The cell viability of group-III for each H_2O_2 treatment duration is significantly greater than the group II. This result indicates that post-PEMF exposure has possible cytoprotective effects against H_2O_2 challenge. ROS levels of group-III are significantly lower than the group-II which indicates preventative effects of post-PEMF exposure against ROS production in H_2O_2 treated U87-MG cells. Overall results have been demonstrated the possible cytoprotective effect of post-PEMF exposure against deleterious effects of oxidative stress triggered by the different time intervals of H_2O_2 treatment in U87-MG cells. We firmly believe the present work may shed light on further works focusing on molecular, biochemical, and cellular effects of PEMF exposure against antioxidant defence mechanisms and their triggered redox-based neuronal pathways.

***Keywords*:** Pulsed electromagnetic field; U87-MG cells; Oxidative stress; Reactive oxygen species

1. Introduction

Pulsed Electromagnetic Field (PEMF) remains a hot topic due to its huge applications such as being an alternative therapy against medical problems in addition to its cytoprotective effects [1,2]. The main reason why it is preferred in cellular biology research is its main characteristic of being an extremely low-frequency non-ionizing form of the electromagnetic field that uses a series of magnetic pulses of electrical energy into exposed biological tissue to accelerate tissue repair without induced thermal effects [3]. Meanwhile, cellular responses vary depending on the cell type, tissue or organism studied. Excessive reactive oxygen species (ROS) production in cells causes cellular damage up to cell death by damaging antioxidant mechanisms. For example in neuronal cells, including microglial cells, it causes cellular damage to proteins, lipids, and nucleic acids, resulting in neuronal death; thus, the quantity of ROS production is crucial for neuronal survival [2]. PEMF exposure depending on dose, timing, and exposure conditions prevents the overproduction of ROS [2,4]. Following a pro-oxidant challenge, short and repeated pre-exposure of neuroblastoma cells (SH-SY5Y, SK-N-BE(2)) to 75 ± 2 Hz frequency, 2 ± 0.2 mT intensity PEMF resulted in a decrease in ROS generation and an increase in MnSOD-based antioxidant production [4,5], long-term exposure, on the other hand, has been shown to decrease antioxidant defences in the brains of aged rats through the generation of reactive oxygen species (ROS) [6].

Previous in vitro studies using various biological techniques have demonstrated that different dosages and periods of pre-exposed PEMF may have different cellular effects on redox status [2,4,5,7]. To date, there has been no report on the post-exposed effects of a specific dose of PEMF with a specific period on human glioblastoma astrocyte-like cell line that

was treated with H_2O_2 at different intervals of time to induce oxidative stress. Here, the focus is to show the possible effects of short-term (15 min) exposure to 75 Hz, 1 ± 0.2 mT of PEMF on H_2O_2 treated U87-MG cell line by determining cell viability and ROS levels.

2. Materials and Methods

2.1. Pulsed Electromagnetic field (PEMF) exposure system

The pulsed electromagnetic field (PEMF) system (Pasco; UI-5000) with its software (Pasco Capstone; UI-5400) was used. The Helmholtz coils, consisting of two 500 turn pairs, 0.64 mm diameter copper wire with a total diameter of 20 cm, were placed parallel to each other to generate a homogeneous magnetic field environment for the cells to be exposed. Each coil was then connected in series and powered by a Pasco 850 Universal Interface pulse generator (Pasco; UI-5000). The software was used to set 75 Hz frequency pulse signal with a pulse duration of 1.3 ms, and the amplitude as a 15 V. 1 ± 0.2 mT of the intensity peak was measured between two coils using the Pasport 2-Axis Magnetic Field Sensor. The coil system was then placed into the cell incubator and the ambient temperature was set to 37°C. The set-up was demonstrated in Fig. 1.

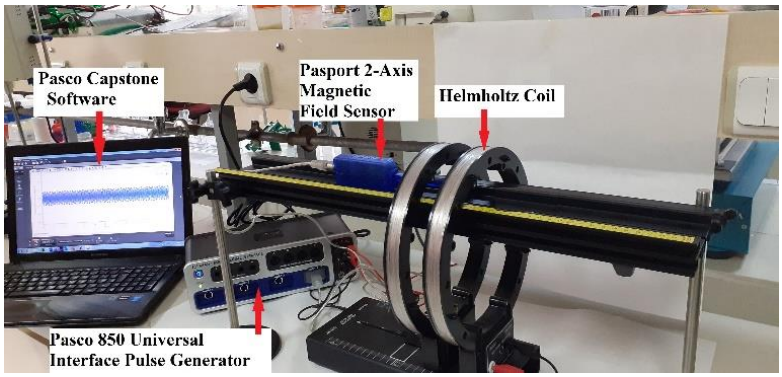

Fig. 1: Representative illustration of PEMF set-up with two-coil Helmholtz set-up.

2.2. Cell culture

U87-MG cell line (ATCC-American Type Culture Collection Manassas, VA, USA) of human glioblastoma cells were cultured in Eagle's Minimum Essential Medium (EMEM; Biological Industries, Cromwell, USA) and supplemented with 10% (v/v) inactivated fetal bovine serum (FBS; Biological Industries, Cromwell, USA), 1% (v/v) penicillin-streptomycin (Pen-Strep; Biological Industries, Cromwell, USA), and 2 mM L-glutamine (Biological Industries, Cromwell, USA), in a 95% humidified atmosphere of 5% CO2 at 37°C. Cells were grouped as: (I) sham-control; (II) cells treated with 0.1 mM H_2O_2 at three different time intervals (30 min, 24 h, and 48 h, respectively); (III) cells post-exposed to 75 Hz-1 ± 0.2 mT PEMF for 15 min following 0.1 mM H_2O_2 treatment at three different time intervals (30 min, 24 h, and 48 h, respectively). Cell cultures were placed in the coil system. The sham-control groups were kept under the same experimental conditions but without PEMF exposure and H_2O_2 challenge.

2.3. Cell viability assay

Cells were trypsinized and counted by Bio-Rad TC20 automatic cell counter (Bio-Rad, California, USA) using 1:1 dilution in 0.04% Trypan Blue (Sigma-Aldrich, Missouri, USA), and then seeded (1x104/well) into 96-well plates as three replicates. The cell viability was measured using an Alamar Blue reagent (Invitrogen, Thermo Fischer Scientific, Waltham, MA, USA) as an indicator with a multi-well scanning spectrophotometer (Multiskan Go; Thermo Scientific Co., Waltham, MA, USA). The sigmoidal plot of inhibition ratio (%) vs log H2O2 concentration was used to measure IC50 values. Results were expressed as a percentage (%) of the cell viability.

2.4. Levels of reactive oxygen species

The levels of reactive oxygen species (ROS) were measured using the fluorescent probe 2',7'-Dichlorodihydrofluorescence diacetate (DCFH-DA; Sigma Aldrich, Missouri, USA) which is based on the principle of oxidation of DCFH to fluorescent 2',7'-Dichlorofluorescein (DCF) in the presence of ROS. In this way, the reactive fluorescence unit (R.F.U) in cells can be determined. A mixture of 10 µM DCFH-DA was prepared (0.19 mg of DCFH-DA dissolved in 40 mL of Dimethyl sulfoxide (DMSO), and then added to petri dishes containing cell groups. The petri dishes were then incubated for 30 min. at 37 °C in 5% CO2. Subsequently, cells were again rinsed with PBS and then seeded into a dark 96-well plate (1.25x103 cells/well). The fluorescent intensity was measured by Synergy H1 Microplate Reader (BioTek, Vermont, USA) with excitation and emission at 504 nm and 529 nm, respectively.

2.5. Statistics

One-Way Analysis of Variance (ANOVA) with a post-hoc Tukey's Honestly Significant Difference (HSD) test, when appropriate was performed. Data were represented as means ± SEM. *$p<0.05$ was considered to be a significant difference. SPSS (v 23.0) was used for statistical analyses.

3. Results

3.1. Post-exposed PEMF increases the cell viability

The IC_{50} value representing the concentration of H_2O_2 required for 50% of cell death was calculated assuming cell viability of the sham-control (group I) as 100%. Treatment with a 0.1 mM concentration of H_2O_2 revealed a time-dependent loss of cell viability in both group II and group III. In group II, the H_2O_2 challenge generated approximately 44%, 68%, and 78% time-dependent reduction in cell viability Fig. 2A. The reduction in cell viability was not as much as in group II when cells were post-exposed to PEMF following the H_2O_2 challenge. In group III, approximately 28%, 49%, and 63% time-dependent reduction in cell viability was observed (Fig. 2B). It has been hypothesized that post-PEMF exposure has a possible cytoprotective effect on cell viability in group III compared to group II.

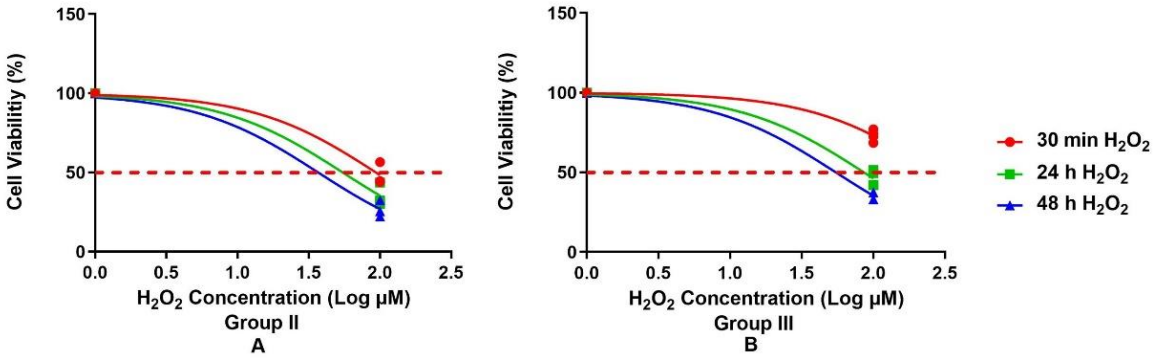

Fig. 2: Effects of **A.** H_2O_2 challenge (Group II); and **B.** post-exposure to PEMF following H_2O_2 challenge (Group III) on percentage (%) of cell viability in U87-MG cell line.

3.2. Post-exposed PEMF reduces intracellular ROS levels

ROS levels in all groups were determined spectrophotometrically and summarized in Fig. 3. As demonstrated in Fig. 3, ROS levels were significantly higher in group II as compared to sham-control and group III ($p\leq0.001$, respectively) which pointing the deleterious effects of oxidative stress. Meanwhile, results suggest that post-exposed PEMF has a potential impact on reducing ROS levels following all the time intervals of H_2O_2 challenges as compared to group II ($p\leq0.001$, respectively)

which once more indicate possible cytoprotective effects of post-exposed PEMF against deleterious effects of oxidative stress in U87-MG cells.

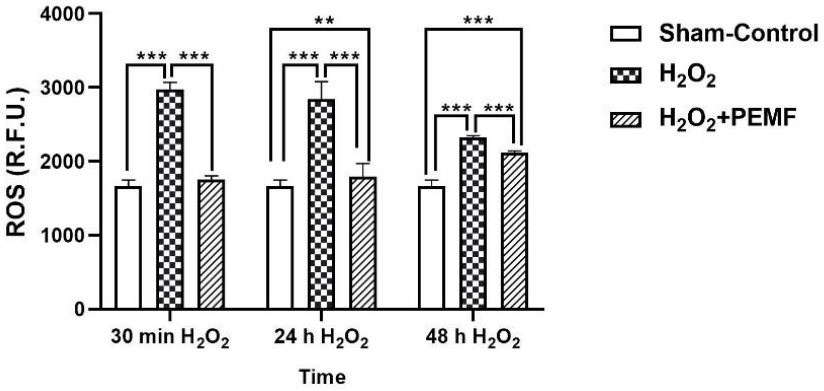

Fig. 3: Comparison of the reactive oxygen species levels (R.F.U.) in U87-MG cells. Data were expressed as the mean ± SEM. Asterisks denote the level of significance: **p<0.01; ***p≤0.001.

4. Discussion

To date, limited studies are present examining possible effects of post-PEMF exposure to levels of reactive oxygen species in neuronal cell lines, notably a cellular model of human glioblastoma, U87-MG, which is an model of astrocytic brain cancer, Glioblastoma multiforme (GBM) [8]. Additionally, there is no report showing possible effects of 75 Hz PEMF post-exposure following 0.1 mM hydrogen peroxide treatment with different periods on cell viability and ROS levels in U87-MG cells. Hence, in this work, the potential effects of 75 Hz PEMF post-exposure on oxidative responses in U87-MG cells were examined.

Overproduction of reactive oxygen species (ROS) or impairment of antioxidant defence mechanisms causes oxidative stress [9]. Because of its high demand for oxygen, an abundance of redox-active metals, and low amounts of endogenous antioxidants such as glutathione (GSH), the brain is particularly vulnerable to oxidative stress [9]. Excessive formation of ROS in neuronal cells, especially microglial cells, causes proteins, lipids, and nucleic acids damages, resulting in neuronal death; therefore, the levels of ROS are crucial for neuronal survival [2]. Following the H_2O_2 treatment with different time intervals, the effect of post-exposed PEMF on both cell viability and basal ROS levels was investigated, and our findings suggest that post-exposed PEMF has an increasing effect on cell viability while reducing ROS levels. This confirms the probable cytoprotective effects of short-term post-exposed PEMF in U87-MG cells against the detrimental effects of oxidative stress. This result is consistent with the results of Vincenzi et al. [2] showing reducing effects of short-term 75 Hz PEMFs exposure on hypoxia-induced ROS generation in N9 microglial cells. In addition, this result is also supported by the studies of Osera et al. [4] and Falone et al. [5], showing that improving antioxidant response effect of short- and repeated PEMF pre-conditioning in drug-sensitive (SH-SY5Y) and drug-resistant (SK-N-BE(2)) human neuroblastoma cell lines against pro-oxidant challenge by restraining H_2O_2-induced ROS production and increasing Mn-dependent superoxide dismutase (MnSOD) activity. The inhibitory effect of PEMF on intracellular ROS levels was also shown to inhibit osteoclastic differentiation [7, 10]. Taken together, our findings could lay the framework for future research into the processes underlying the effects of post-exposed PEMFs at different doses, frequencies, and exposure conditions on intracellular ROS levels in neuronal signaling pathways, allowing us to reach a more thorough conclusion.

5. Conclusion

To conclude, in the current study, it is obvious that the cytoprotective effect of post-conditioning 75 Hz, 15 min PEMF exposure on oxidative stress triggered by the different time intervals of H_2O_2 treatment in U87-MG cells is

mediated by the increase in the cell viability, and the decrease in the ROS level. In the light of these findings, further *in vivo* and/or *in vitro* research on the neurophysiological effects of PEMFs and their underlying molecular mechanisms is needed to elucidate the neurotoxic or neuroprotective role against antioxidant defence mechanisms. We are confident that the findings of this work will contribute to future research on the molecular, biochemical, and cellular processes activated by PEMF exposure.

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