Non thermal effects of radiofrequency electromagnetic waves on olfactory ensheathing cells

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Abstract - The influence of low-intensity, so not inducing thermal effects, RF-EMFs on Olfactory Ensheathing Cell (OEC) cultures, typical glia cells showing characteristics of stem cells, was investigated. Cell cultures were exposed, in far-field condition, at 900 MHz continuous and amplitude modulated EMFs for 10, 15 and 20 min, by maintaining their temperature at 37°C. The expression of OEC marker (S-100), stem cell marker (Nestin), cytoskeletal proteins (GFAP and Vimentin), apoptotic pathway activation by Caspase-3 cleavage and cell viability, were evaluated. Results showed that the exposure to continuous or amplitude modulated radio-frequency electromagnetic fields induces different effects in Olfactory Ensheathing cell viability and in expression level of markers that play a role in cell self-renewal.

INTRODUCTION

Most of the modern communication technologies are based on the use of so-called radio-frequency electromagnetic fields (RF-EMFs) having frequencies ranging from hundreds KHz to some GHz. These fields today have permeated totally our vital environment. Until a few decades ago their biological effects were related only to the overheating of the tissues (the so-called thermal effects). However, more recently, experimental evidence of the ability of RF-EMFs to generate non-thermal biological effects at the cellular level is increasing [1] even if the action mechanisms are not understood, and connection of biological effects with the characteristics of the impinging EMF, as Specific Absorption Rate (SAR), carrier frequency, exposure time (chronic or acute) and type of modulation (if present) should be clarified. Moreover, only few data have been reported about the effect of RF-EMF on self-renewal of neural progenitor cells.

In this study we evaluated the non-thermal effects induced by exposure on primary Olfactory Ensheathing Cells (OECs) to 900 MHz RF-EMF of low intensity and in far field condition, at the different exposure durations 10, 15 and 20 min. We used also an amplitude modulated (at 50 Hz) 900 MHz, in order to assess the effects on cell cultures of sinusoidal amplitude modulation.

OECs are glial cells that accompany the axons from olfactory receptor neurons to the bulb. They exhibit phenotypic properties similar to Schwann cells and astrocytes, they are able to secrete different molecules, such as growth and adhesion factors and molecules of the extracellular matrix. They show specific characteristics of stem cells, expressing nestin (i.e. a marker of neural stem cell precursors). Moreover: (i) an olfactory involvement is present in Alzhèimer disease patients, with a reduced function in their olfactory performance, an early sign of neurodegeneration; (ii) an information transfer has been observed in the olfacto-hippocampal network; (iii) OECs appears to be a promising tool for cellular therapy in spinal cord injury [2] and axonal growth; (iv) OECs are able to stimulate axonal regeneration and functional restoration in the lesion of Nervous System (NS). All these characteristics render them useful as approach in cellular therapy [3].

Biological effects have been investigated by MTT assay to monitor cell viability and immunocytochemistry tests to evaluate the expression of Glial Fibrillary Acidic Protein (GFAP), Vimentin and Nestin, that are proteins implicated in the formation of Intermediate Filaments in cytoskeleton, as well as S100 protein, OEC marker. The activation of apoptotic pathway by analyzing caspase-3 cleavage has been evaluated, too.

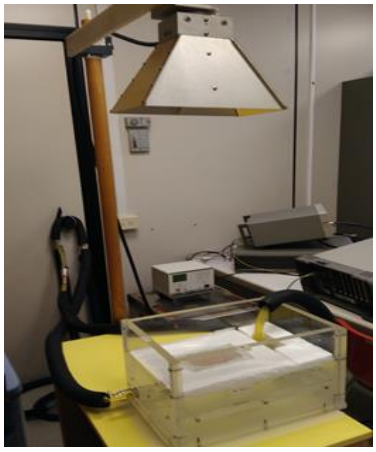

Figure 1: Set-up for RF-EMF exposure

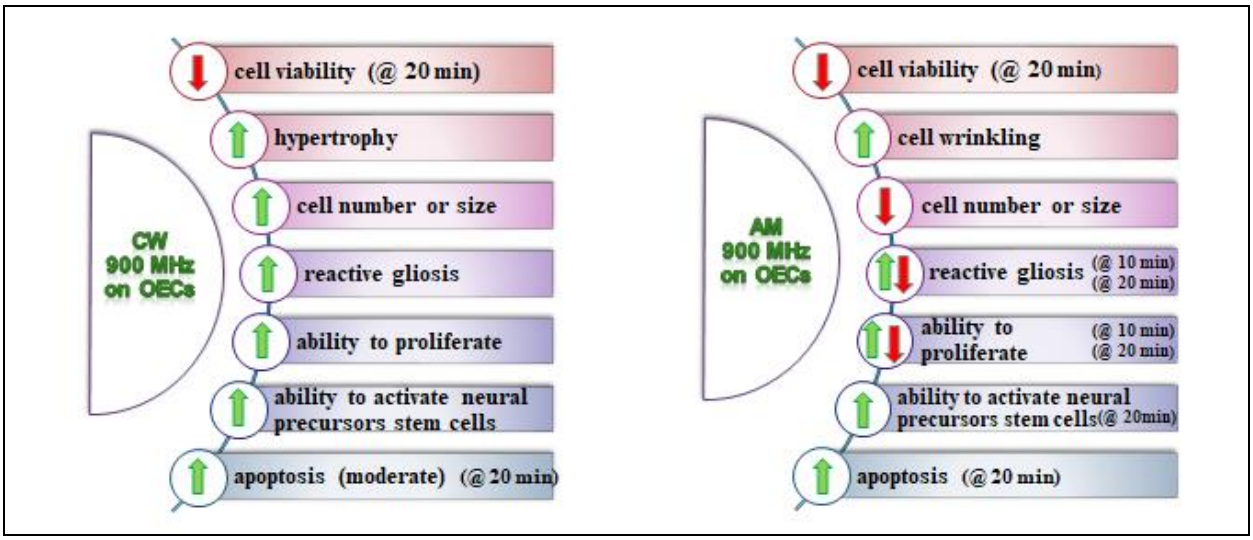

Figure 2: Overview of the biological effects of CW 900 MHz and AM 900 MHz on OECs

MATHERIALS AND METHODS

Biological materials

The primary OEC, used for the experiment were isolated from the olfactory bulb of two-day mice pups (provided by Envigo RMS s.r.l.) and then subjected to a suitable purification procedure [4]. All the experimental procedures were approved by the Ethical Committee at the University of Catania.

RF-EMF exposure

The sample was exposed to an RF-EMF emitted by a double horn antenna (ETS-Emco- 3115) plugged to an Agilent-8648D signal generator (see fig.1). Continuous or amplitude modulated at 50 Hz (modulation index 0.27) 900 MHz wave with amplitude ~ 6V/m was used. During the exposure, the cell cultures were maintained in a thermal bath at 37°C

MTT assay and immunocytochemistry test

OECs cell viability were performed on 96 multiwell flat bottomed 200 µl microplates (1×10^4 cells/well) as described in ref. 2. The expression of cytoskeleton proteins was performed on coverslips (1×10^4 cells/well) as reported in ref. 5. Data were statistically analyzed using One-Way analysis of variance (ANOVA) followed by a post hoc Holm–Sidak test to estimate significant (at $p < 0.05$, $p<0.01$, $p<0.001$) differences among groups.

RESULTS

For the experiments, cells were divided in four groups: control cells maintained into the incubator at 37°C in ambient of humidified air and CO2 (95%-5%), to verify the normal cellular status (Ctrl); cells maintained in water thermal baths at 37°C for 10, 15 and 20 min (Sham); cells maintained in water thermal baths, exposed to CW 900 MHz for 10, 15 and 20 min (CW 900 MHz); cells maintained in water thermal baths, exposed to AM 900 MHz for 10, 15 and 20 min (AM 900 MHz).

Figure 2 shows schematically the most relevant results, shortly described in the following. Detailed results were reported in Ref 6.

MTT test showed that CW 900 MHz and, in particular, AM 900 MHz were able to induce a significant decrease in cell viability respect the corresponding shams only at 20 min exposure.

Exposure of OECs at AM 900 MHz induced a decrease in the number of positive S-100 cells, when compared with control, sham and CW 900 MHz for 15 and 20 min, indicating that the effect leads to cell death.

Exposure to 15 and 20 min at CW 900 MHz induces, in contrast to AM 900 MHz exposure, an increase the number of positive GFAP cells, showing that the 900 MHz CWs, differently from AM 900 MHz, stimulate the activation of gliosis (differentiation in astrocytes).

The activation of the apoptotic pathway was assessed through Caspase-3 cleavage. An increase was observed in CW 900 MHz and, more significantly, in AM 900 MHz for 20 min exposure.

In conclusion, the exposure of OECs to RF-EMF at 900 MHZ in continuous and amplitude modulated, at low intensity, was able to induce effects on cell viability and differently alter the cytoskeletal proteins at various levels. These effects are strongly dependent on the exposure duration and, more interestingly, on the presence of modulation of the electromagnetic wave.

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