Ecotoxicology and Environmental Safety 154 (2018) 268–279

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Measurement of the 100 MHz EMF radiation *in vivo* effects on zebrafish *D. rerio* embryonic development: A multidisciplinary study

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ARTICLE INFO

Keywords:
Electromagnetic field radiation
Biological effects
Zebrafish
Development

ABSTRACT

The augmented exposure of both environment and human being to electromagnetic waves and the concomitant lack of an unequivocal knowledge about biological consequences of these radiations, raised public interest on electromagnetic pollution. In this context, the present study aims to evaluate the biological effects on zebrafish (ZF) embryos of 100 MHz radiofrequency electromagnetic field (RF-EMF) exposure through a multidisciplinary protocol.

Because of the shared synteny between human and ZF genomes that validated its use in biomedical research, toxicology and developmental biology studies, ZF was here selected as experimental model and a measurement protocol and biological analyses have been set up to clearly discriminate between RF-EMF biological and thermal effects.

The results showed that a 100 MHz EMF was able to affect ZF embryonic development, from 24 to 72 h post fertilization (hpf) in all the analyzed pathways. Particularly, at the 48 hpf stage, a reduced growth, an increased transcription of oxidative stress genes, the onset of apoptotic/autophagic processes and a modification in cholesterol metabolism were detected. ZF embryos faced stress induced by EMF radiation by triggering detoxification mechanisms and at 72 hpf they partially recovered from stress reaching the hatching time in a comparable way respect to the control group.

Data here obtained showed unequivocally the *in vivo* effects of RF-EMF on an animal model, excluding thermal outcomes and thus represents the starting point for more comprehensive studies on dose response effects of electromagnetic fields radiations consequences.

1. Introduction

Over the last decades, the population exposure to electromagnetic fields (EMFs) has progressively increased with the concomitant intensification of technological tools from both domestic and industrial uses (Tomitsch and Dechant, 2012; Gajšek et al., 2015). More recently, the rapid development of wireless technologies together with the EMFs use in diagnostic and therapeutic medicine (magnetic resonance imaging, radiofrequency ablations, etc.) amplified human exposure to radiofrequency fields (RF) (Consales et al., 2012; Vijayalaxmi, 2016).

Although these new technologies appear to improve the quality of life, doubts about their effect on human health, as well as on the environment, are on the rise, producing a growing number of studies on possible biological effects of EMF exposure (Funk and Monsees, 2006; Tenorio et al., 2012; Gajšek et al., 2015).

Various effects have been described on cell biology, mainly related to DNA damages and its consequences including primary DNA damage in the form of single and double strand breaks, chromosomal aberrations and mutations (Phillips et al., 2009; Choy and Brannigan, 2012; Zalata et al., 2015; Dasdag and Akdag, 2016), but, to date, evidences of a clear association between EMF exposure and cancer in human and animals are limited. On the other hand, public anxiety about the potential health consequences of electromagnetic pollution is getting higher and authorities have set safety boundaries on the subject to protect the community against EMFs exposure. Particularly, the international reference guidelines for time-varying electromagnetic fields [International Commission on Non-Ionizing Radiation Protection (ICNIRP, 1998, 2010)] ruled out that limits are not constant with the frequency since they are related to the dimensions of a human being.

ICNIRP differentiates between low frequencies (LF, 1 Hz to 100 kHz)

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https://doi.org/10.1016/j.ecoenv.2018.02.053
Received 13 November 2017; Received in revised form 12 February 2018; Accepted 15 February 2018

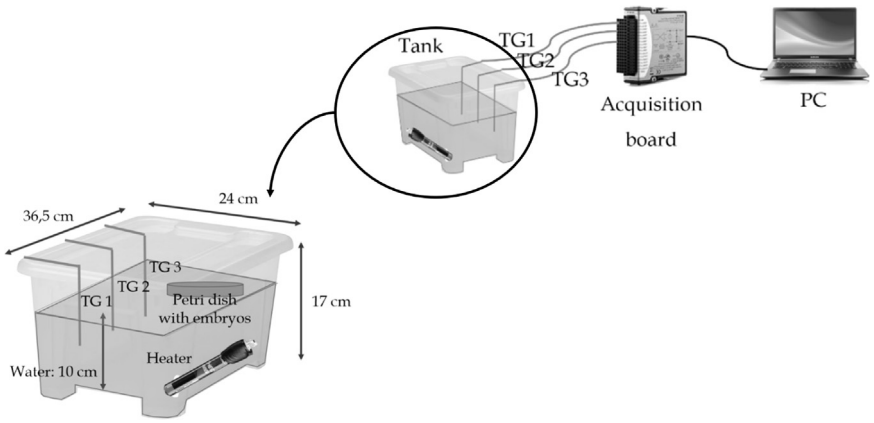

Fig. 1. Acquisition system. Water tank with heater, temperature sensors, Petri dishes and data acquisition system - tridimensional view (example for tank no. 1).

where the quantity used to specify the restrictions is the induced current density (J), and high frequencies (HF, 100 kHz to 300 GHz), where the monitored parameter is the Specific Absorption Rate (SAR).

The Italian exposure limits [Decreto del Presidente dei Ministri (DPCM, 2003)] are instead frequency independent: the quality objective for new installations states that the electric field strength must be lower than 6 V/m from 100 kHz to 300 GHz.

Several studies were performed to investigate the effects at both LF and HF. Unfortunately, data so far available for *in vivo* and *in vitro* studies are conflicting, especially regarding their biological effects and still need to be unequivocally interpreted also considering that they are primarily related to the arising thermal effects and not on the radiation per se. The heterogeneity of exposure systems, the different magnetic stimulation parameters, the inadequate dosimetry and the variability of responses depending on the used experimental models are often responsible for this kind of contrasting results (Paffi et al., 2010; Vijayalaxmi, 2016).

Nevertheless, most of the studies conducted so far, concurred that the crucial event in biological responses elicited by EMFs exposure is the onset of oxidative stress with overproduction of ROS (reactive oxygen species), which may result in cell death, reproductive decline or even cancer induction in both mammalian and non-mammalian species (Harakawa et al., 2005; Juutilainen, 2005; Phillips et al., 2009; Kong and Lin, 2010; Li et al., 2014; Dasdag and Akdag, 2016).

Particularly, the establishment of both stress response and oxidative stress, is strictly conserved in vertebrates and relies on specific pathways defined as hypothalamus-pituitary-adrenal axis (HPA) in mammals and hypothalamus-pituitary-interrenal (HPI) axis in teleosts (Alsop and Vijayan 2008, 2009a, 2009b; Alsop and Aluru, 2011; Farrell, 2011; Spiers et al., 2015). As a consequence, several cellulars and physiological modulations ranging from apoptosis/autophagy trigger (Kannan and Jain, 2000; Kroemer et al., 2010) to changes in growth, lipid metabolism and reproduction are carried out by vertebrates to cope with stress and survive it.

Thus, considering the crucial importance of these issues for human health and environmental safety, the present study aims to better understand the biological effects induced by EMFs during the embryonic development of zebrafish (ZF) *Danio rerio* (Cornet et al., 2017) by performing a specific protocol that unequivocally defines instrumental methods and biological analysis. In particular, the EMF here adopted is a 100 MHz radio-broadcasting frequency, with a field strength of 6 V/m, (ICNIRP limits are higher (61 V/m for occupational and 28 V/m for general public) and were produced by means of the use of a Transverse ElectroMagnetic (TEM) cell, that allowed to generate a well-defined electromagnetic field. Zebrafish embryos were exposed to the above described EMF and oxidative stress pathways, cholesterol metabolism and apoptotic/autophagic processes were analyzed. Finally, in order to unequivocally discriminate between RF-EMF biological effects and thermal or indirect effects, measurements of temperature were made during the whole experiment by means of K-type thermocouples.

2. Materials and methods

2.1. Ethics

All procedures involving animals were conducted in line with European and Italian legislation on experimental animals (Directive, 2010/63/EU, 2010; Decreto Legislativo 26/14, 2014), optimal rearing conditions were applied throughout the study and all efforts were made to minimize animal suffering in accordance with recommendations by “Ministero della Salute” applying the guidelines for the Care and Use of Laboratory Animals. As stated by *Article* 1.3 of the Directive 2010/63/EU, teleosts embryos are not included in the list of organisms that require a specific authorization, so no approval was requested.

2.2. Fish maintenance and embryo collection

Adult AB wild-type ZF > 5 months old (0.85 ± 0.07 g) were kept and bred in a ZF system (Tecniplast, Italy) at 28 °C, 14 L/10D photoperiod, and fed a commercial diet (Blueline, Italy) twice daily (2% body weight). After spawning, 0 h post fertilization (hpf) embryos were collected from breeding tanks, carefully rinsed and vitality and stage of development were confirmed under a dissecting microscope (Leica wild M3B).

2.3. Measurement procedure and experimental design

Newly fertilized ZF embryos (3000 ± 30) 0 hpf were assigned to a control group and a 100 MHz EMF-exposed group.

The experiment was carried out in triplicate so embryos were randomly placed in different Petri dishes assigned to each experimental group (9 Petri dishes for control and 9 for exposed group) and, as illustrated in Fig. 1, Petri dishes were located on plastic parallelepiped tanks (dimensions: 36.5 cm length, 24 cm width, 17 cm depth), filled with water up to a height of 10 cm.

Exposed groups were kept in a TEM cell and exposed to a 100 MHz EMF from 0 hpf to hatching time (72 hpf) and control groups were reared under the same conditions but without EMF exposure. Rearing parameters of each experimental group were maintained constant in terms of photoperiod (24/0 h of light and dark, respectively) and temperature (28 ± 1 °C). Inside each Petri dish, ZF embryos were kept in 30 ml of sterilized (autoclaved) water containing Methylene Blue accordingly to Jarvis and Knowles (2003).

Twice a day, 2/3 of the Petri dishes water was changed, the bottom was syphoned and cleaned, and dead embryos were collected and counted for survival rate analysis. Embryos were sampled at 24, 48 and