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An evaluation of genotoxicity in human neuronal-type cells subjected to oxidative stress under an extremely low frequency pulsed magnetic field

Gianfranco Giorgi[a,g,1], Mariangela Lecciso[b,1], Miriam Capri[c,g], Stella Lukas Yani[d], Angela Virelli[e,g], Ferdinando Bersani[e,g], Brunella Del Re[f,g,*]

[a] Department of Biology, Geology, and Environmental Sciences, University of Bologna, via Selmi 3, 40126 Bologna, Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, via Massarenti 9, 40138 Bologna, Italy
[c] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Via S. Giacomo 12, 40126 Bologna, Italy
[d] Institute for Biomedical Aging Research, University of Innsbruck, Rennweg 10, A-6020 Innsbruck
[e] Department of Physics and Astronomy, University of Bologna, via Berti Pichat 6/2, 40127 Bologna, Italy
[f] Department of Pharmacy and Biotechnology, University of Bologna, via Selmi 3, 40126 Bologna, Italy
[g] Interdepartmental Center "L.Galvani", University of Bologna, via Berti Pichat 6/2, 40127 Bologna, Italy

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ABSTRACT

The possible genotoxicity of extremely low frequency magnetic field (ELF-MF) exposure is still a controversial topic. The most of the reported data suggests that it alone does not affect DNA integrity, but several recent reports have suggested that sinusoidal ELF-MF may increase the effect of known genotoxic agents. Only a few studies deal with non sinusoidal ELF-MF, including pulsed magnetic field (PMF), which are produced by several devices. The aim of this study is to investigate whether PMF exposure can interfere with DNA damage and repair in the presence of a genotoxic oxidative agent in neuronal type cells. To this purpose gamma-H2AX foci formation, which is a sensitive marker of DNA double strand breaks (DSB), was investigated at different points of time (1, 24, 48, 72 h) after the H2O2 treatment (300 μM for 1 h) under PMF exposure (1 mT, 50 Hz) in human neuroblastoma BE(2)C cells. Moreover, cytotoxicity evaluation, by MTT assay and cell cycle analysis, was performed at various points of time after the treatment. Taken together, results suggest that PMF exposure does not interfere with genotoxicity and cytotoxicity induced by oxidative stress.

1. Introduction

In 2002, extremely low frequency magnetic fields (ELF-MF) were classified as possibly carcinogenic to humans by the International Agency for Research in Cancer (IARC) [1]. Since then, many investigations dealing with the interaction between of ELF-MF and biological systems have been carried out using various approaches. However, the potential genotoxicity of extremely ELF-MF exposure is still a controversial topic. According to the review by Vijayalaxmi and Prihoda [2] the major number of papers reported negative results, others described positive results but showed weaknesses of the experimental approach, few reliable papers showed moderate, often reversible, effects. In any case the subject is so relevant for the possible consequence regarding health protection and medical applications that further research is welcome. The most recent findings do not change significantly the situation outlined in the above-mentioned review. Reaching a definite conclusion is hard due in part to the difficulty in comparing different experiments, using diverse culture conditions, signal characteristics and biological systems besides its specific physiological conditions (proliferation or quiescence state, cell cycle etc.). A further reason is that possible genotoxic effects, if any, unlike other physical stressors like ionizing radiations, seem to be moderate, and, thus, quickly counterbalanced by the cell response.

Recently it was reported that although in some systems ELF-MF exposure does not affect DNA integrity, it can increase the DNA damage in the presence of co-exposure with a genotoxic agent [3–5]. This topic is particularly interesting because combined exposure is similar to real life conditions of any living being, where simultaneous exposure to ELF fields and other stress agents such as

* Corresponding author at: Department of Pharmacy and Biotechnology, University of Bologna, via Selmi 3, 40126 Bologna, Italy. Tel.: +39 51 2094202.
E-mail address: brunella.delre@unibo.it (B. Del Re).
[1] These authors contributed equally to this work.

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toxic pollutants and oxidative stress is the rule, opening the possibility that, in some instances, MF may influence the effects of other agents, even if neutral when applied alone.

Oxidative stress is caused by exposure to reactive oxygen intermediates, such a superoxide anion, hydrogen peroxide, and hydroxyl radical, which are generated both within the cell – as a part of cell respiration and metabolism – and by various external environmental factors. Oxidative stress seems to be involved in tumorigenesis, tumor progression, neurodegenerative diseases, and nervous system aging [6,7].

The study of possible interaction between ELF-MF and oxidative stress may be useful to define the potentially dangerous role of ELF-MF exposure as well as its possible applications. Indeed, epidemiological analysis has shown the possible association of the increased risk of brain tumors, and Alzheimer's disease with exposure to ELF-EMF from power lines and various consumer electric devices [8,9]. On the other hand, some studies have suggested that ELF-MFs could be useful in several therapeutic applications including the treatment of nervous diseases [10,11].

Numerous studies have been carried out to verify whether ELF-MF exposure can alter the cellular oxidative status of nervous cells, which are known to be particularly vulnerable to oxidative stress due to their limited antioxidant defense mechanisms, as compared to other tissues. In particular, a sinusoidal, ELF-MF-induced oxidative stress has been observed in rat brain [12,13], mouse brain [14,15], in gerbil brain [16], and in human neuroblastoma cells [17]. Moreover, an impaired antioxidant response has been observed in nerve cells that were co-exposed to ELF-MF and other oxidative stress inducers [18].

The most of the studies dealing with genotoxicity and combined exposure have been carried out using sinusoidal signal ELF-MF, while only a few studies have dealt with non-sinusoidal ELF-MF. In any case, humans are exposed to ELF-MF fields which vary in flux density, duration, and waveform depending on the emission source (power lines, cables, industrial devices, household appliances, etc.). Since the biological effect of the ELF-MF exposure varies along with varying field parameters, including the waveform type [19–21], studies on non-sinusoidal MF would be useful. Moreover, pulsed magnetic fields (PMF) seem to be efficacious for a number of clinical applications, and potential genotoxicity in nervous cells needs to be investigated.

In a previous paper we reported that PMF, characterized by a square wave signal, induced a decrease of retrotransposon mobility in neuronal human cells [22]. The aim of this study is to assess whether the exposure to PMF, can affect DNA damage and repair in neuronal cells subjected to oxidative stress. To this purpose we used BE(2)C cells, which are representative of neuronal cell type [23], since they have been shown to be sensitive to ELF-MF exposure, as reported in previous works [22,24].

2. Materials and methods

2.1. Cell culture and treatments

Neuroblastoma BE(2)C cells were provided by Prof. Della Valle (University of Bologna, Italy), who obtained them from the America Type Culture Collection (ATCC), and were maintained in Dulbecco's modified Eagle's medium (DMEM, EuroClone, Milano, Italy), supplemented with 10% heat-inactivated fetal bovine serum (FBS, EuroClone), 100 UI/ml penicillin (Sigma, Ronkonkoma, NY, USA) and 100 μg/ml streptomycin (Sigma), in a humidified 5% carbon dioxide air atmosphere at 37 °C.

24 h before PMF/Sham exposure BE(2)C cells were seeded into 3 cm Petri dish at the density of 75,000 cells/dish. After 48 h exposure, cells were treated with H_2O_2 (Sigma) (300 μM) for 1 h. Control cultures were treated with equivalent volumes of distilled water.

The dose of 300 μM was chosen because it has been largely used in studies dealing with oxidative stress, and it causes a significant DNA damage, inducing DSBs, without greatly affecting the cell viability of our cellular model, as previously reported [21].

For cell viability experiments BE(2)C cells were plated in 96-well plates at a density of 2000 cells/well.

For γ-H2AX detection experiments cells were placed on glass coverslips into 3 cm Petri dish at the density of 100,000 cells/dish.

2.2. Exposure system and field characteristics

The exposure system has been previously described [22]. It consisted of two sets of horizontal Helmholtz coils, each of 25 cm diameter, with 40 (20 + 20) turns that were double-wrapped in order to obtain wound (active coil) or counter-wound configuration. In the counter-wound configuration, the current is the same as in the active coil but the MF is zero (sham). The coils are powered by a homemade DC current amplifier, connected with a signal generator Model 33120A (Agilent Technologies, Loveland, CO). Both the active and the sham coils were maintained in the same 5%CO_2 incubator (B-5060, Heraeus, Hanau, Germany) at a constant temperature of 37 °C, and at a sufficient distance in order to minimize the stray field from the active coil in such a way as to have in the Sham coils a magnetic field ≤ 1/50 of the field in the active system. The background field within the incubator was also measured: the static component of the local magnetic field was 16.9 μT (horizontal component 10.8 μT, vertical component 13.0 μT), the AC component was on the order of 0.1 μT, as measured with a very sensitive probe (EMDEX II, Enertech Consultants, Campbell, CA).

The system was controlled by means of a PC which, through an appropriate software, chooses randomly, through a switching system, which of the two coil systems is active and which acts as sham. Only at the end of the experiments the code was decrypted so that all the experiments were conducted blind. In order to have a field uniformity within 5%, the samples were placed within a virtual cylinder

a)

day 0	day 1	day 2	day 3	day 3	day 4	day 5	day 6
Cell seeding			H_2O_2 treatment (1^h)	1^h from treatment	24^h from treatment	48^h from treatment	72^h from treatment
	Exposure to: PMF o Sham						
				- Foci evaluation - FACS analysis	- Foci evaluation - FACS analysis	- Foci evaluation - FACS analysis	- Foci evaluation - FACS analysis

b)

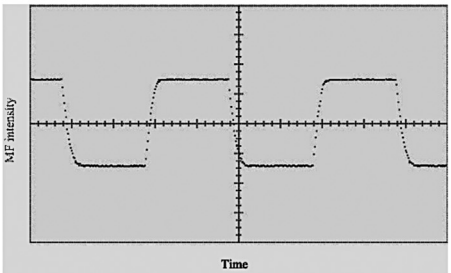

Fig. 1. Experimental scheme: (a) timeline; (b) PMF signal shape (for details see Section 2).