Life Sciences 121 (2015) 117–123

Contents lists available at ScienceDirect

Life Sciences

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

Effects of extremely low frequency electromagnetic field (ELF-EMF) on catalase, cytochrome P450 and nitric oxide synthase in erythro-leukemic cells ☆

Antonia Patruno [a], Shams Tabrez [b], Mirko Pesce [a], Shazi Shakil [c], Mohammad A. Kamal [b], Marcella Reale [d,*]

[a] Department of Medicine and Aging Science, University 'G. d'Annunzio' of Chieti-Pescara, Via dei Vestini, Chieti, Italy
[b] King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[c] Department of Bioengineering, Integral University, Kursi Road, Lucknow, UP, India
[d] Department of Experimental and Clinical Sciences, University "G.d'Annunzio" Chieti-Pescara, Chieti, Italy

ARTICLE INFO

Article history:
Received 14 September 2014
Accepted 1 December 2014
Available online 11 December 2014

Keywords:
K562
Catalase
Cytochrome P450
INOS

ABSTRACT

Aims: Extremely low frequency electromagnetic fields (ELF-EMFs) are widely employed in electrical appliances and different equipment such as television sets, mobile phones, computers and microwaves. The molecular mechanism through which ELF-EMFs can influence cellular behavior is still unclear. A hypothesis is that ELF-EMFs could interfere with chemical reactions involving free radical production. Under physiologic conditions, cells maintain redox balance through production of ROS/RNS and antioxidant molecules. The altered balance between ROS generation and elimination plays a critical role in a variety of pathologic conditions including neurodegenerative diseases, aging and cancer. Actually, there is a disagreement as to whether there is a causal or coincidental relationship between ELF-EMF exposure and leukemia development. Increased ROS levels have been observed in several hematopoietic malignancies including acute and chronic myeloid leukemias.
Main methods: In our study, the effect of ELF-EMF exposure on catalase, cytochrome P450 and inducible nitric oxide synthase activity and their expression by Western blot analysis in myelogenous leukemia cell line K562 was evaluated.
Key findings: A significant modulation of iNOS, CAT and Cyt P450 protein expression was recorded as a result of ELF-EMF exposure in both phorbol 12-myristate 13-acetate (PMA)-stimulated and non-stimulated cell lines. Modulation in kinetic parameters of CAT, CYP-450 and iNOS enzymes in response to ELF-EMF indicates an interaction between the ELF-EMF and the enzymological system.
Significance: These new insights might be important in establishing a mechanistic framework at the molecular level within which the possible effects of ELF-EMF on health can be understood.

Introduction

There has been considerable concern and controversy about the effects related with extremely low-frequency electromagnetic fields (ELF-EMFs) on the health of human populations [36]. Power lines and almost all kinds of household electrical appliances such as television sets, computers, hair dryers, mobile phones and many more emit ELF-EMFs. In view of its large application in everyday life, great attention is focused on the effects of the ELF-EMFs. The biological effects of ELF-EMFs have been the subject of more extensive studies since they can penetrate deeper into tissues [6,13,14,55]. Several epidemiological studies linked ELF-EMFs with an increased risk of cancer, for instance childhood leukemia, brain cancer, breast cancer, kidney cancer, cancer of the nervous system, lymphoma as well as cardiovascular diseases [7,32,45,48].

Despite the large number of studies performed, a causal relationship and biological mechanisms for potential effects of ELF-EMFs on carcinogenesis have not been clearly identified as yet. The main cause of skepticism is the ability of low amount of energy transfer by these fields to DNA [28]. Moreover, epidemiological associations observed between ELF-EMFs and cancer are believed to be mainly due to promoter, co-promoter or progressor effects rather than initiator [31].

On the other hand, appropriately controlled application of ELF-EMFs have therapeutic applications as well. Low frequency and low intensity fields have been used extensively for the treatment of non-union fractures and can accelerate wound healing [20,50,51]. Pain and spasticity reduction is another area in which pulsed electromagnetic therapy has been reported to be very effective [8,16].

☆ This research was partially supported by the grants from the Italian MIUR (60% in 2009).
* Corresponding author at: Dept. of Experimental and Clinical Sciences, University "G d'Annunzio" Chieti-Pescara, Via Dei Vestini, 31 66100 Chieti, Italy.
E-mail address: mreale@unich.it (M. Reale).

http://dx.doi.org/10.1016/j.lfs.2014.12.003
0024-3205/

Table 1
Kinetic characterization of CAT activity in K562 cell line.

Condition	Total υ	↑®	↓®	pT (h)	Pυ
Control	122 ± 2	6.9	0.74 ± 0.3	3.5	24.2
PMA	279 ± 13	0.83	0.18 ± 0.04	11.4	9.4
ELF-EMF	148 ± 2	2.5	0.92 ± 0.3	3.5	8.9
PMA + ELF-EMF	291 ± 10	1.5	0.49 ± 0.1	7.8	11.4

υ, activity; ↑®, Upward υ rate; ↓®, downward υ rate; pT, peak time; pυ, peak activity.

Currently, very little is known about how ELF-EMF modifies the biological systems. It can initiate a number of biochemical and physiological alterations in biological systems of different species [18,21,40]. The biological effects in cell lines exposed to ELF-EMFs have been frequently noted [19,24,35,33,42]. However, despite the large number of studies, an understanding is still lacking [12,25,46]. The time span of application is an important factor which governs the physiological response of cells towards ELF-EMF exposure. In our earlier study, we have reported that ELF-EMFs applied at different time lengths modulate chemokine production and keratinocyte growth via inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signaling pathway and might inhibit inflammatory processes [54]. Another study reported that a single exposure to ELF-EMF results in a decrease in K562 differentiation, while continous ELF-EMF exposure caused an increase in differentiation [2].

In this study, we used the K562 cell line, that are considered to be a reliable in vitro model of the hematopoietic system and of oxidative stress to assess the biological effects of ELF-EMF exposure. The purpose of this study was to evaluate activity and expression of catalase, cytochrome P450 and inducible nitric oxide synthase and their kinetic parameters in K562 cell line exposed to a well-defined and controlled ELF-EMF to add new knowledge about the mechanisms responsible for the biological effects of ELF-EMFs.

Material and methods

Magnetic field exposure system and exposure conditions of cell cultures

The experimental setup and ELF-EMF exposure system have been previously described by [54]. Briefly, the oscillating magnetic field (AC MF) consisted of: 1) a generator of sinusoidal signal at 50 Hz (Agilent mod. 33220A, Loveland, USA); 2) a power amplifier (216; NAD Electronics, London, UK); 3) an oscilloscope (ISR658; ISO-TECH, Vicenza, Italy) dedicated to monitoring output signals from the gaussmeter (MG-3D, Walker Scientific, Worcester, MA, USA); 4) a 160 turn solenoid (22 cm in length, 6 cm in radius, copper wire diameter of 1.25×10^{-5} cm) generating a horizontal magnetic field. The solenoid was then placed inside the incubator. The geomagnetic field and magnetic field generated by solenoid have the same orientation. The achieved MF intensity (1 mT (rms)) was measured continuously using the hall-effect probe, situated adjacent to the specimen located in the central part of the solenoid, and connected to the gaussmeter.

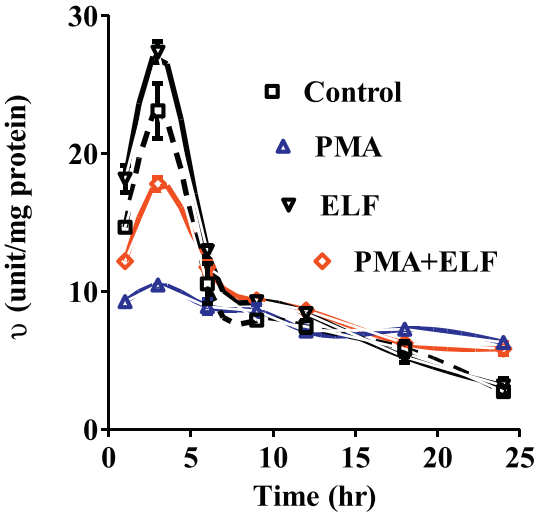

Fig. 1. CAT activity (υ) versus exposure time for control (C), PMA, ELF-EMF and PMA + ELF-EMF. A spline curve was created with 28 points calculated with the x-values ranging from 1.0 to 24.0.

Exposure condition of cell cultures

Cell culture was located in the central part of the solenoid, which was characterized by the greatest field homogeneity (98%). This setting was placed inside the incubator with a 5% CO_2 atmosphere. The incubator built-in digital thermometer monitored the internal temperature, which was set constant at 37 ± 0.3 °C. In addition, another digital thermometer (HD 2107.2, Delta OHM, Padova, Italy) was placed inside the solenoid and near the cell culture to record local temperature variations, while the temperature of the cell medium was measured using a specially designed thermoresistor (HD 9216; Delta OHM, Padova, Italy). No significant temperature changes related to ap; °C). However, no thermal effects on cells can be hypothesized for temperatures around 37 °C, since ELF-EMF interactions with biological molecules are known to be non-thermal in nature [53]. The low-level Joule heating was efficiently dissipated by the fan system inside the incubator and was <0.1308 °C in the medium of exposed cells.

K562 cell culture

Human erythro-leukemic cell line K562 was grown in RPMI-1640 containing 10% heat inactivated fetal calf serum (FCS, Sigma, Milano, Italy), 5 mM of (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 1.5 mg/ml of gentamicin, 200 μg/ml of penicillin and streptomycin and maintained at 37 °C in an atmosphere of 5% CO_2 in air. Before each experiment, cell viability was assessed by Trypan blue exclusion method. K562 cells were incubated with or without PMA (100 nM) at 37 °C, 5% CO_2 for 1, 3, 6, 9, 12, 18 and 24 h and exposed or not to ELF-EMF. Control non-exposed cells were placed in a different incubator, located in the same room. At the end of the incubation time, the cells were harvested and viability was evaluated by Trypan blue dye exclusion and counted in a Burker chamber. For each experiment the cultures were carefully matched in terms of all conditions including cell density, passage number and batch of medium. Each measurement was performed blind.

Measurement of catalase (CAT) activity

Catalase activity was measured spectrophotometrically as followed by [52] at different time points (1–3–6–9–12–18–24 h). The decomposition of H_2O_2 was monitored continuously at 240 nm for 3 min. The assay mixture in a final volume of 3 ml contained 10 mM of potassium phosphate buffer (pH 7.4), 10 mM of H_2O_2 and 10 μg of protein of enzymatic extract. CAT units were defined as 1 μmol H_2O_2 decomposed/min at 25 °C.

Determination of O_2^-

Production of O_2^- was determined spectrophotometrically (Hewlett Packard 8452 A, Palo Alto, CA, USA) by monitoring the reduction of ferricytochrome c (Type VI, Sigma, Milano, Italy) at 550 nm, as described by [39] at different time points (1–3–6–9–12–18–24 h). Briefly, ferricytochrome c (50 μmol/l) was added to the cuvette containing the cells and PBS (final volume 1 ml), either in the presence or absence of superoxide dismutase (SOD, 350 U/ml), subsequent changes in absorbance were followed for 10 min. Rates of O_2^- production were calculated