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Genotoxicity of intermediate frequency magnetic fields *in vitro* and *in vivo*

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ABSTRACT

We assessed genotoxic effects of intermediate frequency magnetic fields (MF) *in vitro* and *in vivo*. Rat primary astrocytes were exposed for 24 h to a 7.5 kHz MF at a magnetic flux density of 30 or 300 μT. Male C57BL/6 J mice were exposed continuously for 5 weeks to a 7.5 kHz MF at 12 or 120 μT, and blood samples were collected for the genotoxicity assays. To evaluate possible co-genotoxicity, the *in vitro* experiments included combined exposure with menadione (an agent that induces mitochondrial superoxide production and DNA damage) and methyl methanesulfonate (an alkylating agent). DNA damage and DNA repair (*in vitro*) were measured using the alkaline Comet assay and formation of micronuclei was assessed microscopically (*in vivo*) or using flow cytometry (*in vitro*). The results did not support genotoxicity or co-genotoxicity of 7.5 kHz MFs at magnetic flux densities up to 300 μT *in vitro* or *in vivo*. On the contrary, there was some evidence that exposure to 7.5 kHz MFs might reduce the level of genetic damage. Strongest indication of any biological effects was obtained from measurements of relative cell number, which was significantly and consistently increased after MF exposure in all *in vitro* experiments. Health implications of this finding are unclear, but it suggests that 7.5 kHz MFs may stimulate cell proliferation or suppress cell death.

1. Introduction

Health effects of electromagnetic fields have been studied for decades, mainly focusing on extremely low frequency (ELF) magnetic fields (MFs) and radiofrequency (RF) fields which have been classified as possibly carcinogenic to humans (Class 2B) (IARC, 2002, 2013). Less attention has been paid to intermediate frequency (IF) MFs even though human exposure in this frequency range is increasing due to new applications. Magnetic fields at frequencies from 300 Hz to 100 kHz or to 10 MHz (upper limit depends on how RF is defined) are classified as IF MFs (Ahlbom et al., 2008). Common occupational sources of IF MFs are industrial induction and plasma heaters, electronic article surveillance systems, wireless power transfer and various medical equipment (Litvak et al., 2002; Roivainen et al., 2014). In households, sources of IF MFs are *e.g.* induction heating cookers, LCD screens, compact fluorescent lighting, laundry machines and different power tools (Aerts et al., 2017). Data concerning possible biological and health effects of IF MFs is limited (Ahlbom et al., 2008), even though the earliest studies were carried out decades ago (*e.g.* Juutilainen and Saali, 1986, Huuskonen et al., 1998).

The present study was conducted to assess genotoxicity of IF MFs. Because of the low photon energy, electromagnetic fields in the ELF, IF and RF ranges are not likely to cause direct DNA damage. Therefore, combined effects with known genotoxic chemicals were studied in rat primary astrocytes. A similar approach has been used in numerous studies on ELF and RF fields. Among studies on ELF MFs, positive findings have been reported in several studies involving combined exposure with other physical or chemical agents (Juutilainen et al., 2006; IARC, 2002; WHO, 2007). However, frequency dependency of these effects has not been explored, and experiments in the IF range are therefore needed. We chose to conduct the study on primary astrocytes for two reasons. First, genotoxic effects in astrocytes would be highly relevant for assessing risk of brain cancer, as gliomas originating from astrocytes are the most common primary brain tumors in humans. Second, data in another frequency range suggest that primary astrocytes might be particularly sensitive to electromagnetic fields (Höytö et al., 2007). We have previously shown that pre-exposure to 50 Hz MFs at 100–300 μT modifies the responses of cultured cell lines to DNA damage induced by menadione, an agent that increases the intracellular production of reactive oxygen species (Markkanen et al., 2008;

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Luukkonen et al., 2011). Menadione was therefore used also in this study on IF MFs. To explore generalizability of the results to other DNA damaging agents with different mechanisms of action, the alkylating agent methyl methanesulfonate (MMS) was also used in combination with IF MFs.

To study genotoxicity *in vivo*, male mice were exposed to IF MFs and genotoxicity was evaluated in blood cells. DNA strand breaks and micronuclei were used to quantify genetic damage both *in vitro* and *in vivo*. The experiments were conducted using 7.5 kHz MFs similar to those emitted by common electronic article surveillance systems. Magnetic flux densities of 30 and 300 μT were used in the *in vitro* experiments. Somewhat lower fields (12 and 120 μT) were used in the *in vivo* experiments, as achieving a strong homogenous MF is difficult in the large volume required by animal cages. In both cases, the highest magnetic flux density exceeded the International Commission on Non-Ionizing Radiation Protection reference level (100 μT in the frequency range 3 kHz–10 MHz) for occupational exposure (International Commission on Non-Ionizing Radiation Protection, 2010). The highest fields used were also higher than the maximum exposure levels (up to 60 μT) found around electronic article surveillance systems used in supermarkets and libraries (Roivainen et al., 2014).

2. Materials and methods

2.1. In vitro studies

2.1.1. In vitro exposure system

The IF MF exposure system consisted of a signal generator (BK Precision 4052 dual channel function/arbitrary waveform generator, B &K Precision Corp., USA), an amplifier (Behringer Europower EP 4000, MUSIC Group Services US Inc., USA), coils and two 1 Ω (tolerance ± 5%) resistors (Sfernice RPS500 DH) connected in series with the coils. Helmholtz coils (a pair of identical circular coils placed along a common axis, separated by a distance equal to the 10.5 cm radius of the coils) were used to produce a homogenous magnetic field. The homogeneity of Helmholtz coil fields is well characterized (Beiranvand, 2013). The exposure coils were placed inside cell culture incubators (Heraeus, HERAcell, Germany) to maintain the samples under proper cell culture conditions with respect to temperature (37 °C), humidity and CO_2 (5%) concentration (Fig. 1).

Current through the coils was determined by measuring voltage over the resistor connected in series with the coils. The voltage was measured with an oscilloscope (Tektronics 2445, Tektronix Guernsey Ltd, USA) (the peak voltage is divided by $2^{½}$ to get the RMS value of the sinusoidal voltage). Continuous measurement of current (voltmeter connected over the resistors) was used to monitor the functioning and stability of the system during exposures.

2.1.1.1. Evaluation of the exposure system. To measure magnetic fields in the coil, a small measuring coil was constructed (diameter 2.0 cm, length 1.3 cm). The measuring coil was connected to a multimeter (Agilent U1241B, Agilent Technologies, Malaysia) to measure voltage induced in the coil. Measurements were done only along one line across the coil system; because of circular symmetry of the coil and the field generated by it, similar results should be obtained along any line in the xy-plane (x and y are here the horizontal axes when the common axis of the Helmholz coil pair is vertical). Measurements were repeated at three levels along the z-axis, at 2.0, 5.25 and 8.5 cm above the lower coil. As expected for a Helmholz coil pair, the homogeneity was excellent. In the xy-plane in which distance from both coils is equal (5.25 cm), deviation from the midpoint value is less than 5% in all points that are < 6 cm from the center of the coils (Fig. 2). Deviations from theoretical values most likely result from errors in determining the location of the measurement coil and manufacturing mismatches (small deviations from optimal shape of the Helmholz coils). The latter might explain why the vertical homogeneity in the upper part of the coil system appears to be even better than expected (Fig. 2), although this may also be within measurement errors, as the difference between measured and theoretical value is only about 1%. Also the size of the measuring coil (not indefinitely small) may affect the results in the areas where the field is not homogenous. Overall, it can be concluded that a homogenous field is available in a volume that allows simultaneous exposure of several multiwell plates or petri dishes.

Due to the low resistance of the Helmholtz coil system, the power dissipated in it is low, about 2.5 W at the highest magnetic field (300 μT) used in this study. Heating of the cell cultures should therefore be negligible. To confirm this, temperature inside the coils placed in incubators was measured with Fluke 52 K/J Thermometer (John Fluke Mfg. Co. Inc., USA) for 24 h. No temperature difference between the exposure and sham-exposure systems was observed. No change in mechanical vibration level, measured by a Bruel et Kjaer accelerometer probe type 4366 (Copenhagen, Denmark) connected to a Wärtsilä 7178D sound level meter (Wärtsilä, Helsinki, Finland), was observed at the location of the cell cultures when the MF exposure system was switched on. The background 50 Hz MF levels were below 2 μT inside the incubators and the static magnetic field was 27–30 μT (inclination 80–85°) in the incubators measured with a Hirst GM08 meter and Hirst Axial Fluxgate Probe AFG100 (Hirst Magnetic Instruments Ltd., Cornwall, UK). The static MF is weaker than the outdoor natural geomagnetic field because the incubator is made of steel and is located in a steel-reinforced concrete building.

2.1.2. Electric fields induced in cell cultures

As effects of IF MFs could, in principle, result either from direct magnetic interaction or from electric fields induced in the cell cultures, we evaluated the electric fields induced in the cell culture medium. The

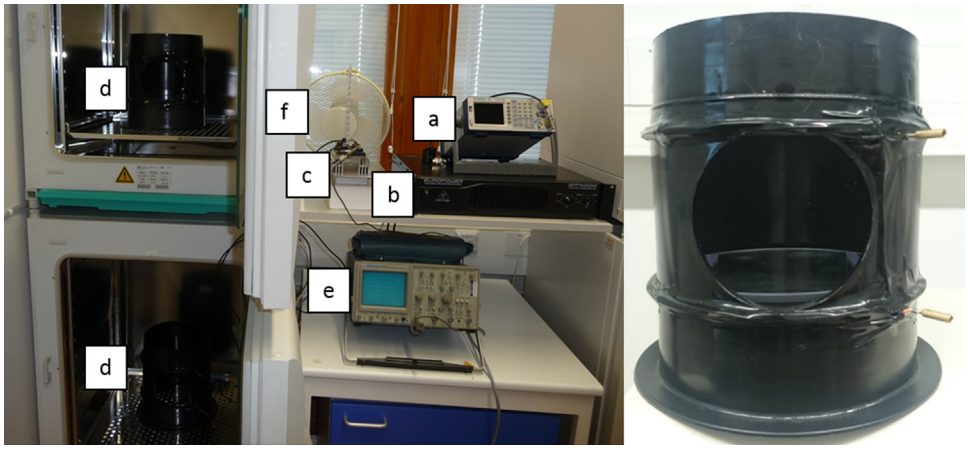

Fig. 1. The *in vitro* exposure system consisted of a signal generator (a), an amplifier (b) and resistors (c) connected in series with one of the coils (d) inside the incubators. An oscilloscope (e) was used to measure voltage and a fan (f) for cooling the resistors. On the right side is a larger picture of the Helmholtz coil used for exposing (or sham-exposing) cell cultures to magnetic fields.