EFFECTS OF 2.45 GHZ ELECTROMAGNETIC FIELDS WITH A WIDE RANGE OF SARS ON MICRONUCLEUS FORMATION IN CHO-K1 CELL

S. Koyama (1), Y. Isozumi (2), Y. Suzuki (3), M. Taki (4) and J. Miyakoshi (5)

(1) Department of Radiological Technology, School of Health Sciences, Faculty of Medicine, Hirosaki University, 66-1 Hon-cho, Hirosaki, 036-8564, Japan. s-koyama@eagle.ocn.ne.jp
(2) Department of Molecular Environment of Life and Nature, Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan. yasuhiro@barium.rirc.kyoto-u.ac.jp
(3) Department of Electrical Engineering, Graduate School of Engineering, Tokyo Metropolitan University, 1-1 Minami Ohsawa, Hachioji, Tokyo 192-0397, Japan. suzuki@eei.metro-u.ac.jp
(4) As (3) above. taki@eei.metro-u.ac.jp
(5) As (1) above. miyakosh@cc.hirosaki-u.ac.jp

ABSTRACT

In the modern era, people are exposed to many kinds of electromagnetic fields (EMFs), and exposure occurs almost all day long. The use of domestic electrical appliances has increased and mobile telephones are used extensively all over the world. Several epidemiological studies have indicated that exposure to EMFs may be associated with an increased risk of childhood leukemia and other cancers, and this has caused great interest in the field. Some positive data have indicated a correlation between exposure to environmental low frequency EMFs and DNA damage or chromosomal aberrations, which may induce certain kinds of disease. However, it has also been strongly suggested that no such relationship exists between EMF exposure and carcinogenesis, or that, if there is a relationship, it is only a weak one. In addition to the conflicting and contradictory views on extremely low frequency EMFs, there is also controversy surrounding the effects of high frequency electromagnetic fields (HFEMFs). HFEMFs are particularly used in mobile communication, and hence there is considerable interest in the possible effects of HFEMFs on human health.

In order to investigate the properties of HFEMFs, we have examined the effects of 2.45 GHz electromagnetic fields on micronucleus (MN) formation in Chinese hamster ovary (CHO)-K1 cells. MN formation is induced by chromosomal breakage or inhibition of spindles during cell division and leads to cell damage. We also examined the influence of heat on MN formation, since HFEMF exposure causes a rise in temperature.

CHO-K1 cells were exposed to a HFEMF for 2h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100 and 200 W/kg, and the effects on these cells were compared with those in sham-exposed control cells. The cells were also treated with bleomycin alone as a positive control or with combined treatment of HFEMF exposure and bleomycin. Heat treatment was performed at temperatures of 37, 38, 39, 40, 41 and 42 °C.

The MN frequency in cells exposed to a HFEMF at a SAR of lower than 50 W/kg did not differ from the sham-exposed controls, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls. There was no apparent combined effect of HFEMF exposure and bleomycin treatment. Upon heat treatment at temperatures from 38 to 42 °C, the MN frequency increased in a temperature-dependent manner. We also showed that an increase in SAR causes a rise in temperature, and this may be connected to the increase in MN formation generated by exposure to a HFEMF.

INTRODUCTION

The potential hazards of exposure to high frequency electromagnetic fields (HFEMFs) have been addressed in many epidemiological studies [1]. However, it remains unclear whether there is an association between exposure to HFEMFs and human health or development. Some reports have suggested that effects caused by increased temperature cannot be ruled out as an explanation of the relationship between HFEMFs and adverse outcomes [2]. On the other hand, numerous reports have shown that HFEMFs exert significant effects under isothermal conditions [3]. Considering these previous data, we have examined the frequency of micronucleus (MN) formation in Chinese hamster ovary (CHO)-K1 cells following HFEMF exposure. MN are derived from clastogenic activity arising from chromosomal fragments that are not incorporated into daughter nuclei at mitosis. This occurs because of the lack of a kinetochore. MN are also derived from an aneugenic process when the whole chromosome is not correctly integrated.
into the two daughter nuclei at the time of cell division [4].

**MATERIALS AND METHODS**

**Cells and Culture Conditions**
CHO-K1 cells were obtained from the Japanese Cancer Research Bank, Tokyo. Cells were maintained in Ham’s F-12 medium (Nikken) supplemented with 10% fetal bovine serum (Gibco, BRL) at 37 °C in 95% air and 5% CO₂. Following incubation for 23 hours, the cells were treated as described below.

**Exposure System**
The setup is based on a rectangular wave-guide, with the size of the wave-guide determined to be 110[mm](width) X 55[mm](height) X 310[mm](depth), since microwaves of frequency 2.45 GHz are used. The electromagnetic wave is propagated along the wave-guide in TE₁₀ modal.

The specific absorption rates (SARs) distribution in the exposure apparatus was calculated using the FDTD method. The relationship between the SARs and temperature was measured, and a line graph was drawn.

**HFEMF Exposure with or without Bleomycin**
After incubation for 23 h, the medium was changed, either to similar fresh medium or to a new medium containing bleomycin at a final concentration of 10 µg/ml. In each case, a further 1–hour incubation was then performed. Cells treated with or without bleomycin were then washed three times with PBS, cultured in new medium, and then exposed to a HFEMF of SAR 5, 10, 20, 50, 100 or 200 W/kg for 2 h.

**Heat Treatment**
After treatment with or without bleomycin, the cells were washed three times with PBS, cultured in new medium, and then the cells were incubated at 38, 39, 40, 41 or 42°C for 2 h.

**Sham Exposure and Bleomycin Treatment**
A sham-exposure experiment was performed as a negative control. Cells were treated in the same way to those that underwent HFEMF exposure, except that HFEMF exposure itself was omitted. Instead, the cells were incubated for 2 h in a conventional incubator. In addition, the cells were treated with bleomycin alone as a positive control, using the same conditions as those used for sham exposure.

**Inhibition of Cytokinesis for Detection of Binucleated Cells and Scoring Procedure**
After each 2 h treatment, the cells were trypsinized and seeded in 10 cm culture dishes in medium containing cytochalasin B at a final concentration of 3 µg/ml for 18 h, in order to prevent cell division.

The cells were collected at a concentration of 2.7 × 10⁵ cells/ml, and samples of 0.2 ml were centrifuged onto slides, using a Cytospin centrifuge (Shandon Southern Ltd.) at 100 × g for 5 min. The cells were then fixed with 80% cold ethanol for 30 min. The slides were washed gently with PBS and soaked in new PBS for 5 min to completely remove the ethanol.

The cells on the slides were stained with 20 µl of propidium iodide (PI) diluted in glycerol (to a concentration of 0.2 µg/ml), and kept in the dark until counting was performed. A total of 1000 binucleated cells were scored for evaluation of the frequency of induction of MN, using fluorescence microscopy (Olympus). Cells were counted as having MN formation when they contained at least one micronucleus. The procedure was performed in a double-blind manner, and followed the method described by Countryman and Heddle [4]. Statistical analysis of the data in the control and experimental groups was conducted using ANOVA (analysis of variance) followed by Fisher’s PLSD test.

**RESULTS**
The formation of MN is shown in Fig. 1. Fig. 1A shows a binucleated cell which does not have a micronucleus and Fig. 1B shows a binucleated cell with a micronucleus.

The effect on MN formation of exposure to a HFEMF is shown in Fig. 2. There was no increase in MN formation in cells exposed to HFEMFs at SARs from 5 to 50 W/kg. However, the frequency of MN formation in cells exposed to HFEMFs at SARs of 100 and 200 W/kg were statistically significantly different to that observed following sham exposure.

The frequency of MN formation following treatment with bleomycin alone was statistically higher, compared with the
sham-exposed control (Fig. 2). Although there was no increase in MN formation in cells treated with a combination of bleomycin and HFEMF exposure at SARs from 5 to 100 W/kg, there was a statistically significant difference in MN formation between treatment with bleomycin alone and combined treatment with bleomycin and HFEMF exposure at a SAR of 200 W/kg.

The effects of heat and change of temperature on MN formation are shown in Fig. 3. The frequency of MN formation with heat treatment increased in a temperature-dependent manner. However, there was no difference between bleomycin-treated cells and cells treated with a combination of heat and bleomycin for temperatures from 38 to 41°C. MN formation in cells that underwent combined treatment with heat and bleomycin at 42°C was statistically different to that in cells treated with bleomycin alone, but there was no difference in MN formation compared to that in cells treated with heat only at 42°C.
The relationship between the effect on MN formation of exposure to a HFEMF and temperature is shown in Fig. 4. Columns indicate the frequency of MN formation and the line graph indicates the change of temperature generated by exposure to the HFEMF. Temperatures higher than 39 °C caused a statistically significant increase in MN frequency, similarly to that shown in Fig. 3. The frequency of MN formation increased in a temperature-dependent manner as the temperature was raised. A correlation coefficient of 0.978239 was obtained between the frequency of MN formation and the temperature.

DISCUSSION

In the present study, there was also no difference in MN formation in CHO-K1 cells exposed to an HFEMF at a SAR lower than 50 W/kg, but there were statistically significant increases in MN frequency at SARs of 100 and 200 W/kg, compared with the sham-exposed control (Fig. 2). However, we failed to detect an increase in MN formation in cells exposed to an HFEMF at a SAR of 100 W/kg after bleomycin treatment, compared with bleomycin treatment alone, and combined effects of bleomycin and HFEMF exposure at SARs lower than 100 W/kg were not detected. Although we detected enhancement of MN formation with combined treatment of HFEMF exposure at a SAR of 200 W/kg and bleomycin, an appreciable rise in temperature occurs under such conditions (Fig. 3 and 4). The MN frequency following combined treatment of HFEMF exposure at a SAR of 200 W/kg and bleomycin did not differ from that with HFEMF exposure at a SAR of 200 W/kg alone. This implies that HFEMF exposure at a SAR of 200 W/kg does not enhance the effect of bleomycin.

In this study, we showed that MN formation increased in a temperature-dependent manner (Fig. 3), in agreement with several previous reports, which have also indicated that a rise in temperature can induce MN formation [5]. Other studies have indicated that significant effects on various cellular activities are obtained upon exposure to radiofrequency (RF) radiation or microwaves under isothermal conditions [6]. However, in the present study, the increase in MN formation following exposure to HFEMFs seems to depend on temperature rise, based on the correlation coefficient (R²=0.978239) for the data in Fig. 4. This result is supported by a literature report based on use of a pulsed electromagnetic field [7].

The present study has shown that MN formation upon HFEMF exposure at SARs from 5 to 200 W/kg is connected to the temperature rise caused by the HFEMF.

ACKNOWLEDGMENTS

This work was supported in part by the Ministry of Internal Affairs and Communications, Japan.

REFERENCES