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Effects of 835-MHz Radiofrequency Radiation on the Chromosomal DNA of Mouse Thymic Lymphoma L5178Y $Tk^{+/-}$ Cells

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Abstract - This study was focused on the risk assessment of whether radiofrequency electromagnetic fields generated by mobile phone is cytogenetically toxic or not. We conducted the effects of 835-MHz electromagnetic field (EMF) on DNA strand breaks in mouse thymic lymphoma L5178Y $Tk^{+/-}$ cells using alkaline comet assay. EMF frequency 835-MHz we chosen is one of the most popular communication frequency bands in Korean code-division multiple-access (CDMA) mobile phone system. The cells were exposed to 835-MHz EMF alone or 835-MHz EMF combined with cyclophosamide (CPA) or 4-nitroquinoline-1-oxide (4NQO) at specific absorption rate (SAR) of 4.0 W kg^{-1} for 24 and 48 hrs. DNA damage expressed as tail moment was increased more than two-fold after exposure to 835-MHz EMF for 24 and 48 hr. In particular, CPA for 48 hr and 4NQO for 24 hr enhanced notably the tail moment to 9-fold and 16-fold in the presence of 835-MHz EMF, respectively, compared to each single treatment. From these results, it appears that exposure to CDMA-mobile phone radiation at 835-MHz frequency may potentiate DNA strand breaks of mouse thymic lymphoma L5178Y $Tk^{+/-}$ cells under the defined conditions of this study.

Key words : electromagnetic field, alkaline comet assay, thymic lymphoma L5178Y $Tk^{+/-}$, DNA strand breaks

INTRODUCTION

Frequent exposure to radiofrequency (300 kHz-300 GHz) of household electrical appliances, telecommunications and navigational equipments is known to be a risk factor to public health. So far, several contradictory reports regarding the potential toxicity of electromagnetic fields (EMFs) have been documented. Recently, the number of people subscribing to use portable hand-held mobile phone is exponentially increasing but little has been done about the epidemiological and biological investigations in mobile phone communication bands. There are some studies of bio-safety at frequencies used in mobile phone communication. Malyapa *et al.* (1997) measured DNA damage of cultured cells U87MG and C3H 10T½ after exposure to 835.62- (frequency-division multiple-access, FDMA) and 847.74-MHz (code-division multiple-access, CDMA) at an SAR of 0.6 W kg^{-1}, but found no effect. In addition, neither 835.62-MHz FDMA nor 847.74-MHz CDMA had any significant effect at the same SAR indicated above on proto-oncogene expression during cellular proliferation (Goswami *et al.* 1999) as well as neoplastic transformation frequency (Roti Roti *et al.* 2001). Recently, Zeni *et al.* (2003) reported no correlation of genotoxic effects in

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human leucocytes exposed *in vitro* to 900 MHz, a European mobile communication band. Contrary to no harmful effects of mobile phone communication radiation, acute exposure to rats with either a 60 Hz magnetic field with flux densities 0.1-0.5 mT or 2450-MHz microwave radiation at SAR 1.2 W kg^{-1} for 2 hr caused a dose-dependent increase in DNA strand breaks in rat brain cells (Lai and Singh 1996, 1997). When the exposure to extremely-low-frequency (ELF) EMFs was applied to human leukemia cell HL60, nucleosomal fragmentation or apoptotic DNA fragmentation was significantly induced (Narita *et al.* 1997). In addition, intermittent exposure to ELF EMFs in human diploid fibroblasts induced chromosomal DNA strand breaks greatly (Ivancsits *et al.* 2002).

In this study, we investigate the possible cytotoxic effects due to radio-frequency 835 MHz (one of the Korean mobile phone bands) to mouse thymic lymphoma L5178Y $Tk^{+/-}$ cells using the alkaline comet assay. SAR we examined was set at levels higher than 1.6 W kg^{-1} body weight specified in ANI/IEEE safety guidelines (Ghandi and Kang 2002).

MATERIALS AND METHODS

1. Cell and Culture

L5178Y $Tk^{+/-}$ mouse lymphoma cells were obtained from LGCI Ltd (Daejeon, Korea). This cell line was derived from the L5178 thymic lymphoma induced by methylcholanthrene in a DBA/2 mouse. The cells were cultured in RPMI 1640 medium supplemented with 100 U mL^{-1} penicillin, 100 U mL^{-1} streptomycin, 2 mM L-glutamine and 10% heat-inactivated horse serum. Subculture was conducted every 2-3 days so as not to exceed 2×10^6 cells mL^{-1}.

2. Exposure Facility Setup

Culture cells were exposed to an 835-MHz EMF in a specially designed apparatus based on the TEM wave theory, in which Wave Exposure V10 (Model CTI835, Patent #0311892, Republic of Korea) irradiated cultivating cells in a CO_2 gas incubator with an 835-MHz CDMA wave. The characteristics of Wave Exposure V10 are summarized in Table 1. The TEM cell was positioned horizontally to allow uniform radiation to all plates and was placed inside CO_2 incubator with constant temperature throughout the radiation. There are two selectable modes-for blood, conductivity $\delta = 1.19$, relative dielectric constant $\varepsilon_r = 74$ and for skin, $\delta = 0.92$, $\varepsilon_r = 57$-by a rotary switch on the front panel. The power level at each mode is controlled by an SAR-level switch on the control panel: each mode has four SAR levels (1.6, 2.0, 3.0 and 4.0 W kg^{-1}). Two petri-dishes, 10 cm in diameter and 2 cm high, can be layered in the TEM cell apparatus by mounting them in a jig fixture as shown in Figure 1. The space in the TEM cell was maintained under controlled conditions similar to those in an incubator; i.e. an atmosphere of 5% CO_2 and 95% air and a temperature of 37°C. To test the increase of temperature during the radiation of 835-MHz at 4.0 W kg^{-1}, preliminary measurements were carried out with digital thermometer every one hour. The temperature change constantly resulted in 36.9 ± 0.1°C throughout the 24-hr radiation in both exposed and unexposed groups (data not shown).

For 24 hr EMF exposure, cell concentrations were adjusted to 3×10^5 cells mL^{-1} in RPMI 1640 medium.

Table 1. Characteristics of wave exposure V10

Manufacturer	Dept. of IT Eng. Soonchunhyang Univ. Rep. of Korea
Cell mode	CTI835
Patent	No. 0311892, Rep. of Korea
Frequency	CDMA 835-MHz
RF power	1.7, 2.1, 3.2, 4.2 W for blood mode 2.2, 2.7, 4.2, 5.4 W for skin mode
Selectable SAR	1.6, 2.0, 3.0, 4.0 W kg^{-1}
Max. size of Petri-dish	10 cm (diameter) × 2 cm (height)
Main power	220V AC, 6A, 60Hz
CO_2 incubator	
Main control	VFD display, micro processor controller
Temperature range	+5 to +60°C
CO_2 range	0 to 20%, ±0.1% at 5% Digital PID controller, IR sensor
Humidification	up to 96% RH
Programmability	10 steps per 10 memories
Capacity	130 liter
Water jacket	65 liter
Dimension	445 × 470 × 620 mm, inside 582 × 580 × 1,015 mm, outside

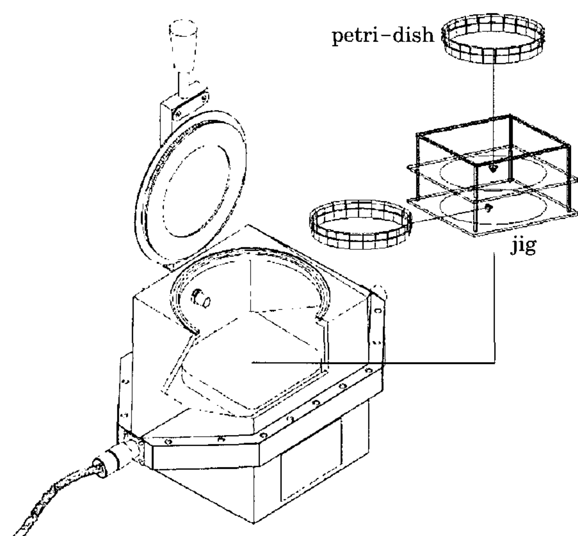

Fig. 1. Electromagnetic field apparatus. Cultivated cells on three Petri-dishes stacked in the jig fixture are exposed to uniform TEM-mode EMFs in the apparatus.

Two mL of cells were dispensed to each well of 12-well plate. For 48 hr-EMF exposure, cells were incubated at concentration of 1.5×10^5 cells mL^{-1} in 2 mL per well in 12-well plate. The cells were exposed to EMF for 24 hr or 48 hr. The positive control items were treated 4 hr prior to harvesting for comet assay. Viability was determined by tryphan blue exclusion.

3. Alkaline comet assay

The comet assay was performed as described by Tice *et al.* (2000) and manufacturer's instruction. Briefly, cell suspension (25 μL) was mixed 1 : 10 with 250 μL molten low melting point (LMP) agarose, and samples of 75 μL of the mixture were rapidly spread on CometSlide (Trevigen). After gelling for 20 min at 4°C in the dark, slides were put in a tank filled with lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris base, 1% sodium lauryl sarcosinate and 1% Triton X-100) for 1 hr at 4°C in the dark. Slides were then washed three times with neutralization buffer (0.4 M Tris, pH 7.5) for 5 min and incubated in fresh alkaline buffer (0.3 M NaOH and 1 mM EDTA, pH > 13) for 30 min at room temperature to allow unwinding of DNA. Electrophoresis was then carried out at room temperature in fresh ice-cold alkaline electrophoresis buffer for 30 min (1 V cm^{-1}; 300 mA). After electrophoresis, slides were gently washed three times for 5 min in fresh neutralization buffer and exposed to 70% ethanol for 5 min. After drying at room temperature, slides were stained with 25 μL of ethidium bromide solution (20 μg mL^{-1}) and covered with a coverslip. Comets were examined at 200X magnification using a fluorescence microscope (Nikon E600) connected to a CCD camera and an image analysis system (Komet 5.0, Kinetic Imaging, Liverpool, UK).

4. Evaluation and interpretation of DNA damage

For quantitative evaluation, Comet imaging system calculated tail moment (tail length × %DNA in tail) from two parameters-an absolute DNA intensity of tail and its distance to the center position of the head. Under a fluorescence microscope 50 cells per slide were evaluated and the tail moment of each cell measured. Results were expressed as the mean tail moment ± S.D. During alkaline gel electrophoresis, the cell lysis and subsequent tailing of L5178Y $Tk^{+/-}$ mouse lymphoma cells showed relatively random pattern rather than a non-random pattern of %DNA in tail. Thus, we could not apply the statistical analysis of tail moment in our measurement system. It was judged to be positive, based on the distribution tendency of tail moment frequency, if there was a relative increase of more than two-fold mean tail moment of experiment group compared to control.

RESULTS AND DISCUSSION

Recently, social concerns on the potent toxicity of EMFs exposure generated by mobile phone have been focused on the possibility of causative linkage with neuro-pathological disease. Whereas the wide survey of experiments have conducted to demonstrate the possible toxicity of microwave, low-frequency EMFs and static magnetic fields (Salford *et al.* 2003), little has been done about cytotoxic study of mobile phone frequency bands in the range of 300 KHz to 3 GHz. For example, exposure to magnetic fields at 5-400 mTesla potentiates X-ray-induced DNA strand breakages in human glioma MO54 cells (Miyakoshi *et al.* 2000). Epidemiological evidences have been reported that the exposure

to extremely-low-frequency EMFs is associated with childhood leukemia (Hone *et al.* 2003). In addition, application of 2450-MHz EMFs to human glioma MO54 cells induced heat shock protein hsp70 expression followed by thermal increase to the cells (Tian *et al.* 2002). Therefore, we challenged to examine the potential hazards of 835-MHz EMFs in mouse thymic lymphoma L5178Y $Tk^{+/-}$ in the absence or presence of positive mutagens by *in vitro* alkaline comet assay. The frequency band we chose is one of the most popular mobile phone frequency in Korea, to induce primary DNA-breakage in individual cells. As seen in Table 2, cell viability was not much changed as 98-103% between control and EMF-treated group, irrespective of the presence of microsomal S9 fraction. However, EMF induced a conspicuous increase in tail moment compared to the control for 24 or 48 hr at SAR 4 W kg^{-1}. The relative tail moment induced by EMF treatment was greatly increased to 2.6-2.9 fold in the presence of S9 fraction, whereas EMF produced remarkably 10.7 fold increase of tail moment for only 24 hr exposure. When the potent mutagen, CPA was treated in combination with EMF, CPA-induced DNA strand breaks were unexpectedly increased to 9.3 fold for only 48 hr exposure, compared to 0.9 and 2.6 fold increase by the single treatment with CPA and EMF, respectively. However, such an unexpected result was not repeated when treated CPA with EMF for 24 hr as shown after 48 hr irradiation. We suspect that CPA is a cytochrome P450-dependent metabolizable drug (Hengstler *et al.* 1997) and requires long incubation time for the maximal increase of tail moment in mouse lymphoma cells. In contrast to CPA treatment, co-treatment of 4NQO and 835-MHz EMFs exerted an additive effect for 24-hr exposure (15.7-fold increase) compared to single treatment of 4NQO (4.6-fold) and EMF (10.7-fold). Slightly enhanced effects (5-fold) of 4NQO and EMF were shown compared to the simply additive effect of single treatment of 4NQO (2.5-fold) and EMF (1.3-fold) for 48 hr exposure. Therefore, 24 hr incubation time with 4NQO is sufficient to induce toxicity of DNA strand breakage since 4NQO plays toxic effect via an independent pathway of drug metabolism (Valentin-Severin *et al.* 2003). It is consistent with our preliminary experiment, that co-mutagenic genotoxicity of EMFs combined with 4NQO was increased to 45% in Ames test (Chang *et al.* 2004). Our finding about toxic effect of EMFs on mouse thymic lymphoma cell is consistent with other reports that EMF induced DNA strand breaks in rat brain cells (Lai and Singh 1997) and human diploid fibroblasts (Ivancsits *et al.* 2002) using comet assay. Since the inherently wide variation of tail lengths of L5178Y $Tk^{+/-}$ cells induced by potent muta-

Table 2. Alkaline comet assay results using L5178Y $Tk^{+/-}$ mouse lymphoma cells with 835-MHz EMFs

Treatment groups	S9 mix	EMF freq. (MHz)	EMF exposure time (h)	Viability[a] (%)	Median tail moment	Relative tail moment[b]
Control	+	0	0	100	0.35±0.48	100
CPA	+	0	0	80	1.09±1.74	311
EMF	+	835	24	98	1.01±1.70	289
EMF+CPA	+	835	24	85	0.71±0.96	203
Control	+	0	0	100	0.30±0.86	100
CPA	+	0	0	87	0.28±0.33	93
EMF	+	835	48	103	0.78±0.79	260
EMF+CPA	+	835	48	95	2.80±2.85	933
Control	−	0	0	100	0.15±0.19	100
CPA	−	0	0	90	0.69±0.82	460
EMF	−	835	24	102	1.61±2.05	1073
EMF+CPA	−	835	24	96	2.35±2.40	1567
Control	−	0	0	100	0.42±0.44	100
CPA	−	0	0	92	0.42±0.44	252
EMF	−	835	48	99	1.06±1.15	129
EMF+CPA	−	835	48	94	2.10±2.06	500

4NQO: 4-nitroquinoline-1-oxide, 0.05 μg mL^{-1}; CPA: Cyclophosphamide, 5 μg mL^{-1}; [a] Viability measured by tryphan blue exclusion, expressed as % viability of control; [b] Relative tail moment = tail moment of experiment group/tail moment of control group.

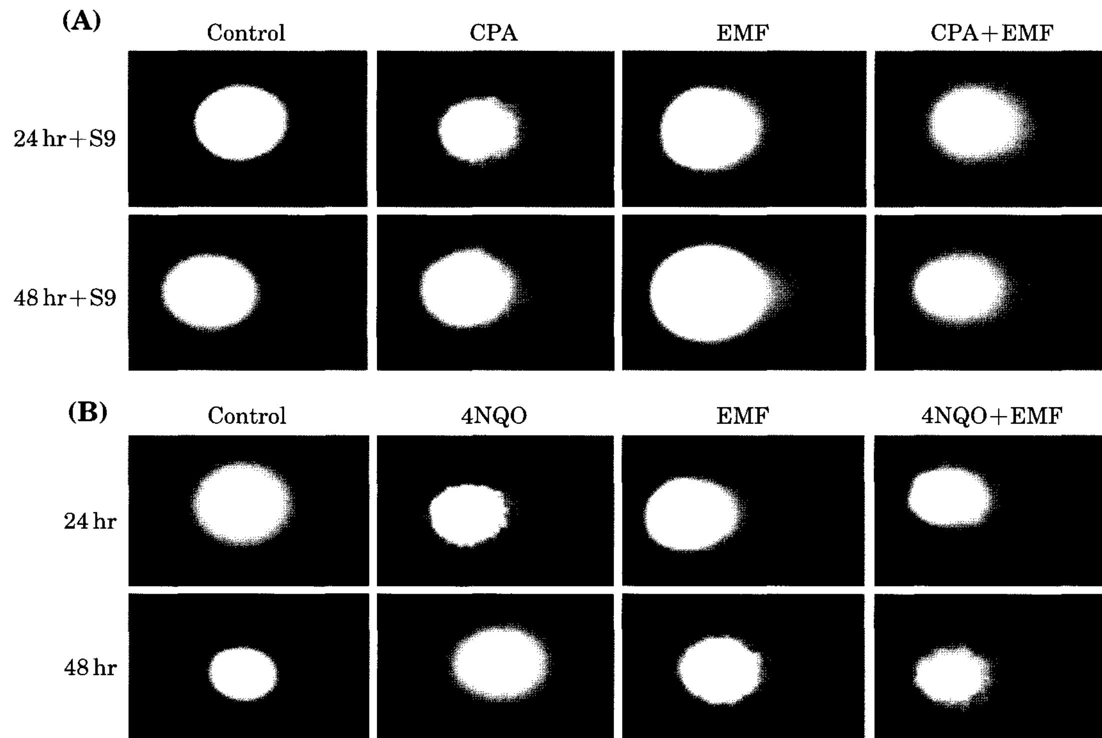

Fig. 2. Microscopic picture of tail-moments of L5178Y $Tk^{+/-}$ mouse lymphoma cells after the exposure to 835-MHz EMFs. Chromosomal DNA fragments of alkali lysis cells are electrophoresed to the right side (anode) on slide glass.

gen was observed in the presence or absence of 835-MHz, it can not be ruled out whether the statistical relevance of tail moments observed in our system is significant or not. Instead, we adopted the relative tail moment of experiment versus control as a tendency evaluation-cytotoxicity indication of 835-MHz EMFs. Microscopically damaged lymphoma cells in 48-hr EMFs exposure with CPA and 24- or 48-hr EMFs exposure with CPA were clearly observed in Figure 2. In conclusion, it can be stated that exposure to CDMA-mobile phone radiation at 835-MHz frequency may potentiate DNA strand breaks of mouse thymic lymphoma L5178Y $Tk^{+/-}$ cells under the defined conditions of this study. The cumulative reports of basic biological safety study will be useful for the prevention and diagnosis of arguable risk factor evaluation from mobile phone radiation.

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