Effects of 2.45 GHz Electromagnetic Fields with a Wide Range of SARs on Bacterial and HPRT Gene Mutations

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High-frequency electromagnetic fields (HFEMF)/Bacterial mutation/Hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation/Chinese hamster ovary (CHO)-K1 cells.

Present day use of mobile phones is ubiquitous. This causes some concern for human health due to exposure to high-frequency electromagnetic fields (HFEMF) from mobile phones. Consequently, we have examined the effects of 2.45 GHz electromagnetic fields on bacterial mutations and the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutations. Using the Ames test, bacteria were exposed to HFEMF for 30 min at specific absorption rates (SARs) from 5 to 200 W/kg. In all strains, there was no significant difference in the frequency of revertant colonies between sham exposure and HFEMF-exposed groups. In examination of mutations of the HPRT gene, Chinese hamster ovary (CHO)-K1 cells were exposed to HFEMF for 2 h at SARs from 5 to 200 W/kg. We detected a combination effect of simultaneous exposure to HFEMF and bleomycin at the respective SARs. A statistically significant difference was observed between the cells exposed to HFEMF at the SAR of 200 W/kg. Cells treated with the combination of HFEMF at SARs from 50 to 200 W/kg and bleomycin exhibited increased HPRT mutations. As the exposure to HFEMF induced an increase in temperature, these increases of mutation frequency may be a result of activation of bleomycin by heat. We consider that the increase of mutation frequency may be due to a thermal effect.

INTRODUCTION

In industrialized countries, people are exposed to many kinds of electromagnetic fields. Exposure to high-frequency electromagnetic fields (HFEMF) or radiofrequency (RF) electromagnetic fields has spread through radio communications in various areas. In particular, the use of mobile phones that emit HFEMF is increasing rapidly. However, it remains controversial as to whether or not exposure to HFEMF elicits adverse results towards human health. Richter et al.1) found that HFEMF exposure exhibits an epidemiologic effect on cancer risk. No effects of HFEMF have been demonstrated on human health2–5); thus, if there are any effects, they are very weak. Moulder et al.6) reviewed the current state of evidence concerning the carcinogenic-potential of HFEMF energy used for wireless communication. In this review, most studies indicated no relationship between the HFEMF energy and cancer, although further investigations of this field are required. In this decade, the world-wide spread of mobile phones is accelerating. Consequently, concern about potential health risk by HFEMF exposure from mobile phone is increasing. Epidemiological studies have produced contradictory results7–11) that were likely due to the short-term mobile phone use and little evidence.

Laboratory studies have also indicated contradictory results. Lai and Singh12,13) demonstrated that acute, low-intensity microwave (whole body average SAR (specific absorption rate) 1.2W/kg) exposure and radiofrequency electromagnetic radiation increased DNA strand breaks. Marinelli et al.14) reported that unmodulated 900 MHz electromagnetic field at the SAR of 3.5 mW/kg induced DNA breaks and early activation of both p53-dependent and – independent apoptotic pathways. Similarly, a positive finding was reported by Erdinc et al.15) that exposure to a 900 MHz electromagnetic field might affect the developing brain in prepubertal mice injected with pentylenetetrazole. An increase in frequency of micronuclei has also been demonstrated following exposure to radiofrequency electromagnet-
ic field and the potential hazards of HFEMF at a SAR of 2.1 μW/kg exposure have been discussed under non-thermal condition. However, Vijayalaxmi et al. suggested that 2450 MHz radiofrequency exposure at a whole body average SAR of 12 W/kg exhibited no effect on the induction of micronuclei in peripheral blood and bone marrow cells of rats. Malyapa et al. also investigated the effect of exposure to 2450 MHz radiofrequency (SARs; 0.7 and 1.9 W/kg) by comet assay and demonstrated no significant differences between the tested groups and the controls after irradiation. Moulder et al. suggested in their review, that in the laboratory studies cell phone HFEMF radiation generally exhibits no genotoxic or epigenetic activity, and that the cell phone HFEMF radiation-cancer connection is physically implausible. While, Leszczynski reported that mobile phone radiation (average SAR 2 W/kg) caused activation of the HSP27/p38MAPK stress pathway. They hypothesized that this activation may facilitate the development of brain cancer or cause an increase in blood-brain barrier permeability through stabilization of endothelial cell fibers. Nylund and Leszczynski reported that mobile phone radiation at an average SAR of 2.4 W/kg affected protein expression. However, it has also been reported that 1800 MHz radiofrequency radiation at the SARs of 1.4 and 2.0 W/kg does not affect apoptosis or HSP70 levels in peripheral blood. Vijayalaxmi and Obe reviewed that a large number of investigations were conducted using rodents, cultured rodent and human cells, and freshly collected human blood lymphocytes to determine the genotoxic potential of exposure to HFEMF. The results of most of these studies did not indicate increased damage to the genetic material (assessed from DNA strand breaks, incidence of chromosomal aberrations, micronuclei and sister chromatid exchanges) in cells exposed to HFEMF compared to sham-exposed and/or unexposed cells. Some investigations reported an increase in such damage in cells exposed to HFEMF.

Thus, although many studies have indicated that there is no relationship between radiofrequency radiation and adverse outcomes, such as genotoxicity, mutagenicity, etc., an epidemiological study has indicated that HFEMF from mobile phones may exhibit adverse effects on humans and the mechanism of the effects of HFEMF on living organisms remains uncertain. National Radiological Protectiopn Board (NRPB) published the advice on limiting exposure to electromagnetic fields from 0 to 300 GHz and summarized the exposure guidelines of International Commission on Non-Ionizing Radiation Protection (ICNIRP). In the guidelines, occupational and general public people are recommended to be restricted to 0.4 or 0.08 W/kg whole-body average SAR (specific absorption rate), respectively. Although we are exposed to HFEMF much less than ICNIRP guidelines, public anxiety about exposure to HFEMF still persists. To investigate these controversial issues, we examined bacterial mutagenicity and 6-thioguanine (6-TG)-resistant mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene following exposure to HFEMF. We have chosen 2.45 GHz electromagnetic fields because of the near frequency to the mobile phone type which is going to be used in next generation. In our previous study, we detected that an increase in micronucleus formation following exposure to continuous wave of 2.45 GHz at the SAR of from 50 to 200 W/kg might be connected to a rise in temperature by the exposure of HFEMF. Mutagenicity was determined by standard bacterial mutation assays that are commonly used for screening of chemicals and environmental contaminants. Mutation frequency was detected by counting 6-TG resistant colonies in Chinese hamster ovary (CHO)-K1 cells. This method also commonly used for genotoxicity assay. Simultaneously, the effect of heat on mutation frequency was examined with the rise in temperature during HFEMF exposure.

**MATERIALS AND METHODS**

**Bacterial strains**

Five routinely used Ames testing strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and Escherichia coli (WP2 uvrA) were used (Table 1). S. typhimurium were originally provided by Dr. B. N. Ames of the University of California, Berkeley while E. coli was originally provided by Japan Bioassay center, Kanagawa, Japan and these strains were provided by Dr. M. Ikehata of the Railway Technical Research Institute of Tokyo, Japan. Table 1 shows the strain genotypes.

Cells were grown on nutrient broth medium (Oxoid, Nutrient broth No. 2; Unipath, Hampshire, UK) at 37°C to the late-logarithmic growth phase. Cells were harvested and resuspended in phosphate buffer at a density of 1 × 10⁸ cells/ml. Aliquots (20.1 ml) of the cell suspension were placed into a special culture dish designed to allow exposure to an electromagnetic field, and were exposed to HFEMF for 30 min according to the protocol of pre-incubation method of Ames test.

Three chemical mutagens were used as positive control. Sodium azide and 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and 4-nitroquinoline-N-oxide (4-NQO) was purchased from Sigma-Aldrich Co. (St. Louis, MO). For E. coli WP2 uvrA, 0.01 μg/plate of AF-2 was used. For S. typhimurium TA1535, 0.5 μg/plate of sodium azide was used. For S. typhimurium TA98 and TA1537, 0.2 μg/plate of 4-NQO were used while 0.025 μg/plate of 4-NQO were used for S. typhimurium TA100.

**Mammalian cell line**

CHO-K1 cells were obtained from the Japanese Cancer Research Bank, Tokyo. Cells were maintained in Ham’s F-12 medium (Nikken, Kyoto, Japan) supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) at 37°C.
in 95% air and 5% CO₂. Aliquots (20.1 ml) of the cellular suspensions (concentration of 5 × 10⁵ cells/ml) were seeded on a section of a special culture dish designed to allow exposure to an electromagnetic field, and were exposed to HFEMF for 2 h.27

**Exposure system**

Details of the exposure system have been described previously.27 In brief, the exposure setup is based on the rectangular wave-guide. The electromagnetic wave is propagated along the waveguide with TE₁₀ mode. To generate standing waves in the wave-guide, one end of it is terminated with a short-circuiting plate. A frequency of 2.45 GHz is used for exposure to HFEMF in an acrylic CO₂ incubator, in which the atmosphere is humidified 95% air and 5% CO₂, that is installed into inner space of the core. A culture dish is placed on two slits that are bored on the wave-guide. Cells are exposed to the HFEMF through these slits. SAR distribution of the exposure setup was measured.

**Mutagenicity assay in bacteria**

For the detection of mutagenicity following exposure to HFEMF, the plate incubation method was used.28 Bacteria were inoculated and incubated for 1 h, in which most bacteria were sinking on the bottom of culture dishes. Bacteria were exposed to HFEMF for 30 min at SARs from 5 to 200 W/kg. Exposed cells were mixed with phosphate buffer and top agar, plated onto minimal glucose agar plates and incubated at 37°C in a conventional incubator for 48 h. Revertant colonies were then counted.

**HPRT assay in mammalian cells**

CHO-K1 cells were seeded at a concentration of 5 × 10⁵ cells/ml onto special culture plates before exposure and cultured for 12 h. Cells were exposed to HFEMF for 2 h at SARs from 5 to 200 W/kg. Exposed cells were washed with Phosphate Buffered Saline (PBS) three times and cultured for 24 h with new medium. The cells were re-plated in 75 cm² flasks and subcultured to subconfluence for 3 days. However, as a positive control or co-mutagenic treatment, bleomycin-treated cells were exposed to bleomycin (final concentration 10 µg/ml) for 1 h before HFEMF-exposure. After the exposure, cells were washed three times with PBS and were re-plated in 75 cm² flasks for subculture to subconfluence for 3 days. Cells (2 × 10⁵ cells) were then plated in 10 cm dishes containing medium with 15 µM of 6-TG. Ten days later, the cells were stained with Giemsa’s solution. For the determination of cloning efficiency, about 100 cells were seeded on 6 cm dishes after 3 days-subculturing and colonies were counted after 6 days. Mutation frequencies were calculated according to the formula of mean scored colonies/2 × 10⁵/mean scored colonies in cloning efficiency × 100.

**Heat treatment**

CHO-K1 cells for heat treatment were prepared as the same as for HFEMF exposure method. Cells were incubated for 2 h at 39, 41 and 44°C corresponding to the heat induction of about 50, 100 and 200 W/kg, respectively. Co-mutagenic treatment, such as heat and bleomycin, was also performed. HPRT gene assay was done the same way as the above-described method.

**Statistical analysis**

Statistical analysis of the data was conducted using ANOVA (analysis of variance) followed by Fisher’s PLSD test from the three independent experiments.

## RESULTS

### Mutagenicity of HFEMF

Mutagenicity of HFEMFs were tested using five different strains; _E.coli_ WP _uvrA_, _S.typhimurium_ TA98, TA100, TA1535 and TA1537. In all strains, there was no significant difference in the frequency of revertant colonies between sham control and HFEMF-exposed groups; although posi-

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### Table 1. Genotypes of the tester strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Amino acid marker</th>
<th>Mutation</th>
<th>Type of mutation</th>
<th>Main DNA target</th>
<th>Other relevant mutations</th>
<th>Plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA repair</td>
<td>Cell wall</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA98</td>
<td>his D 3052</td>
<td>Frameshift</td>
<td>GC</td>
<td><em>uvrB</em></td>
<td>rfa</td>
<td>pKM101</td>
</tr>
<tr>
<td>TA100</td>
<td>his G 46</td>
<td>Base pair substitution</td>
<td>GC</td>
<td><em>uvrB</em></td>
<td>rfa</td>
<td>pKM101</td>
</tr>
<tr>
<td>TA1535</td>
<td>his G 46</td>
<td>Base pair substitution</td>
<td>GC</td>
<td><em>uvrB</em></td>
<td>rfa</td>
<td>–</td>
</tr>
<tr>
<td>TA1537</td>
<td>his C 3076</td>
<td>Frameshift</td>
<td>GC</td>
<td><em>uvrB</em></td>
<td>rfa</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP2 <em>uvrA</em> trp E 56</td>
<td>Base pair substitution</td>
<td>AT</td>
<td><em>uvrA</em></td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>
tive controls showed statistically significant differences compared with sham exposure (Table 2). These results suggested that exposure to HFEMF ranging from 5 to 200 W/kg did not effect mutagenicity in these five experimental strains.

**Mutation frequency of the HPRT gene following exposure to HFEMF and heat treatment**

Mutation frequency of the HPRT gene by HFEMF was determined and the results are presented in Fig. 1. An effect of temperature on HPRT was also detected, due to the

### Table 2. Mutagenicity of HFEMFs by Ames test

<table>
<thead>
<tr>
<th>Strain</th>
<th>Revertant colonies/plate</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Sham</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td></td>
</tr>
<tr>
<td>TA98</td>
<td>30±5</td>
</tr>
<tr>
<td>TA100</td>
<td>111±28</td>
</tr>
<tr>
<td>TA1535</td>
<td>18±7</td>
</tr>
<tr>
<td>TA1537</td>
<td>32±5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>WP2 uvrA</td>
<td>40±11</td>
</tr>
</tbody>
</table>

\(^a\)Data are given as mean ± S.D. from the three independent experiments. Statistical analysis of the data in the sham control and experimental groups was conducted using ANOVA (analysis of variance) followed by Fisher’s PLSD test.

\(^b\)Chemical mutagen was used as positive control. For *S. typhimurium* TA 98, TA 100 and TA 1537, 4NQO was used. For *S. typhimurium* TA 1535, sodium azide was used. For *E. coli* WP2 uvrA, AF 2 was used.

\(*\)Statistically significant differences compared with sham control (p < 0.01).

![Mutation frequency of the HPRT gene following exposure to HFEMF](image.png)

**Fig. 1.** Mutation frequency of the HPRT gene following exposure to HFEMF. Columns represent the mean of mutation frequency calculated from 6-TG resistant colonies. Bars represent the standard deviation from at least three independent experiments. Statistical analysis of the data in the sham exposure and experimental groups was conducted using ANOVA (analysis of variance) followed by Fisher’s PLSD test. * Statistically significant differences compared with sham exposure (p < 0.05). # Statistically significant differences compared with bleomycin treatment alone (p < 0.01).
increase in temperature induced by HFEMF-exposure. The effects of 39, 41 and 44°C exposure were investigated and the combination of the effects of heat and bleomycin were determined simultaneously (Fig. 2). Statistically significant differences were conducted with comparing of HFEMF-exposed and sham exposed samples, and also comparing of HFEMF + bleomycin treatment and bleomycin treatment alone samples. Statistically significant differences were also conducted with comparing of heat treated samples and sham exposed samples, and also comparing of heat + bleomycin treatment and bleomycin treatment alone samples. There was no difference between HFEMF-exposure ranging from 5 to 100 W/kg and sham exposure using the HPRT mutation assay. Nevertheless HFEMF-exposure of 200W/kg elicited a statistically significant induction of HPRT mutants. In the heat experiment, an increase in temperature to 44°C, corresponding to the 200W/kg HFEMF-exposure, induced statistically significant mutation compared with sham exposure. However, exposure to 39 and 41°C did not exhibit any effect on the HPRT gene compared with the sham exposure control (Fig. 1). HFEMF exposure up to SAR 100 W/kg did not affect the mutation frequency. However, at 200 W/kg SAR the increase of mutation frequency was statistically significant compared with bleomycin treatment alone. In the experiment of combined treatment of heat and bleomycin, the mutation frequencies at 39°C were the same as sham controls. However, combined treatment of 41 or 44°C + bleomycin induced statistically significant mutation compared with bleomycin treatment alone.

**DISCUSSION**

In the current study, to determine the effect of HFEMF, we performed Ames test to detect the mutagenic activity of a mutagen and measured the mutation frequency of the HPRT gene. Using the Ames test, there was no effect of HFEMF. Mineta et al. concluded that bacterial mutation is not increased by 270 MHz radiofrequency radiation. We also observed no mutagenic effect of HFEMF using the bacterial mutation assay.

In this study, we determined the mutation frequency induced by HFEMF and heat. We treated cells with a radiomimetic agent, bleomycin, that elicits DNA damage by free radical mechanism(s), as a positive control. Bleomycin also functions as a positive control for the HPRT test. Cells treated with bleomycin in this assay exhibited mutation of the HPRT gene, compared with the sham exposure control (Fig. 1). HFEMF exposure up to SAR 100 W/kg did not affect the mutation frequency. However, at 200 W/kg SAR the increase of mutation frequency was statistically significant.
significant. In heat treatment, we demonstrated an increase of mutation frequency at 44°C, but not at 39 and 41°C (Fig. 2). We have previously demonstrated the relationship between increases in temperature and 2.45 GHz HFEMF exposure. Exposure to HFEMF at the 200 W/kg SAR corresponds to an increase in temperature of about 44°C. This suggests that increasing the temperature > 44°C may induce HPRT gene mutation. Although there have been few investigations concerning HPRT mutation and heat, high temperature may induce increased HPRT gene mutation, indicating that the mutational process in the presence of azaserine was influenced by temperature. Recently, Dewhirst et al. summarized the carcinogenic effects of hyperthermia considering the induction of heat by HFEMF exposure. Barnett et al. suggested that the frequency of HPRT mutants was increased ~3fold when Chinese hamster V79 cells were heated at 45°C for 10 min or at 43.5°C for 30 min.

The combined effects of HFEMF and bleomycin were also detected. The mutation frequency increased in a dose-dependent manner from SARs of 50 W/kg and higher (Fig. 1). We suggested that this was caused by the activation of bleomycin itself by the increase in temperature. This tendency was also detected with the combination of bleomycin and heat (Fig. 2). With heat treatment, it would appear that the mutation frequency is higher than treatment with HFEMF exposure. This may be a result of conditions in the dish. The increase of temperature in the special HFEMF exposure dish was heterogeneous, primarily in the middle of exposed area, whereas the heat treatment increased the temperature constantly at every part of the culture dish.

In the present investigation, we could not detect an increase of bacterial mutation after exposure to HFEMF even the temperature elevated by the exposure. Barnett et al. that no changes were observed in survival, in the incidence of mutants following irradiation with ultrasound intensities of 4.5 W/cm² at temperatures ranging from 37 degree to 43 degree C. They demonstrated that rise in temperature also might not affect bacterial mutations. Several studies also indicated that no increase in mutation was observed by heating or microwave radiation in bacteria. We also concluded that the increase of mutation frequency may be induced by the increase in temperature. The reason for the increase of mutation frequency may be the thermal effect.

During the past decade, mobile phone use has spread world-wide and a huge number of people are now exposed to radiofrequency radiation from mobile phones or base stations. Almost all investigations have indicated the absence of any relation between exposure to RF radiation and adverse health effects. However, there may still be some positive data. In this study, we could not demonstrate any effect of HFEMF exposure using the Ames test. We also could not demonstrate any mutation of the HPRT gene as a result of exposure to HFEMF alone, except for 200 W/kg. However, we were able to demonstrate a combined effect of HFEMF exposure and bleomycin. We concluded that the effects of HFEMF on HPRT gene mutation exposed to high SARs were increased by heat induced by the HFEMF exposure. However, experiments should still be performed to confirm the safety of high-frequency radiation.

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