Exposure to Strong Magnetic Fields at Power Frequency Potentiates X-ray-induced DNA Strand Breaks

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We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4°C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37°C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to 400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109–114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75–79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

INTRODUCTION

There has been considerable concern about possible health effects of extremely low-frequency magnetic field (ELFMF) produced by power lines and household electric appliances. A possible association between ELFMF exposure and cancer has been suggested, but not unequivocally demonstrated\(^1\)–\(^3\). Several studies have been reported concerning the effect of ELFMF on cellular DNA damage. Lai and Singh\(^4\) reported that acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25 and 0.5 mT) caused a dose-dependent increase in DNA strand breaks.
in brain cells using the comet assay. When HL-60 cells were exposed to 45 mT ELFMF, apoptosis, such as nuclear fragmentation and cleavage of intranucleosomal DNA to yield fragments, was induced\(^5\). On the other hand, there are several reports which demonstrate that ELFMF does not cause DNA damage, especially DNA strand breaks\(^6–8\). Part of this inconsistency in the results may be attributed to a difference in the flux density. ELFMF with a higher flux density should cause effects more clearly. Therefore, it is worth investigating whether exposures to high-density ELFMF induce cellular DNA damage. For the genetic effect of a static magnetic field (SMF), exposure to a strong SMF (6.34 T) for 24 h did not induce any significant microsatellite changes in both HCT116 (mismatch repair deficient) and HeLa S3 (mismatch repair proficient) cells\(^9\).

The densities of the magnetic flux in the present study ranged from 5 to 400 mT; the highest density was approximately more than one hundred-times higher than the densities used in other studies. To identify potentially genotoxic effects of ELFMF exposure, we examined whether exposure to ELFMF at different densities (5, 50 and 400 mT) (1) induced DNA strand breaks directly and (2) modified X-ray-induced DNA strand breaks in human glioma MO54 cells, by using the method of alkaline comet assay.

**MATERIALS AND METHODS**

**Cell culture**

MO54 cells, derived from a human malignant glioma, were kindly supplied by Dr. Rufus S. Day III (Cross Cancer Institute, Edmonton, AB, Canada). Cells were cultured in Dulbecco’s modified Eagle’s medium (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 10% fetal bovine serum (GIBCO, BRL) at 37°C in an atmosphere of 95% air and 5% CO₂. Exponentially, growing cells were used for the experiment.

**ELFMF exposure units, X-ray irradiation and survival assay**

Details of the exposure units for 400 mT, 50 mT and 5 mT ELFMF have been described elsewhere\(^10–12\). The frequency of ELFMF was 50 Hz for 400 mT and 60 Hz for 50 and 5 mT. The duration of ELFMF exposure was 30 min. For the control experiments, a conventional incubator in a separate room was used. The measured ELFMF in the conventional incubator was less than 0.5 μT. Static magnetic fields other than geomagnetism were undetectable at these exposure and non-exposure units (<0.1μT) and residual geomagnetism was less than 1μT. X-ray irradiation methods and the analysis of cell survival have been described previously\(^13\). X-ray irradiation was performed using a Hitachi MBR-1520 at 150 kVp, 20 mA with 0.5 mm Al and 0.1 mm Cu filters with a dose-rate of 0.98–1.02 Gy min\(^{-1}\). To inhibit repair of DNA strand breaks, the cells were exposed to ELFMF and/or X-rays at a temperature under 4°C or on ice. Part of these exposures was performed at 37°C. For a cell-survival assay, cells after the treatment were incubated at 37°C for 2–3 weeks, and then colonies were stained with crystal violet and scored. Five replicate 10 cm φ plates per experiment were used and the experiments were repeated at least three times.
Alkaline comet assay

To detect DNA damage (DNA strand breaks and alkali labile DNA adducts) at the single-cell level, an alkaline comet assay was performed. The comet assay has been described previously\textsuperscript{14,15}. We used a Trevigen Comet Assay system (Trevigen, Inc., Gaithersburg, MD). Cell samples were handled under dimmed light to prevent DNA damage from ultraviolet light. The cells after X-ray irradiation (5 Gy) and/or ELFMF for 30 min were suspended and harvested by centrifugation. Cell suspension (1 × 10\textsuperscript{5}/ml in 1× phosphate buffer saline (Ca\textsuperscript{++} and Mg\textsuperscript{++} free; PBS) on ice was mixed with low melting agarose at 42\textdegree C (cell suspension: agarose = 10 : 1). The cell suspension agarose mixture on a slide was kept in the dark at 4\textdegree C for fixation, and then immersed in a lysis solution at 4\textdegree C for 30 min. The lysis solution contained 2.5 M sodium chloride, 100 mM EDTA (pH 10), 10 mM Tris base, 1% sodium lauryl sarcosinate, and 0.01% Trioton X-100. The slide was placed in the alkali buffer for 40 min. The electrophoresis was performed as previously reported\textsuperscript{16}. In brief, a fresh solution of 0.03 mol/liter NaOH and 0.02 mol/liter EDTA was added to the chamber; electrophoresis was then con-

(a) 

(b) 

Fig. 1. Photographs of single-strand break DNA migration pattern of an MO54 cell. (a) control and (b) X-rays with 5 Gy.
ducted at 0.5 V/cm for 20 min. After fixation and drying, the agarose including cells was stained with SYBR Green.

DNA strand breaks were analyzed by using IDL5.0.3 (Adamnet, Tokyo, Japan). Figure 1 shows typical photographs of single cell DNA migration pattern. The tail moment was calculated from the following equation:

\[
\text{Tail moment} = \text{Differential between means} \times \% \text{ in Tail},
\]

in which “Differential between means” and “% in Tail” represent “the distance between a center of comet head and a center of comet tail” and “a ratio of tail DNA content and total DNA content”, respectively.

Statistics

For a statistical evaluation of the tail moment, tail length and % in tail, we used an analysis of variance (ANOVA) test for intergroup differences. When a significant \( F \) value was found (\( p < 0.05 \)), the Bonferroni/Dunn test was used for a multiple comparison.

RESULTS

In order to determine whether exposure to ELFMF affects X-ray-induced cytotoxicity, we examined the survival of MO54 cells after X-irradiation followed by ELFMF exposures at 5, 50, and 400 mT for 30 min. These exposures were performed at 37°C. Figure 2 shows the X-ray dose-survival curves of cells exposed to X-rays and the ELFMF. Cell survival did not change significantly between X-rays alone and X-rays followed by ELFMF.

Figure 3 shows the representative tail moment histograms of control MO54 cells and those exposed to ELFMF at 5, 50, and 400 mT. The treatments were done at 4°C. Most of the tail moment (more than 80%) showed a distribution of less than 5. Figure 4 shows the representative tail moment histograms of cells treated with X-rays alone (5 Gy) or X-rays followed by ELFMF at 5, 50 and 400 mT under a cold condition. Figure 5 shows the average DNA tail moment in the various treatment groups. No significant difference in the tail moment was observed between the control and ELFMF exposures. X-ray irradiation with 5 Gy increased the DNA single-strand breaks in the cells (\( P < 0.0001 \), between control and X-rays alone). The tail moment of cells irradiated with X-rays plus exposure to ELFMF at 50 and 400 mT increased significantly (\( P < 0.02 \) for 50 mT and \( P < 0.01 \) for 400 mT), as compared with that for X-rays alone. However, no significant difference was observed between X-rays plus 5 mT and X-rays alone. The tail length and % in tail of cells in these treatments are given in Table 1. The difference in % in tail was similar to the data for tail moment. The tail length of cells exposed to the ELFMF alone increased slightly. Both the tail length and % in tail increased significantly in cells exposed to X-rays followed by 400 mT ELFMF.

Figure 6 shows the average DNA tail moment of cells exposed to X-rays and/or 400 mT at 37°C. The tail moment in X-rays alone increased slightly compared with that in the control;
Fig. 2. Survival curves of MO54 cells after X-irradiation with or without post-ELFMF exposures for 30 min. The experiments were conducted at 37°C. The error bars represent the standard error.

Fig. 3. Representative tail moment histograms of MO54 cells. The exposure time to ELFMF was 30 min at 4°C. The number of comets scored was 65 for (a) control, 37 for (b) 5 mT, 30 for (c) 50 mT, and 51 for (d) 400 mT.
Fig. 4. Representative tail moment histograms of MO54 cells. X-ray dose was 5 Gy and exposure time to ELF-MF was 30 min at 4°C. The number of comets scored was 63 for (a) X-rays alone, 52 for (b) X-rays+5 mT, 29 for (c) X-rays+50 mT, and 49 for (d) X-rays+400 mT.

Fig. 5. DNA tail moment in the various treatment groups. MO54 cells were exposed to X-rays with 5 Gy and/or ELF-MF for 30 min at 4°C. The data are the average from three independent experiments. The error bars represent the standard error. The asterisks show the statistic significance between the treatment groups as follows: * P<0.0001, **P<0.02 and ***P<0.01.
Table 1. Tail length and % in Tail of MO54 cells exposed to X-rays and/or ELFMF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tail Length(^a)</th>
<th>% In Tail(^b)</th>
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<tbody>
<tr>
<td>control</td>
<td>54.56 ± 1.24</td>
<td>12.71 ± 0.58</td>
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<tr>
<td>5 mT</td>
<td>58.54 ± 1.28(^c)</td>
<td>12.25 ± 0.57</td>
</tr>
<tr>
<td>50 mT</td>
<td>59.82 ± 1.24(^*)</td>
<td>12.29 ± 0.73</td>
</tr>
<tr>
<td>400 mT</td>
<td>60.12 ± 1.29(^*)</td>
<td>12.34 ± 0.69</td>
</tr>
<tr>
<td>X-rays(^b)</td>
<td>91.91 ± 1.07</td>
<td>35.50 ± 0.75</td>
</tr>
<tr>
<td>X-rays(^b) + 5 mT</td>
<td>90.86 ± 1.41</td>
<td>35.07 ± 1.10</td>
</tr>
<tr>
<td>X-rays(^b) + 50 mT</td>
<td>92.45 ± 1.60</td>
<td>41.69 ± 1.31(^****)</td>
</tr>
<tr>
<td>X-rays(^b) + 400 mT</td>
<td>97.03 ± 1.15(^****)</td>
<td>40.59 ± 0.91(^****)</td>
</tr>
</tbody>
</table>

\(^a\) Asterisks show the statistical significance in comparison to control or X-rays alone as follows: *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

\(^b\) X-ray dose is 5 Gy.

Fig. 6. DNA tail moment in the various treatment groups. MO54 cells were exposed to X-rays with 5 Gy and/or 400 mT ELFMF for 30 min at 37°C. The data are the average from three independent experiments. The number of comets scored was at least 40 in each treatment group in a single experiment. The error bars represent the standard error.

However, no significant change was observed between X-rays alone and X-rays plus 400 mT. The tail length and % in tail in those treatments at 37°C were also similar to the data of tail moment (data not shown).

**DISCUSSION**

Our results show that acute exposure (30 min) to ELFMF at 5 to 400 mT did not increase the mean tail moment (Figs. 3 and 5) and the % in tail (Table 1). However, the tail length of
cells increased slightly upon exposure to ELFMF (Table 1). Lai and Singh\textsuperscript{4} reported that exposure to a 60 Hz magnetic field at 0.25 and 0.5 mT increased both the single and double DNA strand breaks in rat brain by using a comet assay system. They also reported that a treatment with melatonin and the spin-trap compound N-tert-butyl-α-phenylnitrone (PBN), as efficient radical scavengers, blocked the ELFMF-induced DNA strand breaks in rat brain cells\textsuperscript{17}, suggesting that free radicals may play a role in magnetic field-induced DNA damage. We previously observed that longer exposure (42 h) of mouse m5S cells to 400 mT ELFMF elevated the frequency of sister chromatid exchanges\textsuperscript{11}. However the two results cannot be compared, because the experimental conditions were very different. Previous reports\textsuperscript{18,19} have shown that ELFMF does not have sufficient quantum energy to break a DNA strand directly. Therefore, acute exposure to ELFMF, even at a high-density of 400 mT, may have no, or very little, activity to induce DNA strand breaks directly in cultured mammalian cells.

DNA damage induced by ionizing radiation, especially DNA strand breaks, can be clearly detected by the method of alkaline comet assay in \textit{in vitro} system\textsuperscript{16,20,21}. We could also detect X-ray-induced DNA strand breaks in single cells by this method (Figs. 1 and 5). In the present study, the average value of tail moment increased significantly during a combined treatment of X-rays followed by ELFMF exposure at 50 and 400 mT as compared with that of X-rays alone (Fig. 5), in which the exposures to X-rays and ELFMF were performed under a cold condition, and therefore rejoining of DNA strand breaks was inhibited. However, no increase was observed at 5 mT. Both tail length and % in tail increased significantly in cells exposed to X-rays followed by 400 mT ELFMF (Table 1). In our comet assay system, the value of the tail length increased with the dose from 0 up to 12 Gy\textsuperscript{16}. Our previous report showed that exposure to 400 mT ELFMF for 2 h immediately after X-ray irradiation enhanced the X-ray-induced mutations\textsuperscript{22}. Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during the DNA-synthesizing phase\textsuperscript{23}. Therefore, the present results suggest that exposure to the high density ELFMF, at more than 50 mT may potentiate X-ray-induced DNA strand breaks, especially in cells at the S phase.

In our previous report, long-term exposure (up to 6 weeks) to an ELFMF at 5 mT was found to increase X-ray-induced mutations in Chinese hamster ovary (CHO) cells\textsuperscript{24}. A similar result was also observed by Walleczek et al.\textsuperscript{25}, where exposure to a magnetic field (0.7 mT at 60 Hz) increased γ-ray-induced mutations in CHO cells. The exposure time of 30 min used in this study was much more shorter than that used in our previous study. Our results show that acute (30 min) post-exposure to the ELFMF might potentiate X-ray-induced DNA strand breaks. Although most of the X-ray-induced DNA strand breaks were rejoined during post-exposure to ELFMF at 37°C (Fig. 6), there is a possibility that longer exposure to ELFMF at a density of 5 mT can affect DNA damage repair, such as an induction of the DNA rejoining error, resulting in an increase in X-ray-induced mutations.

The density of environmental ELFMF from high-voltage power-lines would be several thousand times less than the ELF densities used in this study. Unless exposure to very low-density ELFMF causes DNA damage by unknown mechanisms, the possibility of carcinogenesis due to environmental ELFMF is very low, or unlikely.
ACKNOWLEDGEMENTS

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REFERENCES