CELLULAR AND MOLECULAR EFFECTS OF ELF ELECTROMAGNETIC FIELDS

Junji Miyakoshi

Department of Radiological Technology, School of Health Sciences, Faculty of Medicine, Hirosaki University, 66-1 Hon-cho, Hirosaki, 036-8564, Japan

Summary

Most of the published experimental results suggest that very low-density exposure to extremely low frequency (ELF) electromagnetic fields do not have a clearly demonstrated potential to cause genotoxic effects. We designed and manufactured several equipments for exposure of cells to high-density (5 to 400 mT) ELF electromagnetic fields. This paper reviews our studies on the effects of ELF electromagnetic fields with or without other factors, such as chemical agents or ionizing radiation.

Introduction

The increased use of electrical energy in modern society has subjected the general and working population to unprecedented levels of ELF electromagnetic fields. There has been speculation that ELF electromagnetic fields can act as promoters or co-promoters of cancer. Some epidemiological studies have revealed a positive association between exposure to ELF electromagnetic fields and the incidence of several types of cancer, particularly leukemia and brain tumors. However, other studies have failed to discern any association between ELF electromagnetic fields and the incidence of cancer. In *in vitro* studies, the existance of the effects of the electromagnetic fields at low flux densities has been contradictory among reports. We designed and manufactured several equipments for exposure of cells to high-density (5 to 400 mT) ELF electromagnetic fields [1ă 3]. This paper reviews our studies on the effects of ELF electromagnetic fields. Genotoxicity (micronucleus formation, chromosomal aberration, and mutation), gene expression and apoptosis by exposure to ELF electromagnetic fields were analysed.

Micronucleus formation and chromosomal aberration

To test the genotoxic effects of ELF electromagnetic fields, the induction of micronuclei by exposure to ELF electromagnetic fields and/or X-rays was investigated in cultured Chinese hamster ovary (CHO) cells, using the cytokinesis block method [4]. Micronuclei derived from acentric fragments or from whole chromosomes were evaluated by immunofluorescent staining using anti-kinetochore antibodies. No statistically significant difference in the frequency of micronuclei in CHO cells was observed between a sham exposure (no exposure to an ELF electromagnetic field) and a 24 h ELF electromagnetic field exposure. Exposure to an ELF electromagnetic field before or after X-ray irradiation did not affect the frequency of X-ray-induced micronuclei. However, the number of kinetochore-positive micronuclei was significantly increased in the cells subjected to X-ray irradiation followed by ELF electromagnetic field exposure, but not in the cells treated with ELF electromagnetic field before X-ray irradiation, compared with X-rays alone.

Mouse m5S cells were either untreated or pretreated with mitomycine C (MMC, 1 μM) for 1h or X-rays with 3Gy, and then exposed to ELF electromagnetic field at three different flux densities (5, 50 and 400mT) for 40 h [5]. Chromosomal aberrations were analysed in the first post-treatment metaphases. ELF electromagnetic field enhanced the formation of spontaneous and MMC- or X-ray-induced chromosomal aberrations, in a

flux-density-dependent manner. Statistically significant increases in the frequency of chromosomal aberrations were observed in cells exposed to 400 mT ELF electromagnetic field with respect to unexposed controls. The aberrations induced by ELF electromagnetic field were mostly chromatid-type, not chromosome-type. Flow cytometric and mitotic index analyses revealed that the S or G2 arrest following MMC or X-irradiation was more profound in ELF electromagnetic field-exposed cells than in unexposed cells.

Mutation

For the mutation induction, exposure to ELF electromagnetic field at 400 mT induced mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene of human melanoma MeWo cells [6ă 8]. The mutant frequency was enhanced both by increasing the exposure period and the induced current intensity. Mutations induced by X-rays were enhanced by the ELF electromagnetic field exposure. No significant increase in mutant frequency occurred when DNA replication was inhibited during ELF electromagnetic field exposure. Mutation induced by the ELF electromagnetic field increased during the DNA-synthesis phase in synchronously growing phase. DNA replication error is suspected of causing the mutations produced by ELF electromagnetic field exposure.

We investigated the effect of long-term exposure to 5 mT ELF electromagnetic field at 60 Hz on mutant frequency. CHO-K1 cells were exposed or sham-exposed to the 5 mT ELF electromagnetic field for up to 6 weeks with or without X-irradiation (3 Gy), and the mutant frequency of the HPRT gene was analyzed [9]. Long-term exposure to 5 mT ELF electromagnetic field did not increase mutations, suggesting a threshold for mutation induction greater than a magnetic density of 5 mT. However, enhancement of the X ray-induced mutation rate was observed after treatment with X-irradiation followed by long term exposure to 5 mT ELF electromagnetic field. These results suggest thtat exposre to more than 5 mT ELF electromagnetic field may promote X-ray-induced mutations.

Gene expression

For the gene expression, the effect of 5 mT ELF electromagnetic field on *c-myc* mRNA expression was examined in CHO cells. No significant difference in the *c-myc* expression of CHO cells was observed with the ELF electromagnetic field exposure, sham exposure and incubation in a conventional incubator [2]. Exposure of PC12-VG cells to 400 mT electromagnetic field enhanced the β-galactosidase gene expression stimulated by treatment of the cells with forskolin [10, 11]. The enhancing effect of the ELF electromagnetic field was inhibited by treatment of the cells with a specific inhibitor of PKC, calphostin C, as well as with the Ca^{2+} entry blockers, nifedipin and dantrolen.

Effect of ELF electromagnetic field at 50 mT on heat-induced expression of heat shock protein 70 (hsp-70) was examined in HL60RG cells [3]. No increase in hsp-70 production was observed in the cells after exposure to 50 mT ELF electromagnetic field alone. Simultaneous exposure to 50 mT ELF electromagnetic field in combination with mild heat at 42 and 40℃ suppressed heat-induced hsp-70 expression. This result suggests that exposure to 50 mT ELF electromagnetic field may act on a protection against the concomitant mild heat stress in HL60RG cells. We investigated the distribution and expression of growth associated protein-43 (GAP-43) in human glioma cells (MO54) after exposure to a magnetic field (60 Hz, 5 mT), with or without initial X-ray radiation (2 Gy), by using immunocytochemistry and the reverse transcription polymerase chain reaction (RT-PCR) [12]. GAP-43 was present in the cytoplasm, accumulating in the perinuclear area. An increase in GAP-43 expression was observed with a peak at 10 h at the mRNA level and at 12 h at the protein level, after exposure to the magnetic field. The increased level of GAP-43 protein returned to a normal level within 24 h of exposure to a 5 mT magnetic field.

Enhanced expression of neuron derived orphan receptor (NOR-1) gene was also observed by exposure of CHO-K1 cells to 400 mT ELF electromagnetic field, but not to the 5 mT field [13]. The enhanced expression, reaching the maximum at 6 h, was transient and reduced to the control level after exposure to 400 mT ELF electromagnetic field for 24 h. The NOR-1 expression induced by treatment with forskolin and TPA was further enhanced by the

simultaneous treatment with 400 mT ELF electromagnetic field, in which the maximum response was at 3 h.

Apoptosis

Cell cycle distribution, apoptosis, and the expression of related proteins (p21, Bax, and Bcl-2) were determined in MCF-7 cells following exposure to ELF electromagnetic field (5 mT) alone or in combination with X rays [14]. Exposure of MCF-7 cells to the ELF electromagnetic field for 4, 8 and 24 h had no effect on cell cycle distribution. Furthermore, the ELF electromagnetic field failed to affect cell growth arrest and p21 expression induced by X rays (4 Gy). Similarly, the ELF electromagnetic field did not induce apoptosis or the expression of Bax and Bcl-2, two proteins related to apoptosis. However, exposure of cells to the ELF electromagnetic field for 24 h after irradiation by X rays (12 Gy) significantly decreased apoptosis and Bax expression but increased Bcl-2 expression. These data suggest that exposure to the ELF electromagnetic field has no effects on the growth of MCF-7 cells, but it might transiently suppress X-ray-induced apoptosis through increasing the Bcl-2/Bax ratio.

Ku80-deficient cells (xrs5) and Ku80-proficient cells (CHO-K1) were exposed to ELF electromagnetic fields at 5mT. Cell survival, and the levels of the apoptosis-related genes p21, p53, phospho-p53 (Ser^{15}), caspase-3 and the anti-apoptosis gene bcl-2 were determined in xrs5 and CHO-K1 cells following exposure to ELF electromagnetic fields and X-rays [15]. Exposure of xrs5 and CHO-K1 cells to ELF electromagnetic fields had no effect on cell survival, cell cycle distribution and protein expression. Exposure of xrs5 cells to 60 Hz ELF electromagnetic fields for 5 h after irradiation significantly inhibited G1 cell cyle arrest induced by X-rays (1 Gy), resulting in elevated bcl-2 expression. A significant decrease in the induction of p53, phospho-p53, caspase-3 and p21 proteins was observed in xrs5 cells when irradiation by X-rays (8 Gy) was followed by exposure to 5 mT ELF magnetic fields. Exposure of xrs5 cells to the ELF electromagnetic fields for 10 h following irradiation significantly decreased X-ray-induced apoptosis from about 1.7% to 0.7%. However, this effect was not found in CHO-K1 cells within 24 h of irradiation by X-rays alone and by X-rays combined with ELF electromagnetic fields. Exposure of xrs5 cells to ELF electromagnetic fields following irradiation can transiently suppress apoptosis by decreasing the levels of caspase-3, p21, p53 and phospho-p53 and by increasing bcl-2 expression.

Conclusion

From most of in vitro studies, there maybe no or very little effect of ELF electromagnetic field at very low density, such as environmental level. The genotoxic effect of ELF electromagnetic field was not also observed at lower than 5 mT. However, some positive effects (Mutation, gene expression and apoptosis) were observed, when the ELF electromagnetic field was combined with other agents, such as chemical, heat or ionizing radiation. These results suggest that the exposure to ELF electromagnetic field may be modifying the cellular damage induced by the known other agents, chemical and radiation.

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