Estimation of Mutagenic Effects of Intermediate Frequency Magnetic Field using Mammalian Cells

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Abstract

Since the opportunities that people are exposed to intermediate frequency (IF) magnetic fields (MF) are increasing, the health risk assessment of IF-MF has now become important. Because there have been few studies about long-term exposure to IF-MF with high magnetic flux density, we have developed a new apparatus capable of IF-MF exposure up to 3.9 mT for *in vitro* study. In this study, we found out that IF-MF did not affect both cell growth and mutagenicity using the mammalian cell line CHO-K1 and its DNA repair deficient derivatives.

1. Introduction

In recent years, the opportunities that people are exposed to intermediate frequency (IF) magnetic fields (MF) have been increasing through staying close to such devices as IH cooker, inverter installed on electric train and so on, bringing about the public concerns for IF-MF. However, only a few studies on biological effects of IF-MF have been reported to date, even though there have been a lot of studies related to extremely low frequency (ELF)-MF and radiofrequency (RF)-electromagnetic fields (EMF), reflecting social anxiety about explosive spread of mobile telephony and so on. In such a situation, the health risk assessment of IF-MF has now become important. The World Health Organization (WHO) also recommended that more research to reveal scientific evidence for health risk of IF-MF should be conducted [1]. Since there have been few reports about exposure to IF-MF with high magnetic flux density, especially long term exposure, it is important to evaluate biological effect of long-term IF-MF exposure. Therefore, we have newly developed an exposure apparatus of IF-MF, which is designed for long-term exposure for *in vitro* study [2].

In this study, we investigated the effect of IF-MF on growth rate of various cell lines which were deficient in DNA repair using newly developed IF-MF exposure apparatus. Additionally, mutagenicity of IF-MF was assessed by *HPRT* mutation assay using the CHO-K1 cells, because mutagenicity, which is a part of initiation step of cancer development, is one of the important indices to evaluate the health risk of IF-MF.

2. Materials and Methods

2.1 Exposure Devices

Figure 1 shows the exposure apparatus of IF-MF. This exposure apparatus employed a meritt type coil as a basic structure [2], which can generate up to 3.9 mT homogeneous IF-MF (±5%), at the frequency of around 20 kHz in the cubic space of 150 mm on a side. A CO_2 incubator was installed in that homogeneous IF-MF space to be able to expose MF to cultured cells at 37±1ºC under 5% CO_2 conditions. We made two identical apparatus combined a CO_2 incubator and a merit coil were made and used with eneregizing (exposure) or without energizing (sham exposure: no-magnetic field) simultaneously. In this study, IF-MF exposure condition was set to be 21 kHz, 2, 3 and 3.9 mT for from 24 to 72 hrs.

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Fig. 1 The exposure apparatus for IF-MF exposure.

2.2 Cell Growth

Chinese hamster ovary cells, the CHO-K1 and its DNA repair deficient derivatives, xrs5 (*Ku86*), V3 (*DNA-PK*), irs1SF (*XRCC3*) and EM9 (*XRCC1*) were used in this study. The CHO-K1 was incubated in the Ham's F12 medium and other cell lines were the alpha minimum essential medium. To investigate the growth rates, 100 µl aliquot of pre-cultured these cell lines (10^4 cells/ml) was poured into each well of 96-well plates and incubated for 24 hrs prior to the exposure period, then the plates were exposed to sham or IF-MF (2 or 3.9 mT). After 0, 24, 48 and 72 hrs, 10 µl of the WST-1 premix solution (TaKaRa Bio Inc.) was added to 4 well each and then put back to the apparatus. The WST-1 method is colorimetric assay based on cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. Absorbance at 450 and 630 (for reference) nm was measured using microplate reader (Bio-Rad laboratories, Inc.) after incubation for 3 hrs.

2.3 Mutation Assay

In this study, *HPRT* mutation assay was done using the CHO-K1 cell line. The CHO-K1 cells (1.25×10^5 cells) in 60 mm culture plate were pre-cultured for 24hrs prior to the exposure period, and then exposed to sham or IF-MF (2, 3 or 3.9 mT) for 24hrs. As a positive control, cells were added to culture medium including 5 µg/ml of methyl methanesulfonate (MMS) and incubated for 24 hrs. After exposure, medium was changed to new medium and cells were incubated another 24 hrs. Exposed cells were subcultured once a several days for 5 days to fix mutation. After 5 days, these cells were subcultured in the Ham's F12 medium for cloning efficiency and the medium containing 6-thioguanine (TG) for 6-TG resistant mutant detection. Colonies were counted after 6 and 10 days for survival cells and 6-TG resistant cells, respectively. Based on these data, mutation frequency was calculated as 6-TG resistant cells per 10^6 survival cells.

3. Results and Discussion

3.1 Cell Growth

Figure 2 shows the results of cell growth using the WST-1 method in the CHO-K1 cell lines and its DNA repair deficient derivatives exposed to 2 mT IF-MF. Comparing growth rate between sham and IF-MF exposures, there was no statistically significant difference in cell growth of any of the cell lines used in this study for up to 72 hrs-exposure. For 3.9 mT IF-MF, no significant differences were also observed (Data not shown). These results suggested IF-MF exposure does not affect cell growth regardless of lack of the ability of DNA repair, although some studies relating static magnetic field or ELF-MF suggested some effects on DNA repair deficient organisms or cells [3, 4]. The cell lines used in this study were deficient in genes related to repair of single strand break or double strand break such as non-homologous end-joining and homologous recombination. Therefore, these results suggested that IF-MF did not affect the repair pathway that was involved these genes at least.

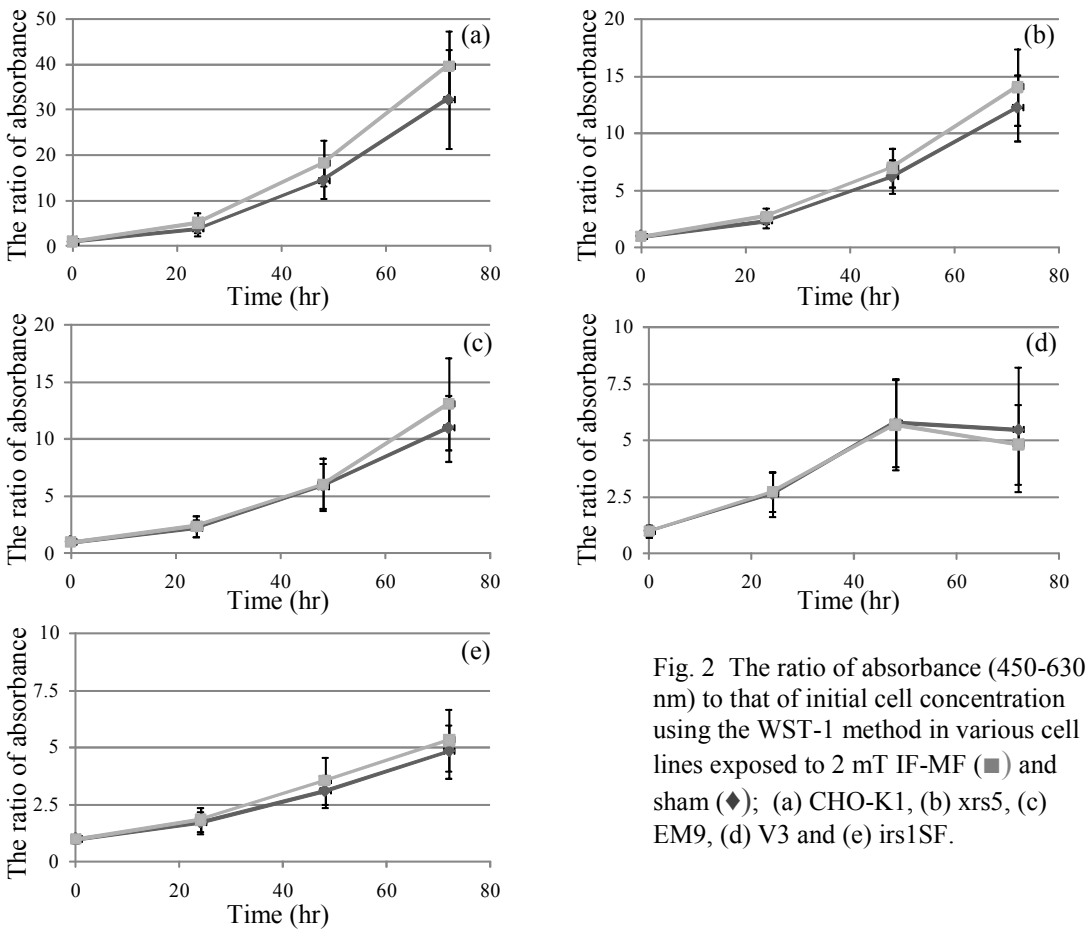

Fig. 2 The ratio of absorbance (450-630 nm) to that of initial cell concentration using the WST-1 method in various cell lines exposed to 2 mT IF-MF (■) and sham (♦); (a) CHO-K1, (b) xrs5, (c) EM9, (d) V3 and (e) irs1SF.

3.2 Mutation Assay

Figure 3 shows the ratio of *HPRT* mutation frequencies in the CHO-K1 cell line exposed to 2 mT IF-MF to sham exposure. As the result of mutation assay, there were no significant differences between sham and 2 mT IF-MF exposures. In contrast, MMS (5 µg/ml) as a positive control significantly induced 6-TG resistant mutant. For 3 or 3.9 mT of IF-MF, no statistically significant differences were observed between sham and IF-MF exposures. Previous studies about IF-MF exposures under both short-term/high magnetic flux density (23kHz, 2 hrs, 6.05 mT for mammalian cells) [5] and long-term/low magnetic flux density (2, 20 or 60 kHz, 48hrs, 1.1 mT for bacterial cells) [6] conditions showed that IF-MF did not have mutagenicity. The results in this study indicated that IF-MF exposure under long-term/high magnetic flux density condition did not cause mutation also.

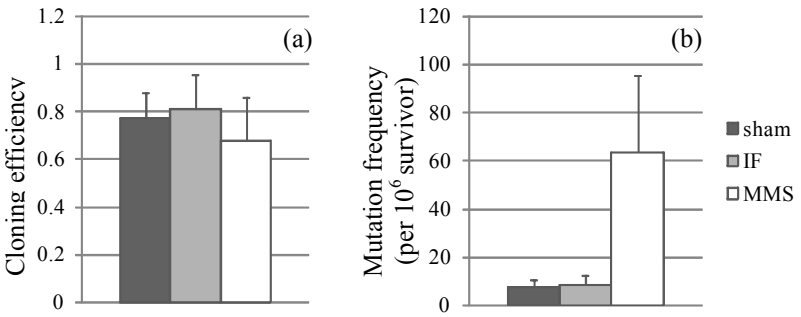

Fig. 3 The result of mutation assay in the CHO-K1 exposed to 2 mT IF-MF; (a) cloning efficiency, (b) mutation frequency.

The magnetic flux density of IF-MF in this study was more than 100 times reference level of international commission of non-ionizing radiation protection (ICNIRP) [7]. Since the magnetic flux density, which was exposed in the general environment, is lower than that exposed in this study, it is indicated that IF-MF generated in the general environment did not have the effect on cell growth and mutagenicity.

4. Conclusion

In this study, long-term IF-MF exposure with high magnetic flux density was conducted using a newly developed exposure apparatus for *in vitro* to investigate biological effects of the IF-MF. The effect on cell growth and mutagenicity of the IF-MF (21 kHz, up to 3.9 mT) was evaluated using the mammalian cell lines CHO-K1 and its DNA repair deficient derivatives. The results indicated that there were no significant differences in cell growth of any cell lines (CHO-K1, xrs5, EM9, V3 and irs1SF) between sham and IF-MF (2 or 3.9 mT) exposures for 72 hrs, suggesting that IF-MF did not affect cell growth regardless of lack of DNA repair ability. Moreover, *HPRT* mutation assay using the CHO-K1 cell line indicated that 2, 3 and 3.9 mT IF-MF exposure for 24 hrs did not have mutagenicity. These results suggested that the IF-MF to which general public was exposed through IH cooktop, electric railway environment and so on would not have mutagenicity and cytotoxicity.

5. Acknowledgments

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