Effects of exposure to intermediate frequency magnetic fields on gene expression of estrogen-regulated gene in MCF-7 cells

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Abstract

To evaluate biological effects of intermediate frequency magnetic fields (IF-MF), estrogen-regulated gene expression under magnetic fields were studied. Genetically modified MCF-7 cells that transformed with ERE-luc fusion gene was used. Cells which endogenous estrogen depleted by estrogen free media are exposed to 21 kHz IF-MF for 24 hr. Then, luciferase activity was measured as estrogen-regulated gene expression.

In this study, we have observed no significant difference in luciferase activity between exposed and sham-exposed cells by exposure to up to 3.9mT, 21 kHz IF-MF for 24hr. These results suggest that IF-MF is unlikely to affect directly on estrogen-regulated gene expression.

1. Introduction

Recently, IH hobs are becoming popular and production of IH hobs is over a million units per year in Japan. The IH hobs are very effective and safe to cook because of using IF-MF, not flame. On the other hand, the fact remains that people concern about health risk of IF-MF because assessment of health risk is not enough to date.

To respond this, the World Health Organization (WHO) is conducting the International EMF Project to evaluate possible health risk by exposure to electromagnetic fields (EMF). WHO already published two Environmental Health Criteria monographs for static fields (No. 232, in 2006) [1] and time-varying (<100 kHz) extremely low frequency fields (No. 238, in 2007)[2]. However, health risk of IF-MF is inconclusive because there is few researches to date, thus further research in frequencies between 300 Hz to 100 kHz are recommended by WHO.

To evaluate a possible health risk of IF-MF, it is important to investigate various biological effects of exposure to IF-MFs. In case of using IH cooking hob, effect on the pregnant woman and the fetus should be carefully investigated in addition to evaluate its carcinogenic and toxic effect. In this study, estrogen-regulated gene expression under IF-MFS was studied in order to evaluate the effect on women who are considered as a main user of the IH hobs.

2. Materials and Methods

Genetically modified MCF-7 cells (human breast adenocarcinoma cell line) that were transfected with the ERE-Trans Lucent Reporter Vector (Panomics) using Lipofectamine were used in this study. As the result of transfection, EREs-luc fusion gene (Estrogen Response Elements with luciferase gene) was translocated on genome of the cells. Thus, estrogen induces expression of the luciferase in these cells.

For IF-MFs exposure, an original exposure system that is capable of generating 21 kHz, IF-MF up to 3.9mT (144 times higher than reference level for public in the ICNIRP guideline published 2010) within exposure space (150mm×150mm×150mm) within ± 5% deviation was used [3].

Pre-cultured cells are re-inoculated in estradiol free media (phenol red free MEM media with insulin and activated charcoal treated FBS) for three days to deprive cellular estradiol. After this treatment, cells were harvested and re-suspend in estradiol free media. Then, cell aliquot was divided three 6-well plates. One is for IF-MF exposure, second group is for sham exposure and third group for control. In each plate, three wells contained 10^{-11} M estradiol. For exposure period, exposure group incubated in exposure device at 0, 2, 3 and 3.9mT with 21 kHz IF-MF, respectively, while sham exposure group incubated in identical exposure device

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without power unit. Control group incubated in a conventional CO_2 incubator.

After exposure to IF-MF for 24 hrs, cells were harvested and rough total protein solution was collected using cell culture lysis reagent (Promega Co., U.S.A.) on ice. The extract was directly used for chemiluminescence assay using Luciferase assay kit (Promega Co., U.S.A) and for quantification of total protein concentration by Lowry method using Bio-Rad DC protein Assay Kit (Bio-Rad, U.S.A.). Measurement of chemiluminescence was performed by manufacturer's protocol using chemiluminescence meter (Lumat LB 9507, Berthold Technologies, U.S.A).

3. Results and Discussion

Because of its high sensitivity and uncertainty, there is large deviation of measurement data in chemiluminescence over experiments. Therefore, analysis of the data was performed in each experiment with three wells per treatment, not inter experiments. Fig. 1 shows a typical result of an experiment of 0mT IF exposure (sham exposure), sham exposure and an incubator control. There is no significant difference between sham and sham with/without 10^{-11}M estradiol. This indicates both identical exposure device provides almost same cultivate environment for MCF-7 cells. We performed two to four independent tests in sham-sham, 2mT-sham, 3mT-sham and 3.9mT-sham condition. Fig. 2 shows a typical result of an experiment of 2mT-sham and an incubator control. It is almost similar to sham-sham experiment and there is no significant difference among an incubator control, sham and 2mT with/without 10^{-11}M estradiol. In 3mT-sham and 3.9mT-sham experiments, no significant differences were also observed (Data not shown). These results indicate that exposure to 21 kHz, up to 3.9mT IF-MF for 24 hrs did not have potential to induce estrogen regulated gene expression on the estimation of our reporter gene assay.

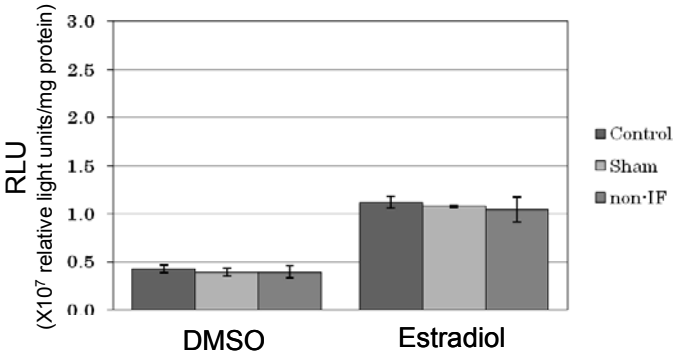

Fig. 1 Result of luciferase assay by IF (0mT:sham) and sham exposure.

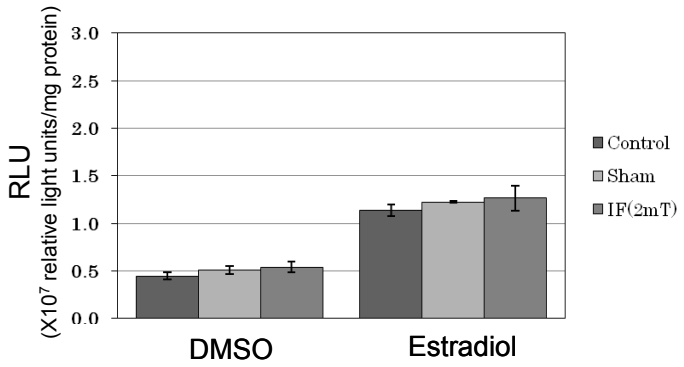

Fig. 2 Result of luciferase assay by IF (2mT) and sham exposure.

4. Conclusion

To evaluate biological effects of intermediate frequency magnetic fields (IF-MF), estrogen-regulated gene expression under magnetic fields were studied. From the results presented here, we have not found any significant difference in luciferase activity between exposed and sham-exposed cells by exposure from 2.0 to 3.9 mT, 21 kHz IF-MF at least for 24hr. To evaluate the effect of IF-MF on the pregnant woman and the fetus in detail, we will investigate the effect of longer period of exposure to IF-MF on the expression of estrogen-regulated gene in MCF-7 cells in further study.

5. Acknowledgement

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6. Reference

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