Effects of long-term exposure to 60 GHz millimeter-wave on genotoxicities in cells derived from human eye

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Abstract

Two kinds of cells (HCE-T and SRA) derived from human eye were exposed to a millimeter-wave for 24 hours. The micronucleus (MN) frequency of cells treated with bleomycin for 1 hour was high enough as a positive control. However there is no statistically significant increase in the MN frequencies of the cells exposed to 60 GHz millimeter-wave at 1 mW/cm^2 compared with sham-exposed controls and incubator controls. The comet assay to detect DNA strand breaks also showed that the exposure of the millimeter-wave for 24 hours did not have statistically significance. These results indicate that the exposure to the millimeter-wave would be considered to have no effect on the genotoxicity to the human eye cells.

1. Introduction

Since the rapid introduction of wireless device has increased the use of millimeter-wave (30-300 GHz) technologies, public concern about possible adverse effect on human health has been discussed. Previously it is reported that wide-band millimeter-wave exposure at 53-78 GHz significantly inhibited the proliferation of human skin melanoma cells [1]. Then it is necessary to evaluate the influence on human body by the low-level exposure of millimeter-wave. As the energy of millimeter-wave is absorbed by the body surface, the skin and the eye are the main target for discussion [2]. To investigate the athermal effects of millimeter-wave, we manufactured a device which can expose 60 GHz millimeter-wave to cells. In this study, we assessed the frequency of micronucleus (MN) formation and comet assay on the cells derived from human eye exposed to 60 GHz millimeter-wave.

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2. Materials and Methods

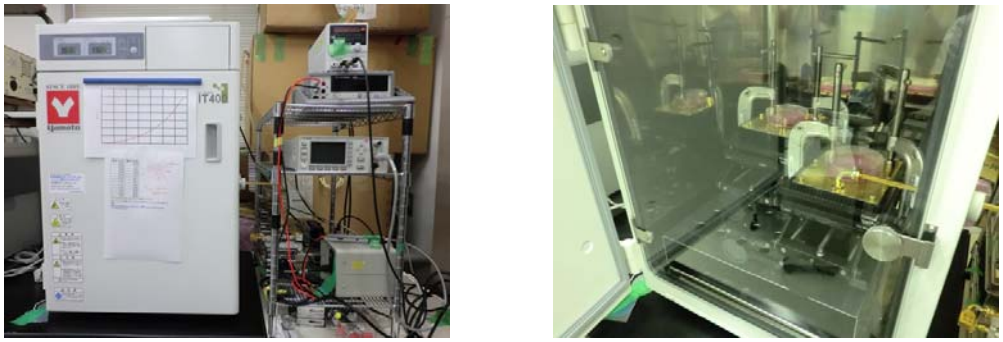
We used a specially designed exposure apparatus which emit 60 GHz millimeter-wave. The system was developed by researchers from Tokyo Metropolitan University (Figures 1 (a), (b)). The cavity in the exposure system was maintained under controlled conditions similar to those in an incubator, i.e., an atmosphere of 95% air and 5% CO_2 at a relative humidity of >95% and a temperature of 37°C. The exposure apparatus was based on the disc-shaped post-wall waveguide. A culture dish of 100 mm in diameter was put on the top of apparatus. Sixty GHz electromagnetic field, emitted from the fine slots opened on the top of apparatus, coupled with culture medium with adherent cells at the bottom of the culture dish. Spatially averaged power density was set for 1mW/cm^2 at the bottom of culture dish. Temperature elevation was suppressed less than 0.1ºC for that condition. HCE-T derived from human corneal epithelial cells were maintained in medium supplemented with 5% fetal bovine serum (FBS), insulin at a final concentration of 5 μg/ml and human epidermal growth factor at a final concentration of 10 ng/ml. SRA derived from human lens epithelial cells were maintained in medium supplemented with 20% FBS. At 24 hours after of millimeter-wave exposure, the cells were collected. For positive controls, the cells were treated with 10 μg/ml bleomycin for 1 h.

The methodology of MN frequency has been described previously [3]. Briefly, after millimeter-wave exposure or bleomycin treatment, the cells were cultured in medium supplemented with 3 μg/ml cytochalasin B in a conventional incubator for 24 h, centrifuged onto slides using a Cytospin centrifuge at 100 g for 5 min, fixed with cold 80 % ethanol for 30 min, and stained with 0.2 μg/ml propidium iodide. A total of 1000 binucleated cells and frequency of micronucleus formation were counted according to the criteria described previously using fluorescence microscope. Three independent tests were performed.

The comet assay was performed to detect DNA strand breaks at the single-cell level, and the methodology of comet assay has been described previously [3]. Briefly, cells were exposed to millimeter-wave radiation for 24 h, collected by trypsinaization and centrifugation immediately after exposure, and mixed with low melting point agarose to prepare a cell suspension in 0.1 % agarose/phosphate buffered saline (PBS). After gelation of the agarose, the cells were lysed, and the resulting DNA samples were electrophoresed at 1 V/cm for 30 min in a 0.3 M NaOH and 1 mM ethylenediamine-N, N, N',N'-tetraacetic acidsolution for the alkaline comet assay, which detects single strand breaks. After the DNA was stained with SYBR Green I, immunofluorescence images were captured using a fluorescence microscope. DNA strand breaks were analysed using Comet software. At least 100 comets from each gel were analysed, and three independent experiments were performed. Tail length indicates the pixel length of the comet tail. Tail percent indicates the percentage of tail content relative to comet content. Tail moment was calculated as follows: Tail moment=(the distance between the centre of the comet head and the centre of the comet tail) × (Tail percent)/100.

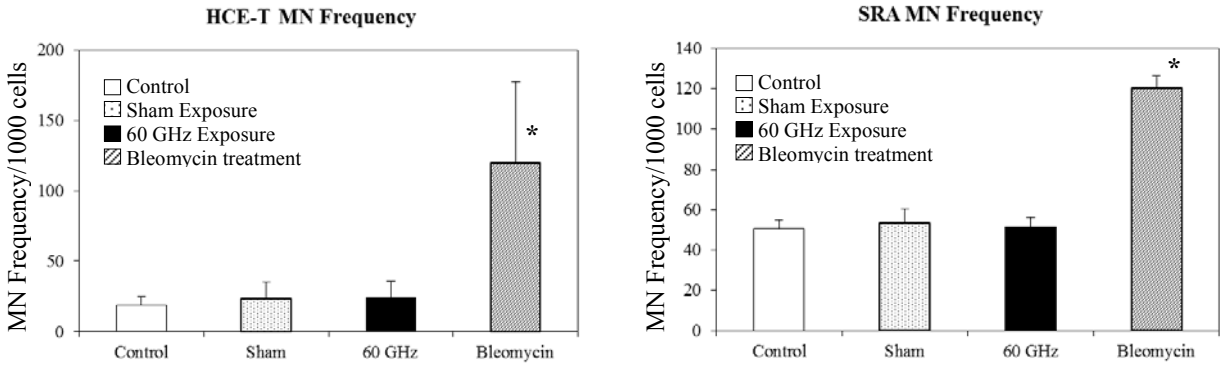
3. Results and Discussion

The MN frequency of HCE-T and SRA are shown in Figures 2 (a) and (b), respectively. Both MN frequency of HCE-T and SRA were increasing with the bleomycin treatment significantly. However, no significant difference in both MN frequencies was observed between incubator control, sham-exposure and the millimeter-wave exposures. The results from these experiments suggest that 24-hour exposure to a 60 GHz millimeter-wave might have no significant effect on MN frequency on HCE-T and SRA.



(a) (b)

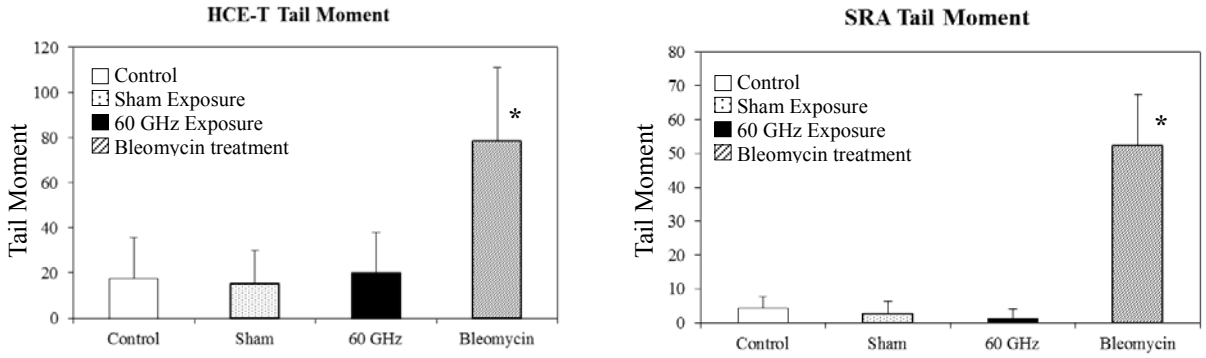
Figure 1. The built-in incubator for 60 GHz exposure system (a) and the inside view of the incubator (b).



(a) (b)

Figure 2. The micronucleus frequency exposed to millimeter-wave of 60 GHz for 24 hours on HCE-T (a) and SRA (b). For positive control, treatments of bleomycin (10μg/ml) were used. Data are presented as the mean ± SD from three independent experiments. Asterisks indicate $p < 0.05$.

The tail moment of HCE-T and SRA are shown in Figures 3 (a) and (b), respectively. The tail moment indicates the degree of genotoxicity on DNA. Both tail moment of HCE-T and SRA were increasing with the bleomycin treatment significantly. However, no significant difference in both tail moment was observed between incubator control, sham-exposure and the millimeter-wave exposures. The results from these experiments suggest that 24-hour exposure to a 60 GHz millimeter-wave might have no significant effect on comet assay on HCE-T and SRA.



(a) (b)

Figure 3. Values of the comet parameter of tail moment exposed to millimeter-wave of 60 GHz for 24 hours on HCE-T (a) and SRA (b). For positive control, treatments of bleomycin (10μg/ml) were used. Data are presented as the mean ± SD from three independent experiments. Asterisks indicate $p < 0.05$.

4. Conclusions

The data observed in the present study suggest that the exposure to a millimeter-wave of 60 GHz for 24 hours might have no significant effect of MN frequency and comet assay on HCE-T and SRA. In conclusions, we do not consider that the exposure of millimeter-wave of 60 GHz could contribute to some adverse effects on genotoxicity in cultured human eye cells under our experimental conditions. However the possibility of the response to stress is not clear and further studies are required.

5. Acknowledgment

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6. References

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