Effects of Ultrahigh-Frequency Electromagnetic Field at 28 GHz on Micronucleus Formation in Human Cell Lines

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Abstract – The relationship between exposure to electromagnetic fields (EMF) and health risks is of increasing interest. Although general concern regarding the potential hazards of EMF exposure have led to many epidemiological investigations, the effects of EMF exposure on human cell lines remain controversial. In this study, we developed a new exposure system to generate an ultrahigh-frequency (UHF) EMF of 28 GHz and confirmed the device retains a normal culture environment without any artifacts by cell proliferation tests. We then investigated the effects of the UHF EMF by micronucleus (MN) formation test, which is an internationally recognized method to evaluate safety. Our results showed that exposure to UHF at 28 GHz and a power density of 0.5 mW/cm^2 to 2.5 mW/cm^2 for 24 h does not have a significant effect on MN formation in CCD32Sk, XP2SA, XP2OSSV, and AT2KY cells.

1. Introduction

Fifth-generation wireless technologies for digital cellular communication networks and ultrahigh-speed wireless local area network systems are expected to spread globally in the near future, although clear data on the effects of ultrahigh frequency (UHF) on the human body have yet to be evaluated. The introduction of mobile telecommunication devices is a public concern with potential health risks associated with RF radiation emitted when using these devices and the base station antennas [1, 2]. The International Agency for Research on Cancer classifies RF in group 2B, which is "possibly carcinogenic to humans" [3, 4].

Many studies have discussed the effects of high frequencies on human health [5–10]. A recent study reported that exposure to UHF at 834 MHz affected erythrocytes in rat offspring [11]. Because the relationship between exposure to electromagnetic fields (EMF) and human health is very important, research on the safety to the human body is urgently required. In this study, we used human cell lines and assessed the frequency of micronucleus (MN) formation, which is a widely recognized method for analyzing DNA breaks [12–14]. To investigate the nonthermal effects of UHF radiation, we developed a device to expose human cell lines to UHF of 28 GHz EMF and assessed the frequency of MN formation.

Manuscript received 12 July 2020.

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2. Materials and Methods

We used a specially designed exposure apparatus, which uses a transmitting antenna of UHF radio wave, as shown in Figure 1. A uniform UHF of 28 GHz is emitted from a horn antenna at the top of the applicator. It penetrates the culture medium and cells adhering to the bottom of the culture dishes placed on the cooling water jacket. The inside of the incubator, which contained the exposure system, was maintained under controlled conditions in an atmosphere of 95% air and 5% CO_2 at a relative humidity of >95% and a temperature of 37°C.

Prior to the exposure experiment, we conducted a study on basic cell kinetics. We measured the cell proliferation test, colony-forming ability test, and cell cycle distribution by using HeLa cells and WI38VA13 cells. As a result, no differences were observed between the conventional normal incubator and the incubator with built-in the exposure apparatus. It was confirmed that our newly developed UHF exposure apparatus of 28 GHz retains the normal culture environment without any artifacts.

A continuous UHF signal of 28 GHz was produced by a signal generator (N5173B; Keysight Technologies, Santa Rosa, CA) through a power amplifier (AMP6034-40; Exodus Advanced Communications, Las Vegas, NV). The dosimetry of the RF field was performed by using both numerical and experimental approaches. A model, which was created from the dimensions of the exposure equipment used in this experiment, simulated the power density of the cell exposure calculated from the input level of the RF. The fundamental mode of 20 dBm was inputted from the upper square waveguide in this device. Five dishes with a diameter of 35 mm containing culture medium (real component of relative permittivity 36.6 and imaginary component of relative permittivity 31) were placed at the bottom of the device, and absorber sheets with a reflectance of –20 dB were placed on all boundaries. The time domain solver of CST Studio Suite 2019 (AET, Kawasaki, Kanagawa, Japan) was used in this simulation. The induced current density had a distribution in which the strength was repeated circularly from the center of the dishes. The maximum value was 80 A/m^2 at the edge of the dishes. The induced current density between the culture dishes was approximately

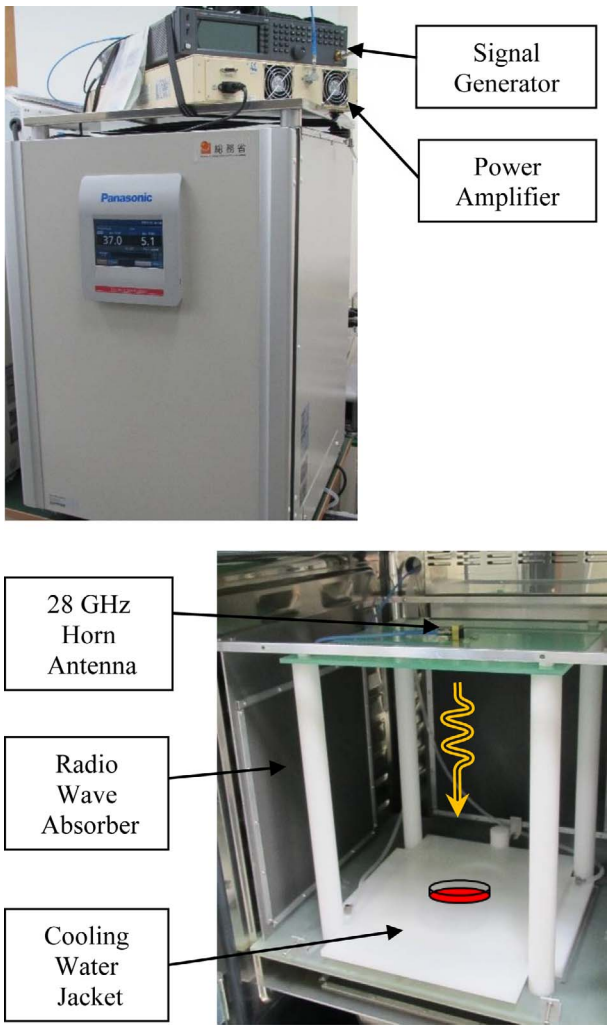

Figure 1. Newly developed exposure system that generates UHF of 28 GHz EMF.

uniform [15]. To measure the power level of UHF with a 28 GHz signal, a power sensor was connected to the probe antenna at the bottom of the device, where the culture dishes were placed. The measurement range was a 120 mm^2 at 10 mm intervals. The variation in the power density was within 6%. These results agreed fairly well with an exposure intensity of up to 2.5 mW/cm^2 at the bottom of the medium, where the cells were located.

The temperature of the medium in the culture dishes was maintained at 37°C by using a constant temperature water bath (SA-100; Sansyo, Tokyo, Japan). The temperature change of the exposure system was monitored during the experiment. It ranged within 37.0°C ± 0.2°C when the cooling device was operated continuously with an exposure level of 2.5 mW/cm^2 for 24 h.

The MN formation test was performed after exposure to the UHF of 28 GHz EMF at 0.5 mW/cm^2, 1.0 mW/cm^2, and 2.5 mW/cm^2 for 24 h. An exposure at 0.5 mW/cm^2 is approximately half the power density of the reference level for general public exposure for RF fields at 10 GHz to 300 GHz stated in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines [16]. We exposed samples at up to 2.5 mW/cm^2, which is more than twice the power density of the reference level. In this study, we used CCD32Sk (normal skin of a newborn infant), XP2SA, XP2OSSV (hereditary disease with xeroderma pigmentosum), and AT2KY (hereditary disease with ataxia telangiectasia) cells. Cells were exposed to UV at 1.0 J/m^2 or x-ray at 1.0 Gy, or a bleomycin treatment (10 $\mu g/mL$) for 1 h as positive controls. A total of 500 binucleated cells were counted, and the frequency of MN formation was determined by using a fluorescence microscope (AX-70; Olympus, Tokyo, Japan) according to the criteria described previously [17]. In detail, cells were counted as having MN formation when they contained at least one MN. The procedure was performed in a double-blind manner following the method described in [18]. Statistical analysis of the data was carried out by analysis of variance, followed by Dunnett's test with IBM SPSS Statistics Version 22 (SPSS Inc., Chicago, IL) from three independent experiments.

3. Results and Discussion

In this study, cells derived from healthy humans, UV-sensitive cells (XP), and radiation-sensitive cells (AT) derived from genetic diseases were used. The XP cells are deficient in the UV-induced thymine dimer and 6–4 product repair [19–21], and AT cells are defective in repair of DNA damage, including DNA strand breaks [22–24]. The MN formation was used as an index to search for the effects of radio waves on carcinogenicity. The frequencies of MN formation in CCD32Sk, XP2SA, XP2OSSV, and AT2KY cells are shown in Figure 2. Although we gradually increased the exposure level of EMF, it was unnecessary, as the results suggest that 24 h exposure to UHF of 28 GHz at 2.5 mW/cm^2 did not have a significant effect on MN frequency in CCD32Sk cells and XP2OSSV cells. The MN frequency increased significantly following the bleomycin treatment, whereas a significant difference was not observed between incubator control, sham exposure, and the UHF of 28 GHz EMF–exposed cells. These results indicate that the UHF of 28 GHz radio wave exposures in our experimental conditions did not affect the repair process of cell damage caused by UV rays or radiation.

4. Conclusions

The data observed in the present study suggest that the exposure to UHF of 28 GHz at 0.5 mW/cm^2 for 24 h does not have a significant effect on MN formation in any of the cells derived from a physically unimpaired person (CCD32Sk) or in the cells derived from a patient with a genetic disease (XP2SA, XP2OSSV, and AT2KY). We conclude that exposure to UHF at 28

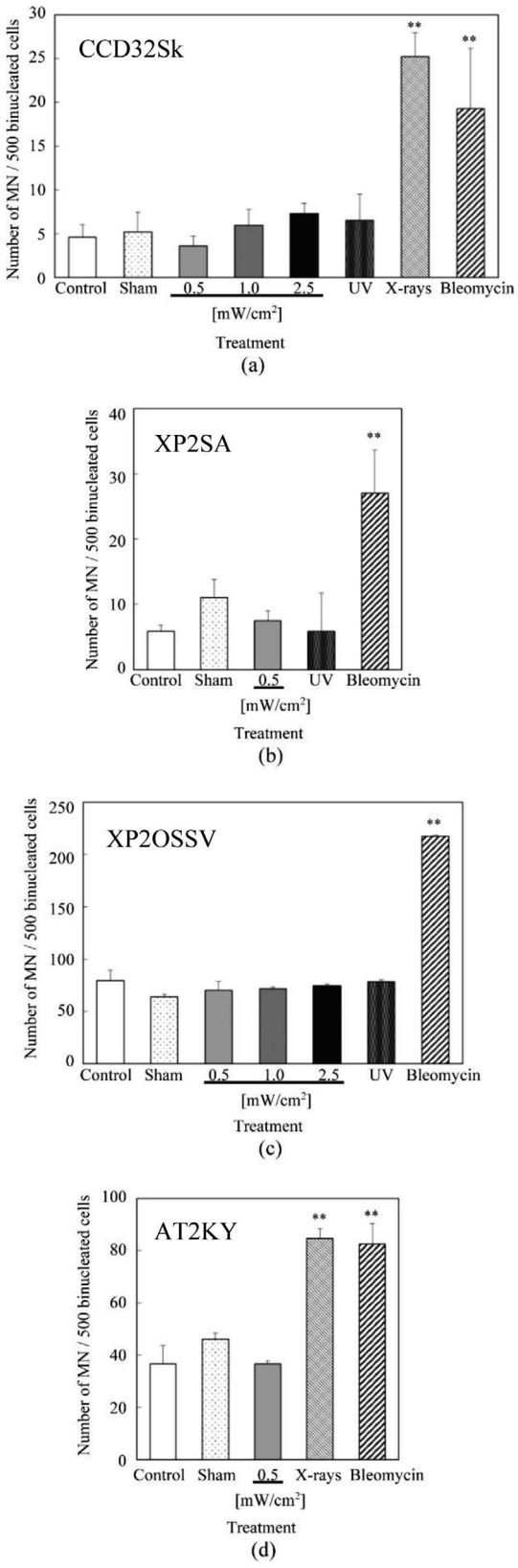

Figure 2. The MN frequency in (a) CCD32Sk, (b) XP2SA, (c) XP2OSSV, and (d) AT2KY cells exposed to 28 GHz EMF at up to 2.5 mW/cm^2 for 24 h. Treatment with UV (1.0 J/m^2), x-ray (1.0 Gy), and bleomycin (10 μg/mL) served as positive controls. Data are given as mean $\pm$ standard deviation from at least three independent experiments (** indicate $p < 0.01$).

GHz radiation for 24 h at 0.5 mW/cm^2 does not influence the genotoxicity, as determined by MN formation. Therefore, the ICNIRP guidelines are valid. However, the possibility of epigenetic effects of the UHF EMF wave in human cell lines remains, and further studies are required.

5. Acknowledgment

This work is partially supported by a grant from the Ministry of Internal Affairs and Communications, Tokyo, Japan.

6. References

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