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Acute Low-Intensity Microwave Exposure Increases DNA Single-Strand Breaks in Rat Brain Cells

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Levels of DNA single-strand break were assayed in brain cells from rats acutely exposed to low-intensity 2450 MHz microwaves using an alkaline microgel electrophoresis method. Immediately after 2 h of exposure to pulsed (2 μs width, 500 pulses/s) microwaves, no significant effect was observed, whereas a dose rate-dependent [0.6 and 1.2 W/kg whole body specific absorption rate (SAR)] increase in DNA single-strand breaks was found in brain cells of rats at 4 h postexposure. Furthermore, in rats exposed for 2 h to continuous-wave 2450 MHz microwaves (SAR 1.2 W/kg), increases in brain cell DNA single-strand breaks were observed immediately as well as at 4 h postexposure.

Key words: microwaves, brain cells, DNA damage, rats, single-strand

INTRODUCTION

In this paper, we report the results from an experiment to study the effect of acute exposure to low-intensity microwaves on DNA damage in brain cells of the rat. Well-characterized damages to DNA include DNA single and double strand breaks, alkali labile sites (apurinic, apyrimidinic, alkylation, and phosphotriester formation), base damage, base modification, DNA-DNA and DNA-protein cross links, pymidine dimers, and DNA adducts. Among all of these, the most commonly used marker for DNA damage is single-strand break. DNA single-strand breaks can lead to carcinogenicity [Tice, 1978; Cerutti, 1985; Ames, 1989a,b], cell death [Walker et al., 1991; Onishi et al., 1993; Prigent et al., 1993], and aging [Hart and Setlow, 1974; Tice, 1978]. We used the alkaline microgel electrophoresis method [Singh et al., 1988, 1991, 1994] to assay for DNA single-strand breaks in individual brain cells. This is the most sensitive method available for assaying DNA single-strand breaks and can detect one break per 2×10^{10} daltons of DNA in lymphocytes.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–300 g) purchased from B and K Laboratory (Bellevue, WA) were used in this research. They were housed three in a cage in a vivarium on a 12 h light-dark cycle (light on 7 AM to 7 PM) at an ambient temperature of 22 °C and a relative humidity of 65%. They were given food and water ad libitum.

The cylindrical waveguide system developed by Guy et al. [1979] was used for microwave exposure. Our waveguide system consists of eight individual cylindrical exposure tubes connected though a power-divider network to a single microwave power source. Each tube consists of a section of circular waveguide constructed of galvanized wire screen in which a circularly polarized TE_{11} mode field configuration is excited. The tube also contains a plastic chamber to house a rat. This waveguide system, using circularly polarized radiation, enables efficient coupling of microwave energy to the animal exposed. For example, a spatially averaged power density of 1 mW/cm^2 in the waveguide produces a whole body specific absorption rate (SAR) of 0.6 W/kg in the rat. The range of power densities for a linearly polarized plane wave associated with an SAR of 0.6 W/kg would approximate 3–6 mW/cm^2. Local SAR in eight brain regions measured in rats exposed in this waveguide

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system varied from 0.5 to 2.0 W/kg per mW/cm^2 [Chou et al., 1985]. With this system, rats can be irradiated with either continuous-wave (CW) or pulsed (2 μs pulses, 500 pps) 2450 MHz microwaves at different spatially averaged power densities.

In our experiments, animals were subjected to either microwave or sham exposure in the waveguide system for 2 h. Immediately or at 4 h after exposure, rats were placed in a closed box containing dry ice for 60 s and then decapitated. All procedures from this time onward were done in minimum light. Brains were removed immediately and dissected on ice for assay of DNA single-strand breaks. Removal of the brain from the skull took approximately 30 s, and dissection of the hippocampus took an additional 40–45 s. All experiments were run blind. Two workers were involved: One conducted the animal exposure and brain dissection, and the other conducted the DNA single-strand break assay. Neither worker knew the treatment condition (microwave or sham exposed) of the rats.

The method of Singh et al. [1994] was used to assay for DNA single-strand breaks in brain cells with minor modifications. All chemicals used in the assay were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Immediately after dissection, brain tissues were immersed in ice-cold phosphate-buffered saline (PBS; NaCl, 8.01 g; KCl, 0.20 g; Na_2HPO_4, 1.15 g; KH_2PO_4, 0.20 g per liter) with 200 μM N-t-butyl-α-phenylnitrone at pH 7.4. Tissue was washed four times with the same buffer to remove most of the red blood cells. A pair of sharp scissors was used to mince (approximately 200 times) the tissue in a 50 ml polypropylene centrifuge tube containing 5 ml of ice-cold PBS to obtain pieces of approximately 1 mm^3. Four more washings with the cold buffer removed most of the remaining red blood cells. Finally, in 5 ml of PBS, these tissue pieces were dispersed into single-cell suspension using a 5000 liter Pipetman. Therefore, this cell suspension consisted of different types of cells in the brain.

A small volume (10 μl) of this cell suspension was mixed with 0.2 ml of 0.5% agarose maintained at 37 °C, and 75 μl of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24 × 50 mm^2 No. 1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed, and 75 μl of agarose solution was layered as before. This layering was done very quickly, because agarose solidifies rapidly on top of a cold agarose layer.

Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium N-lauroyl sacosinate, 100 mM disodium EDTA, 10 mM Tris base, and 1% Triton-X 100, pH 10). After 1 h of lysing at 0 °C, slides were treated with DNAase-free proteinase K (Boehringer Mannheim Corp., Indianapolis, IN) in lysing solution for 2 h at 37 °C. The proteinase K solution was preincubated for 1 h at 37 °C to ensure DNAase inactivation. Slides were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA) modified so that both ends of each electrode are connected to the power supply. Nine hundred milliliters of an electrophoretic buffer (300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide, and 10 mM tetrasodium EDTA) were gently poured into the assembly to cover the slides to a height of 6 mm above their surface.

After 20 min to allow for DNA unwinding, electrophoresis was started (18 V, approximately 300 mA, for 60 min), and the buffer was recirculated at a rate of 100 ml/min. At the end of the electrophoresis, extra electrophoretic buffer was removed from the top of the slides. The slides were gently removed from the electrophoretic apparatus and immersed for neutralization in 35 ml of 0.4 M Tris, pH 7.4, in a Coplin jar (two slides per jar) for 30 min. After two more similar steps of neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar three times for 30 min and blow dried. One slide at a time was taken out and stained with 50 μl of 1 μM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50 mm coverglass.

Slides were examined and analyzed with a Reichert vertical fluorescent microscope (model 2071) equipped with a filter combination for fluorescence isothiocyanate (excitation at 490 nm, emission filter at 515 nm, and dichromic filter at 500 nm). We measured the length (in microns) from the beginning of the nuclear area to the last pixel of DNA at the leading edge. The migration length is used as the index of DNA single-strand breaks. (As a reference, lymphocytes exposed to 25 rads of X-rays show an increase of 50–60 μm of DNA migration when assayed with this method.) Fifty representative cells were measured per slide. Data were analyzed by ANOVA, and difference between groups was compared by the Newman- Keuls test. A difference at $P < .05$ was considered statistically significant.

RESULTS

In the first experiment, we exposed rats for 2 h to 2450 MHz pulsed (2 μs pulses, 500 pps) microwaves at spatially averaged power density of either 1 or 2 mW/cm^2 inside the waveguide. These power densities give an average whole-body SAR of 0.6 and 1.2 W/kg, respectively, in the exposed animals [Chou et al., 1984]. Amounts of DNA single-strand breaks in cells from the hippocampus and the rest of the brain were assayed

immediately and at 4 h postexposure. Figures 1 and 2 show an increase in DNA single-strand breaks (expressed as microns of migration) in cells of the hippocampus and the rest of the brain, respectively, of the microwave-exposed rats at 4 h after exposure (Figs. 1, 2; right), whereas no significant effect was seen immediately after 2 h of microwave exposure (Figs. 1, 2; left).

In a second experiment, the effect of CW microwave radiation was investigated. Rats were exposed to CW 2450 MHz microwaves for 2 h in the circular waveguide at a power density of 2 mW/cm^2 (average whole-body SAR of 1.2 W/kg). DNA single-strand breaks were assayed in cells obtained from the whole brain of the animals immediately and at 4 h postexposure. Data in Figure 3 show a significant increase in DNA single-strand breaks in brain cells of the microwave-exposed rats compared to those of sham-exposed animals. Increase in breaks was observed immediately after microwave exposure and at 4 h postexposure. It should be noted that the baseline of DNA single-strand breaks in the sham-exposed samples in this experiment is lower than that in the previous experiment. This difference could be due to the use of whole brain in this study, whereas, in the previous study, the hippocampus was dissected out and assayed separately from the rest of the brain. The dissection process took 40–45 s more before the tissues could be put into ice-cold buffer for processing. Such a delay and the additional disturbance to the tissue during dissection could increase the baseline level of DNA breaks.

DISCUSSION

Our data indicate that acute microwave exposure increases DNA single-strand breaks in brain cells of the rat. Previous studies have also suggested effects of microwave exposure on chromosome and DNA. Garaj-Vrhovac et al. [1991] showed that acute (15–60 min) exposure to 7.7 GHz microwaves at 0.5 mW/cm^2 caused higher incidence of chromosome aberrations in Chinese hamster fibroblasts. Maes et al. [1993] reported that acute exposure to 2450 MHz microwaves at SAR of 75 W/kg and constant temperature increased dicentric chromosome and acentric chromosomal fragments in human lymphocytes. Both studies suggest damage of chromosomal DNA in cells after microwave exposure. In an in

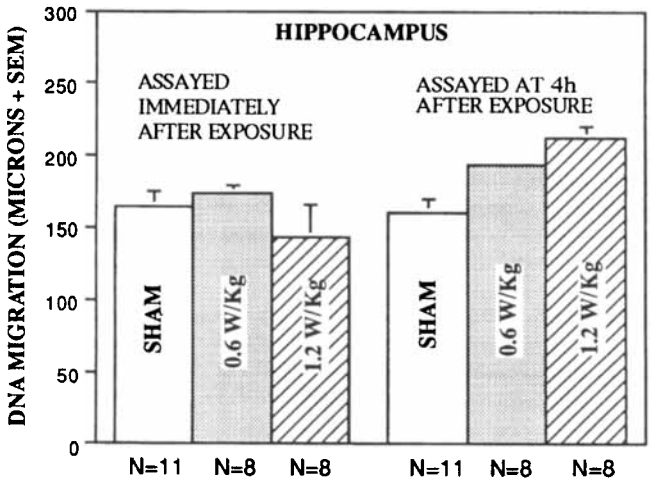

Fig. 1. DNA single-strand breaks (shown as micrometers of migration during electrophoresis) in hippocampal cells of rats subjected to 2 h of exposure to pulsed microwaves at an average whole-body SAR of 0.6 or 1.2 W/kg or sham exposure. Assay was done immediately after exposure (**left**) or at 4 h postexposure (**right**). N is the number of rats studied. One-way ANOVA showed no significant treatment (microwave) effect on DNA migration immediately after exposure ($F[2,24] = 1.239$; nonsignificant), whereas a significant treatment effect was observed at 4 h postexposure ($F[2,25] = 12.22$; $P < .001$). Values of 0.6 and 1.2 W/kg are significantly different from sham exposure at $P < .01$ (Newman-Keuls test). No significant difference was found between exposures at 0.6 and 1.2 W/kg.

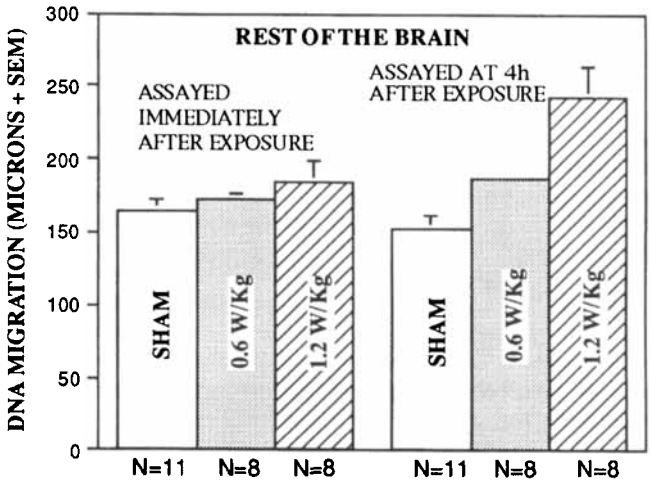

Fig. 2. DNA single-strand breaks (shown as micrometers of migration during electrophoresis) in cells from the rest of the brain (i.e., whole brain minus hippocampus) of rats subjected to 2 h of exposure to pulsed microwaves at an average whole body SAR of 0.6 or 1.2 W/kg or sham exposure. N is the number of rats studied. Assay was done immediately after exposure (**left**) or at 4 h postexposure (**right**). One-way ANOVA showed no significant treatment (microwave) effect on DNA migration immediately after exposure ($F[2,24] = 1.288$; nonsignificant), whereas a significant treatment effect was observed at 4 h postexposure ($F[2,24] = 14.04$; $P < .001$). Values of 0.6 and 1.2 W/kg are significantly different from sham exposure at $P < .05$ and .01 (Newman-Keuls test), respectively. A significant difference at $P < .01$ was found between exposures at 0.6 and 1.2 W/kg.

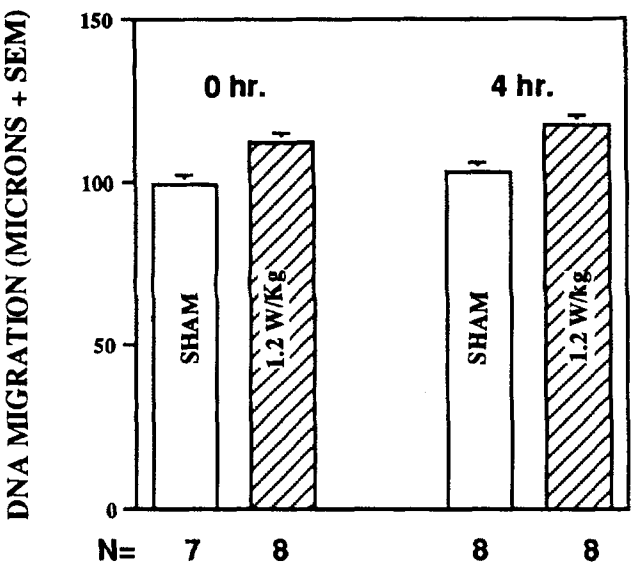

Fig. 3. Effect of acute exposure (2 h) to continuous-wave 2,450 MHz microwaves (SAR 1.2 W/kg) on DNA single-strand breaks in cells of the rat brain. Brain cells from microwave- and sham-exposed rats were assayed immediately and at 4 h postexposure. Two-way ANOVA showed a significant effect of microwaves ($F[1,27] = 25.18$, $P < .005$). Differences between microwave- and sham-exposed rats were significant at $P < .01$, both immediately and at 4 h after exposure, when compared using the Newman-Keuls test. There is no significant effect due to time of assay ($F[1,27] = 1.79$; nonsignificant) nor time of assay × microwave interaction effect ($F[1,27] = 1.582$; nonsignificant).

vitro study, Sagripanti and Swicord [1986] reported an increase in single- and double-strand breaks in isolated DNA after acute microwave exposure. Recently, Sarkar et al. *[1994]* reported a change in the sizes of DNA fragments isolated from the brain and testis of mice given repeated exposure to 2450 MHz microwaves at 1 mW/cm^2 (SAR 1.18 W/kg).

The mechanism of interaction between microwaves and DNA is unknown. An increase in DNA single-strand breaks could be due to an increase in the rate of breaking or a reduction in the DNA damage repair processes in the cell. It is also puzzling that brain cell DNA responded differently to CW and pulsed microwaves. A significant increase in DNA single-strand breaks was observed immediately after exposure to CW but not to pulsed microwaves. This further supports our previous conclusion that biological responses to microwaves depend on the parameters of the radiation [Lai, 1992].

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