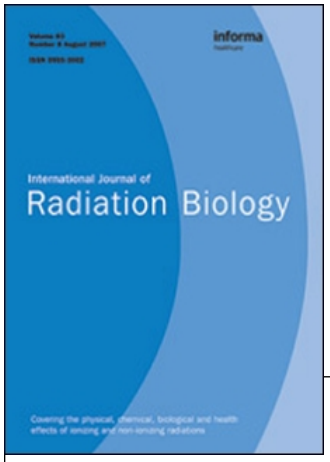

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Single-and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation

H. Lai

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Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation

H. LAI* and N. P. SINGH

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Abstract. We investigated the effects of acute (2-h) exposure to pulsed (2-μs pulse width, 500 pulses s^{-1}) and continuous-wave 2450-MHz radiofrequency electromagnetic radiation on DNA strand breaks in brain cells of rat. The spatial averaged power density of the radiation was 2 mW/cm^2, which produced a whole-body average-specific absorption rate of 1·2 W/kg. Single- and double-strand DNA breaks in individual brain cells were measured at 4 h post-exposure using a microgel electrophoresis assay. An increase in both types of DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation. No significant difference was observed between the effects of the two forms of radiation. We speculate that these effects could result from a direct effect of radiofrequency electromagnetic energy on DNA molecules and/or impairment of DNA-damage repair mechanisms in brain cells. Our data further support the results of earlier in vitro and in vivo studies showing effects of radiofrequency electromagnetic radiation on DNA.

1. Introduction

High power continuous-wave as well as modulated radiofrequency electromagnetic radiation (RFR) (in the non-ionizing range 10 kHz–300 GHz) are a source of heating for industrial processing of materials and food, in microwave ovens, and in medical therapy. Low- to high-power continuous-wave, modulated, and pulsed RFR are extensively used for air-traffic control systems, police and military radars, earth-to satellite television broadcast systems, and long distance telephone communication. In recent years, the tremendous increase in the use of cellular telephones, which emit the RFR at the 800–900 and 1800–2200 MHz ranges, has further raised concerns on the possible health effects of RFR.

Exposure to RFR has been shown to affect the nervous system and its functions. Effects include changes in the neural tissue morphology, neuroelectrophysiology, neurochemistry, neuropharmacology, and behavior (cf. review by Lai 1994). In the present study we investigated the effects of acute whole-body exposure to 2450 MHz RFR on DNA damage in brain cells of rat. Two common forms of DNA damage, single- and double-strand breaks, were studied using a microgel electrophoresis assay. This assay has been used extensively in toxicological studies for DNA damage (cf. reviews by Fairbairn *et al.* 1995, McKelvey-Martin *et al.* 1993).

In most research on the biological effects of RFR, the frequency at 2450 MHz was studied: it is the frequency used in microwave ovens and diathermy. In the present study, 2450 MHz RFR at a radiation power density of 2 mW/cm^2 was studied. In our exposure system this intensity gives an average whole-body-specific absorption rate (*SAR*) of 1·2 W/kg for the size of animals used in our study (Chou *et al.* 1984). This absorption rate does not induce a detectable change in body (colonic) temperature in a conscious rat, apparently the thermoregulatory system of the animal is sufficient to dissipate the heat load from the radiation (Lai *et al.* 1984). Animals were exposed for 2 h to the radiation and single- and double-strand DNA breaks were studied at 4 h post-exposure. This experimental procedure was used because previous similar experiments showed induced single-strand DNA breaks in brain cells (Lai and Singh 1995).

2. Materials and methods

2.1. *Animals*

Male Sprague-Dawley rats (250–300 g) purchased from the B & K Laboratory (Bellevue, WA, USA) were used in this research. They were housed three to a cage in a room adjacent to the microwave exposure room for 48 h before experiment. The laboratory was maintained on a 12-h light–dark cycle (light on 07:00–19:00 hours), at

* Author for correspondence.
Bioelectromagnetics Research Laboratory, Center for Bioengineering, Box 357962 University of Washington, Seattle, WA 98195, USA.

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an ambient temperature of 22°C and a relative humidity of 65%. Animals were given food and water ad libitum.

2.2. *RFR exposure system and exposure conditions*

The cylindrical waveguide system developed by Guy *et al.* (1979) was used for RFR exposure. The system consists of individual cylindrical waveguide tubes connected though a power divider network to a single RFR power source. Each waveguide can be activated individually. Waveguides were calibrated and checked from time to time. An animal can be subjected to either RFR- or sham-exposure in a waveguide depending on whether it is activated or not. Both RFR- and sham-exposed animals were included in each exposure session.

Each tube consists of a section of circular waveguide constructed with galvanized wire screen in which a circularly polarized TE_{11} mode field configuration is excited. The tube contains a plastic chamber to house a rat with enough space inside for it to move freely. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside of the waveguide. This waveguide system using circularly polarized radiation enables efficient coupling of radiation energy to the animal exposed. For example, a spatially averaged power density of 1 mW/cm^2 in the circular waveguide produces a whole-body-specific absorption rate (*SAR*) of 0·6 W/kg in a rat (Chou *et al.* 1984). The range of power densities for exposure to a linearly polarized plane-wave associated with an *SAR* of 0·6 W/kg approximate 3–6 mW/cm^2. Local SAR in eight brain regions measured in rat exposed in this waveguide system varies from 0·5 to 2·0 W/kg per mW/cm^2 (Chou *et al.* 1985). By connecting this system to different signal sources (Applied Microwave microwaves pulse signal source model PG5KB for pulse radiation and Hewlett Packard 8616A signal generator for continuous-wave radiation), rats can be irradiated with either pulsed (2 μs pulse width, 500 pulses s^{-1}) or continuous-wave (CW) 2450 MHz radiation at different spatially averaged power densities. In this study, rats were subjected either to pulsed or CW RFR or sham exposure in the waveguides for 2 h. The averaged power density within the waveguide was set at 2 mW/cm^2, which gave an averaged whole-body *SAR* of 1·2 W/kg. This *SAR* is not expected to produce a significant change in body (colonic) temperature in a conscious rat (Lai *et al.* 1984). Furthermore, since animals can move freely in the waveguide during exposure, local 'hot spots' in the body are not expected to raise local temperature significantly at the intensity of RFR used in our experiment.

Animals were returned to their home cages after exposure. Four hours later, each rat was placed for 60 s in a closed foam box containing dry-ice (a card board was put on top of the dry ice to prevent its direct contact with the animal) and then decapitated with a small animal guillotine. All procedures from this step onward were done in minimum indirect light. Brains were removed immediately and dissected on ice for assay of DNA strand breaks. Removal of the brain from a skull took approximately 30 s. All experiments were run blind. The on/off conditions of the waveguides were determined by an experimenter before an experiment. Two other experimenters, who did the animal exposure and brain dissection and DNA strand-break assays, respectively, did not know the treatment condition (RFR or sham exposure) of the rats. Since each experiment included both RFR- and sham-exposed rats, individual control (sham-exposed) group was obtained for both pulsed and CW RFR-exposure. Therefore, there were four treatment groups: pulsed-RFR, control for pulsed-RFR, CW-RFR, and control for CW-RFR. There were eight rats studied in each treatment condition.

2.3. *Assay methods for DNA strand breaks*

The microgel electrophoresis method of Singh *et al.* (1994), with some modifications, was used to study DNA single- and double-strand breaks in rat brain cells. All chemicals used in the assay were purchased from Sigma Chemical Co. (St Louis, MO, USA) 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, pH 7·4) containing 200 μmol dm^{-3} N-t-butyl-α-phenylnitrone, a spin-trap. Tissue was quickly washed four times with the PBS to remove most of the red blood cells. A pair of sharp scissors was used to mince (approximately 200 cuts) the tissue in a 50-ml polypropylene centrifuge tube containing 5 ml ice-cold PBS to obtain pieces of approximately 1 mm^3. Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 ml PBS, tissue pieces were dispersed into single-cell suspensions using a 5000 λ pipetman. This cell

suspension consisted of different types of brain cells. Of this cell suspension, 10 μl was mixed with 0·2 ml 0·5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH, USA) maintained at 37°C, and 30 μl of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH, USA) and immediately covered with a 24 × 50 mm square #1 coverglass (Corning Glass Works, Corning, NY, USA) 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 100 μl agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2·5 mol dm^{-3} NaCl, 1% sodium *N*-lauroyl sacosinate, 100 mmol dm^{-3} disodium EDTA, 10 mmol dm^{-3} Tris base, pH 10) containing 1% Triton X-100.

To measure single-strand DNA breaks, after lysing overnight at 4°C, slides were treated with DNAase-free proteinase K/mg/ml (Boehringer Mannheim Corp., Indianapolis, IN, USA) in the lysing solution for 2 h at 37°C. They were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Fansisco, CA, USA) modified so that both ends of each electrode are connected to the power supply. Of an electrophoresis buffer (300 mmol dm^{-3} NaOH, 0·1% 8-hydroxyquinoline, 2% dimethyl sulphoxide, 10 mmol dm^{-3} tetra-sodium EDTA,) pH 13, 1 litre was gently poured into the assembly to cover the slides to a height of 6·5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0·4 V/cm, approximately 250 mA, for 60 min) and the buffer was recirculated. At the end of the electrophoresis electrophoretic buffer above the slides was gently removed. Slides were then removed from the electrophoresis apparatus and immersed in an excess amount of neutralization buffer (0·4 mol dm^{-3} Tris at 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 for 30 min and then dried.

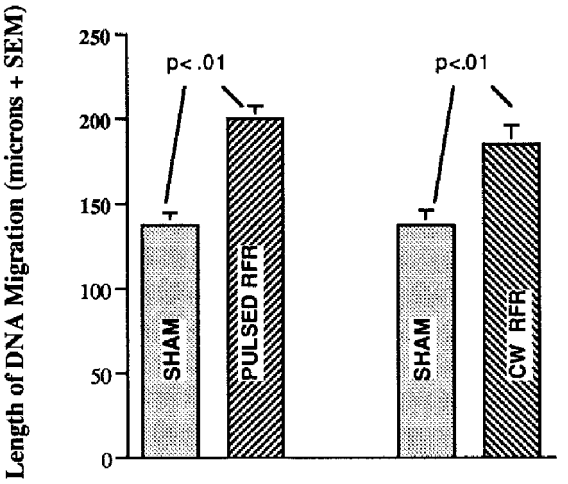

Figure 1. Single-strand DNA breaks in brain cells of rat after exposure to either pulsed- or continuous-wave RFR. Each bar represents data from eight animals (plotted mean + SEM).

For double-strand breaks, microgel preparation and cell lysis were done as mentioned above. Slides were then treated with ribonuclease A (Boehringer Mannheim) (10 μg/ml in the lysing solution) for 2 h and then with proteinase K (1 mg/ml in the lysing solution) for 2 h at 37°C. They were then placed for 20 min in an electrophoretic buffer (100 mmol dm^{-3} Tris, 300 mmol dm^{-3} sodium acetate, acetic acid, pH 9·0), and then elecrophorezed for 1 h at 0·4 V/cm (approximately 100 mA). The slides were treated with 300 mmol dm^{-3} NaOH for 10 min and neutralized as before with 0·4 mol dm^{-3} Tris (pH 7·4). Slides were then dehydrated in absolute ethanol for 30 min and dried.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was taken out and stained with 50 μl 1 μmol dm^{-3} solution of YOYO-1 (stock, 1 mmol dm^{-3} in DMSO; Molecular Probes, Eugene, OR, USA) and then covered with a 24 × 50-mm cloverglass. Slides were examined and analyzed with a Reichert vertical fluorescent microscope (model 2071) equipped with a filter combination for fluorescence isothyocynate (excitation at 490 nm, emission filter at 515 nm, dichromic filter at 500 nm). We measured the length of DNA migration (μm) from the beginning of the nuclear area to the last three pixels of DNA perpendicular to the direction of migration at the leading edge. The migration length is used as the index of DNA strand breaks. Four slides were prepared from the brain sample of each animal. Two slides were assayed for single-strand DNA breaks and two were assayed for double-strand breaks. Fifty representative cells were scored from each slide. Therefore, from each animal, 100 cells each were scored for single- and double-strand DNA breaks.

2.4. *Data analysis*

The average length of DNA migration from the 100 cells measured for single- and double-strand

breaks in each rat were used in data analysis using the two-tailed Student's *t*-test comparing data from RFR- and sham-exposed animals. Percent of cells with respect to DNA migration length (in intervals of 10 μm) were plotted. Difference in distribution between treatment groups were compared by the χ^2-test. A difference at $p < 0{\cdot}05$ was considered statistically significant.

3. Results

Data on the effects of RFR exposure on single-strand DNA breaks in brain cells are shown in Figures 1–3. Figure 1 shows the average length of DNA migration of brain cells from rats in the different treatment groups. Both pulsed and CW RFR exposures significantly increased single-strand DNA breaks in brain cells. However, there was no significant difference in mean migration lengths between the pulsed and CW RFR exposure treatments (two-tailed Student's *t*-test). Figures 2 and 3 are plots of the percent distribution of cell versus DNA migration lengths (in intervals of 10 μm) for the pulsed and CW exposure, respectively. In both cases there was a significant shift in the distribution curves of the RFR-exposed cells to the right compared with that of sham-exposure, i.e. more cells in the RFR-exposed rats showed longer DNA migration lengths. (Pattern of cell distribution of pulsed-RFR exposed rats versus that of sham-exposure: $\chi^2 = 921{\cdot}5$, d.f. = 13, $p < 0{\cdot}001$; and pattern of cell distribution of CW-RFR exposed rats versus that of sham-exposure: $\chi^2 = 523{\cdot}0$, d.f. = 12, $p < 0{\cdot}001$.)

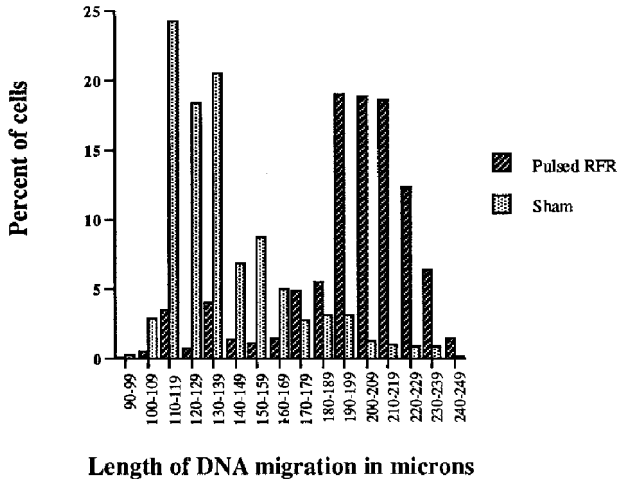

Figure 2. Single-strand DNA breaks frequency distribution graph plotting percent of cells versus length of DNA migration of brain cells from pulsed RFR- and sham-exposed animals. Each treatment plotted consists of data from 800 brain cells: 100 cells per animal. Marks on the *x*-axis designate ranges of migration lengths (μm) of sham and RFR bars touching each other.

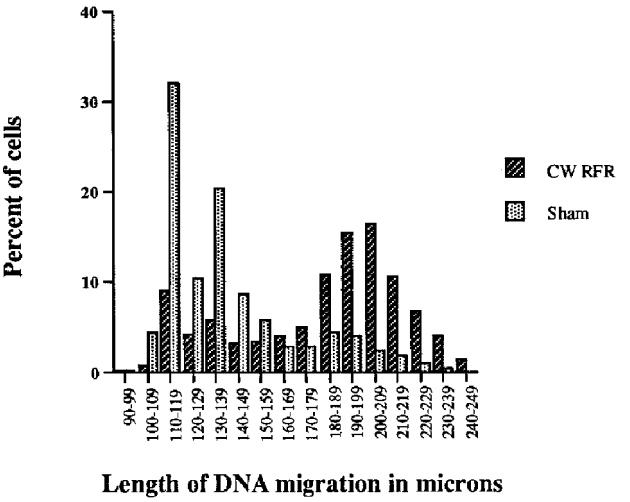

Figure 3. Single-strand DNA breaks frequency distribution graph plotting percent of cells versus length of DNA migration of brain cells from continuous-wave RFR- and sham-exposed animals. Each treatment plotted consists of data from 800 brain cells: 100 cells per animal.

Data on the effects of RFR exposure on double-strand DNA breaks in brain cells are shown in Figures 4–6. Conclusions from these data are similar to these of the single-strand breaks. They indicate an increase in double-strand DNA breaks in brain cells of both pulsed and CW RFR-exposed animals compared with those of sham-exposed controls, and no significant difference in effects

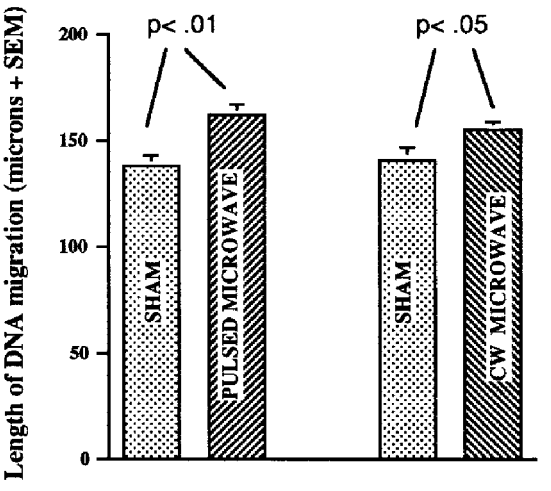

Figure 4. Double-strand DNA breaks in brain cells of rat after exposure to either pulsed or continuous-wave RFR. Each bar represents data from eight animals (plotting mean + SEM).

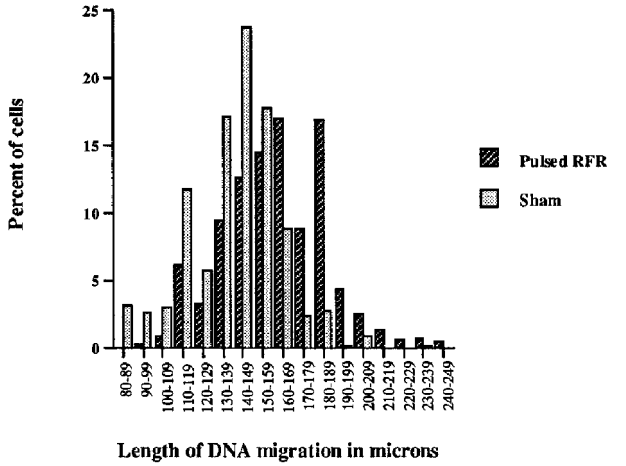

Figure 5. Double-strand DNA breaks frequency distribution graph plotting percent of cells versus length of DNA migration of brain cells from pulsed RFR- and sham-exposed animals. Each treatment plotted consists of data from 800 brain cells: 100 cells per animal.

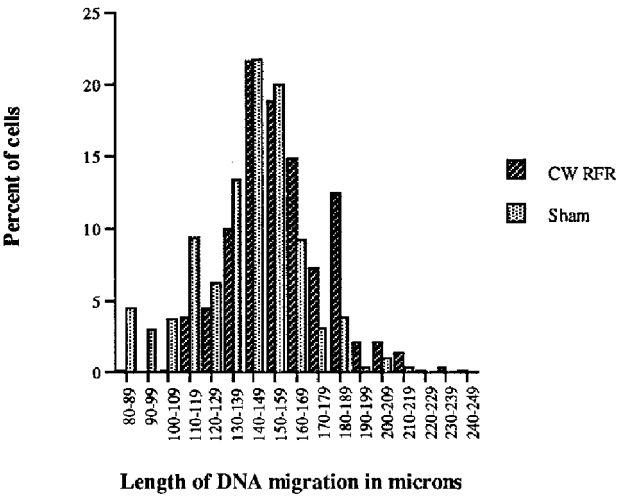

Figure 6. Double-strand DNA breaks frequency distribution graph plotting percent of cells versus length of DNA migration of brain cells from continuous-wave RFR and sham-exposed animals. Each treatment plotted consists of data from 800 brain cells: 100 cells per animal.

between pulsed and CW RFR exposures (Figure 4). A significant shift towards the right was also seen in the cell-frequency distribution curves after RFR exposure (Figures 5 and 6). (Pattern of cell distribution of pulsed-RFR exposed rats versus that of sham-exposure: $\chi^2 = 303{\cdot}5$, d.f. = 9, $p < 0{\cdot}001$; and pattern of cell distribution of CW-RFR exposed rats versus that of sham-exposure: $\chi^2 = 174{\cdot}7$, d.f. = 8, $p < 0{\cdot}001$.)

4. Discussion

Results of the present experiment indicate that acute exposure to RFR at an averaged whole body *SAR* of 1·2 W/kg causes a significant increase in both single- and double-strand DNA breaks in brain cells of the rat. In relation to human exposure to RFR in the environment, the dose rate used in the present study is three times the exposure limit recommended for human exposure by the IEEE Standard Co-ordinating Committee (1992), which is partially based on the threshold of work stoppage effect occurs in animals exposed at a dose-rate of 4 W/kg. On dosimetry study of RFR emitted from cellular telephones, the peak *SAR* in the brain of the user can reach up to 4·7 W/kg W^{-1} over 1 g tissue (Dimbylow and Mann 1994). However, it must be pointed out that cellular telephones emit RFR of a different frequency and users are subjected to intermittent and short periods of exposure than in the present study.

In our experiment, rats were subjected to whole-body exposure. However, the effects of RFR on brain cell DNA most likely resulted from a direct effect of RFR on brain cells, rather than a consequence of energy absorption in other parts of the body. The rat of RFR energy absorption is significantly higher in the head than in the rest of the body of a rat exposed in the circular waveguide (Chou *et al.* 1985). (The average *SAR* in the head is about 30% higher than that in the whole body.) It is not likely that local 'heat load' in other parts of the body can lead to DNA damage in cells of the brain.

The mechanism by which RFR affects DNA is not known. An increase in DNA strand breaks in brain cells after RFR exposure could be due to a direct effect of the radiation on DNA molecules and/or to an effect of the radiation on DNA repair mechanisms. The energy of RFR is too low to cause direct breakage of covalent bonding in DNA molecules. However, an *in vitro* study by Sagripanti and Swicord (1986), showing an increase in single- or double-strand breaks in plasmid DNA molecules after acute RFR exposure, suggested a direct interaction of the radiation with DNA molecules resulting in damages. Kirschvink (1994) proposed that biogenic magnetite crystals contained in cells could absorb RFR with peak resonance in the range 0·1–10 GHz and conversion of the absorbed energy into acoustic waves could lead to disruption of subcellular structures including DNA in the cell. Ferromagnetic materials

have been reported to be present in the human cerebellum and cerebral cortex (Kirschvink *et al.* 1990).

In addition to direct damage to DNA by genetoxic chemicals and physical agents, DNA strand breaks are also generated as intermediate steps during DNA repair, e.g. during repair of damages due to DNA–DNA and protein–DNA cross links, and DNA adduct formation, etc. DNA single-strand breaks are also produced when double-strand breaks are repaired by recombination (King *et al.* 1993). Thus, in our case, the measured single- and double-strand breaks could be a result of these various types of DNA repair processes. In addition, in order for DNA strand breaks to accumulate and remain measurable at 4 h after exposure, one can also postulate that DNA repair mechanisms are impaired by the irradiation. Through a homeostatic mechanism cells maintain a delicate balance between spontaneous and induced DNA damage and repair. DNA damage accumulates if such a balance is altered. In many instances an impairment in DNA repair enzyme synthesis at transcription or translation levels, rather than a significant increase in damages, could lead to a cumulation in DNA damages in cells. In particular, postmitotic cells, such as neurons, lack post-replication repair processes, and have a low capability for DNA repair (Bernstein and Bernstein 1991); this could cause the observed accumulation of DNA breaks after irradiation. In support of the hypothesis that RFR can affect DNA repair is that millimeter waves, a form of RFR, have been shown to affect DNA repair mechanisms in cells (Belyaev *et al.* 1992, Rojavin and Ziskin 1995).

In addition to our study, several other groups of researchers, using different RFR exposure conditions and assay methods, have also reported effects of RFR exposure on DNA and chromosome in various cell types. In an *in vitro* study, Sagripanti and Swicord (1986) reported an increase in single- and double-strand breaks in plasmid DNA molecules exposed to 2550-MHz RFR for 20 min at 20 °C. Effect was observed at a SAR_{max} of 8·5 W/kg. They also reported that elevation of the sample temperature by 8 °C did not produce the same effects as observed after RFR exposure. Narasimhan and Huh (1991) reported altered restriction patterns in λ phage DNA exposed to short pulses (2–20 s) of 2450-MHz RFR. DNA samples were placed in stoppered microcentifuge tubes and suspended in a beaker of ice water at 5–8 °C. The researchers speculated that these DNA changes resulted from single-strand breaks and localized strand separations induced by the radiation. Garaj-Vrhorac *et al.* (1990, 1991) showed that acute (15–60-min) exposure to 7700-MHz RFR (30 mW/cm^2) caused an inhibition of [^{3}H]-thymidine incorporation into DNA and a higher incidence of chromosome aberrations in Chinese hamster fibroblasts. Maes *et al.* (1993) reported that acute (30–120-min) exposure to 2450-MHz RFR at an *SAR* of 75 W/kg and constant temperature (36·1 °C) increased dicentric chromosome and acentric chromosomal fragments and micronuclei formation in human lymophocytes, whereas sister chromatid exchange was not significantly affected. These studies suggest chromosomal DNA damages in cells after RFR exposure.

More recently, Verschaeve *et al.* (1994) showed an increase in single-strand DNA breaks as assayed by the alkaline microgel electrophoresis method in lymphocytes from human blood samples exposed for 1–2 h *in vitro* to 954-MHz RFR at 17 °C (the electric field of exposure reported was 49 V/m, *SAR* = 1·5 W/kg). However, they found no significant effect in lymphocytes obtained from rat exposed *in vivo* to the radiation. In addition, Sarkar *et al.* (1994) reported that repeated exposure (2 h/day for 120–200 days) to 2450-MHz RFR (*SAR* = 1·18 W/kg) caused DNA alterations in brain and testicular cells of mice. Distinct changes in band patterns were observed in the 7–8-kb range of digested DNA from cells of exposed animals when hybridized with a synthetic oligo probe. However, it must be pointed out that other studies have found no significant mutagenic effect from RFR exposure. Meltz *et al.* (1990) reported that RFR exposure (2450-MHz, 4-h exposure at a *SAR* of 40 W/kg) had no mutagenic effect on mouse leukaemia cells and the radiation also did not enhance the mutagenic effect of proflavin. Ciaravino *et al.* (1991) reported that RFR exposure (2450-MHz at a *SAR* of 33·8 W/kg for 2 h) did not significantly affect cell mitotic cycle progression nor change the number of sister chromatid exchange induced by adriamycin in Chinese hamster ovary cells. It is possible that RFR only induces low levels of genetic damage which is not detectable by the assay methods used in these experiments. The microgel electrophoresis assay is capable of detection of low levels of DNA damage (e.g. that induced by 205 mGy of γ-rays; Singh *et al.* 1995), whereas the sensitivity of chromatid aberration assays is considerably lower (approximately 0·5 Gy of X-rays; Kodym and Hoerth 1993).

Increases in DNA strand breaks in cells could lead to important health consequences. DNA damage accumulating in body cells over a period of time can be the cause of slow onset of diseases, such as cancer. On the other hand, cell death occurs when too much DNA damage has accumulated in the cell. Double-strand breaks are a major cause of cell death (Ward 1990). One-to-two unrepaired double-strand breaks in the genome can be lethal to a cell (Frankenberg *et al.* 1981, Blocher and Pohlit 1982) and Radford (1985, 1986a,b) has shown a correlation between double-strand DNA breaks and cell death after X-irradiation.

Furthermore, relevant to our finding in brain cells is that cumulative DNA damage in cells has been shown during ageing (Hart and Setlow 1974, Wheeler and Lett 1974, Chetsanga *et al.* 1977, Targovnik *et al.* 1985, Mullaart *et al.* 1990a, Ames *et al.* 1993). Cumulative DNA damage in cells, particularly in neurons, has been associated with Alzheimer's disease (Robbins *et al.* 1983, Jones *et al.* 1989, Mullaart *et al.* 1990b), Huntington's disease (Bridges 1981, Scudiero *et al.* 1981), and Parkinson's disease (Robbins *et al.* 1983). An increase in DNA strand breaks in body cells also is seen in disorders of premature senility such as xeroderma pigmentosum, Werner's Syndrome, Cockayne Syndrome, ataxia telangiectasia, and retinal dystrophies (Robbins *et al.* 1983).

Thus, DNA strand breaks could lead to disruption of cell functions, carcinogenesis, and cell death. Cumulative DNA damage in cells in the central nervous system could be a cause of accelerated ageing and neurodegenerative disorders. Therefore, it is imperative that the effects of RFR on DNA in brain cells be further studied and understood. Specifically, the long-term effect of repeated exposure to RFR should be studied, since continuous long-term exposure to RFR is unlikely in the environment. Adaptive responses of cells to repeated insults and perturbation have been reported, e.g. a decrease in chromosomal damages in human lymphocytes after repeated low-doses of X-ray exposure (Fan *et al.* 1990), and an increase in resistance of Chinese hamster cells to multiple short-duration exposures to UV radiation (Osmak *et al.* 1990). However, repeated short-duration exposure to low-level RFR does not necessarily guarantee safety compared with exposure of long duration or high intensity, especially if the exposure is not sufficient to elicit adaptive changes. For example, a recent paper indicated that secondary cigarette smoking could be more harmful to health than smoking, because the intermittent low-dose exposure of the secondary smoker to cigarette chemicals is not enough to trigger an adaptive response (Glantz and Parmley 1995). A detailed study of the interaction effect of intensity and duration of exposure and the effect of intermittent exposure, is important in the understanding and evaluation of the possible hazardous effect of exposure to RFR in the environment.

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