DNA Strand Breaks in Rat Brain Cells Exposed to Low level Microwave Radiation

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Abstract

This investigation concerns with the effect of low intensity microwave (2.45 and 16.5 GHz, SAR. 1.0 and 2.01 W/kg respectively) radiation on developing rat brain. Wistar rats were exposed for 35 days at the above mentioned frequencies separately in two different exposure systems. After the exposure period the rats were sacrificed and the whole brain tissue was dissected and used for study of single strand DNA breaks by micro gel electrophoresis (comet assay). Single strand DNA breaks were measured as tail length of comet. This study shows that the chronic exposure to these radiations cause statistically significant ($p<0.001$) increase in DNA single strand breaks in brain cells of rat.

1. Introduction

Non-ionizing radiation has a significant and positive impact on modern society through a number of uses. There has been a growing concern among the public regarding the potential human health hazard of exposure to these frequencies by these appliances [1]. Potential health effects of radiofreqency and microwave radiation generated by telecommunication, industry or other emitters, are reported earlier [2]. In ICNRIP report [3] on possible effects of long term exposure to RF radiation state that there is no convincing evidence of causal relation between RF and any adverse health effects. Epidemiological studies reported that an increased risk of brain tumors among analogue cellular phone users [4].

Cleary et al. [5] reported that RF radiation at 2.45 GHz and 27 MHz increase cell proliferation. Increased blood brain barrier permeability has been reported in rats to rhodomine –ferritin complex at 2.45 GHz [6]. 2.45 GHz radiation causes significant increase in ornithine decarboxylase activity and a decrease in protein kinase activity in rat brain after chronic microwave exposure [7].

Studies on central nervous system (CNS) showed that, exposure to electromagnetic field (EMF) resulted in depressed activities of protein kinase C [8] and different types of neurotransmitter systems such as acetylcholine esterase, acetylcholine, dopamine, serotonin and amino acids in the developing rat brain [9]. Structural and genomic changes have been reported in the brain and testis of rat exposed to 2.45 GHz radiation [10]. Lai and Singh [11] also reported that rats exposed to pulse or continuous wave of 2.45 GHz field for 2 hours, resulted in an increase of single and double stand breaks in the DNA of brain cells. Differences in DNA repair capacity have become the accepted explanation for this range of intrinsic radio sensitivities, since it is generally believed that a subset of the DNA breaks is lethal if unrepaired [12].

Malyapa et al., [13] reported that no DNA damage in cells of the rat cerebral cortex or the hippocampus after a 2 or 4 h exposure to 2450 MHz (CW). Above experimental results refer to acute exposure. In the present investigation it is intended to carry out the effects of chronic exposure (35 days) on developing rats brain.35 days old rats were selected because during this period the brain developments take place in rat. A choice of 2.45 GHz is made because of its wide spread use in Radar, industry, scientific research and medicine, and hence the probability of its leakage in the environment is possible. A choice of second frequency and dose was made to see if the effect is of general nature. Also no data at frequency 16.5 GHz are reported in the literature.

2. Materials and Methods

Male Wistar rats of 35 days old (60-70 gm) were supplied with food and water *ad libitum* and were grouped in to two (control and experimental). Each group consists of six rats (n=6).

2.45 GHz irradiation, six rats were kept in a plexi glass cage (43 x 27 x 15 cm) and placed in an anechoic chamber [7]. Each animal was kept in a pre-specified compartment of the cage throughout the exposure period. The dimension of the cage was made (according to the animal size) in such a way that animals remain restrained. Each animal was thus irradiated homogeneously at the same power level. Temperature in the chamber was maintained at 30° C throughout the period. The cage was constantly aerated to avoid the possibility of any increase in temperature. The cage was placed symmetrically along the midline of the pyramidal horn antenna, having dimensions 13x 9.8 cm. Exposure was given for 2 h /day for a total period of 35 days (excluding the weekends) at power density of 0.344 mW/cm^2 (SAR 1.0 W/Kg). Sham exposure (control group, n = 6) was performed similarly as the exposed group but without power input

Another set of animals were treated similarly but at different frequency i.e., 16.5 GHz amplitude modulated frequency at power density 1.0 mW/cm^2 (SAR 2.01 W/kg,). These rats were housed in a rectangular plexi glass cage having dimensions 32 x 10 x 9 cm. Two rats were kept simultaneously in a cage and placed inside the chamber. Exposure was given for 2 hrs /day for 35 days. Control animals were kept in the same way without power input (sham irradiation). In total six animals were used in each category.

2.1. DNA Strand Breaks

The protocol of Lai and Singh [14] was followed. Briefly, immediately after the exposure period, whole brain was dissected out and a single cell suspension was made using a 5 ml pipette. From the cell suspension 10µl of its suspension was mixed with 0.2 ml, 0.5% agarose. The mixture was pipetted out and poured on to a fully frosted slide, immediately covered with coverglass (24 x 50 mm^2). These slides were kept in an ice-cold steel tray on ice for 1 min. to allow the agarose to gel. After the lysis at 4° C, electrophoresis was started at 250 mA (25 V) for 60 min. and stained with 50µl of 1 mmol dm-3 solution of YOYO-1 [benzoxazolium-4-quinolium oxalo yellow dimer]. Microscopic slides (two slides per animal) were prepared with each individual animal separately [11,15]. Slides were examined and analyzed with a Reichert vertical fluorescent microscope. DNA damage was quantitative as length of the comet tail with the help of an ocular micrometer.

3. Results and Discussion

Our results show, that the prolonged chronic exposure to 2.45 GHz and 16.5 GHz separately causes reproducible increase in single strand DNA breaks in brain cells of rat in all the exposed group animals. A significant increase in the length of DNA migration was observed in rat brain exposed to 2.45 and 16.5 GHz radiation. A corresponding no change in tail length was observed in all the control animals. We did not find any cell death by treating the field mentioned above.

The average values of DNA migration of rat brain cells exposed to 2.45 GHz continuous wave is given in Table 1. It shows that there is a significant increase in the tail length of DNA as compared to the control group. For the control group the average value comes out to be 24.11 ± 4.47 µm whereas for the exposed group it was 41.011 ± 4.625 µm, which were found to be statistically significant ($p<0.001$). A statistically significant increase in the DNA migration was observed. For control group it was 20.46 ± 3.58 µm, whereas for the exposed group it was 31.147 ± 4.66 µm ($p<0.001$) (Table 2).

Table 1. DNA migration (in microns) of individual animals exposed to 2.45 GHz radiation. The values are average (± S.D.) of 100 cells.

Animals	Control	Exposed
1	24.87 ± 4.50	37.47 ± 5.07
2	24.65 ± 3.59	41.23 ± 4.21
3	24.77 ± 4.76	42.20 ± 4.84
4	24.40 ± 4.38	40.28 ± 5.08
5	22.98 ± 5.52	43.42 ± 3.74
6	23.01 ± 4.17	41.46 ± 4.80

Table 2. DNA migration (in microns) of individual animals exposed to 16.5 GHz radiation. The values are average (± S.D.) of 100 cells.

Animals	Control	Exposed
1	20.14 ± 2.46	28.95 ± 4.13
2	19.97 ± 3.03	31.22 ± 5.16
3	20.54 ± 3.69	32.46 ± 4.43
4	20.41 ± 3.50	32.24 ± 4.21
5	20.89 ± 4.35	31.61 ± 4.50
6	20.82 ± 4.46	30.40 ± 5.09

Data so obtained show that prolonged (35 Days) exposure to microwave radiation (2.45 and 16.5 GHz) causes single strand DNA breaks in brain cells of rat. Maes et al. [16] reported that acute 30-120 min exposure to 2450 MHz RFR at an SAR 75 W/kg and constant temperature 36.1° C increased dicentric and acentric chromosomal fragments and micronuclei formation in human lymphocytes. Mitchell et al. [17] observed a decrease in motor activity in rats after 7 hr of exposure to CW 2450-MHz RFR (10 mW/cm^2, average SAR 2.7 W/kg). Lai and Singh [11] reported that acute exposure (2h) to both pulsed and CW (continuous wave) 2.45 GHz radiation (power density, 2 mW/cm^2, SAR 1.2 W/kg) produce a significant increase in the DNA single and double strand breaks in rat brain. The present study is in agreement with these studies showing a significant difference in DNA single strand breaks in the exposed group. DNA damage is closely related to human health risk. Particularly, DNA damage in brain cells could affect neurological functions and also possibly lead to neurodegenerative diseases [11].

The exact mechanism is yet to be elucidated, as to how DNA strand breaks occur due to RF radiation. DNA single strand breaks are also produced when double strand breaks are repaired by recombination [11]. Using the comet assay a diverse types of DNA damage can be determined. Since various tissues or cell types differ in their susceptibility towards EMF exposure, hence conflicting results among the mammalian cell types are also reported [18]. It is hoped that data presented here will help in identifying possible causal connections of exposure to electromagnetic fields and biological effects.

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5. References

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