Biomarkers inducing changes due to microwave exposure effect on rat brain

Kavindra Kumar Kesari[1], Sanjay Kumar[2] and J. Behari[3]*

School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India
Tel: +91-11-26704323, Fax 91-11-26717502, 26715886
[1]kavindra_biotech@yahoo.co.in, [2]sia_cara@yahoo.com, [3]jbehari@hotmail.com
*Corresponding author

Abstract

Animals were exposed for 2 h a day for 45 days at 0.21 mW/cm^2 power density SAR (0.038 W/Kg). After exposure, pineal gland and whole brain tissues were separated for the study of melatonin, creatine kinase and caspase 3. Result showed a significant decrease ($P<0.05$) in melatonin level in the exposed group as compared with sham-exposed. A significant increase ($P<0.05$) in creatine kinase and caspase 3 was also observed in exposed group of whole brain as compared with sham exposed. The study concludes that the chronic exposure to these radiations may be an indication of possible tumor promotion.

1. Introduction

Over three billion peoples in more than 200 countries are daily exposed consciously to electromagnetic fields (EMFs) (Fragopoulou et al., 2009). The effects of EMFs emitted by mobile phones and several other gadgets on the central nervous system (CNS) have become a particular focus of concern owing to the fact that mostly mobile phones are kept near head during talking mode and hence affect brain (Mausset et al., 2001; Odaci et al., 2008).
Previously we have reported that the microwaves at several frequencies are able to induce several changes at the level of DNA and enzyme levels, including an increase in DNA single and double strand breaks in the rat brain (Kesari and Behari, 2009a; Kesari and Behari, 2010b; Paulraj and Behari, 2006b). Lai and Singh (1997) also reported such effects due to microwave exposure. Recently a significant changes in brain antioxidant enzymes level (Kesari and Behari, 2009a; Kesari and Behari, 2010b), sperm count (Kesari and Behari, 2010a, c), alteration in calcium of rat brain (Paulraj et al., 1999), an increase in the glial cell activity were observed at 112 MHz 2.45 GHz and 50 GHz (Paulraj and Behari, 2004, 2006a). Paulraj and Behari, (2004) and Kesari and Behari (2010b) observed a significant decrease in protein kinase C (PKC) level of hippocampus and whole brain at the frequency of 2.45 GHz and 50 GHz microwave respectively. Our study on other biomarkers (caspase, melatonin, creatine kinase) are found to be tumor promoter caused by free radical formation. Free radicals that are derived from oxygen metabolism are known as reactive oxygen species (ROS) (Desai et al., 2008). Recently Kumar et al., (2010a; 2010b) has also recorded possibility of tumor promotion at various frequencies by alteration in antioxidant enzymes, micronuclei, histone kinase and cell cycle due to an increased level of ROS.

2. Material & Methods

2.1 Material

P^{32} radioactive labeled ATP was purchased from BRIT, Hyderabad, India. The rest of the chemicals were purchased from Thomas Baker Chemicals Limited, Marine Drive, Mumbai, India. Caspase 3 assay kit, colorimetric (Cat. No. CASP-3-C) was purchased form Sigma, USA. Creatine assay kit (Cat No. K635-100) from BioVision Research Products (Mountain View, CA, USA), ELISA melatonin kit (Cat. No. E90908Ra) form Uscn Life Science Inc, (Wuhan, China)

2.2 Animals

35 days old male Wister rats (130 ± 10 g) were used in the present study. The animals were maintained as guidelines and protocols, approved by the Institutional Animal Ethics Committee (IAEC-JNU/83/675-687; Code No. 12/2008). The animals were housed in clean polypropylene cages and maintained in a controlled temperature environment with constant 12 h light and 12 h dark schedule. The animals were fed on standardized normal diet (Tetragon Cheime Private Limited, Bangalore) and water *ad libitum*.

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2.3 Exposure System

The rats were divided into two groups: group I - sham exposed and group II - 2.45 GHz exposed. Each group contained six animals and experiment repeated twice. The rats were placed in a Plexiglas cage ventilated with holes of 1 cm diameter; exposure cage was made in such a way that the animals were comfortably placed. For 2.45 GHz exposure, the chamber was lined with radar absorbing material (attenuation, 40 db) to minimize the reflection of the scattered beam. The exposure cage was placed vertically, so that all animals were irradiated homogeneously with the same power level. Rats were exposed with 2.45 GHz radiations source at 50 Hz modulation frequency (Input 1080 W, Output 700 W). Microwave oven [Haier India Co. Ltd, made in PRC (China) (Model No. HR-18MS1)] was used as a source of exposure, through the horn antenna, 2 h a day for 45 days.

2.4 Specific absorption rate (SAR) calculation

The emitted power of microwaves was measured by a power meter which is a peak sensitive device [RF power sensors 6900 series and IFR 6960 B RF power meter; made of Aeroflex, Inc., Wichita, Kansas, USA]. Every day the cage was placed in the same position below the horn antenna and the same numbers of rat positions were reshuffled. A similar experiment was performed with sham-exposed animals without energizing the system. Similar experimental set-up and procedures were earlier adopted by Paulraj and Behari (2004, 2006) and Kesari and Behari (2010b). The full description of the exposure setup with diagram is mentioned earlier (Kesari et al, 2010b). For a plane wave exposure having random polarisation and power density of 0.21 mW/cm^2, the SAR value turns out to be 0.038 W/kg.

2.5 Melatonin Estimation

Melatonin level in pineal gland was estimated by ELISA kit developed by Uscn life science Inc. In this, 50 µl each of dilutions of standard, blank and samples were added to pre coated wells with polyclonal antibody specific for rat melatonin followed by addition of 50 µl detection reagent A to each tube. After incubation for one hour at 37^0C, wells were washed with wash solution three times. 100 µl of detection reagent B was added to each well and incubated for 30 min at 37^0C. Plate was washed again followed by addition of substrate solution to each well and incubation for 15 min at 37^0C. The color development was stopped by addition of stop solution and the intensity of the yellow color measured by spectrophotometer (450nm). Concentrations of the unknown samples were calculated by comparison with a standard curve.

2.6 Creatine kinase assay

The CK level was estimated using the CK kit. Whole brain was homogenized and washed with ice cold imidazole buffer (0.15 M NaCl and 0.03 M imidazole, pH 7.0 at a ratio of 1:15). The supernatant was decanted after centrifugation at 500*g*, and pellet was resuspended in a 0.1% triton X-100 detergent solution using a vortex for 20 seconds. The sample was centrifuged again at 500*g,* and supernatant was analyzed for CK activity. In the assay, creatine is enzymatically converted to sarcosine which is then specifically oxidized to generate a product that converts a colorless probe to an intensely red color product, which is easily detected calorimetrically (λmax = 570 nm).

2.7 Measurement of caspase 3 activity

The activity of caspase 3 was measured using the colorimetric caspase 3 assay kit. Briefly, the homogenized whole brain was centrifuged at 300*g* for 10 min at 4^0C. The pellet was then resuspended in lysis buffer for 20 min and centrifuged at 20,000*g* for 20 min at 4^0C, and the supernatant was collected. The assays were conducted in 96 wells plates, and all the measurements were carried out in duplicate. The caspase 3 colorimetric assay is based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by caspase 3, resulting in the release of the p-nitroaniline (pNA) moiety. To assess the specific contribution of caspase 3 activity, Ac-DEVD-pNA substrate (2 mM), was added to each well, according to the manufacturer's protocol. The plates were incubated overnight at 37^0C to measure caspase 3 activity. Absorbance was measured with a microplate reader (Spectromax M_2) 405 nm. Caspase 3 activity was expressed in µmol pNA released per min per ml of cell lysate at 37^0C.

3. Results

3.1 Melatonin

An average concentration of melatonin in the pineal gland of mobile phone exposed group (53.56 ± 9.20 pg/ml) was found significantly lower ($P < 0.002$) as compared with the average concentration of melatonin in the control group (81.03 ± 8.01 pg/mg of protein).

3.2 Creatine kinase

The mean value of CK level was higher ($P < 0.001$) in exposed group (1.80 ± 0.11 IU/mg of protein) of brain as compared with sham exposed ones (1.22 ± 0.17).

3.3 Caspase 3 activity

A statistically significant activation of caspase-3 was observed in mobile phone exposed animals. Brain caspase activity showed significant increase ($P < 0.015$) in the exposed group (46.83 ± 1.83) as compared to the sham-exposed ones (44.05 ± 1.43).

4. Discussion

This study provides three important findings relating to oxidative damage and tumor promotion due to microwave exposure. Firstly, we demonstrated that microwave radiations decreases the level of melatonin in pineal gland. Secondly an increase in the level of creatine kinase was observed in brain cells. Third and final finding was caspase 3 which showed an increased level in the whole brain. All these parameters are mutually related. The secretion of hormones and enzyme metabolisms from various organs (pineal gland and whole brain) is controlled by central nervous system (CNS). This interaction may lead to free radical formation. When there is an imbalance between production of ROS and its neutralization, it leads to oxidative stress. Such conditions can lead to necrosis or apoptosis leading to tumor promotion. Parameters presented here points toward possibility of variation in level of PKC, melatonin, CK and caspase 3. Melatonin plays a central role in the biologic effects of RF-EMF interaction (Wilson et al. 1989; Stevens et al. 1992). Melatonin is not only known to suppress the growth of certain malignant cells and tissues, but is also a very potent scavenger for oxygen-derived free radicals that cause severe damage to biomolecules (Reiter et al. 2004; Loots et al. 2005; Lee et al. 2005). On the other hand creatine kinase (CK) may catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to ADP, and regenerate ATP. This enzyme activity plays a key role in energy metabolism of tissues with intermittently high and fluctuating energy requirements, such as skeletal, cardiac, and neuronal tissues like brain and retina (Wallimann et al. 1992). There are distinct CK isoenzymes, which are compartmentalized specifically in the places where energy is produced or utilized (Wallimann et al. 1998). Because energy is necessary to maintain the development and regulation of cerebral functions, it has been postulated that alteration in CK activity may participate in a neurodegenerative pathway leading to neuronal loss in the brain (Tomimoto et al. 1993).

It is suggestive that overproduction of reactive oxygen species could be a key factor in all these events. These data clearly have important implications for the safety of radio frequency microwave radiations use and highlights the potential importance of RF-EMF in the etiology of brain physiology.

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