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Biochemical and histological studies on adverse effects of mobile phone radiation on rat's brain

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ABSTRACT

With the rapid development of electronic technologies, the public concern about the potential health hazards induced by radiofrequency (RF) radiation has been grown. To investigate the effect of 1800 MHz RF radiation emitted from mobile phone on the rat's brain, the present study was performed. Forty male rats were randomly divided into two equal groups; control and exposed group. The later one exposed to 1800 MHz emitted from mobile phone with an SAR value of 0.6 W/kg for two hours/day for three months. The brain tissues were collected at the end of the experimental period and separated into hippocampus and cerebellum for subsequent biochemical, histological, immunohistochemical and electron microscopic investigations. The rats that were exposed to RF- radiation had a significant elevation in MDA content and a significant reduction in antioxidant parameters (glutathione, super oxide dismutase and glutathione peroxidase) in both regions. Degenerative changes were observed in the hippocampus pyramidal cells, dark cells and cerebellar Purkinje cells with vascular congestion. In addition a significant DNA fragmentation and over expression of cyclooxygenase-2 apoptotic gene was detected. Those results suggested that, direct chronic exposure to mobile phone caused severe biochemical and histopathological changes in the brain.

1. Introduction

The mobile phone is one of the major inventions which have changed the way of communication in today's world. There is accumulating evidence reported that exposure to the radiofrequency (RF) radiation emitted from mobile phones and or their base stations could affect people's health (Hao et al., 2015). People generally hold their mobile phones close to the head in the talking mode and this causes a higher exposure of the brain to the RF-radiation than other parts of the body. The brain tissue is a major potential route for the absorption of hazardous materials encountered in the environmental place (Irmak et al., 2002). In addition the brain is one of the most sensitive target organs of RF-radiation, where the mitochondrial injury occurs earlier and more severely than in other organs (Hao et al., 2015). The radiation emitted from mobile phone could be absorbed by neural tissue more than other tissues (Irmak et al., 2002). RF-radiation could affect individuals by increasing free radical production, which enhances the lipid peroxidation (LPO) leading to oxidative damage (Ozben, 2007). RF-radiation might disturb reactive oxygen species (ROS) production by decreasing antioxidant enzymes activity or elevating ROS production. It has been reported that RF-radiation generates ROS by stimulating cell membrane nicotinamide adenine dinucleotide (NADH) oxidase and causes production of extracellular superoxide leading to oxidative stress and subsequent cellular damage (Consales et al., 2012). The continuous produced ROS are scavenged by different antioxidant enzymes such as SOD, GPX, and catalase (Ozguner et al., 2005). Under some circumstances, the endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue (Kovacic and Somanathan, 2010). Moreover, the over ROS production can harm cells by depleting enzymatic and/or non-enzymatic antioxidants (Kong and Lin, 2010). The high metabolic rate and the composition rich in polyunsaturated fatty acids, the target for ROS, make the brain more sensitive to oxidative damage (Ozmen et al., 2007). RF-radiation is known to induce oxidative stress, which in turn activates the apoptotic pathway (Ozben, 2007). The hippocampus is the valuable part of the brain cerebrum that controls the behavioral and cognitive functions, including spatial learning and memory (Fortin et al., 2002). Bolla (2015) shown that mobile

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phone radiation can cause damage to hippocampus leading to hyperactivity and difficulty learning. Based on subsequent data presented here the present study was designed to investigate the effect of chronic RF-radiation exposure (frequency 1800 MHz, specific absorption rate 0.6 W/kg) emitted from the mobile phone on oxidative stress and apoptosis through the biochemical, histological, immunohistochemical, electron microscopy in hippocampus and cerebellum of adult rats.

2. Materials and methods

2.1. Animals used

Forty apparently healthy adult male rats (130–150 g) of relatively equal age obtained from the International Institute of Oncology, Cairo University was used in the present study. The rats were kept in the same environmental condition (temperature, 24–26 °C; humidity, 55–60%, on a 12:12-h light/darkness, away from any external noise, fed standard food pellet and water ad libitum) in the Faculty of Veterinary Medicine, Cairo University. All rats were carried out in accordance with the guide to the Care and Use of Laboratory Animals published by the Material Institute of the Health and approved by the Animal Experimental Local Ethics Committee at Cairo University. The rats were divided randomly into two equal groups. The group I: Control group was placed in four cages (five rats per cage) in a separate room away from any mobile phones. Group II: Exposed group was placed also in four cages (five rats per cage). One mobile phone was placed in the bottom of each cage at the center under a wire mesh to give maximum exposure near the brain. At the end of the experiment, the animals sacrificed by cervical dislocation and the brain were carefully dislocated and separated into hippocampus and cerebellum for subsequent analysis.

2.2. Exposure device

The Radiation for this study (1800 MHz) was provided by mobile phone with specific absorption rate (SAR) value of 0.6 W/kg (according to the user guide of the mobile phone, and with electric field 0.87 mw/cm^2 at 5 cm away from the mobile phone) estimated by Radiofrequency meter. During the experimental period, the rats were exposed to RF-radiation emitted by a mobile phone continuously for 120 min/day for three months.

2.3. Oxidative stress parameter measurements

Specimens from brain tissue (hippocampus and cerebellum separately) were weighted and homogenized with Teflon tissue Homogenizer. The samples were homogenized in cold phosphate buffered saline (pH 7.4) using Teflon Homogenizer. The homogenates were centrifuged at 14,000 × *g* for 15 min at 4 °C. The supernatant was used to measure the neuronal MDA (Ohkawa et al., 1979), glutathione peroxidase (GPX) activity (Rotruck et al., 1973), superoxide dismutase (SOD) activity (Marklund and Marklund, 1974), reduced glutathione (GSH) concentration (Ellman, 1959) and estimation of protein content (Bradford, 1976).

2.4. General histological and histochemical study

After sacrificing of the rats, the different parts of the brain (hippocampus **a**nd cerebellum) were immediately dissected out and sectioned into small pieces. These specimens were fixed in neutral buffered formalin. They were processed by dehydration in ascending graded of alcohol, xylene and embedded in periplast. Serial and step-serial sections of 5–6 μm thick were obtained and stained with Haematoxylin and Eosin (H&E) and Mallory's phosphotungstic acid-Haematoxylin. The later stain neurons-salmon and Myelin & glial nerve fiber-blue as adopted by (Bancroft and Gamble, 2008).

2.5. Immunohistochemistry for cyclooxygenase-2 (COX2) protein

Different Brain region sections were deparaffinized in xylene and rehydrated in graded alcohol. Drops of Hydrogen Peroxide Block (Thermo Scientific, USA) were added to block the endogenous peroxidase activity. The tissues were pretreated with 10 mM citrate buffer, pH 6.0 in the microwave oven at 500 W for 10 min for antigenic retrieval. The slides were washed with PBS, and blocked with ultra V Blocking solution (Thermo scientific, USA) for 5 min. Sections were incubated overnight at 4 °C in a humidified chamber with the following primary antibodies rabbit anti-COX2 polyclonal antiserum (Cayman Chemical, Ann Harbor, MI) at a 1:50 dilution. The sections were rinsed again with PBS, then incubated with a biotinylated goat anti-rabbit antibody (Thermo Scientific, USA) for 10 min. The sections were rinsed again with PBS. Finally, sections were incubated with Streptavidin peroxidase (Thermo scientific, USA). To visualize the reaction, slides were incubated for 10 min with 3, 3′ diaminobenzidinetetrahydrochloride (DAB, Sigma). The slides were counterstained with hematoxylin then dehydrated and mounted. Primary antibodies were omitted and replaced by PBS for negative controls.

2.6. Transmission electron microscopy

Small tissue blocks from the different parts of the brain (hippocampus and cerebellum) were fixed in paraformaldehyde-glutaraldehyde in phosphate buffer (Karnovsky, 1965). Specimens were post-fixed in 1% osmium tetraoxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated in graded ethanol and embedded in an open Araldite mixture (Mollenhauer, 1964). Semi-thin sections (1 μm) were cut, stained with Toluidine Blue (Richardson et al., 1960) and examined with the light microscope. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate. The sections were examined with a JEOL 1010 transmission electron microscope at Regional Center for the Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo, Egypt.

2.7. Genomic DNA fragmentation

Quantitative DNA fragmentation percentage assay and DNA laddering were determined calorimetrically by diphenylamine assay and agarose gel electrophoresis as previously described by Ogaly et al. (2015). Hippocampus and cerebellum were lysed separately in hypotonic lysis buffer pH 8.0. Lysates were centrifuged for 10 min. The supernatant containing small DNA fragments was separated from the pellet of intact DNA and divided into two portions one for electrophoretic analysis using agarose gel electrophoresis for DNA laddering. Another portion and pellet were precipitated with trichloroacetic acid and then were centrifuged. After centrifugation, the supernatant was resuspended in two volumes of diphenylamine solution. Samples were stored at 4 °C for 48 h and measured spectrophotometry at 578 nm.

2.8. Statistical analysis

The different analytical determinations in the biological samples were carried out in duplicate and results are expressed as the mean ± SE. Student's *t*-tests were performed to determine whether differences between the two groups were statistically significant ($p < 0.05$) using SPSS version 16 packages for Windows.