HAYATI Journal of Biosciences xxx (2017) 1–8

HOSTED BY

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

Micronuclei Formation and 8-Hydroxy-2-Deoxyguanosine Enzyme Detection in Ovarian Tissues After Radiofrequency Exposure at 1800 MHz in Adult Sprague–Dawley Rats

Ali Saeed Hammoodi Alchalabi,[1,2*] Hasliza Rahim,[3] Mohamed Fareq AbdulMalek,[4] Erkihun Aklilu,[1] Abd Rahman Aziz,[1] Suzanna Harun Ronald,[5] Mohd Azam Khan[1]

[1] *Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag 36, 16100 Kota Bharu, Kelantan, Malaysia.*
[2] *Veterinary Medicine College, Mosul University, Mosul, Iraq.*
[3] *Bioelectromagnetics Research Group (BioEM), School of Computer and Communication Engineering, Universiti Malaysia Perlis (UniMAP), Pauh Putra, Arau, Perlis 02600, Malaysia.*
[4] *University of Wollongong in Dubai, Faculty of Engineering and Information Sciences, Block 15, Dubai Knowledge Park, P. O. Box 20183, Dubai, United Arab Emirates.*
[5] *Bioelectromagnetics Research Group (BioEM), Centre of Diploma Studies, Universiti Malaysia Perlis (UniMAP), S2-L1-26, Kampus UniCITI Alam Sg. Chuchuh, 02100 Padang Besar (U), Perlis, Malaysia.*

ARTICLE INFO

Article history:
Received 5 July 2017
Received in revised form
22 August 2017
Accepted 22 August 2017
Available online xxx

KEYWORDS:
cytological changes,
GSM frequency,
micronuclei formation,
oxidative DNA damage

ABSTRACT

Human fertility and its correlation to ovarian function and cytological changes are linked to ever-increasing use of mobile phones. Wireless communications have become a critical topic of concern because of an increasing number of studies in this field with controversial outcomes. The aim of this study was to assess the genotoxic effect of GSM frequency at 1800 MHz on ovarian function. Sixty female Sprague–Dawley rats were distributed over six groups (control group and the exposure groups with whole-body exposure for 2 h/day, 7 days/week for 15, 30 and 60 continuous days). The study investigated the oxidative stress, 8-hydroxy-2-deoxyguanosine enzyme, micronuclei formation and histopathological changes in ovarian tissue. The results showed an induced oxidative stress via an increase in lipid peroxidation and decreased antioxidant enzyme activity. There was also an elevation in the 8-hydroxy-2-deoxyguanosine enzyme and an increased rate of micronuclei formation in ovarian tissues of exposed animals with 60-day exposure compared with control animals. Cytological changes were recorded such as micronuclei formation, vacuolation, degeneration and impaired folliculogenesis. The study suggests that GSM frequency at 1800 MHz was negatively impacted on female reproductive performances mediated by oxidative stress induction and 8-hydroxy-2-deoxyguanosine formation leading to overall impaired ovarian function.

1. Introduction

Radiofrequency (RF) radiation at 1800 MHz is the worldwide frequency used in mobile phone communication and is characterized by low energy because of the nature of this radiation (non-ionizing radiation). A total of 101 published articles related to the genotoxic effect of RF radiation were reviewed in an analytic study to investigate the genotoxicity of RF radiation on a biological system, including both *in-vitro* and/or *in-vivo* studies (Ruediger 2009). In this review, 48.58% suggested a genotoxic effect, whereas 41.58% indicated that there is no effect, and 9.9% failed to find any influence on genetic material.

The process of carcinogenesis is mediated by changes in hereditary material in somatic cells, and this is why any agent (chemical and non-chemical) which contributes to alterations of DNA or induces DNA damage could be considered as a carcinogenic agent (Verschaeve *et al.* 2010; Verschaeve and Maes 1998). The International Agency for Research on Cancer in 2011 highlighted at their meeting in France that RF electromagnetic fields

* Corresponding author. Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag 36, 16100 Kota Bharu, Kelantan, Malaysia.
E-mail address: alisaeedchalaby@yahoo.com (A.S.H. Alchalabi).
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2017.08.007

(EMFs) are possibly carcinogenic to humans based on intensive studies on brain cancer incidence in humans (Baan *et al.* 2011). The risk of cancer development among wireless communication users can be used as an indicator for risk assessment in human studies, especially with modern wireless technologies (Tsybulin *et al.* 2013).

There are different methods used to assess and evaluate the genotoxicity of RF-EMFs in the biological system. For instance, Khalil *et al.* (2012) investigated the presence of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in the urine of male Sprague–Dawley rats. They found that one major form of oxidative DNA damage is 8-oxodG and that the urine level was higher in exposed rats after 1 h of irradiation, suggesting a repair of the DNA lesions leading to 8-oxodG formation. Formation of free radicals such as hydroxyl radical (HO^-), which is a potent oxygen-free radical, negatively affects large molecules in an animal cell (proteins, lipids and DNA) and induces damage. The interaction of HO^- with DNA nucleoside guanine leads to formation of 8-hydroxy-2-deoxyguanosine enzyme (8-OHdG; Valavanidis *et al.* 2009). Formation of reactive oxygen species in liver tissue because of exposure to RF radiation leads to the production of the 8-OHdG enzyme as a result of DNA damage (Güler *et al.* 2012). However, other team researchers found that mobile phone radiation had no effect on the 8-OHdG level in liver tissue, there was an increase in malondialdehyde (MDA) level as an oxidative stress biomarker in exposed animals (Tomruk *et al.* 2010). Karihtala *et al.* (2009) found that free radical activation, especially OH^- plays an important role in oxidative DNA damage in ovarian carcinoma and DNA 8-OHdG enzyme elevation is a potent prognostic factor in ovarian carcinoma. HL-60 leukaemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts were irradiated by 50-Hz extremely low frequency (ELF) EMF for up to 3 days to induce DNA damage in cell lines and to assess the 8-OHdG as a biomarker for DNA damage (Wolf *et al.* 2005). The researchers found that the effect of ELF-EMF induced DNA damage in a dose-dependent manner was mediated by the activation of free radical formation, and the study suggests that ELF-EMF leads to cell proliferation and DNA damage in studied cell lines via the reactive oxygen species activity.

Wahab (2005) used a micronuclei formation method to evaluate genotoxicity in human peripheral lymphocytes. The author found that ELF-EMF has a clastogenic effect on human chromosomes, which was confirmed by other assessment methods, such as sister chromatid exchange and comet assay. Another study was performed on peripheral blood samples from healthy donors to assess the impact of modulated and unmodulated 2450 MHz RF fields on the induction of micronuclei in human peripheral blood lymphocytes (Reddy *et al.* 2013). They found that modulated and unmodulated 2450 MHz RF exposure did not induce excess micronuclei in human blood lymphocytes, and both types of exposure had the same effect. Amâncio *et al.* (2006) irradiated rats during pregnancy to check whether ultra-high frequency EMF from a cellular phone during their embryogenesis induced chromosomal damage in erythrocytes from rat offspring. The study revealed that ultra-high frequency EMF increased the incidence of micronuclei formation in erythrocytes of offspring. Male rats exposed to 10 GHz of microwave radiation for 2 h/day for a continuous 45 days showed formation of the micronuclei body in lymphocytes detected by a flow cytometry technique (Kumar *et al.* 2013). Different techniques were used to assess the genotoxicity of RF-EMF on the biological system with contradictory results.

The objectives of this study was to investigate the occurrence of micronuclei formation in ovarian tissue and detection of 8-OHdG level in ovaries, a biomarker for genotoxicity from mobile phone radiation in an adult female rat model.

2. Materials and Methods

2.1. Animals

The project was approved by the scientific committee of the Fakuliti Perubatan Veterinar (FPV) of the University Malaysia Kelantan (UMK) and was performed in accordance with the UMK guidelines for animal experiments (FPV-PGSC-2015). The study used 2-month old Sprague–Dawley rats of an average body weight (b.w.) of 200 g. Oestrus synchronization was performed before starting the experiment. Sixty female Sprague–Dawley rats were distributed over six groups (control group and the exposure groups as whole-body exposure for 2 h/day, 7 days/week for 15, 30 and 60 continuous days). The animals were bred in the laboratory animal research unit of the FPV/UMK. Animals were kept in plastic cages in a light/dark cycle 12–12 h at room temperature 25 ± 1°C and humidity 60% ± 10% (relative humidity) with tap water and standard rat pellets that were provided *ad libitum*.

2.2. RF-EMF setup

RF-EMR exposure was performed as whole-body exposure at a 1800 MHz GSM frequency of a mobile phone using PSG vector signal generators (Agilent Technologies E8267D, 250 KHz–20 GHz, Santa Clara, CA, USA) with an integrated pulse modulation unit. The signal source of the mobile phone antenna was a standard horn antenna (A-INFO Standard Gain Horn Antenna 1.7–2.6 GHz WR430, Beijing, China) at an specific absorption rate (SAR) level of 0.974 W/kg that was calculated using the following equation:

$$SAR = (\delta/P)\, E^2$$

where E is the magnitude of the electric field 28.156 V/m, δ is conductivity 1.34 S/m and ρ is the mass density of the tissue-equivalent media 1090 kg/m^3. The exposure setup was as described by a previous publication (Alchalabi *et al.* 2016; Figure 1).

2.3. Sampling

At the end of the experiment, all rats were weighed and anesthetized with an intraperitoneal injection of ketamine and xylazine combination at a dose 0.1 mL/100 g b.w. at a dose 80 mg/kg b.w. ketamine and 5 mg/kg b.w. xylazine. Blood samples were collected via bleeding from the eye angle (Van Herck *et al.* 1998). Blood samples were collected in plain tubes without anticoagulant for serum preparation, spun at 11,000 rpm for 10 min and the sera was placed in serum tubes, labelled and stored at 20°C until later biochemical analysis.

Ovaries were removed, washed by ice-cold phosphate-buffered saline, and right ovary samples were fixed in 10% neutral buffered formalin for 24 h before starting the process of preparation for histological slides. Specimens were dehydrated, cleared with xylene and processed in tissue blocks. Sections at six microns were prepared, stained with H&E stains and examined under a light microscope to count ovarian follicles. Thirty slides/groups were prepared to count ovarian follicles. Each slide contained three sections from each ovary (Gul *et al.* 2009). Digital histopathology using slide scanner was used to evaluate ovarian tissue slides. The diagnosis of micronuclei foci in ovarian follicles was confirmed by a histologist and pathologist in the FPV/UMK.

2.4. Biochemical analysis

For oxidative stress assessment, kits were purchased from Cusabio Biotech Co., Ltd. (Wuhan University Science, Wuhan, China) and Abcam (Cambridge Science Park, Cambridge, UK) for biochemical analysis. MDA was assessed as a lipid peroxidation biomarker by using a Lipid Peroxidation (MDA) Assay Kit (ab119870, Abcam, UK) according to the manufacturer's

Please cite this article in press as: Alchalabi, A.S.H., et al., Micronuclei Formation and 8-Hydroxy-2-Deoxyguanosine Enzyme Detection in Ovarian Tissues After Radiofrequency Exposure at 1800 MHz in Adult Sprague–Dawley Rats, HAYATI J Biosci (2017), http://dx.doi.org/10.1016/j.hjb.2017.08.007