Journal of Chemical Neuroanatomy xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Neurodegenerative changes and apoptosis induced by intrauterine and extrauterine exposure of radiofrequency radiation

Göknur Güler [a], Elcin Ozgur [a,*], Hikmet Keles [b], Arin Tomruk [a], Sevil Atalay Vural [c], Nesrin Seyhan [a]

[a] Department of Biophysics, Gazi University School of Medicine and Gazi Non-Ionizing Radiation Protection Center, 06500 Ankara, Turkey
[b] Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200 Afyonkarahisar, Afyon, Turkey
[c] Department of Pathology, Faculty of Veterinary Medicine, Ankara University, 06110 Ankara, Turkey

ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form 14 October 2015
Accepted 15 October 2015
Available online xxx

Keywords:
Mobile phone
Apoptosis
DNA damage
8-OhdG
Lipid peroxidation
TUNEL

ABSTRACT

Adverse health effects of radiofrequency radiation (RFR) on the ongoing developmental stages of children from conception to childhood are scientifically anticipated subject. This study was performed to identify the effects of global system for mobile communications (GSM) modulated mobile phone like RFR in 1800 MHz frequency on oxidative DNA damage and lipid peroxidation beside the apoptotic cell formation, using histopathological and immunohistochemical methods in the brain tissue of 1-month-old male and female New Zealand White rabbits that were exposed to these fields at their mother's womb and after the birth. Oxidative DNA damage and lipid peroxidation levels were investigated by measuring the 8-hydroxy-2′-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) levels, respectively. Histopathological changes were observed using by hematoxylin and eosin (HE) staining. Apoptotic cells were detected in the examined organs by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining.

For both male and female infants; 8-OHdG levels increased in the group exposed to RFR in both intrauterine and extrauterine periods compared to the infants that were never exposed to RFR and the ones were exposed when they reached one month of age ($p < 0.05$). MDA results were different for male and female rabbits. There was no difference between all female infant groups ($p > 0.05$), while only intrauterine exposure significantly causes MDA level increase for the male infants. HE staining revealed mild lessions in neuronal necrobiosis in brain tissues of female rabbits that had only intaruterine exposure and male rabbits had only extrauterine exposure. Gliosis were mildly positive in brain tissues of rabbits that are exposed only intrauterine period, also the group exposed both intrauterine and extrauterine periods. However, there was no apoptotic change detected by TUNEL staining in the brain tissues of all groups.

1. Introduction

Part of the electromagnetic spectrum comprising the frequency range from 100 kHz to 300 GHz may be named as high frequency (HF) or radiofrequency (RF) radiation. Mobile phones operate in this range of the electromagnetic spectrum, from several hundred MHz to several GHz, to enable wireless phone calls and data transfer, including communication through the internet. The exact frequency band used differs between technologies (GSM, UMTS, 4G, etc.) and between countries (ICNIRP, 2015).

Increasing use of mobile phones cause to ascend the public concern about the possible ill-effects of mobile phone radiation especially on children and teenagers, beside the sensitive people such as pregnant women and the babies. Although it may be stated as if there is scientific uncertainty potential health hazard of low-energy radiofrequency radiation (RFR) emitted by mobile phones, International Agency for Research on Cancer (IARC) published a release in France at May 31, 2011 has classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (Group 2B), based on an increased risk for glioma, a malignant type of brain cancer, associated with wireless phone use (Hietanen, 2006). At the time of the IARC review it was known that when mobile phone use began as a teenager, the risks were higher than when use began as

* Corresponding author at: Gazi Üniversitesi Tıp Fakültesi Biyofizik Abd, Dekanlık Binası 5., Kat 06500 Beşevler, Ankara, Turkey. Tel.: +90 312 202 46 02; fax: +90 312 212 90 23.
E-mail address: elcin.ozgur@gmail.com (E. Ozgur).

http://dx.doi.org/10.1016/j.jchemneu.2015.10.006
0891-0618/

an adult (Hardell and Carlberg, 2009; Hardell et al., 2006). Since then, additional evidence has accrued of an increased risk to children (Morgan et al., 2015).

Our previous studies revealed the evidence on the possible biological effects in several tissues of both non-pregnant and pregnant New Zealand White rabbits and in their newborns (Guler et al., 2010, 2011; Tomruk et al., 2010; Kismali et al., 2012) and 1-month-old infants (Guler et al., 2012; Ozgur et al., 2013) that are exposed to whole body 1800 MHz GSM-like RFR (Fig. 1).

This study is also designed to study the same level of RFR on the oxidative DNA damage, lipid peroxidation levels and the apoptotic cell formation by using histopathological and immunohistochemical methods in the brain tissues of 1-month-old infant rabbits. Here, we focused on two exposure scenarios: intrauterine (IU) (pre-natal) and extrauterine (EU) (postnatal) exposure to mobile phone-like RFR.

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Durackova, 2009; Reuter et al., 2010). Since repair of almost all of the biomolecules depends on the information coded in the DNA, there is a postulated importance of oxidative DNA damage. DNA damage may be quantified by the level of 8-hydroxydeoxyguanosine (8-OhdG), which is most widely used fingerprint of radical attack toward DNA (Marnett, 2000; Wiseman and Halliwell, 1996). Proteins and lipids are also significant targets for oxidative attack, and modification of these molecules can increase the risk of mutagenesis (Schraufstatter et al., 1988). One of the main biomarkers widely used in determination of oxidative destruction on lipids mediated by second messengers is malondialdehyde (MDA) (Nair et al., 1986; Draper and Hadley, 1990). Levels of 8-OhdG and MDA were analyzed in the present study in order to identify the oxidative DNA damage and lipid peroxidation.

Cancer initiation and progression has been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation (Visconti and Grieco, 2009). Dysregulated cell proliferation rate, in other words dysfunction in apoptosis is directly related to tumor development. Apoptosis, also termed "programmed cell death" is the necessary mechanism complementary to proliferation that ensures homeostasis of all tissues (Larsson et al., 2010). Our previous reports showing histopathological changes due to 1800 MHz RFR exposure were observed in the brain, eyes, liver, kidneys, lung, heart, and spleen of non-pregnant and pregnant rabbits and their newly born babies (Guler et al., 2011). In this study, brain tissue was histopathologically examined by haematoxylineosin (HE) staining in the brain tissues of the one-month-old infants of the pregnant rabbits. Apoptotic cell formations were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining.

To clarify the possible link between RFR and health effects, scientists have been investigating this problem more than 20 years. Most of the recent epidemiological and experimental (in vivo/in vitro) studies have indicated that acute or chronic exposure in different frequency ranges may alter biological responses including cell cycle (Cleary et al., 1996), cell proliferation (Cleary et al., 1990; Kwee and Raskmark, 1998; Velizarov et al., 1999), apoptosis (Marinelli et al., 2004; Zhao et al., 2007), and DNA damage (Diem et al., 2005; Lai and Singh, 1995, 1996, 1997; Tice et al., 2002).

In the present study, the principal aim was to design the continual RF exposure and investigate the possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. The levels of lipid peroxidation and DNA damage based on free radical attacks were analyzed, beside the histopathological examination and apoptosis detection carried out in the brain tissues of baby rabbits aged one month.

2. Materials and methods

2.1. Animals

A total of 72 one-month-old female and male New Zealand white rabbits were used in this study. The animals were obtained from the Laboratory Animals Breeding and Experimental Research Center of Gazi University. The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University (G.U.ET-06.027). Thirty-six of the infant rabbits were exposed to 1800 MHz GSM-like RF radiation for 15 min/day during a week in the intrauterine period (between 15th and 22nd days of the gestational period when the transition from embryogenesis to organogenesis takes place) whereas others were not exposed.

After birth, all 72 infant rabbits were kept with their mothers until they reached one month of age. They were breastfed and their optimum growth was obtained during this one-month period. Baby rabbits aged one month were housed under the same conditions in a temperature and humidity-controlled room (20 ± 1 °C, $50 \pm 10\%$ relative humidity) and 14/16 h light/dark cycle conditions. The animals were provided with tap water and standard pelletized food ad libitum except during exposure periods. Only one animal was placed in each cage during each radiofrequency radiation (RFR) exposure period because placing more than one animal in a cage could have created stress.

2.2. Exposure level and quality control

GSM-like signals in 1800 MHz frequency were formed by using a signal generator (Agilent Technologies 8648C, 9 kHz–3.2 GHz) with the integrated pulse modulation unit and horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5–2.8 GHz). The generated power was controlled by a spectrum analyzer (Agilent Technologies N9320A, 9 kHz–3 GHz) integrated to the signal generator. The signals were amplitude-modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 ms), corresponding to the dominant modulation component of the GSM.

RFR generator provided 0.1 W (20 dBm) during the exposure period. The signal was controlled by means of the spectrum analyzer connected to the signal generator, and NARDA EMR 300 and type 26.1 probe were used for measurement of the output radiation. Measurements were taken during the entire experiment

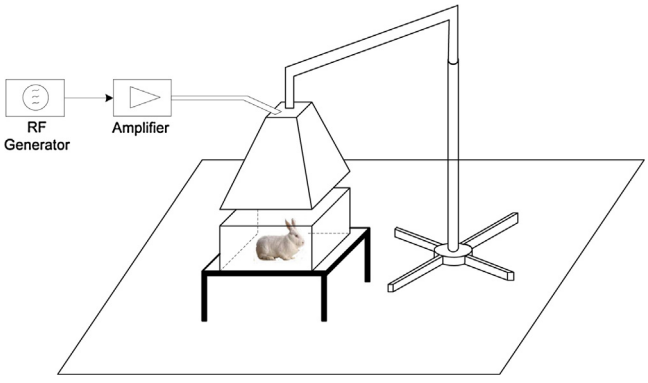

Fig. 1. Schematic view of exposure set-up.