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Adverse effects in lumbar spinal cord morphology and tissue biochemistry in Sprague Dawley male rats following exposure to a continuous 1-h a day 900-MHz electromagnetic field throughout adolescence

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ABSTRACT

Cell phones, an indispensable element of daily life, are today used at almost addictive levels by adolescents. Adolescents are therefore becoming increasingly exposed to the effect of the electromagnetic field (EMF) emitted by cell phones. The purpose of this study was to investigate the effect of exposure to a 900-MHz EMF throughout adolescence on the lumbar spinal cord using histopathological, immunohistochemical and biochemical techniques. Twenty-four Sprague Dawley (28.3–43.9 g) aged 21 days were included in the study. These were divided equally into three groups – control (CG), sham (SG) and electromagnetic (ELMAG). No procedure was performed on the CG rats until the end of the study. SG and ELMAG rats were kept inside an EMF cage (EMFC) for 1 h a day every day at the same time between postnatal days 22 and 60. During this time, ELMAG rats were exposed to the effect of a 900-MHz EMF, while the SG rats were kept in the EMFC without being exposed to EMF. At the end of the study, the lumbar regions of the spinal cords of all rats in all groups were extracted. Half of each extracted tissue was stored at −80 °C for biochemical analysis, while the other half was used for histopathological and immunohistochemical analyses. In terms of histopathology, a lumbar spinal cord with normal morphology was observed in the other groups, while morphological irregularity in gray matter, increased vacuolization and infiltration of white matter into gray matter were pronounced in the ELMAG rats. The cytoplasm of some neurons in the gray matter was shrunken and stained dark, and vacuoles were observed in the cytoplasms. The apoptotic index of glia cells and neurons were significantly higher in ELMAG compared to the other groups. Biochemical analysis revealed a significantly increased MDA value in ELMAG compared to CG, while SOD and GSH levels decreased significantly. In conclusion, our study results suggest that continuous exposure to a 900-MHz EMF for 1 h a day through all stages of adolescence can result in impairments at both morphological and biochemical levels in the lumbar region spinal cords of Sprague Dawley rats.

1. Introduction

Although the telephone has changed in shape and size over the years, it has become and remains an inseparable means of communication and part of daily life. While the use of land lines has decreased with advances in technology, the use of wireless cell phones is increasing all the time. However, the growing use of cell phones, which are popular with all age groups, also causes a number of problems. While adults use cell phones more for work purposes, they have become toys that children and young people are reluctant to be parted from even for a moment (Bianchi and Phillips, 2005; Nikhita et al., 2015). Indeed, half of adolescents even take their cell phones with them to bed (Adachi-Mejia et al., 2014), and college students spend almost 9 h a day on their mobiles

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(Roberts et al., 2014). The increasing use of cell phones under the influence of attractive advertising campaigns and falling prices has led to their becoming the main source of exposure to electromagnetic field (EMF) in children, teenagers and adolescents (Kheifets et al., 2005).

The World Health Organization defines adolescence as the intermediate period between 10 and 19 years when the individual is no longer a child but not yet fully adult (World Health Organization, 2016). It is a transitional period when individuals diversify and mature both physiologically and neurologically and progress from childhood to adulthood (Spear, 2000). As with many systems in the human body, the central nervous system (CNS) that begins developing in the intrauterine period continues to do so after the postnatal (PN) period and throughout adolescence. Cortical and spinal maturation in the nervous system continues until adulthood (Smith et al., 2015; Fietzek et al., 2000). A large number of clinical and experimental studies have therefore investigated the effects of various environmental factors on the CNS in adolescence (Smith et al., 2015; Risher et al., 2015; Weiss et al., 2015; Mitchell et al., 2014). This is particularly because any agent that might impact on normal CNS development during this time may also lead to consequences that affect the rest of life. Although there have been several studies on agents that may cause changes in the CNS in the PN period, the number of studies investigating the effects of exposure to EMF in adolescence is quite limited. Yet EMF emitted by numerous electronic devices, including cell phones, that are overused during adolescence causes oxidative stress and injury in several tissue, such as the liver, heart and testis (Topal et al., 2015; Hancı et al., 2013; Türedi et al., 2015). Rat studies have reported that EMF causes injury leading to oxidative stress in nervous system tissues (İkinci et al., 2016). In addition, the application of 900-MHz EMF has been shown to cause a decrease in neurons in the hippocampus of both adult rats and of pups born to pregnant rats (Bas et al., 2009; Odaci et al., 2008). There are also studies reporting that EMF causes injury in cultured neurons, as well as impairing the blood brain barrier and leading to neuronal degeneration in the brain (Xu et al., 2010; Nittby et al., 2009).

Some authors have reported that the spinal cord can act as an antenna for the effects produced by EMF in the CNS (Balaguru et al., 2012). There is therefore a strong probability that the spinal cord, a component of the CNS that enables constant communication between the brain and peripheral nervous system will also be affected by EMF. Indeed, Odacı et al. (2013) reported impairment in motor function tests and pathological changes in the spinal cord in rat pups exposed to EMF during pregnancy. However, we encountered no studies in the literature investigating the potential effects on the spinal cord of exposure to EMF throughout adolescence.

It has already been established that investigation may provide useful data on the possible adverse effects of 900-MHz EMF on the human child spinal cord since the age of 8–9 weeks in Sprague Dawley rats is comparable to human preadolescence (Tirelli et al., 2003; Şahin et al., 2015). The purpose of this study was therefore to use histological and biochemical methods to investigate changes that may occur in the lumbar region of the spinal cord in male rats following daily 1-h exposure to a 900-MHz throughout adolescence between PN days 21 and 60.

2. Material and methods

2.1. Animals and experimental procedures

The study was performed following approval from the Karadeniz Technical University (KTU) Animal Care and Ethics Committee, Turkey. Twenty-four Sprague Dawley rats aged 21 days and weighing 28.3–43.9 g obtained from the KTU Surgical Research Center were used. These were kept in standard plastic rat cages in the laboratory (room temperature $22 \pm 2\,^{\circ}C$, humidity 50% in a 12-light and 12-h dark cycle) and given standard rat chow and tap water throughout the study. Rats were weighed at the beginning of the study and randomly divided into three equal groups. No procedure was performed on the control group (CG) rats until the end of the study. The sham (SG) and EMF group (ELMAG) rats were placed inside an EMF cage (EMFC) for 1 h a day at the same time every day from PN day to 22 until PN day 60 (SG at 10–11 a.m. and ELMAG at 11–12 a.m.). During this time, the ELMAG rats were exposed to the effect of a 900-MHz EMF, while SG rats were kept in the EMFC but were not exposed to EMF. At the end of the study (on PN day 60), rats from all groups were sacrificed under deep anesthesia (Ketalar® 50 mg/kg, Eczacıbaşı, Turkey). The lumbar region of the spinal cord of all rats was removed. Half of each tissue was stored at $-80\,^{\circ}C$ for biochemical analysis, while the other half was placed in 10% formaldehyde for histopathological investigation. The animals were obtained from the KTU Surgery Research Center, where they were housed during the study and where the tissues were removed.

2.2. EMF application system and EMF exposure

A system we describe as the EMF application system was used for the application of EMF to the ELMAG rats. The EMF application system and the EMFC within it were as described in various previous studies of ours, where all details are available (Türedi et al., 2015; Topal et al., 2015; Odacı and Özyılmaz, 2015). In brief, the cage used in the system and known as the EMFC was made out of Plexiglas and measured $30\,cm \times 42\,cm \times 52\,cm$. A half-wave dipole antenna made out of a 1-mm thick copper rod was inserted to a depth of 11 cm from the center of the open top of the EMFC. The antenna was attached to an ultrahigh-frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, MA, Serial no. 1483) set to an output power of approximately 300 mW and a frequency of 900 MHz via a coaxial cable. The oscillator was attached to an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, serial no. 903) in order to avoid electrical disruptions. The EMF application system was used in order to establish a 900-MHz EMF effect inside the EMFC during EMF application. EMF intensities inside and outside the EMFC were measured using a broadband EMF intensity measuring device (100 kHz–2.5 GHz range, Chauvin-Arnoux C.A 43 Fieldmeter, Chauvin-Arnoux Group, Paris, France) with a range of 100 kHz to 2.5 GHz. Measurements were performed at nine different points on the floor of the EMFC, from both inside and outside the cage, and with the EMFC empty and when it contained rats. These measurements confirmed an uninterrupted 900-MHz EMF effect inside the cage during EMF application.

2.3. Histological staining

Spinal cords set aside for histological staining were decalcified following 1-day fixation in 10% formaldehyde. Tissues were next washed in running water and passed through alcohol series and then made transparent with xylene before finally being embedded in paraffin blocks. For histopathological investigation, 5-μm sections were prepared with the help of a fully automatic microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) and stained with cresyl violet. The sections obtained were analyzed under a light microscope (Olympus BX51; Olympus Co., Tokyo, Japan), and photographs were taken with a digital camera (Olympus DP 71 Olympus Co., Japan) attached to the same microscope.