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Different methods for evaluating the effects of microwave radiation exposure on the nervous system

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ABSTRACT

Microwave radiation (MWR) leads to hazardous effects on he central nervous system (CNS) for both human and animals. The widespread use of mobile phones has increased the risks of health problems in the CNS caused by radiofrequency (RF) electromagnetic fields. To determine these effects various methodological approaches related to neuroscience such as stereology, immunohistochemistry, and electron microscopy have been used. These approaches examine the effects on cells exposed to MWR at the light microscopic and ultrastructural levels, and novel information is obtained. The main aim of this paper is to discuss possible side effects of MWR in the light of current literature with different methodological approaches.

1. Introduction

Current literature asserts that microwave radiation (MWR) leads to hazardous effects on the central nervous system (CNS) for both humans and animals. The most sensitive target tissue for MWR are the brain, and mitochondria, since injury occurs in these tissues earlier than in other organs (Hao et al., 2015). Studies on the effects of MWR brain energy metabolism have aroused great concern. The widespread use of mobile phones causes radiofrequency (RF) and electromagnetic fields (EMFs). Thus, this situation may lead to an increase in health risks to the CNS as well as other organs.

The side effects of MWR occur in two types: thermal and non-thermal. While thermal effects are prominent in cases of high-power and high frequency MWR non-thermal effects predominantly occur in cases of low-power MWR (Adair and Black, 2003; Black and Heynick, 2003; Cao et al., 2004).

The direct effects of the EMF develop thermally by absorbing electromagnetic energy and effecting athermancy after long-term exposure. Also, EMFs are divided into three categories: extremely low frequency, high frequency, and microwave. Microwaves are electromagnetic waves that range between the frequencies of 300 MHz and 300 GHz (Foster and Glaser, 2007; Gaestel, 2010; Yu and Yao, 2010). Similarly, detrimental effects of high-density MWs on health such as the CNS, the cardiovascular system, and the hematopoietic system have been detected by many *in vitro* studies and occur by leading to DNA damage and structural defects to chromatin. Also, it has been reported that prolonged MW exposure may cause neurodegenerative diseases (Deshmukh et al., 2013).

The widespread use of mobile phones has induced the risks of health problems caused by RF and EMFs in the CNS. For example, studies have shown increases in blood–brain permeability, headaches, neuronal loss, glial cell death, impairments in cognitive functions, and abnormalities in neurotransmitters. Whether these effects originated from RF exposure or not, however, is still

Abbreviations: CNS, central nervous system; RF, radiofrequency; EMF, electromagnetic field; MWR, microwave radiation; GHz, gigahertz; MHz, megahertz; CE, coefficient error; CV, coefficient variation; MAPK, mitogen-activated protein kinases; ERK1, extracellular-signal-regulated kinase 1*hsp 27*heat shock protein 27; SDH, succinate dehydrogenase; COX, cytochrome c oxidase; PI3K, phosphatidylinositol 3-kinase*mitochondrial membrane potential*MMP; PC 12, pheochromocytoma cell; DCX, doublecortin; GFAP, glial fibrillary acidic protein; Hz, hertz; SAR, specific absorption rate; W/kg, watts per kilogram; BrdU, 5-bromo-2′-deoxyuridine; dUTP, 2′-deoxyuridine*5′-triphosphate; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling; DNA, deoxyribonucleic acid; CA, cornu ammonis; ELISA, enzyme-linked immunosorbent Assay; PI, propidium iodide; ROS, reactive oxygen specimens; CAT, catalase; SOD, superoxide dismutase; MPO, myeloperoxidase; GSH-Px, glutathione peroxidase; GSH, glutathione; BBB, blood-brain barrier; PUFA, polyunsaturated fatty acids; MWM, Morris Water Maze; OFT, open field test; EPM, elevated-plus maze; TST, tail suspension test; FST, forced swimming test; EMR, electromagnetic radiation..

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controversial (Testylier et al., 2002; Joubert et al., 2008; Nittby et al., 2008; Redmayne et al., 2013). Additionally, the effects of MWR on neuronal development and its results are unknown. What is known is that the developing brain absorbs more MWR than an adult brain, because of higher conductivity since it has higher water content and ion concentration. Hence, studies on the potential effects of RF exposure during the foetal period (including cellular calcium homeostasis, neuronal electrical activity, genomic responses, neurotransmitter release, and blood–brain barrier permeability) become crucial (Zuo et al., 2014; Zhang et al., 2015).

The purpose of this review is to summarise the effects of MW exposure on the CNS due to the widespread use of mobile phones headsets and to emphasise the different methodological approaches by comparing other frequencies of EMF exposure.

2. Stereological approaches to the cellular effects of exposure

The significant relationship between brain morphology and cognitive function is well known. Information about the variability of cortical area size and volume of the hippocampus provides data for evaluating CNS function and determining the degree of neuronal damage in cases of disease or injury-related alterations. For the last 30 years, obtaining information about confidential numerical data has been possible by using unbiased stereological techniques (Pakkenberg and Gundersen, 1997; Andersen et al., 2003; Fabricius et al., 2007, 2013).

Stereology is a histomorphometric technique that provides three-dimensional data from two-dimensional sections, independent of tissue structure and shape (Altunkaynak et al., 2012). Many studies have shown that 900 MHz EMFs affect neurons and glial cells. For example, stereological studies in prenatal and postnatal periods have revealed cell loss following exposure (Odaci et al., 2008; Bas et al., 2009; Sonmez et al., 2010). In comparison to other groups, the EMF-exposed group showed highly significant decreases in the number of Purkinje cells (Sonmez et al., 2010). Additionally, stereological investigation of EMF-exposed groups has shown significant losses of pyramidal neurons in the cornu ammonis of the hippocampal region in comparison to other groups (Bas et al., 2009). These hippocampal results have been supported by analyses in the dentate gyrus (DG) region. It has been suggested that 900 MHz EMF exposures affect the granule cell development in the DG and inhibit neurogenesis (Odaci et al., 2008). These numerical data were obtained by the optical fractionator and dissector methods. These methods, like other stereological analyses, rely on systematic random sampling. During particle counting, each nucleus that intersects with including line of the unbiased counting frame and totally inside of the frame is counted through the *z*-axis of the selected sections. Coefficient error (CE) and coefficient variation (CV) values play a key role in making the appropriate estimation. CE gives adequate information about analysing procedures such as counting frame area, sampling grid area, and sufficient section number (Fig. 1). Also, this CV value shows the variability of the members in a group. In stereological approaches, group variability is affected by multiple factors such as study population and estimation parameters (Altunkaynak et al., 2012). Additionally, the numerical data obtained from stereological analyses have been supported by histopathological images in the studies (Odaci et al., 2008; Bas et al., 2009; Sonmez al., 2010).

Stereological studies, however, are particularly inadequate to demonstrate the effects of MWR. Fukui et al. (1992) studied the long-term effects on mice in cases of prenatal exposure to 2.45 GHz and found brain weight was significantly lower in the group exposed for 15 and 20 min/day compared to the control group. Numerical density in the cerebrum was significantly increased, however, in the case of MWR exposure for 20 min/day compared to the control group. Additionally, in the exposed group a significant number of pyknotic cells were observed in the microscopic examination (Fukui et al., 1992).

3. Evaluation of cell death and pathways

In addition to stereological analysis, immunohistochemical approaches have gained importance to clarify the molecular mechanism of cell death due to exposure. Staining the glial cells and neurons by various antibodies is particularly important for evaluating the histopathological effects at the cellular level. In this context, many researchers have preferred to determine the apoptosis in the neurons after exposure (Orendácová et al., 2009; Carballo-Quintás et al., 2011; Eser et al., 2013).

The essential aim of *in vitro* studies is to explain the effects of EMF exposure on cell structure and the cellular microenvironment. First, the effect of EMF exposure on Ca^{+2} channels in the cell membrane should be mentioned. Ca^{+2} is responsible for regulating cell proliferation, apoptosis, differentiation, and hence, cytotoxicity (Sergeev and Rhoten, 1998; Aldinucci et al., 2000). In the case of phosphorylation, the signal reaches to the nucleus, and gene transcription starts. Mitogen-activated protein kinases (MAPKs) are members of the protein serine/threonine kinases family and play an important role in the response to EMF exposure. MAPK and ERK1/2 MAPK pathways are especially affected by ionizing radiation (Fig. 2). Apoptosis plays a critical role in providing normal tissue homeostasis. Apoptosis is controlled cell death to eliminate damaged cells and is started by an intracellular or extracellular signal and ends with cell phagocytosis (Carballo-Quintás et al., 2011). During this apoptosis process, many remarkable intracellular interactions occurred. Particularly, when physiological steps were evaluated, the cell cytoskeleton is playing a key role for critical intracellular events. Although Simkó et al. (2001) have found negative effects on the cytoskeletons of cell and motor proteins after EMF exposure, the effects on the cytoskeleton have been defined by Santoro et al. (1997). It has also been suggested that an intermediate frequency (100–300 kHz) might discompose the microtubule polymerization. As a result of these deteriorations, EMF exposure may lead to mitosis aberrations (Kirson et al., 2007).

It has been shown that MW radiation leads to damage in the mitochondrial structures of male Wistar albino rats exposed to radiation with average power densities of 2.5, 5, and 10 mW/cm^2 for 6 min/day for 30 days in a dose-dependent manner. Following the experimental process, swollen and vacuolized mitochondria (depending on specific absorption rate (SAR)) were observed in the hippocampus of the group exposed to MWR. Specifically they found that MWR with a power density of 30 mW/cm^2 showed side effects on the mitochondria of the rat's hippocampus or cerebral cortex compared to a power density of 3 mW/cm^2 (Xie et al., 2004). Additionally, Dong et al. (2011) studied Sprague-Dawley rats exposed to MW radiation with a power density of 4.68 mW/cm^2 for 30 days and observed similar changes such as cavitation (swelling of the mitochondria in the hippocampal region, and brain cortex). In addition to these structural effects, MW caused reductions in mitochondrial enzymes such as ATP synthesis, ATPases with a power density of 30 mW/cm^2, for 5 min. In the case of MWR with a frequency of 591 MHz and a power density of 13.8 mW/cm^2, *Sprague-Dawley* rats showed a high brain energy metabolism (Sanders and Joines, 1984). Also of note, the effects of MWR on the enzymatic reaction in the mitochondria and the activity of succinate dehydrogenase (SDH) (one of the key enzymes for energy metabolism in mitochondria) should be discussed. In this context, Zhao et al. (2007) have shown the reduced activity of SDH and, as a result, abnormal energy metabolism in cases of MWR pulsed with a power density of 30 mW/cm^2 for 5 min in Wistar albino male rats. In another study, high-power MWR in different