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The Effects of Mobile Phone Exposure on Mast Cells in Rat Dura Mater

Efectos de la Exposición del Teléfono Móvil en los Mastocitos de la Duramadre de Ratas

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SUMMARY: Mobile phone use has increased rapidly. The central nervous system has been shown to be adversely affected by its electromagnetic field (EMF) resulting in headache and sleep disturbances. How the cells make up the CNS and are affected by EMF is unclear. However, because of their central role in inflammation through diverse stimuli including radiation, this study aimed to investigate the effects of electromagnetic fields induced by mobile phones on mast cells in rat dura mater. A total of 18 adult, female, Sprague-Dawley rats were divided into two groups. The choice of female rats for his study was based on recent surveys demonstrating that mobile phone use is more frequent and prolonged among females. The study group was exposed to 900 MHz electromagnetic field (1 h/day for 45 days). In the end of the study, duramater tissue was extracted and stained using Toluidine blue. Mast cells were counted and results were analysed using Student t test. Mean must cell number was 202.33±9.82 and 456.78±35.01 in the control and study groups, respectively ($p<0.05$). Analysis of serum electrolyte and immunoglobulin E levels showed no statistically significant difference between the two groups ($p>0.05$). The study showed that mobile phone exposure increased mast cell number and degranulation in rat dura mater. Further studies are required to evaluate the clinical implications of these findings.

KEY WORDS: Mobile phone; Mast cell; Electromagnetic field; Dura mater; Rat.

INTRODUCTION

The use of mobile phone is becoming increasingly popular and indispensable in modern daily life. This is one of the fastest growing technological developments today (Kerimoglu *et al*., 2016). However, there is increasing amount of public concern regarding the health risk of radio-frequency electromagnetic fields (EMF) created by mobile phone (MP) (Ikinci *et al*., 2016). MP operate with radiofrequencies ranging mainly from 900 to 1800 MHz (Hancı *et al*., 2013; Bodera *et al*., 2015; Tumkaya *et al*., 2016). These frequencies excite the rotations of the water and some organic molecules and have been attributed to thermal and non-thermal effects (Szentpali, 1999). It is well established that EMF exposure of the entire body to SAR of 1-4 W/kg produces pathological reactions in mammals (World Health Organization & International Programme on Chemical Safety, 1993). EMF are thus suspected of causing biological alterations in living entities.

There is data showing that MP EMF exposure can affect the neural activity (Hamblin *et al*., 2006). Although there is no clear evidence for adverse effects of MP EMF, there is a widespread public concern over its possible harmful potential (Szentpali). As MP is usually held close to the head, it is appropriate to study the effects on the cranial region that is involve in hearing, concentration, sleep and other functions that may be related to fatigue, headache and migraine (Bortkiewicz, 2001; Parazzini *et al*., 2005). MP users often complain of a burning or heating sensation within the most exposed head area (Straume *et al*., 2005). The increase in temperature during MP use may be due to electric power dissipation and EMF exposure (Straume *et al*.). GSM-900 is used in most parts of the world by MP. GSM-900 uses 890-960 MHz and several laboratory studies showed disruptive effects of this range of EMF on the blood-brain barrier (BBB) and on normal brain functioning (Salford *et*

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al., 2003; Curcio *et al*., 2005; Persson *et al*., 2005). Mast cells are key players in the inflammatory response as they can be activated to release a wide variety of inflammatory mediators, by a variety of stimuli including irradiation (Michaloudi *et al*., 2007). Dural and pial mast cells are present along the main meningeal vessels and their branches and therefore in close proximity to the sympathetic dural plexuses (Michaloudi *et al*.). Their presence along the middle meningeal artery and trigeminal nerve branches suggests a role in meningeal blood flow, neurogenic inflammation and headache (Dimlich *et al*., 1991). Mast cells are suspected to play a role in the pathophysiology of migraine headache (Levy *et al*., 2006). To address the issue of EMF exposure in human daily life working with human subjects has great advantages. However, the varying duration of exposure and the heterogeneity of participants would yield less reliable information than inbred animal strains (Söderqvist *et al*., 2008). A further relevant issue is the more frequent and prolonged MP use by females (Söderqvist *et al*., 2007). A significant association between EMF exposure and symptoms like headaches and migraine has not been neurologically demonstrated, suggesting contribution of psychological factors such as the conscious expectation of adverse effect (Söderqvist *et al*., 2008). Since migraine has a higher prevalence in females, who are more exposed to MP EMF, we investigated the effect on dural mast cells in female rats.

MATERIAL AND METHOD

After review and approval of the study protocol by the Local Ethics Committee for Animal Experiments, School of Medicine, Recep Tayyip Erdogan University (Approval date: 30.06.2011, meeting number: 10), 18 adult female Sprague-Dawley rats weighing 250-300 g were obtained from the Animal Laboratory for Experimental Surgery, Trabzon, Turkey. The rats were kept at room temperature (22±2 ˚C) and 40-50 % relative humidity. An automatic light control system provided light between 05:00 and 17:00 h. All animals were fed with standard pelleted rat diet (Bayramoglu, Erzurum, Turkey) and tap water ad libitum. All procedures involving the animals were designed and performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

The rats were equally divided into two groups to be exposed to a working MP on talking mode (study group) or to the same phone turned off (control group). The MP with a carrier frequency of 890-915 MHz (SAR 0.702 W/kg), modulation frequency of 217 Hz, and maximal peak power of 2 W, was placed 10 cm away from the cage for one hour a day (19:00-20:00) for 45 days during experiments.

Tissue Preparation Processes. At the end of the experiment, following intraperitoneal anaesthesia with thiopental sodium 50 mg/kg, blood samples (3 ml) were taken from the left ventricle for the measurement of total serum levels of sodium, chloride, potassium, calcium, and magnesium using a standard autoanalyser technique (Architect c16000 Autoanalyzer, Abbott Diagnostics, MA, USA) and total serum immunoglobulin E using ELISA. Anesthetised rats were terminated by intracardiac injection of 10 % formaline. The duramater were excised, and specimens were prepared for histopathological studies.

The fixed tissues were dehydrated in graded ethyl alcohol series and stained with Toluidine Blue. Sections were examined under a light microscope (BX51, Olympus, Tokyo, Japan) and photographed at relevant magnifications with attached digital camera (DP72, Olympus, Tokyo, Japan). Total and granulated mast cells were counted in 15 different sections in each slides, total of 7 slides, 1200 μm^2 (Fig. 1).

Fig. 1. Mast cell and degranulated mast cell were counted using the Olympus DP2-BSW (Ver.2.1 to Ver.2.2, Build 6212, Olympus Corporation, Tokyo, Japan) software system.

Statistical Analysis. Data were analysed using the SPSS statistical package (version 18 for Windows, IBM, Chicago, IL, USA). All data showed normal distribution according to Kolmogorov-Smirnov and Shapiro-Wilk tests ($p>0.05$). Total and granulated mast cell count and biochemical marker levels were analysed with Student's t test. A p value lower than 0.05 was considered significant.

RESULTS

Analysis of serum electrolyte and immunoglobulin E levels showed no statistically significant difference between the groups (p>0.05). In addition, total mast cell count) in the study group was statistically significantly higher (mean: 31.37±15.47) than that of the control group (13.36±7.70; p=0.00; Figs. 2 and 3; Table I). Granulated mast cell count in study group (3.07±1.73) was statistically significantly higher compared to control group (7.03±1.62; p=0.00; Figs. 2 and 3; Table I).

Table I. Quantative analysis results.

Group	Mast cell number (mean±standart deviation)	Degranulated Mast cell number (mean±standart deviation)
Control	13.36±7.70	3.07±1.73
EMF	31.37±15.47*	7.03±1.62*

*P=0.00 versus Control group, Mann-Whitney U test

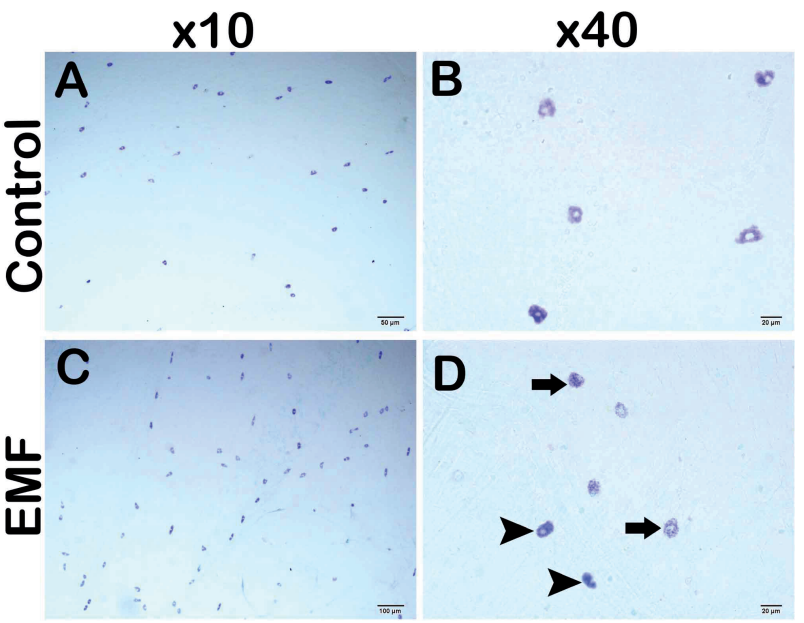

Fig. 2. Light microscopic photographs from dura mater tissue sections stained with Toluidine blue. A(x10)-B(x40) Control Group: Typical Mast cell. C(x10)-D(x40): Typical mast cell (arrow) and degranulated mast cell (arrowhead).

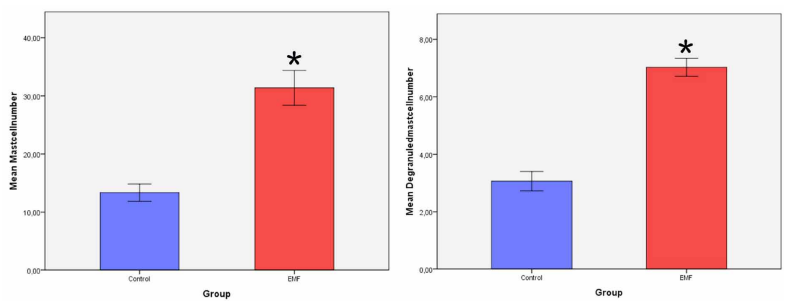

Fig. 3. Quantative measurements result box chart graphs.

DISCUSSION

Mast cells stem from a bone marrow precursor. They migrate to mucosal and connective tissues and undergo tissue-specific maturation (Levy *et al*.). The wide variety of substances they respond to and those they secrete suggest that mast cells may have a role in neurogenic inflammation, migraine, angiogenesis and tumor growth (Hiromatsu & Toda, 2003). Mast cells in the meninges were shown to be activated by stimulation of trigeminal nerve and neuropeptides secreted by nociceptive nerve endings such as Substance P and bradykinin (Levy *et al*.). Therefore some investigators proposed that mast cells may be involved in neurogenic inflammation and migraine headache (Rozniecki *et al*., 1999).

The main mechanism of mast cell degranulation is through surface IgE cross-linking (Benoist & Mathis, 2002). In addition, it has been shown that high-affinity IgE receptor FceRI may also sensitize mast cells and cause degranulation in the absence of antigen (Kawakami & Kitaura, 2005). This study showerd that mast cells both increased in numbers in the dura mater and degranulated following MP EMF exposure. The data showed no significant difference in total IgE. This suggests that mast cells migrated to dura mater due to tissue injury or dysfunction in BBB. Previous studies proposed that mast cell degranulation may cause BBB dysfunction via histamine release, edema formation and migration of polymorphonuclear leucocytes (Lindsberg *et al*., 2010). Similarly, some researchers showed that 900 MHz MP EMF disrupts the function of BBB. They showed that EMF may disrupt the barrier function of the BBB, and if strong enough, may even denaturate proteins and cause fibrin accumulation in the brain (Salford *et al*., 2003; Persson *et al*.).

The dsyfunction of BBB, whether caused by mast cell activation or EMF, may predispose individuals to migraine.

A previous study by Theoharides and colleagues showed that restraint stress alone

may cause mast cell degranulation in rat brains through stimulation of the hypothalamic-pituitary-adrenal axis (Theoharides *et al*., 1995). The same investigators showed that stress may trigger migraine type headaches via mast cell activation through corticotropin releasing hormone mediated mechanism (Theoharides *et al*.). Given the fact that the rats used in our study were housed in cages, yet not immobilized, it is unlikely that immobilization stress alone induced mast cell migration and degranulation. However, MP EMF may have triggered a stress response in rats.

In conclusion, this study shows for the first time that mast cell number and degranulation increase in the duramater of female rats in response to prolonged MP EMF exposure. This occurs in the absence of any significant changes in body/brain weight, serum electrolyte and immunoglobulin E levels. Studies are required to investigate possible cellular and inflammatory alterations in the human duramater with regard to chronic MP EMF exposure. Further studies investigating hormone levels and inflammatory mediators in rats are also needed to determine the relationship between mobile phone use and cranial complaints.

This study showed that exposure to 900 MHz EMF nearly doubled the mast cell number in the dura mater of rats, with an associated increase in their degranulation.

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RESUMEN: El uso del teléfono móvil ha aumentado rápidamente. Se ha demostrado que el sistema nervioso central (SNC) se ve afectado de manera adversa debido al campo electromagnético (CEM) que produce dolor de cabeza y trastornos del sueño. No está claro cómo se ve afectada la composición celular del SNC por el CEM. Sin embargo, debido a su función principal en la inflamación a través de diversos estímulos que incluyen la radiación, este estudio tuvo como objetivo investigar los efectos de los campos electromagnéticos inducidos por los teléfonos móviles en los mastocitos de la duramadre de ratas. Un total de 18 ratas Sprague-Dawley adultas, hembras, se dividieron en dos grupos. Se usaron ratas hembras para este estudio en base a investigaciones recientes que han demostrado que el uso de teléfonos móviles es más frecuente y prolongado en las mujeres. Los grupos de estudio fueron expuestos a un campo electromagnético de 900 MHz (1 h / día durante 45 días). Al término del estudio, fue extirpado el tejido de la duramadre y teñido con azul de toluidina. Se contaron los mastocitos y se analizaron los resultados utilizando la prueba t de Student. La cantidad media de células cebadas fue de 202,33 ± 982 y 456,78 ± 35,01 en los grupos control y estudio, respectivamente ($p < 0,05$). El análisis del electrolito sérico y los niveles de inmunoglobulina E no mostraron diferencias estadísticamente significativas entre los dos grupos ($p > 0,05$). El estudio mostró que la exposición a teléfonos móviles aumentó el número de mastocitos y la desgranulación en la duramadre de las ratas. Se requieren estudios adicionales para evaluar las implicaciones clínicas de estos hallazgos.

PALABRAS CLAVE: Teléfono móvil; Mastocito; Campo electromagnético; Dura madre; Rata.

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