Effect of Electromagnetic Pulse Exposure on Brain Micro Vascular Permeability in Rats[1]

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Objective To observe the effect of electromagnetic pulse (EMP) exposure on cerebral micro vascular permeability in rats. **Methods** The whole-body of male Sprague–Dawley rats were exposed or sham exposed to 200 pulses or 400 pulses (1 Hz) of EMP at 200 kV/m. At 0.5, 1, 3, 6, and 12 h after EMP exposure, the permeability of cerebral micro vascular was detected by transmission electron microscopy and immunohistochemistry using lanthanum nitrate and endogenous albumin as vascular tracers, respectively. **Results** The lanthanum nitrate tracer was limited to the micro vascular lumen with no lanthanum nitrate or albumin tracer extravasation in control rat brain. After EMP exposure, the lanthanum nitrate ions reached the tight junction, basal lamina and pericapillary tissue. Similarly, the albumin immunopositive staining was identified in pericapillary tissue. The changes in brain micro vascular permeability were transient, the leakage of micro vascular vessels appeared at 1 h, and reached its peak at 3 h, and nearly recovered at 12 h, after EMP exposure. In addition, the leakage of micro vascular was more obvious after exposure of EMP at 400 pulses than after exposure of EMP at 200 pulses. **Conclusion** Exposure to 200 and 400 pulses (1 Hz) of EMP at 200 kV/m can increase cerebral micro vascular permeability in rats, which is recoverable.

Key words: Electromagnetic pulse; Permeability; Cerebral micro vascular; Rats

INTRODUCTION

With the increasing use of modern techniques including electromagnetic pulses，the potential health risks for human body and brain in particular have become a public concern. It was reported that radiofrequency field exposure from mobile telephones can result in a significantly increased risk of developing brain tumors[1], which has not been confirmed by laboratory studies. It is well known that blood-brain barrier (BBB) plays an important role in maintaining the homeostasis of brain microenvironment, which is essential for the normal function of brain. A variety of pathological conditions can adversely affect BBB and lead to its disruption in humans and laboratory animals[2-3]. It was reported that exposure to electromagnetic fields (EMF) can significantly increase leakage of albumin through BBB of exposed rats compared to non-exposed animals[4-5]. However, no increase in BBB permeability has been reported in other studies of EMF exposure[6-7]. Usually, disturbances of brain micro vascular permeability are assessed by observing the extravasation of external tracers such as Evans blue (EB) and lanthanum nitrate or internal serum constituents such as albumin. In this study, we used both endogenous albumin and lanthanum nitrate to assess the changes in brain micro vascular permeability in rats after exposure to electromagnetic pulses (EMP).

MATERIALS AND METHODS

Animals and EMP Exposure

Male Sprague-Dawley rats, weighing 200-250 g, were obtained from Animal Center of Fourth Military Medical University (Xi'an, China). The rats were kept separately in a specific pathogen-free environment with free access to sterile laboratory pellets and water.

[1]This study was supported by National Natural Science Foundation of China (No. 30670492), Foundation for Authors of National Excellent Doctoral Dissertation of China (200465), and National 863 Project (No. 2006 AA02Z4C3).

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0895-3988/2009
CN 11-2816/Q

The animals were sham or whole-body exposed to 200 or 400 pulses of 1 Hz EMP at 200 kV/m. During exposure, the rats were awake and not restrained in the exposure chamber. Their rectal temperature was increased less than 0.2 ℃ after the exposure.

Albumin Immunohistochemistry

Half an hour, 1, 3, 6, and 12 h after EMP exposure, five animals from each group were anesthetized with 40 mg/kg sodium pentobarbital, i.p. The heart was exposed and the left ventricles were perfused with 0.9% saline, followed by perfusion with 200 mL fixative (4% paraformaldehyde in phosphate-buffered saline, pH 7.4) for 15 min. After the perfusion, brains were fixed for more than 24 h, then coronal slices were embedded in paraffin and cut into 4-μm thick sections. The accurate histological detection of any extravasated endogenous albumin was performed using goat anti-rat albumin (Bethyl Laboratories, Inc) as the primary antibody and 2-step plus poly-HRP anti-goat IgG detection system (ZSGB-Bio). The sections were counterstained with hematoxylin to enhance the nuclear staining. The efficacy of vascular tracer was confirmed with a positive control group exposed to adrenaline known to increase vascular permeability in brain. For negative controls, adjacent sections were processed with the same steps except for primary antibodies. Pictures taken from the sections were photoed with a digital camera attached to Nikon light microscope.

TEM Study of Brain Micro Vascular Permeability

Transmission electronic microscopic (TEM) studies were conducted at Electronic Microscope Center, Fourth Military Medical University, using the JEM-100SX electronic microscope (Hitachi, Tokyo, Japan). After EMP exposure, the animals were anesthetized with 40 mg/kg sodium pentobarbital, i.p. The heart was exposed and the left ventricles were perfused with 0.9% saline, followed by perfusion with a fixative consisting of one part 4% lanthanum nitrate and two parts 6% glutaraldehyde-0.1 mol/L sodium cacodylate (pH 7.40-7.50) for 2 h. At the end of brain perfusion, frontal cerebral cortex were isolated and cut into 1 mm^3 pieces. The isolated tissues were immersed in 4% glutaraldehyde for 2 h, and then washed with PBS. The tissues were immersed in 1% osmium tetroxide for 2 h, and then washed with PBS for 5 min. After embedded, the specimens were heated at 60 ℃ for 48 h. The sections were stained with acetic acid uranium and lead, and then observed under TEM.

RESULTS

Effect of EMP on Permeability of Brain Micro Vascular Using Albumin Tracer

In sham exposure rats, no micro vascular of brains showed albumin leakage. Half an hour after EMP exposure, little extravasated serum albumin was found around capillaries. However, 1 h after EMP exposure, extravagated serum albumin was found in a few capillaries, the albumin leakage of micro vascular vessels reached its peak at 3 h (Fig. 1), and then decreased at 6 h, which nearly recovered at 12 h after EMP exposure. In addition, the leakage of micro vascular vascular vessels was more obvious after exposure to EMP at 400 pulses than at 200 pulses.

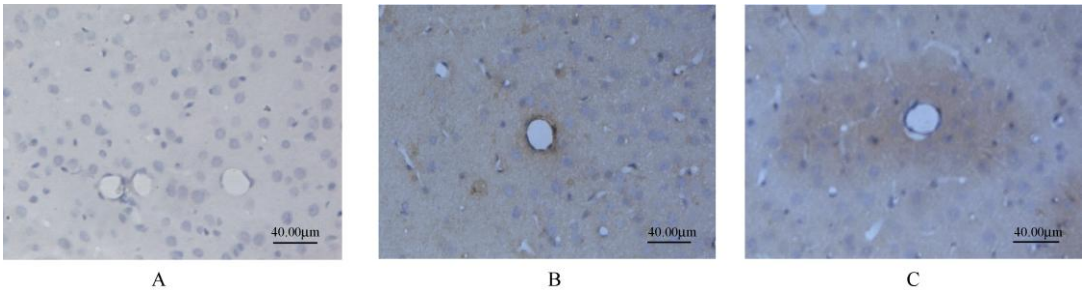
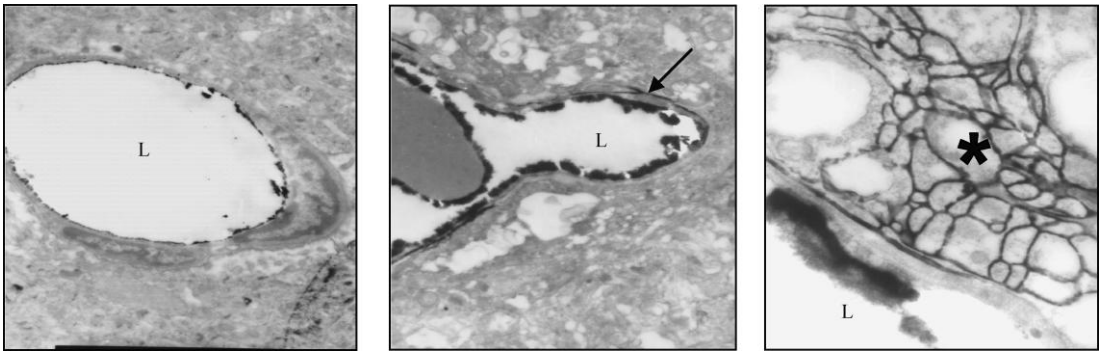


FIG. 1. Extravasated serum albumin in frontal lobe of rat cortex after sham EMP exposure (A) in sham exposure, 1 h after EMP exposure (B), and 3 h after EMP exposure (C) (200 kV/m, 400 pulses). Bar=40 μm.

Brain Micro Vascular Permeability after EMP Exposure by TEM

It has been shown that lanthanum nitrate is not able to penetrate brain micro vascular and has thus been widely used as a marker to examine the integrity of BBB by TEM[8-9]. The lanthanum stains were exclusively located in cerebral capillaries of sham exposed rat brain. EMP exposure resulted in the leakage of capillary lanthanum stains to the

surrounding of cerebral capillaries, and lanthanum stains could be found in the tight junction and basal lamina 1 h after EMP exposure (Fig. 2). Three hours after EMP exposure, lanthanum nitrate invaded the parenchyma area, and the extravasation of lanthanum nitrate decreased 6 h after EMP exposure (Fig. 2).



Sham exposure 1 h after EMP exposure 3 h after EMP exposure

FIG. 2. Electron micrographs of representative frontal lobe cortex micro vessels from sham exposed rats showing lanthanum nitrate circumscribed at the luminal (L) space with no lanthanum tracer reached the basal lamina. One hour after EMP exposure (200 kV/m, 400 pulses), the extracellular tracer appeared in base membranes (arrow), and invaded the parenchyma area 1 h after EMP exposure (asterisk).

DISCUSSION

BBB is a specialized structure responsible for the maintenance of neuronal microenvironment. It protects brain from foreign toxic substances but allows passage of molecules that are necessary for metabolism. A wide variety of toxic conditions, such as anoxia, hypertension, and ionizing radiation, can increase the permeability of BBB. Since Frey[10] reported that 1.2 GHz continuous wave exposure induces a significant increase in BBB permeability, many studies have been performed, but the results are controversial. Most researchers believe that permeability change is associated with increased temperature induced by the electromagnetic field. In recent years, the effect of nonthermal radio frequency (RF) exposure on BBB permeability has also been investigated, with both positive and negative findings[11-12]. Salford group using more than 1800 Fisher rats confirmed that subthermal power levels from both pulse-modulated and continuous RF fields have the potency to significantly open the BBB for animals' own albumin (but not fibrinogen) to pass out into the brain and to accumulate in neurons and glial cells surrounding capillaries[13]. However, their results could not be replicated by other laboratories[14]. One study in which rats were exposed to a 1439MHz TDMA field at a high SAR (2000 mW/kg) did not report any significant albumin leakage[15]. Recently, Grafström *et al.*[16] investigated the effects of repeated exposures for a long period (55 weeks) to 900MHz GSM radiation on brain histopathology in a rat model in order to mimic the real life situation. After exposure, the brains were evaluated for histopathological alterations such as albumin extravasation, dark neurons, and lipofuscin aggregation. However, no significant alterations in any of these histopathological parameters were identified. Up to now, no data about the effect of exposure to EMP on the permeability of BBB are available. Recently, Wang *et al.*[17] investigated the effect of EMP exposure on the permeability of blood-testicle barrier (BTB) in mice using EB as a vascular tracer, and found that the permeability of BTB is increased after exposure to 200 pulses of EMP at 200 kV/m.

In this study, we investigated the micro vascular permeability after EMP exposure using lanthanum nitrate and endogenous albumin as vascular tracers. The results showed that EMP exposure at 200 kV/m increased the brain micro vascular permeability in rats, which was more obvious after exposure to 400 pulses of EMP. The brain micro vascular permeability increased at 1 h and reached its peak at 3 h, then began to recover at 6 h, and almost completely recovered at 12 h, after EMP exposure, indicating that EMP exposure under such conditions can transiently alter the micro vascular permeability in rats.

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(*Received December* 28, 2008 *Accepted May* 25, 2009)