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Effects of 1.84 GHz radio-frequency electromagnetic field on sperm maturation in epididymis micro-environment

Guangzhou An[1]*, Jing Li[1], Shenglong Xu[1], Tao Zhao[1], Kangchu Li[1], Jiajin Lin[1], Yan Zhou[1], Lihua Zeng[2], Guozhen Guo[2] and Guirong Ding[1]

[1]Department of Radiation Biology, Faculty of Preventive Medicine, The Fourth Military Medical University, Xi'an 710032, China.
[2]Department of Radiation Medicine, Faculty of Preventive Medicine, The Fourth Military Medical University, Xi'an, 710032, China.

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In recent years, intense interest has been drawn to the effects of radio-frequency electromagnetic field (RF-EMF) on reproduction. To explore the effects of RF-EMF on sperm maturation in epididymis microenvironment, 24 male Sprague Dawley (SD) rats were randomly divided into three exposed groups (SAR 1, 2 and 4 W/kg) and one sham group. The rats in exposed group were exposed to 1.84 GHz RF-EMF for 5 days (1 h/day). After that, the rats were anaesthetized immediately and epididymis were taken out. Half of them were fixed in 4% formalin and the others were placed in tissue homogenate. The morphology of sperm and microstructure of epididymis were observed under microscope after hematoxylin-eosin (HE) staining. Expression of Bin1b protein was detected by immunohistochemistry; the level of glutathione (GSH) and enzymes including superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase (ALP) and disaccharidase were determined by commercial kits. It was found that, compared with sham group, the sperm morphology and microstructure of epididymis did not change obviously; similarly, there was no significant change in Bin1b protein expression and the levels of GSH, SOD, ACP and ALP in exposure group. These results suggest that 1.84 GHz RF-EMF under this experimental condition could not affect the sperm maturation in epididymis micro-environment of SD rats.

Key words: 1.84 GHz, radio-frequency electromagnetic field (RF-EMF), epididymis, sperm maturation, Bin1b.

INTRODUCTION

As human society and technology develops fast, electrical products are applied widely, and the potential harmful effects of electromagnetic fields emitted from these products, especially communication devices are

*Corresponding author. E-mail: dingzhao@fmmu.edu.cn.

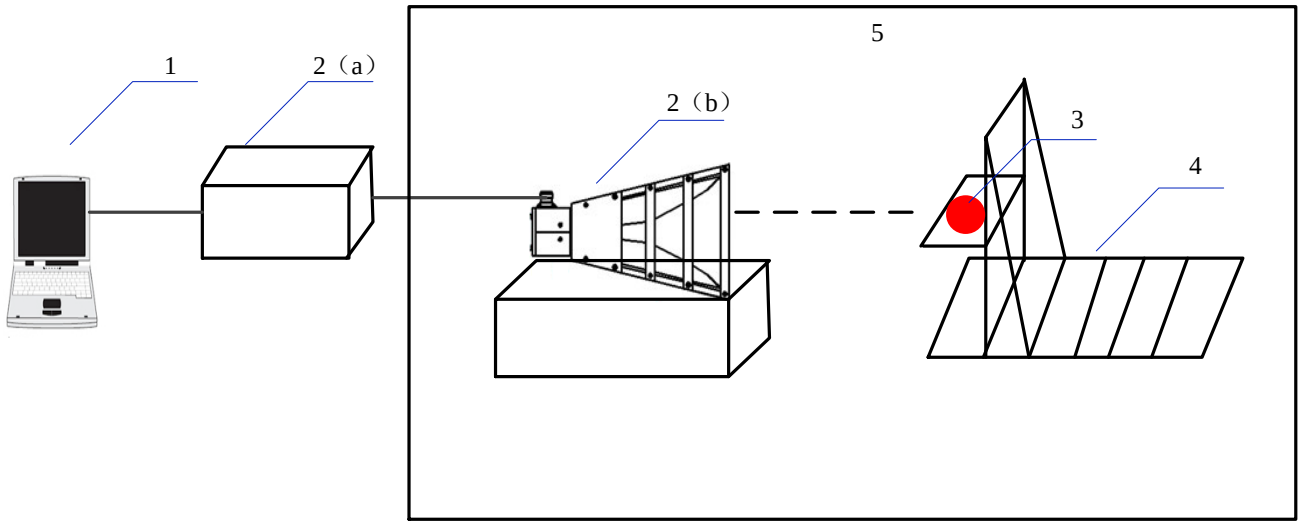

Figure 1. Schematic diagram of the irradiation platform. 1, Computer; 2, microwave irradiator; 2(a), microwave source; 2(b), antenna; 3, animal; 4, 2D objective table; 5, microwave anechoic chamber.

already given high attention. It was found that the RF-EMF (spectral bandwidth ranging from 300KHz-300 GHz) used in communication devices had various biological effects (Megha et al., 2015; Wang et al., 2015; Trivino et al., 2012). However, the biological effects of 1.84 GHz RF-EMF, which are widely selected for mobile communication in China are rarely reported.

As we know, epididymis provide an important micro-environment for sperm maturation. Epididymis consisting of many tortuous tiny ducts can be divided into three main regions (caput, corpus and tail); the caput is connected to ductus deferens and the tail is connected to testis. Once sperm leave testis, they enter epididymis for maturation and then stay here. Epididymis micro-environment includes the length and patency of ductus, epididymis secretion ability, and immune barrier function. Some important enzymes in epididymal fluid such as ACP could promote sperm maturation and motility (Li et al., 2005; Wang et al., 2006). Once the epididymis micro-environment is damaged, sperm maturation could be harmed and result in infertility.

Bin1b is a small peptide specially expressed and secreted from the caput region of rat epididymis (Li et al., 2001). Bin1b not only kills bacteria as the epididymis-specific β-defensin, but also mediates the induction of sperm motility by inducing uptake of Ca^{2+}; therefore, it can help sperm maturation through initiating sperm motility (Zhou et al., 2004). If the expression of Bin1b is interfered, the sperm motility may be impaired and finally result in infertility and sub-fertility.

It was reported that RF-EMF influenced reproductive system (Gutschi et al., 2011; Kesari et al., 2011), but at present, few studies have been performed to investigate the effects of RF-EMF on sperm maturation in epididymis microenvironment. In this study, the expression of Bin1b protein and some other important components playing an important role in maintaining the stability of microenvironment of epididymis have been examined.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the ethical committee and conformed to internationally accepted ethical standards. The experimental 24 male SD rats of 6 weeks were supplied by the Laboratory Animal Centre, the Fourth Military Medical University, Xi'an, China. Rats were randomly divided into three exposed groups by specific SAR value (SAR 1, 2 or 4 W/kg) and one sham group. The rats in exposed group were continuously exposed to 1.84 GHz RF-EMF for 5 days (1 h/day, continuous wave). When 5 days exposure finished, rats were anaesthetized immediately and all epididymis were taken out; half of them were for fixed in 4% formalin and the others were involved in tissue homogenate and protein extraction.

Exposure system

The schematic diagram of the animal irradiation platform with controllable electromagnetic irradiation dose is shown in Figure 1. The platform consists of a microwave irradiating subsystem and a control subsystem of irradiation dose in animal organ. In detail, the microwave irradiating subsystem consists of a microwave irradiator and a 2D objective table. The microwave irradiator could operate with the frequency of 1.84 GHz and the maximum power of 200 W. Moreover, the control subsystem could realize two functions. One displays the irradiation dose in the important organs in real time. The other controls the operation of the microwave irradiation source.

In this experiment, 24 male SD rats were randomly divided into three exposed groups (SAR 1 g, 2 and 4 W/kg) and one sham group. The rats in exposed group were whole-body exposed to be 1.84 GHz RF-EMF for 5 days (1 h/day, continuous wave).

HE staining

The rats were anaesthetized and given a limited gross necropsy with a focus on the reproductive organs. The epididymis were removed from the scrotum, freed from adherent tissues, weighed on analytical scales (Sartorius Co., Ltd, Gottingen, Germany), and fixed in 4% formalin solution. Each portion was dehydrated in a graded series of ethanol, saturated in benzene, benzene-paraffin, embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin-eosin (Toman et al., 2012). Six sections of each rat were randomly chosen for light microscopy evaluation (Leica Microsystems, Wetzlar, Germany) at 400 fold magnification to detect qualitative histological changes induced by being exposed to 1.84 GHz RF-EMF.

Immunohistochemistry staining

The epididymis samples fixed in 4% formalin solution were dehydrated and embedded in paraffin as described in HE staining. After sectioned at 5 µm, paraffin was removed. Then sections were immersed in distilled water following routine methods. Afterwards, all operations were conducted according to SABC immuno-histochemical staining kit (BosterCompany, Wuhan, China) instructions. In short, after sectioning, endogenous enzymes were inactivated, thermal antigen repair was done; they were incubated with Bin1b antibody (1:500 dilution, Bioss Company, Beijing, China) and biotinylated goat antibody (Boster Company) in that order. The sections were incubated with SABC for 20 min at 37□C followed by PBS rinsing (pH 7.2-7.6) four times. After that, sections were stained using DAB kit (Boster Company). At last, sections were mildly stained with hematoxylin, and after dehydration, transparency, neutral gum mounting, photos were taken using light microscope (Leica Microsystems).

Detection of GSH, SOD, ACP, ALP and disaccharidase

The epididymis samples were homogenized by a Polytron homogenizer followed by centrifugation at 3000 rpm/min for 15 min; supernatant was collected for GSH or enzyme level measurement.

The superoxide dismutase detection kit (A001; Jiancheng Bioengineering Institute, Nanjing, China) was selected for SOD measurement; A GSH Detection Kit (A006-1; Jiancheng Bioengineering Institute) was selected to determine the GSH level; An ACP detection kit (A060; Jiancheng Bioengineering Institute) was selected to determine the ACP level; A disaccharidase detection kit (A082-1; Jiancheng Bioengineering Institute) was selected to determine the disaccharidase level; AnALP Detection Kit (A059-1; Jiancheng Bioengineering Institute) was selected to determine the ALP level.

All assays were conducted according to the manufacturer's instruction.

Statistical analysis

All experiments were conducted at least in triplicate, and data analysis was performed using SPSS software (SPSS 16.0, SPSS Inc.,Chicago, USA). Data were analyzed by ANOVA with $P<0.05$ as the criterion for significance in all statistical comparisons.

RESULTS

The morphology of epididymis in SD rats after being exposed to RF-EMF

Compared with sham group, the epididymal tubules as well as the morphology of mature sperm in their lumen showed no differences in exposed group (Figure 2).

The protein level of Bin1b in epididymis after being exposed to RF-EMF

Compared with sham group, the protein levels of Bin1b in different SAR groups did not change significantly. These results suggest that 1.84 GHz RF-EMF could not affect Bin1b protein expression in epididymis (Figure 3).

The Level of GSH, SOD, ACP, ALP and disaccharidase after being exposed to RF-EMF

Compared with sham group, the level of GSH, SOD, ACP, ALP and disaccharidase in different SAR groups did not show significant difference ($p>0.05$).These results suggest that 1.84 GHz RF-EMF could not affect the levels of these enzymes (Figure 4).

DISCUSSION

The integrity of epididymis structure is essential for maintaining the stability of sperm mature micro-environment. From HE staining results, we found 1.84 GHz RF-EMF did not affect epididymis structure under this experimental condition.

Epididymis microenvironment is vital for sperm maturation. Some representative components have been examined in this study, such as Bin1b, GSH and some enzymes. It was reported that Bin1b protein, ACP and disaccharidase could promote sperm maturation or motility (Li et al., 2005; Wang et al., 2006; Zhou et al., 2004). SOD and GSH belong to the anti-oxidant system that can antagonize oxidant system to maintain the balance between the two sides, and once the balance was disrupted, the epididymis microenvironment would be disturbed and further influence sperm maturation.

Based on RF-EMF exposure limit (2 W/Kg) in China, three doses (<2 W/Kg, =2 W/Kg, >2 W/Kg) were designed in corresponding exposed group to compare the biological effects caused by different doses. From the results, we did not find any significant change in Bin1b protein, GSH, SOD, ACP and ALP levels between exposure group and sham group. In conclusion, 1.84 GHzRF-EMF under these experimental conditions has no influence on sperm maturation in SD rats.

Our results are consistent with those reports that did not find the positive effects of RF-EMF on reproduction or sperm maturation (Adams et al., 2014; Falzone et al., 2010; Lee et al., 2010) but there are some reports that found positive effects of RF-EMF on reproduction. For example, Mortazavi reported that RF-EMF from mobile jammers significantly decreased sperm motility (Mortazavi et al., 2013). Kesari et al. (2011) exposed the

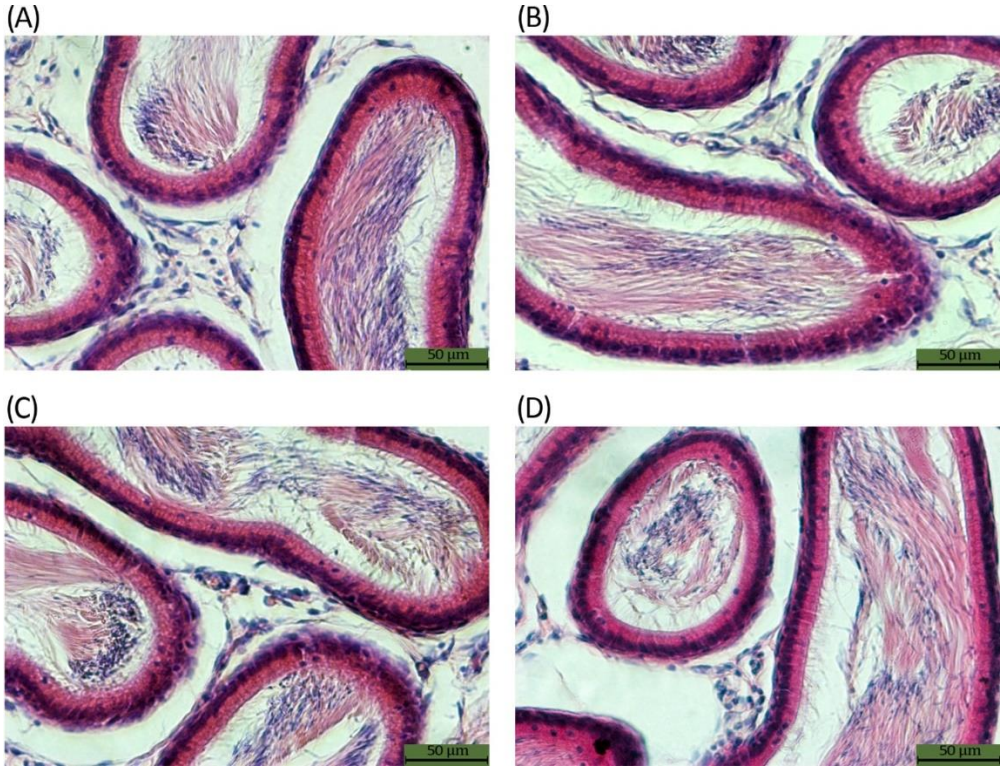

Figure 2. The epididymis morphology under microscope in different groups (images, ×400 magnification). **(A)** sham group. **(B)** 1 W/kg group. **(C)** 2 W/kg group. **(D)** 4 W/kg group.

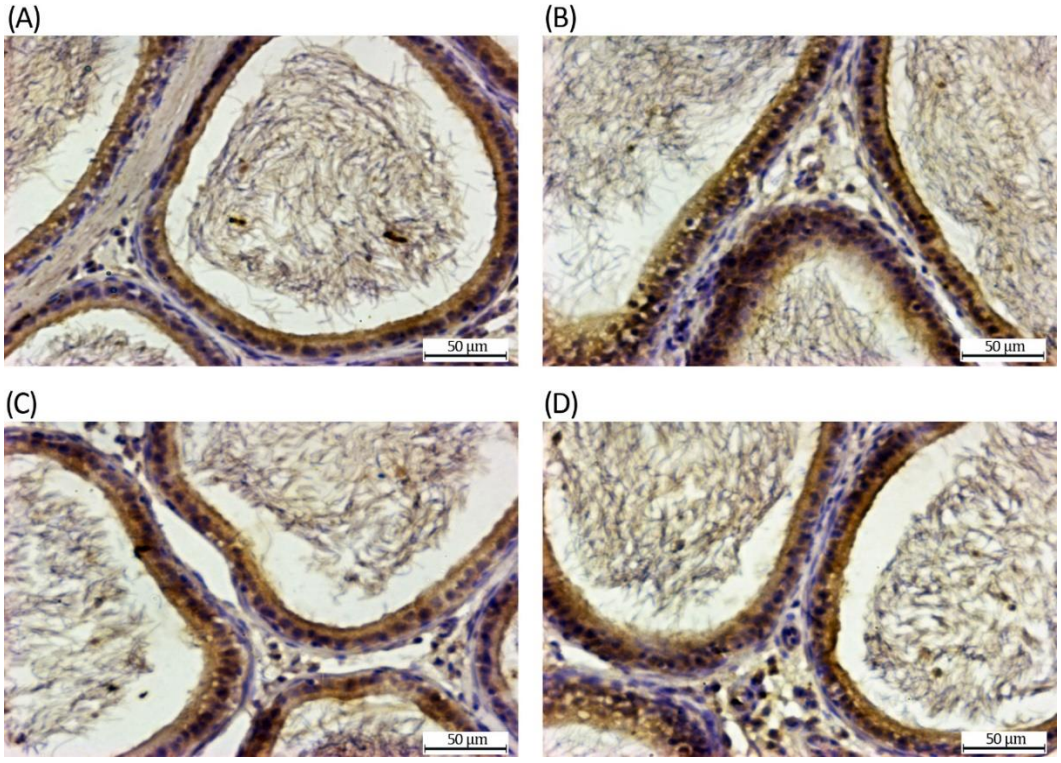

Figure 3. The Bin1b protein expression level in different groups (images, ×400 magnification). (A) sham group (B) 1 W/kg group (C) 2 W/kg group (D) 4 W/kg group.

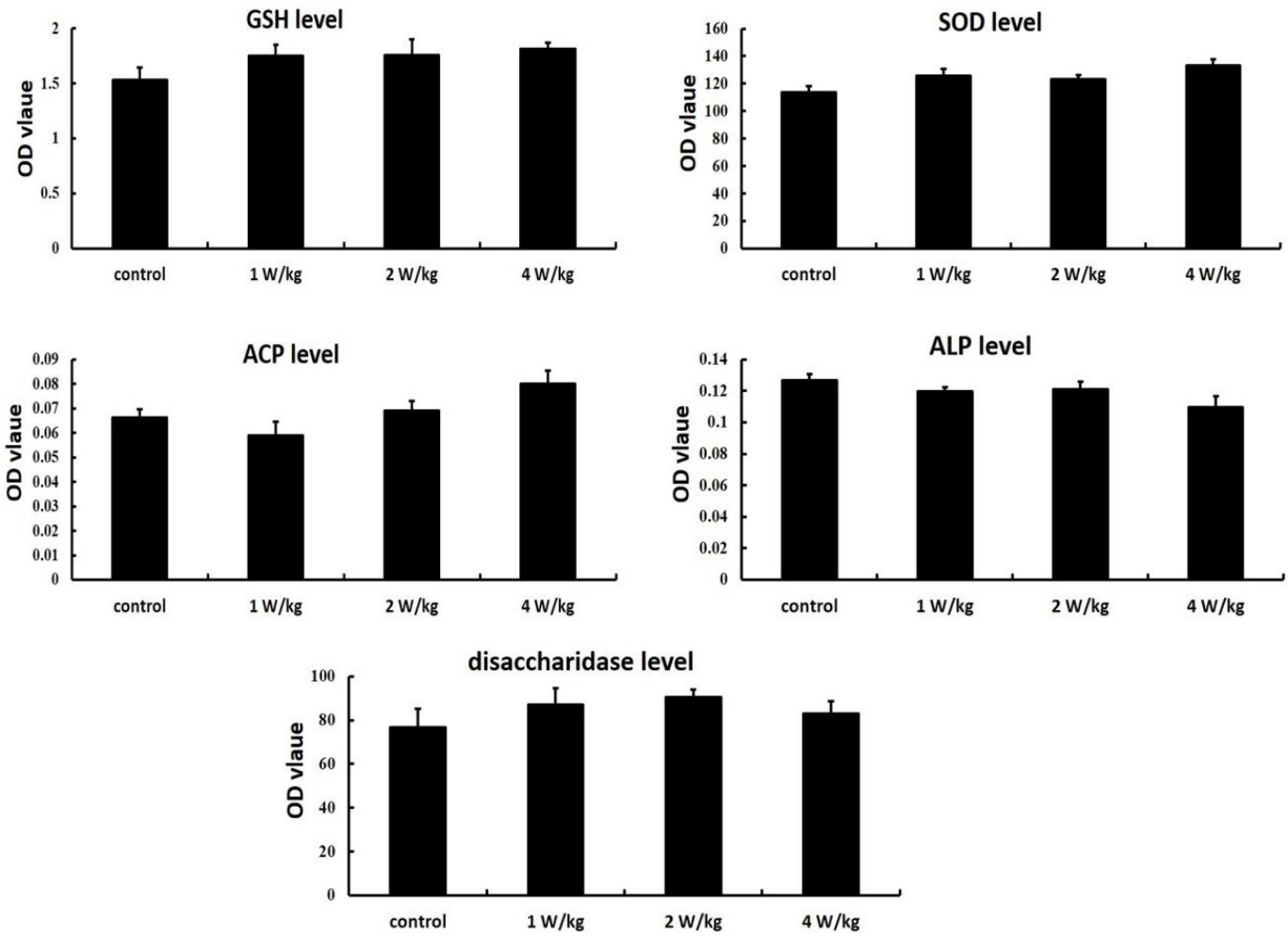

Figure 4. The GSH, SOD, ACP, ALP and disaccharidase level in different groups.

rats for 2 h a day in 35 days to mobile phone frequency electromagnetic field. They found a significant decrease in GSH peroxidase and SOD dismutase in the exposed rats compared to control group (Kesari et al., 2011). Gorpinchenko et al. (2014) found a correlation exists between mobile phone exposure, DNA-fragmentation level and decreased sperm motility. The contradiction among these reports may be ascribed to differences in exposure parameters, experimental animals and experimental conditions. For example, under the same experimental conditions, the transcription level of HSP70 increased after intermittent RF-EMF exposure, but not continuous RF-EMF exposure (Valbonesi et al., 2014). In addition, the accumulated exposure time is another factor that influences RF-EMF biological effects.

Some limitations of the current study are relatively short exposure time and small sample size.

Although the results were negative in this study, it provides some information for further study. Moreover, this is the first study to report the effects of RF-EMF on Bin1b protein in this filed. Since the exposure time chosen in this study was limited compared with that of mobile phone users in our daily life, it is too early to conclude that RF-EMF exposure is safe or not for male reproduction. Whether higher SAR value and longer exposure duration could influence epididymis microenvironment needs further study.

Conflict of Interests

The authors have not declared any conflict of interests.

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