Effect of Electromagnetic Pulse Exposure on Permeability of Blood-testicle Barrier in Mice[1]

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Objective To study the effect of electromagnetic pulse (EMP) exposure on the permeability of blood-testicle barrier (BTB) in mice. **Methods** Adult male BALB/c mice were exposed to EMP at 200 kV/m for 200 pulses with 2 seconds interval. The mice were injected with 2% Evans Blue solution through caudal vein at different time points after exposure, and the permeability of BTB was monitored using a fluorescence microscope. The testis sample for the transmission electron microscopy was prepared at 2 h after EMP exposure. The permeability of BTB in mice was observed by using Evans Blue tracer and lanthanum nitrate tracer. **Results** After exposure, cloudy Evans Blue was found in the testicle convoluted seminiferous tubule of mice. Lanthanum nitrate was observed not only between testicle spermatogonia near seminiferous tubule wall and sertoli cells, but also between sertoli cells and primary spermatocyte or secondary spermatocyte. In contrast, lanthanum nitrate in control group was only found in the testicle sertoli cells between seminiferous tubule and near seminiferous tubule wall. **Conclusion** EMP exposure could increase the permeability of BTB in the mice.

Key Words: Electromagnetic pulse (EMP); Blood-testicle barrier (BTB); Lanthanum; Evans Blue; Permeability; Mice

INTRODUCTION

With the increasing electromagnetic pollution, more and more attention have been drawn to the effect of electromagnetic pulse (EMP) on the procreative ability of male[1-2]. It is well known that blood-testicle barrier (BTB) plays an important physiological role in male procreative system[3-4]. Could EMP exposure increase the permeability of BTB, and then affect sperm cell maturity, or lead anti-sperm antibody, and finally affect the ability of male genitality? These issues remain unsolved and are worth an investigation.

MATERIALS AND METHODS

Animals

BALB/c male mice were obtained from the Animal Center of the Fourth Military Medical University. The mice were 6 to 8 weeks old and weighed 20±2 g. All the animals were whole-body exposed to EMP at 200 kV/m for 200 pulses with 2 seconds interval.

Effect of EMP on Permeability of BTB Using Evans Blue Tracer

Twenty four mice were randomly divided into four groups (n=6), namely three exposure groups and control group. After exposure, the mice were injected with 2% Evans Blue solution through caudal vein at 1, 2, and 6 h. The mice were euthanized and their testis removed 10 min later. The tissue specimens were stored at -80℃ and snap-frozen. The permeability of BTB in both the control and EMP-exposed mice was monitored under a fluorescence microscope.

Effect of EMP on Permeability of BTB Using Lanthanum Nitrate Tracer

Mice were randomly divided into two groups (n=6 each) with one group being exposed to EMP. After 2 h, the mice were anaesthetized with 2%

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pentobarbital (ip, 40 mg/kg), and perfused with 20 mL normal saline by heart, followed by lanthanum nitrate solution. The mice were sacrificed and their testis removed. The tissue samples were immersion-fixed in lanthanum nitrate solution and prepared for the transmission electron microscopy observation. The permeability of BTB of mice was observed by using lanthanum nitrate tracer.

RESULTS

Effect of EMP on Permeability of BTB Using Evans Blue Tracer

In the control mice, Evans Blue was not found in the testicle convoluted seminiferous tubule, but 1 h after EMP exposure Evans Blue began to exude, and 2 h after exposure, cloudy Evans Blue was observed. Evans Blue was hardly found exudation 6 h after exposure.

Effect of EMP on Permeability of BTB Using Lanthanum Nitrate Tracer

Lanthanum nitrate in the control mice was only found in the testicle stertoli cells between seminiferous tubule and near seminiferous tubule wall, while 2 h after EMP exposure, lanthanum nitrate was observed not only in the place between mice testicle spermatogonia of near seminiferous tubule wall, sertoli cells, but also in the places between sertoli cell and primary spermatocyte or secondary spermatocyte.

DISCUSSION

It was reported that different intensities of EMP exposure not only injured seminiferous epithelia in mice[5], but also damaged leydig cells[6]. BTB has important physiological function on male procreative system, while little information is known about the influence of EMP exposure on BTB permeability.

The present results showed that EMP exposure could temporarily increase the permeability of BTB in mice. Several changes involved in the opening of BTB, including the changes of sertoli cell tight junction dynamics, the permeability of cell membrane, and hormone level. Four different tight junctions (TJs) integral membrane proteins had been identified in the sertoli, occludins, claudins, ZO-1, and JAM (junctional adhesion molecules)[7-9]. These proteins could regulate tight junction dynamics and affect the development of sertoli.

It has been shown that the assembly of sertoli cell TJ coincides with a transient plummeting of the levels of TGFβ3 protein and mRNA. Addition of TGFβ3 to sertoli cell epithelium could effectively inhibit the expression of occludins, ZO-1, and claudin-11, which also apparently affected the sertoli cell TJ barrier[10]. Tyrosine phosphorylation of TJ-associated protein plays a crucial role in modulating TJ dynamics. Both occludins and ZO-1 could be phosphorylated and were substrates of tyrosine kinase. In addition, occludins found at the site of TJs was highly phosphorylated[11]. Recent studies strongly suggested that protein kinases A and C played a critical role in regulating Sertoli cell TJ barrier *in vitro*[12-13]. For example, both adenosine 3,5-cycli monophosphothioate (a PKA inhibitor), chelerythrine chloride (a PKC inhibitor), and D-erythro-sphingosine (a PKC inhibitor and a calmodulin-dependent kinase inhibitor) were shown to modulate the assembly and maintenance of the sertoli cell TJ barrier *in vitro*[14]. The changes at the levels of molecule and protein which were induced by the opening of BTB after EMP exposure and the regulation mechanism need to be further studied.

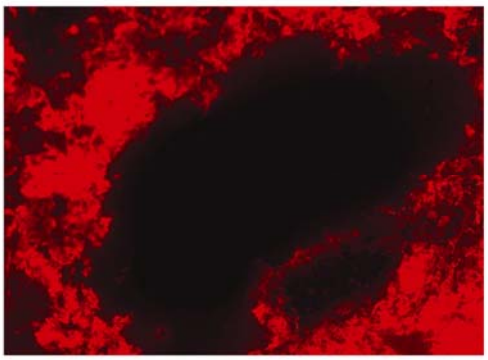

FIG. 1. In control group Evans Blue was not found in the testicle convoluted seminiferous tubule in mice (×40).

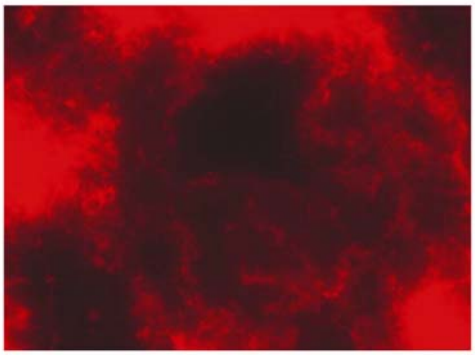

FIG. 2. One hour after EMP exposure, Evans Blue began to exude in the testicle convoluted seminiferous tubule in mice (×40).

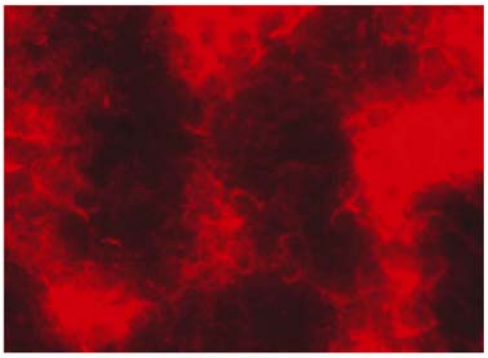

FIG. 3. Two hours after EMP exposure, cloudy Evans Blue appeared in the testicle convoluted seminiferous tubule in mice (×40).

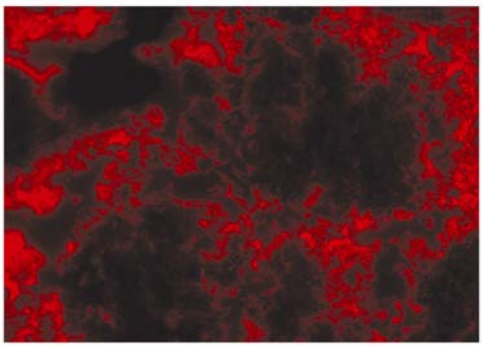

FIG. 4. Six hours after EMP exposure Evans Blue was hardly found in the testicle convoluted seminiferous tubule in mice (×40).

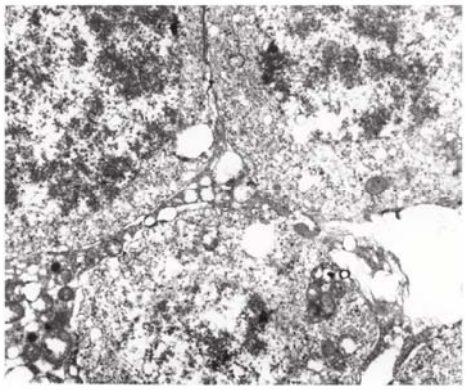

FIG. 5. In control group lanthanum nitrate was only found in the mice testicle sertoli cells between seminiferous tubule and near seminiferous tubule wall (×5000).

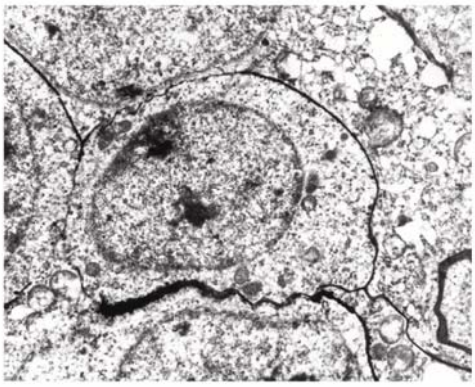

FIG. 6. Two hours after EMP exposure, lanthanum nitrate was observed not only in the place between mice testicle spermatogonia of near seminiferous tubule wall, sertoli cells, but also in the places between sertoli cell and primary spermatocyte or secondary spermatocyte (×5000).

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