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# Electromagnetic-pulse-induced activation of p38 MAPK pathway and disruption of blood–retinal barrier

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## HIGHLIGHTS

- EMP (200 kV/m for 200 pulses) exposure induced transient breakdown of BRB.
- The decrease of occludin and claudin-5 protein played an important role in the disruption of tight junctions.
- JNK, ERK, and p38 MAPK pathways were activated after EMP exposure.
- P38 MAPK pathway was involved in this procedure through phosphorylations of signaling molecules.

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## ABSTRACT

The blood–retinal barrier (BRB) is critical for maintaining retina homeostasis and low permeability. In this study, we evaluated the effects of electromagnetic pulse (EMP) exposure on the permeability of BRB, alterations of tight junction (TJ) proteins of BRB and if any, involvement of mitogen-activated protein kinase (MAPK) pathway. Male Sprague–Dawley (SD) rats and RF/6A cells which were pretreated with or without MAPKs inhibitors were sham exposed or exposed to EMP at 200 kV/m for 200 pulses. The alteration of BRB permeability was examined through fluorescence microscope and quantitatively assessed using Evans blue (EB) and endogenous albumin as tracers. The expressions of TJ proteins and some signaling molecules of MAPK pathway were measured by Western blots. The observations were that EMP exposure resulted in increased BRB permeability concurrent with the decreased expressions of occludin and claudin-5, which were correlated with the increased expressions of phospho-p38, phospho-JNK and phospho-ERK and could be blocked when pretreated with p38 MAPK inhibitor. Thus, the results suggested that the alterations of occludin and claudin-5 may play an important role in the disruption of TJs, which may lead to the transient breakdown of BRB after EMP exposure with the involvement of p38 MAPK pathway through phosphorylation of signaling molecules.



## 1. Introduction

The increased exposure of the population to electromagnetic fields has become a public health concern. Eyes are hotspots of exposure to electromagnetic radiation because of their anatomical position and composition. The blood–retinal barrier (BRB) serves as a selective barrier that regulates osmotic balance, ionic concentration, and transport of nutrients, including sugars, lipids, and amino acids and hence, plays an important role in maintaining internal environment homeostasis in retinal function. Additionally, BRB defines retina as an immunologically privileged site by acting as a barrier against immunoglobulins and circulating immune cells (Erickson et al., 2007). Accumulated evidences indicated that BRB breakdown related to almost all retinal diseases (Cunha-Vaz, 1976). The strict control of fluids and solutes that cross BRB is achieved through well-developed TJs (Farquhar and Palade, 1963; Erickson et al., 2007). TJs are composed of multiple transmembrane proteins such as occludin, claudins, cytoplasmic accessory proteins such as zonula occludens (ZO-1/-2/-3) and cytoskeletal proteins (Huber et al., 2001). Changes of TJ proteins have been observed in a

*Abbreviations:* BRB, blood–retinal barrier; EMP, electromagnetic pulse; TJ, tight junction; MAPK, mitogen-activated protein kinase; EB, Evans blue; FBS, fetal bovine serum; ZO, zonula occludens proteins; BCA, bicinchoninic acid; SDS, sodium dodecyl sulfate; PVDF, polyvinylidene difluoride; TBST, Tris buffered saline with Tween 20; GAPDH, glyceraldehyde phosphate dehydrogenase.

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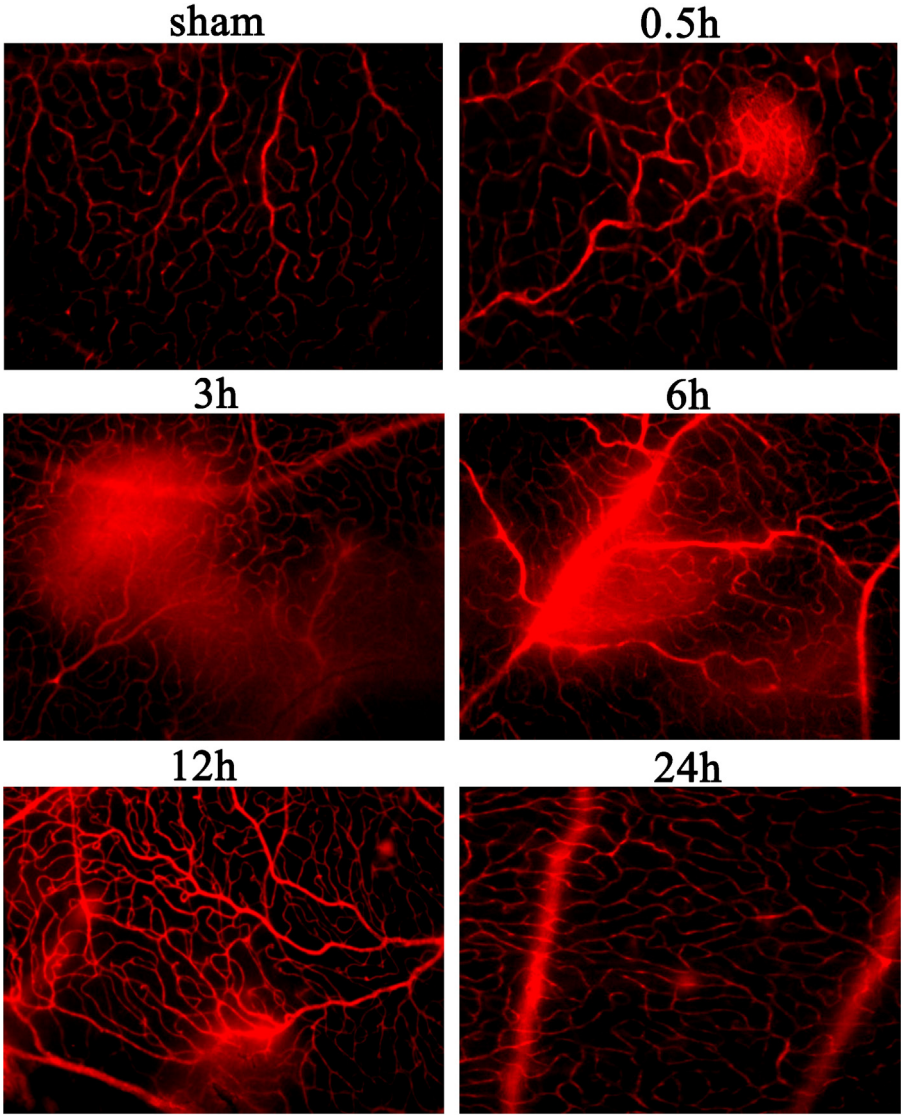


**Fig. 1.** Fluorescence microscope images of SD rats' retinal stretched preparations of the sham group and EMP (200 kV/m, 200 pulses) exposure groups (at 0.5 h, 3 h, 6 h, 12 h and 24 h after the exposure).

wide variety of retinal diseases associated with the breakdown of BRB. Furthermore, MAPK signaling pathway was suggested to regulate the TJs by modulating expressions of TJ proteins and altering the interactions between TJ integral proteins and other membrane proteins (Gonzalez-Mariscal et al., 2008). Results of previous studies have shown that exposures to electromagnetic pulses (EMP) can induce optical injuries including cataracts, corneal edema, endothelial cell loss and retinal degeneration, but the mechanisms are poorly understood. Lu et al. (2009) reported an increased BRB permeability in Sprague–Dawley rats exposed to EMP (400 kV/m, 200 pulses) and the effect was dependent on the duration of exposure. Hence, in this study, we have selected different durations of EMP exposure (200 kV/m, 200 pulses) to investigate the effects on BRB, alterations in TJs and whether MAPK pathway was involved in this process.

## 2. Materials and methods

### 2.1. Animals and groups

Male Sprague–Dawley rats weighing 200–250 g were obtained from the Animal Center of Fourth Military Medical University. All experiments were performed with the approval of the experimental animal committee of Fourth Military Medical University. To observe EB leakage in the retinas as a result of BRB breakdown, 18 rats were randomly divided into sham-exposed group and EMP exposure (200 kV/m for 200 pulses) groups of different time points (0.5 h, 3 h, 6 h, 12 h and 24 h after EMP exposure). To measure EB extravasation in the retinas, 35 rats were randomly divided into sham group and exposure groups of different time points (0.5 h, 3 h, 6 h, 12 h, 24 h and 48 h after EMP exposure). To examine the amount of endogenous albumin leaked from retinal capillary lumens through BRB, 18 rats were randomly divided into sham group and exposure groups of different time points (0.5 h, 3 h, 6 h, 12 h and 24 h after EMP exposure). To measure the expressions of TJ proteins and some signaling molecules of BRB, 24 SD rats were randomly divided into sham group and exposure groups of different time points (0.5 h, 3 h, 6 h, 12 h and 24 h after EMP exposure).

### 2.2. Cell culture and groups

RF/6A cells were cultured in DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 95% air and 5% $CO_2$ (v/v). Three groups were set including sham group, EMP exposure group and EMP exposure group pretreated with MAPKs inhibitors.

### 2.3. Chemical reagents

EB dye (E2129) was purchased from Sigma–Aldrich. Sheep anti-albumin antibody (A110-134A) was obtained from Bethyl Lab. Rabbit anti-ZO-1 antibody (61-7300) was purchased from Zymed Biotechnology. Rabbit anti-occludin antibody (C0424) was purchased from Santa Cruz Biotechnology. Rabbit anti-claudin-5 (BS1069), anti-JNKs (BS1544), anti-phospho-JNKs (BS4763), anti-ERKs (BS5517), anti-phospho-ERKs (BS5016), anti-phospho-HSP27 (BS4762) antibodies