Kaohsiung Journal of Medical Sciences (2018) 34, 134–141

Available online at www.sciencedirect.com

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journal homepage: http://www.kjms-online.com

Original Article

Brain-derived neurotrophic factor preserves intestinal mucosal barrier function and alters gut microbiota in mice

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Received 10 May 2017; accepted 3 November 2017

KEYWORDS
Brain-derived neurotrophic factor;
Intestinal mucosal barrier function;
Gut microbiota;
ZO-1;
Occludin

Abstract The intestinal mucosal barrier (IMB) enables the intestine to provide adequate containment of luminal microorganisms and molecules while preserving the ability to absorb nutrients. In this study, we explored the effect of brain-derived neurotrophic factor (BDNF) on IMB function and gut microbiota in mice. BDNF gene knock-out mice (the $BDNF^{+/-}$ group) and wild-type mice (the $BDNF^{+/+}$ group) were selected. The gut microbiota of these mice was analyzed by denaturing gradient gel electrophoresis (DGGE) assay. The ultrastructure of the ileum and the colonic epithelium obtained from decapitated mice were observed by transmission electron microscopy. The protein expression of epithelial tight junction proteins, zonula occludens-1 (ZO-1) and occludin was detected by immunohistochemistry staining. The protein expression of claudin-1 and claudin-2 was determined by Western blotting. The DGGE band patterns of gut microbiota in the $BDNF^{+/-}$ group were significantly different from that in the $BDNF^{+/+}$ group, which indicated that the BDNF expression alters the gut microbiota in mice. Compared with the $BDNF^{+/+}$ group, the $BDNF^{+/-}$ group presented no significant difference in the ultrastructure of ileal epithelium; however, a significant difference was observed in the colonic epithelial barrier, manifested by decreased microvilli, widening intercellular space and bacterial invasion. Compared with the $BDNF^{+/+}$ group, the expression of ZO-1 and occludin in the $BDNF^{+/-}$ group was significantly decreased. The expression of claudin-1 in the $BDNF^{+/-}$ group was significantly reduced, while the expression of claudin-2 was elevated. These findings indicate that BDNF preserves IMB function and modulates gut microbiota in mice.

Conflicts of interest: All authors declare no conflicts of interest.

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https://doi.org/10.1016/j.kjms.2017.11.002

Introduction

The intestinal mucosal barrier (IMB) represents the first barrier against hostile environment, consisting of inter-epithelial tight junctions and a single layer of columnar epithelium. To move toward and adhere to mucosal epithelium cells, bacterial enteropathogens must traverse the first mucosa barrier of defense [1]. IMB functions by selectively absorbing nutrients and resisting the invasion of toxins, pathogens and inflammatory factors [2]. IMB dysfunction can lead to multiple intestinal diseases, such as inflammatory bowel disease, food allergy and ischemic disease [3]. Intestinal microflora is recognized as a likely source to induce immune deviation in infancy [4]. Additionally, intestinal dysbacteriosis has been demonstrated to result in decreased IMB function and increased bacterial translocation [5].

The IMB is mainly formed by the tight junctions of epithelial cells. Tight junctions consist of two proteins: the transmembrane proteins (occludins and claudins-5), which can interact with adjacent endothelial cells to make up a physical barrier against paracellular diffusion, and the accessory proteins (Zonula occludens [ZO] family), which anchor the transmembrane proteins to the cytoskeleton [6]. ZO-1 and occludin form a high resistance barrier in the bladder epithelium, and deterioration of these proteins marks the disruption of the pulmonary epithelial barrier [7—10]. Studies report that in human minor salivary glands, as claudin-1 and claudin-4 increase, ZO-1 and occludin decrease [11]. Additionally, the expression of the epithelial tight junction proteins ZO-1, occludin, claudin-1 and claudin-2 can be regulated by brain-derived neurotrophic factor (BDNF) [12]. BDNF, a member of the neurotrophin polypeptide family, is abundantly and widely expressed in the nervous system [13]. BDNF plays an important role in neuroplasticity and neurogenesis by regulating the growth of neurons and influencing synaptic efficiency and plasticity [14,15]. BDNF also reportedly acts as a bridge between neuroplasticity and inflammation and can have a detrimental effect on pro-inflammatory cytokines [16,17]. Reduced BDNF expression can be related to inflammation and stress [18]. Studies have also demonstrated that BDNF plays an important role in inhibiting cell apoptosis during gut inflammation [19].

A previous study indicated that gut microbiota can influence BDNF levels and behaviors in mice [20]. However, there is little research on the roles of BDNF in regulating IMB functions and gut microbiota. Therefore, by detecting and analyzing tight junction proteins, this study explores the effect of BDNF in IMB function and gut microbiota in mice, with hope to provide new insight for future studies.

Material and method

Ethics statement

Animal experiments were conducted in strict accordance with the approved animal protocols and guidelines established by the Medicine Ethics Review Committee for animal experiments at our hospital. All efforts were made to minimize the suffering of animals.

Subjects

BDNF knock-out mice ($BDNF^{+/-}$, $n = 9$) and wild-type mice ($BDNF^{+/+}$, $n = 9$) were obtained from the Shanghai Laboratory Animal Center (Shanghai, China). All mice in the current study were males, with an average age of 5—7 weeks and an average weight of 20.3 ± 5.4 g. The Animal Use Permit Number was SYXK (Min) 2004-0007 and all mice were fed in a specific pathogen-free (SPF) environment with free access to water and food. In the third week, excrement obtained within 24 h from mice in the two groups was preserved at −80 °C for further use. After being fed for three weeks, all mice were decapitated, and intestinal tissues were collected for immunohistochemical staining and Western blotting.

Denaturing gradient gel electrophoresis

QIAamDNA Mini Kit (QIAGEN, Hilden, Germany) was applied to extract the bacterial genomic DNA in strict accordance with the manufacturer's instructions. The A_{260} value of the extracted DNA was measured using an ultraviolet spectrophotometer, and the concentration was calculated. The purity of the extracted DNA was identified based on the A_{260}/A_{280} ratio, and 0.8% agarose gel electrophoresis was used to confirm its integrity. The V3 regions of the 16S rDNA gene were amplified with PCR using the following primers: 357f (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGC-CTACGGGAGGCAGCAG-3′) and 518r (5′-ATTACCGCGGCTG-CTCC-3′). Polymerase chain reaction (PCR) was conducted based on following conditions: pre-denaturation at 94 °C for 3 min, then 31 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by extension at 72 °C for 7 min. The PCR product was subject to DGGE analysis using the Diode Mutation Detection System with within and between $BDNF^{+/+}$ and $BDNF^{+/-}$ and an 8% polyacrylamide gel (20 μL). The denaturing gradient was approximately 25—55% at 150 V with a constant temperature of 60 °C. The PCR product was subject to electrophoresis in 1 × TAE buffer solution (containing Tris base, acetic acid and EDTA) for 4.5 h, followed by ethidium bromide (EB) staining for 8 min. The Bio-Rad imaging system (Bio-Rad, Inc., Hercules, CA, USA) was used for photographing.

Transmission electron microscopy

Proximal ileum tissue and distal colonic tissue from the abovementioned intestinal tissues were sliced into 1-mm^3 cubes and immediately fixed in 2.5% glutaraldehyde solution. After 12 h, samples were taken out and immersed in phosphate buffered saline (PBS) for 30 min, followed by fixation in osmium tetroxide (OsO_4) for 2 h, dehydration using graded ethanol and embedding in epoxy resin. The embedded samples were then sliced, stained by methylene blue dye and positioned under an optical microscope. The slices were observed and photographed under a