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Berberine increases the expression of NHE3 and AQP4 in sennosideA-induced diarrhoea model

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ABSTRACT

Berberine, a compound isolated from Chinese Goldthread Rhizome, has been widely used as a non-prescription drug to treat diarrhoea in China. Previous studies have demonstrated multiple pharmacological activities for berberine, including its significant role in antimicrobial activity. However, its effect on ion exchange and water transfer remains unclear. The present study aims to explore the effect of berberine on the expression of Na^+/H^+ exchanger3 (NHE3) and aquaporin4 (AQP4) in both diarrhoea mouse model induced by sennosideA and human intestinal epithelium cell line (HIEC). Semi-quantitative RT-PCR, immunohistochemistry and western blotting were adopted to detect the mRNA and protein expression levels of NHE3 and AQP4. Furthermore, the absorption of berberine and the PKC activity were detected by HPLC and PepTag® Assay to elucidate the underlying mechanisms. It was shown that the expression levels of NHE3 and AQP4 were significantly increased in the diarrhoea mice treated with berberine compared with the untreated diarrhoea mice. Similarly, the expression levels of NHE3 and AQP4 were strikingly enhanced in HIEC co-treated with sennosideA and berberine compared with samples treated with sennosideA only. We also found the maximal absorption of berberine to be approximately 0.01%. In addition, no significant change of PKC activity was observed in the different HIEC treated groups. These results showed that berberine was able to increase the expression of NHE3 and AQP4, suggesting that berberine might exhibit its anti-diarrhoeal effect partially by enhancing the absorption of Na^+ and water.

1. Introduction

Berberine, as a traditional Chinese medicine isolated from Chinese Goldthread Rhizome, has been used extensively to treat diarrhoea. Many studies have demonstrated its significant antimicrobial activity [1], but antimicrobial activity cannot fully account for its anti-diarrhoeal effect, especially in terms of secretory diarrhoea. Tai YH et al. [2] found berberine could reverse secretory diarrhoea induced by the cholera toxin. A randomised controlled trial [3] also indicated that berberine was an effective and safe anti-secretory drug for diarrhoea caused by enterotoxigenic *Escherichia coli* and *Vibrio cholera*, indicating a need to further explore the underlying mechanisms.

The Na^+/H^+ exchanger (NHE) is a transmembrane protein that mediates the exchange of extracellular Na^+ with intracellular H^+ [4]. NHE3 is mainly expressed on the brush border of the colon epithelial cells [5], which plays critical roles in neutral sodium absorption and in a variety of physiological processes, including the fine-tuning of intracellular pH, cell volume control and fluid volume homeostasis [6]. In NHE3-knockout mice, the basal fluid absorption was severely decreased and the mice suffered from diarrhoea [7]. It has been reported that NHE3 activity and expression are down-regulated in most diarrhoeal diseases. For example, NHE3 is suppressed in cases of acute T

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cell-mediated diarrhoea [8], in active IBD patients [9], and in cholera toxin (CT)-induced diarrhoea [10].

The aquaporins (AQP) are a family of homologous membrane proteins that serves as water channels. AQP4 is an important one which is involved in saliva formation, gastric acid secretion, bile production, pancreatic juice production, and regulation of transepithelial fluid transport in the gastrointestinal tract [11–13]. In AQP4-deficient mice, a high water content in faeces and a reduced water osmotic permeability were observed [14], and AQP4 was involved in the rapid return of luminal water back into the body, thus dehydrating the faecal contents [15]. Many studies have demonstrated the association between AQP4 and diarrhoea. For example, the expression of AQP4 was significantly decreased in cholera toxin induced diarrhoea [16], IBD patients [17], and allergic diarrhoea [18].

Protein kinase C (PKC) serves as a second messenger for G-protein receptors that sits at the crossroads of many signal transduction pathways and is involved in a wide range of cellular responses [19]. It has been well established that NHE3 and AQP4 contain consensus sequences for phosphorylation by protein kinase C [20], and the expression of NHE3 and AQP4 can be regulated by protein kinase C [21,22].

In the present study, we investigated the effect of berberine on the expression of NHE3 and AQP4 in a diarrhoea animal model, and the results were further confirmed in human intestinal epithelium cells (HIEC). Furthermore, the absorption of berberine and PKC activity were detected to elucidate the underlying mechanisms.

2. Materials and methods

2.1. Animal model

Mice were treated according to the Animal Care and Use Committee of the Fourth Military Medical University and the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). BALB/c mice (male or female, 20–22 g) were used in this study. Sennoside, a well-known laxative, has been widely used to induce diarrhoea in mice, rats and dogs [23–26]. Mice were repeatedly given 20 mg/kg sennosideA (diluted in ddH_2O, ChenDu mansite pharmaceutical co) with intragastric feeding needles twice a day in the diarrhoea group, and those with profuse liquid stools were recorded as diarrhoea-positive animals. In the treatment group, 30 min after administration of sennosideA, each mouse was administered with 80 mg/kg berberine (diluted in ddH_2O, purity ≥98%, Sigma-Aldrich) with intragastric feeding needles twice a day. In the control group, ddH_2O was administered. Each mouse was fed in a single cage lined with white filter paper. The frequency of liquid stool was counted per 12 h for 7 days (9:00 am and 9:00 pm), and the average frequency of liquid stools (per 12 h) was calculated. After assessment for 7 days, animals were sacrificed by cervical dislocation. The proximal colon was removed and used for mRNA examination and immunohistochemistry analysis.

2.2. Cell culture

Human intestinal epithelial cell line (HIEC) was cultured as described previously [27]. When the cells became 50% confluent, 20 mg/L sennosideA (diluted in 1640 medium), 80 mg/kg berberine (diluted in 1640 medium) or both were administered, respectively. After culture for 48 h, the total RNA and proteins were extracted for semi-quantitative RT-PCR and western blotting analysis.

2.3. Semi-quantitative RT-PCR

The animals were sacrificed, and their proximal colons were removed. Epithelial cells were scraped from proximal colon, and then, 1 mL Trizol (Invitrogen) was added immediately. Total RNA was extracted according to the guidelines, and the total RNA of HIEC was acquired as well. Reverse transcription PCR was performed using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas) according to the manufacturer's guidelines. PCR amplification was then performed (for primer sequences, see Table 1).

2.4. Immunohistochemistry

Mice proximal colons were fixed in 4% paraformaldehyde for 24 h. The tissue was embedded in paraffin and sectioned at 3 μm. Sections were incubated with anti-NHE3 serum (goat polyclonal, 1:100; SANTA CRUZ) or anti-AQP4 serum (mouse monoclonal, 1:200; SANTA CRUZ), and then incubated with rabbit anti-goat or goat anti-mouse immunoglobulins conjugated to peroxidise-labelled polymer. Finally, the sections were stained using a diaminobenzidine reaction and counterstained with hematoxylin.

2.5. Western blotting

The total HIEC cellular proteins were prepared and then quantified by the Bradford method. Eighty micrograms of the lysates were resolved by SDS-PAGE and electrotransferred to a nitrocellulose membrane. Non-specific binding was blocked with TBS with 10% non-fat dry milk. Subsequently, the membrane was incubated with primary antibodies: a goat polyclonal antibody against NHE3 (1:200), a mouse monoclonal antibody against AQP4 (1:300), AMPKα Rabbit mAb (1:1000) or Phospho-AMPKα Rabbit mAb (1:600), overnight at 4 °C. The membrane was washed by TBST three times for 10 min each time, then incubated with HRP-conjugated secondary antibody (1:2000) for 1 h. After thorough washing, the membrane was imaged by ECL.

Table 1
Primer sequences used for RT-PCR (m mouse, h human).

PCR product	Primer sequences	TM(°C)
mAQP4	Forward: 5′-CTTTCTGGAAGGCAGTCTCAG-3′ Reverse: 5′-CAGTCACAGCGGGATTGATGT-3′	62 °C
mNHE3	Forward: 5′-CCACACACTGCAACAGTACC-3′ Reverse :5′-ATAGGCAGTTTCCCATTAGG-3′	58 °C
mGAPDH	Forward: 5′-TGACCACAGTCCATGCCATC-3′ Reverse: 5′-GACGGACACATTGGGGGTAG-3′	62 °C
hAQP4	Forward: 5′-ATAGGAGCTGTCCTCGCTGGT-3′ Reverse: 5′-TCTGCTTTCAGTGCGATCTTC-3′	58 °C
hNHE3	Forward: 5′-CCTGGCTAGTGTCACCAAGGA-3′ Reverse: 5′-GAAGGAGTCCACGGACTTGCT-3′	57 °C
hGAPDH	Forward: 5′-TGGGTGTGAACCATGAGAAGTA-3′ Reverse: 5′-CGCTGTTGAAGTCAGAGGAGA-3′	60 °C