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The laxative effect of emodin is attributable to increased aquaporin 3 expression in the colon of mice and HT-29 cells

Yan-Fang Zheng [a,1], Chun-Fang Liu [b,1], Wen-Fang Lai [a], Qing Xiang [a], Zuan-Fang Li [a], Hui Wang [a,b], Na Lin [a,b,*]

[a] Fujian University of Traditional Chinese Medicine, No. 1, Huatuo Road, Minhoushangjie, Fuzhou 350122, China
[b] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, No. 16, Nanxiaojie, Dongzhimennei, Beijing 100700, China

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ABSTRACT

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a stimulant laxative and used to treat constipation. Aquaporin 3 (AQP3) plays an important role in regulating water transfer in the colon. In the study, we investigated whether the laxative effect of emodin is associated with the regulation of AQP3 in the colon. Our results showed that treatment with emodin increased the fecal water content in the colon of mice and evaluation index of defecation in a dose-dependent manner. More interestingly, emodin significantly increased the AQP3 protein and mRNA expression both in the colon of mice and in human intestinal epithelial cells (HT-29). Mechanistically, emodin obviously up-regulated the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A catalytic subunits α (PKA C-α) and phosphorylated cAMP response element-binding protein (p-CREB Ser133) expression in HT-29 cells. These results suggest that the laxative effect of emodin is associated with the increased expression of AQP3 by up-regulating PKA/p-CREB signal pathway.

1. Introduction

Aquaporins (AQPs) are water and water/glycerol channels that are responsible for the rapid transport of water across membranes. So far, there are 13 types of AQPs that have been discovered in mammals [1]. At least 9 types of AQPs exist in the intestinal tract, such as AQP1, AQP2, AQP3, AQP4, AQP7, AQP8, AQP9, AQP10 and AQP11 [2,3]. Among these, AQP3 plays an important role in the colon, and it is restricted to the villus epithelial cells, which implicated in water reabsorption across colonic surface cells [4]. It has been reported that bacterial pathogens, gut hormone vasoactive intestinal polypeptide and laxative magnesium sulphate can induce diarrhea by altering the AQP3 expression to prevent the water reabsorption in colon [5–7].

Emodin (Fig. 1) (1,3,8-trihydroxy-6-methylanthraquinone, Pubchem CID:3220), the major bioactive component of herbal laxative rhubarb, is known to treat constipation [8]. It has a variety of biological effects such as laxative, diuretic, antibacterial, anticancer, immunosuppressive and vasorelaxant activities [9–11]. In laxative effect, a previous study has indicated that emodin triggers the release of endogenous acetylcholine in gastrointestinal tract, which significantly promotes intestinal peristalsis and accelerates colonic transit [12]. Emodin also has been demonstrated to increase the paracellular permeability across the colonic mucosa by an inhibition of Na^+-K^+-ATPase or by an activation of chloride channels [13]. Moreover, emodin down-regulates AQP4 expression in loVo cells, which related to the laxative effect of emodin [14]. However, many details

Abbreviations: AQP, aquaporin; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; p-CREB, phosphorylated cAMP response element-binding protein.

* Corresponding author at: Fujian University of Traditional Chinese Medicine, No. 1, Huatuo Road, Minhoushangjie, Fuzhou 350122, China. Tel.: +86 10 64014411–2869; fax: +86 10 64013996.
E-mail address: linna888@163.com (N. Lin).

[1] Equal contributors.

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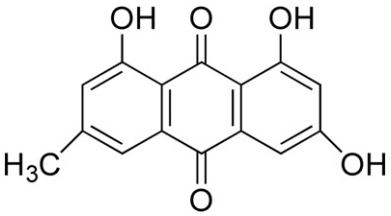

Fig. 1. Structure of emodin.

regarding water transfer in the colon have not yet been elucidated. Since water transfer is primarily mediated by AQP3 and emodin can down-regulate AQP4 expression, we hypothesized that the laxative effect of emodin was associated with the regulation of AQP3 in the colon.

In the present study, we investigated the effect of emodin on AQP3 protein and mRNA expression in the colon of mice, and the results were further confirmed in human intestinal epithelial cells (HT-29 cells). In addition, the mechanism of the changes induced by emodin in the expression of AQP3 was examined.

2. Materials and methods

2.1. Animals and treatment

Twenty-four male ICR mice (18–22 g) were obtained from Laboratory Animal Center of Fujian Medical University, Fujian, China (License No: SCXK Min 2012-0001). They were kept in a temperature controlled environment (22 ± 2 °C), 55% ± 5% relative humidity with a 12-h: 12-h light–dark cycle and fed with standard chow, for at least 1 week before any manipulations. Before all experiments, mice were fasted for 12 h with free access to water. ICR mice were divided separately into 4 groups randomly with the equal number ($n = 6$): the normal control group and the emodin (1, 2 and 3 g/kg/day, respectively)-treated mice groups. Four groups of mice were orally administrated by syringe feeding with distilled water (20 ml/kg/day) or emodin once a day for 2 days. After 5 h of the first administration of emodin or water, mice were fed with standard chow and then fasted for 12 h with free access to water again. In the second day, mice were administrated with distilled water or emodin for the second time. The assay was repeated 3 times. Emodin was purchased from Sciphar Ltd. (Shanxi, China). The experiments were approved by the Institutional Animal Care and Use Committee of Fujian University of Traditional Chinese Medicine, Fuzhou, China. All animal treatments were strictly in accordance with International Ethics Guidelines and the National Institutes of Health Guidelines Concerning the Care and Use of Laboratory Animals.

2.2. Fecal water content

Detailed procedures have been previously described [5]. Briefly, fecal samples from mice were collected for up to 5 h after the administration of emodin suspension and were placed in silica gel (Yubao, Shandong, China) followed by drying for 48 h in a desiccator. The fecal water content per gram of feces was calculated based on the difference between the wet and dry fecal weights. This was then used to calculate the percentage of water in fecal samples. These percentages were averaged among six animals for each group and the assay was repeated 3 times.

2.3. Evacuation index of mice defecation

The method as previously reported [15] was used to assess the defecation function of mice. A numerical score based on stool consistency was assigned: 1 = normal stool, 2 = semi-solid stool and 3 = watery stool. Each mouse received an evacuation index (EI) expressed according to the formula: EI = 1× (number of normal stool) + 2 × (number of semi-solid stool) + 3 × (number of watery stool). The data were expressed as mean EI for each group ($n = 6$). The assay was repeated 3 times.

2.4. Cell culture

Human colon cancer HT-29 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. HT-29 cells were maintained in sterile Dulbecco's modified Eagle medium (DMEM, Keygen Biotech, Nanjin, China) supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM Gln-glutamine at 37 °C. Cells were plated on a 24-well plate, 96-well plate or 100-mm dish at a density of 2×10^5 cells/cm^2, incubated in a CO_2 incubator at 37 °C for 24 h and then treated with compounds dissolved in culture medium. Experiments were done using cells that had previously been passaged five to fifteen times.

2.5. Cell viability assay

HT-29 cells were seeded in 96-well plates and incubated in serum free sterile DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM Gln-glutamine for 24 h. Cells were then incubated with medium containing emodin (20, 40 and 80 μM, respectively) for 24 h. After treatment, cells were washed twice with phosphate-buffered saline (PBS, pH 7.4), and then cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method using Cell Titer 96® Non-Radioactive Cell Proliferation Assay (Promega, Madison, USA) according to the manufacturer's instructions. All absorbances at 570 nm were measured with a Tecan Infinite M200 Pro microplate reader (Tecan, Mannedorf, Swizerland). The experiments were carried out 3 times in triplicate measurements.

2.6. Immunolocalization

Mice were sacrificed at 2 h after the last administration of the emodin suspension, and the colons were removed. After washing the colons with PBS, the samples were fixed immediately for 3 h in 4% paraformaldehyde and embedded in paraffin and tissue sections (5 μm) of colon were prepared for immunofluorescence analysis.

For *in vitro* experiments, the medium used to culture HT-29 cells in 24-well plates for 24 h was replaced with media containing emodin (20 and 40 μM, respectively) or forskolin (50 μM, Beyotime Biotech, Jiangshu, China), and these cells were further cultured at 37 °C for 24 h. The control was treated with the original medium. After washing with PBS twice, cells