Journal of Functional Foods 45 (2018) 348–354

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Phloridzin alleviate colitis in mice by protecting the intestinal brush border and improving the expression of sodium glycogen transporter 1

Ying-yu Lu[a,1], Jian Liang[a,b,1], Shu-Xian Chen[c,a], Bing-xin Wang[a], Han Yuan[a,b], Can-tao Li[a,b], Ya-yun Wu[a,b], Yan-fang Wu[a,b], Xu-guang Shi[a], Jie Gao[a,*], Shao-zhen Hou[a,b,**]

[a] School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou 510006, PR China
[b] Guangdong Provincial Key Laboratory of New Drug Development and Research of Chinese Medicine, Guangzhou 510006, PR China
[c] Department of Hepatobiliary Surgery, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510000, Guangdong, PR China

ARTICLE INFO

Keywords:
Phloridzin
Ulcerative colitis
NHE3
SGLT1
Food additive

Chemical compounds:
Phlorizin (PubChem CID: 6072)
Dextran hydrogen sulfate sodium salt (PubChem CID: 3084656)
Mesalazine (PubChem: 4075)

ABSTRACT

Increasing reports showed that artificial food additives might increase the incidence of ulcerative colitis (UC). Phloridzin is a natural food additive and a pharmacologic agent, whether phloridzin could induce or treat UC is not clear. We investigated the effects of phloridzin on acute colitis in mice and elucidated the potential mechanism. Phloridzin was given to mice with acute UC induced by 4% dextran sulfate sodium orally (60 mg/kg) and daily. Body weight, food and water intake and disease activity index scores were recorded every day. The colon length and thickness, and histopathological changes were determined after mice being sacrificed. Data showed that phloridzin could improve the symptoms of acute colitis, protect the intestinal brush border, and may raise the expression of sodium glycogen transporter 1, phospho-p38MAPK and Ezrin, resulting in promoting the activation and transportation of Na^+/H^+ exchanger. Therefore, phloridzin may be a food additive and dietary supplementary of UC.

1. Introduction

Ulcerative colitis (UC) is one of the inflammatory bowel diseases, and its causes are not fully understood yet (Lu, Lan, Din, Chen, & Chen, 2017). The symptoms of ulcerative colitis are usually diarrhea, hematochezia, and weight loss. These symptoms are the same as the symptoms of acute colitis induced by 4% dextran sulfate sodium (DSS) in mice, so the laboratory used 4% DSS to establish a model of acute UC (Chang et al., 2017; Shin et al., 2017). UC is prevalent in Europe and North America, and in recent years, it has a growing tendency in Asian area (Forrester, Cai, Mbanje, Rinderknecht, & Wren, 2017; Li et al., 2017; Ng et al., 2017; Park et al., 2007). More studies have shown that UC has become a high risk group for colorectal cancer (Chen et al., 2016; Tanaka, 2012).

With the discovery and development of artificial synthesis technology, many artificial food additives, such as artificial sweeteners and carrageenan, are widely used in food industry (Bian et al., 2017a; Suez et al., 2014). However, it has been found that artificial sweeteners and carrageenan could imbalance the gut microbiota (Bian et al., 2017b; Suez et al., 2014) and may increase the body's inflammatory response or as a direct result of UC (Martino, Van Limbergen, and Cahill, 2017; Qin, 2016), which not only put the healthy population at risk of UC but also is a vile for UC patients indeed.

Phloridzin is a special natural food additive, which has various pharmacologic effects (Ridgway and Tucker, 1997; Ridgway, O'Reilly, West, Tucker, & Wiseman, 1996). Phloridzin is a well-known competitive inhibitor of sodium glycogen transporter 1 (SGLT1) (Gonzales, 2017; Raja, Tyagi, & Kinne, 2003). The suppression on intestinal SGLT1 can decrease of the translocation of Na^+/H^+ exchanger (NHE3) and induce the disruption of intestinal brush border, finally cause the diarrhea, which is a typical symptom of UC (He and Yun, 2010; Kiela, Xu, and Ghishan, 2006; Turk, Zabel, Mundlos, Dyer, & Wright, 1991; Turner and Black, 2001). So, phloridzin might increase the risk of UC theoretically. However, in previous study, we found that phloridzin could attenuate intestinal injury induced by 4% DSS, but its mechanism was unclear. We wondered if phloridzin might exhibit protective effect for UC in mice though different pathway.

As what were shown above, UC may bring many adverse effects to people's daily life and the effects of food additives on UC objects deserve to be carefully evaluated. Thus, this experiment was aimed to

* Corresponding author at: School of Chinese Pharmaceutical Science, Guangzhou University of Chinese Medicine, University Town, Guangzhou, Guangdong 510006, China.
** Corresponding author at: School of Chinese Pharmaceutical Science, Guangzhou University of Chinese Medicine, University Town, Guangzhou, Guangdong 510006, China.
E-mail addresses: gj@gzucm.edu.cn (J. Gao), hsz0214@gzucm.edu.cn (S.-z. Hou).
[1] Ying-yu Lu and Jian Liang contributed equally to this work.

https://doi.org/10.1016/j.jff.2018.02.006
Received 6 December 2017; Received in revised form 5 February 2018; Accepted 6 February 2018

study the effects of phloridzin on acute colitis and to evaluate whether phloridzin could be a potential food additives or dietary supplementary in UC patients' diet to improve the quality of life.

2. Material and methods

2.1. Chemicals and reagents

Phlorizin (99%) was provided by Chemlin Chemical Industry Co. Ltd (Nanjing, China). The dextran sulfate sodium (MW, 36,000–50,000) was purchased from MP Biomedicals (USA). The β-actin (beta-actin mouse monoclonal antibody, Lot.8565e62), p-p38 (phospho-p38MAPK (Thr180/Tyr182) antibody, Lot.2498184), p38 (p38MAPK, Lot.2997d62), PDZK1 (PDZK1 antibody, Lot.9589g88), NHERF1 (SLC9A3R1, Lot.7962164) primary antibodies and peroxidase-conjugated secondary antibodies were bought from Affinity Biosciences (USA). The SGLT1 (SLC5A1 polyclonal antibody, Cat.A3126) primary antibody was bought from ABclonal Inc (Wuhan, China). The NHE3 (NHE3 rabbit polyclonal antibody, Cat.27190-1-AP) and Ezrin (Ezrin rabbit polyclonal antibody, Cat.26056-1-AP) primary antibodies were bought from Proteintech Grou, Inc (USA). The BCA kit (BCA Protein Assay Kit, Lot.00071501) was obtained from CoWin Biosciences (China). The RIPA buffer, Phenylmethanesulfonyl fluoride (PMSF) inhibitors, phosphates inhibitors, SDS-PAGE loading buffer and Maker were purchased from Beyotime Biotechnology (China). The SDS-PAGE kit (Lot.20170801) was purchased from Fu De Biological Technology Co. Ltd (Hangzhou, China). The PVDF membrane (pore size: 0.45 μm) was bought from Millipore (USA). The bovine serum albumin (BSA, Lot.CA24172601) and Tween 20 were bought from Rui Shu iological Technology Co. Ltd (Guangzhou, China). The ECL kit (WESTAR ULTRA2.0, Lot.HF27C-MZ) was bought from Cyanagen Srl (Italy). The IHC kit (sp-0022 HistostainTM-Plus Kits, Lot.AG03311135) was provided by Biosynthesis Biotechnology Co. Ltd (Beijing, China). The DAB kit (SignalStain® DAB Substrate Kit, Lot.4) was purchased from Cell Signaling Technology (USA).

2.2. Experimental animals

40 KM male mice, weight of 20–24 g, were provided by the Guangdong Experimental Animal Center. Being free to standard food intake and sterilized tap water, they were fed under the temperature of 20–25 °C and humidity of 55 ± 10% with 12 h light and 12 h dark cycle. All mice were studied with the guidance of institutional and National Institutes of Health (NIH) guidelines for humane animal use. The experimental protocols were given permission by the Animal Ethics Committee of Guangzhou University of Chinese Medicine.

2.3. Inducement of experimental active ulcerative colitis

After the adaptive feeding of experimental mice for 3 days, 40 mice were randomly divided into four groups (8–12 mice per each group): the normal control group, the model group (4% DSS), the positive control group (4% DSS + mesalazine 60 mg/kg), the phloridzin group (4% DSS + phloridzin 60 mg/kg). Phloridzin or mesalazin were given to mice in the administration group by oral gavage, while distilled water (10 ml/kg) was given to mice in the normal and the model group. The normal group was given distilled water every day, the mice in other groups were given 4% DSS for free drinking. The body weight, activity, status of faeces and severity of hematochezia in each group were recorded every day. These data were used to calculate the value of disease activity index (DAI). 7 days later, all the mice were fasted for 18 h with free drinking. Then all animals were sacrificed by over dose of sodium pentobarbital (70 mg/kg, i.p.). Spleens, livers, thymuses, colons were carefully separated. The length and the thickness of the colons and the weight of organs were accurately measured. Tissues were partially stored at −80 °C for protein extraction and biochemical assays and partially stored in 4% paraformaldehyde for histopathologic examination.

2.4. Assessment of active ulcerative colitis

2.4.1. Disease activity index

Mice suffered from acute colitis induced by 4% DSS have the symptoms of diarrhea, hematochezia and loss of weight. DAI values were calculated in each group referring the method of Tohru (Funakoshi et al., 2012) with some adjustment. Briefly, the formula for calculating DAI score is one third of the sum of weight loss score, fecal trait score and bloody stool score. The weight loss scores were determined by the standard as follow: 0: no weight loss, 1: 0–5% loss, 2: 5–10% loss, 3: 10–15% loss, 4: over 15% loss. The fecal trait scores were ranked from 0 to 4 that 0 was normal faeces, 1–2 were wet and soft faeces, 3–4 were loose. The order of severity of bloody stool were ranked among 0 to 4 that 0 was normal or occult blood, 1–2 were dim blood, 3–4 were obvious blood.

2.4.2. Histopathology of colon tissues

The colon tissues were immersed in 4% paraformaldehyde for 48 h. Then they underwent dehydration and embedded in paraffin and cut into slices. These slices were dewaxed and stained with hematoxylin and eosin. The main histological signs of active UC are inflammatory cells infiltration, vascular and epithelial changes. The basic lesions of activity period perform the lamina of neutrophils, chronic inflammatory cells and eosinophils diffusely infiltrated. In the crypts, especially epithelial cells, increased inflammatory cells contributes to the formation of cryptitis, even visible crypt abscess which can rupture into the lamina propria and is regarded as the main sign of active phase. Epithelial cell injury, and increased neutrophils and plasma cells in the basal layer have been suggested as a marker of disease activity. Histopathological scores were graded from 0 to 8: Grade 0 meant no inflammation and normal tissues; Grade 1–2 meant mild inflammation of mucous membrane in which monocytes were predominant with epithelial less damage; Grade 3–4 meant multiple inflammation including monocyte, a small amount of neutrophils, crypt glands being far from the basement membrane, decreased mucin in goblet cells; Grade 5–6 meant multiple inflammation including infiltration of submucosal mononuclear and neutrophil, crypt abscesses and mucin depletion, epithelial destruction and some ulceration formation; Grade 7–8 meant crypt disappearance and serious mucosal inflammation where neutrophils were predominant.

2.5. Western blot of SGLT1, p-p38, Ezrin, PDZK1, NHERF1, NHE3

Colon tissue samples were immersed in 1 ml pre-cool RIPA buffer containing Phenylmethanesulfonyl fluoride (PMSF) and phosphates inhibitors, then were homogenized at 4 °C. The colon tissue homogenate was centrifuged at 12,000*g* for 10 min at 4 °C. The BCA kit was used to measure protein concentration. On the basis of the ratio of 4:1, protein samples and loading buffer were evenly mixed, and the mix was boiled for 6 min at 100 °C. The separation of the protein samples was carried out by 8% SDS-PAGE gel. After separating, the protein on the gel was transferred to PVDF membrane. When the transfer finished, the membranes were rinsed by deionized water and then blocked with 5% bovine serum albumin (BSA) for 2 h at 25 °C. After completing the above steps, primary antibodies, including SGTL1, p38, p-p38, Ezrin, NHE3 were diluted in the different ratios referring to specification for overnight incubation. After three times washing with TBST buffer, blots underwent 1 h incubation with peroxidase-conjugated secondary antibodies at room temperature. The membranes were washed with TBST for three times. Finally, the membranes were developed to show bands by ECL kit and the bands were quantitatively analyzed by using ImageJ software.