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Rhapontin ameliorates colonic epithelial dysfunction in experimental colitis through SIRT1 signaling

Wencheng Wei [a], Lei Wang [a,1], Kai Zhou [a], Haifeng Xie [b], Mian Zhang [a], Chaofeng Zhang [a,*]

[a] Research Department of Pharmacognosy, China Pharmcaeutical University, Nanjing, 211198, PR China
[b] Chengdu biopurity phytochemicals Ltd., Chengdu 611131, PR China

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ABSTRACT

Rhapontin (3, 3′, 5-trihydroxy-4′-methoxystilbene-3-O-glucoside) has anti-thrombotic, anti-allergic and anti-diabetic activities. This study aimed to assess the protective effects of rhapontin on intestinal damage *in vivo* and *in vitro*. In a dextran sodium sulfate (DSS)-induced mouse model, oral administration of rhapontin (100 mg/kg) significantly attenuated colonic pathological damage and remarkably inhibited infiltration by inflammatory cells, myeloperoxidase (MPO) activity, NLRP3 inflammasome activation and SIRT1 expression in the colon. Moreover, rhapontin prevented DSS-induced impairment in the colon epithelium barrier by increasing the expression of tight junction proteins, such as zonula occludens-1(ZO-1) and occludin, and reduced apoptosis-associated protein (cyt-c, the ratio of bcl-2/bax and cleaved-capase9) expression in the colon. The *in vitro* results showed that rhapontin significantly reduced NLRP3 inflammasome activation and cleaved caspase-1 expression as well as lowered IL-1β secretion in LPS-stimulated human-THP-1-derived macrophages. Further study revealed that compound EX257 (an SIRT1 inhibitor) blocked the inhibitory effects of rhapontin on NLRP3-dependent caspase-1 activation and IL-β production in activated macrophages. In addition, in TNF-α-stimulated intestinal epithelial NCM460 cells, rhapontin significantly increased the expressions of occludin and ZO-1 and notably reduced the ratio of bcl-2/bax and cleaved-capase9 expression through SRIT1 signaling. In sum, the protective effect of rhapontin is from blocking the NLRP3 priming cascade reaction and is dependent on SIRT1 activation. Our findings demonstrate that rhapontin might be a potential agent for the treatment of colitis by targeting SIRT1.
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1. Introduction

Ulcerative colitis is an inflammatory bowel disease characterized by chronic, relapsing and remitting inflammation [1]. It not only impairs the quality of life of colitis patients but also contributes to the risk of colon cancer. The number of colitis patients in China has increased recently, and non-conventional remedies are being used to treat ulcerative colitis [2,3]. Some functional foods and phytonutrients, such as epigallocatechin gallate [4], blueberry polyphenol [5] and resveratrol [6,7], have been reported in the treatment of ulcerative colitis.

Although the precise pathogenesis of colitis remains unclear, the breakdown of the intestinal epithelial barrier and sustained production of pro-inflammatory cytokines have been identified as playing a key role in the process of ulcerative colitis [8]. The epithelial barrier acts as the first line of defense against varieties of harmful substances such as luminal antigens and bacteria in the intestines, of which damage results in increased intestinal permeability and the development of inflammation [9]. Moreover, persistently elevating mucosal cytokine expression and leukocyte infiltration could lead to the apoptosis of intestinal epithelial cells and further result in the destruction of intestinal homeostasis [10]. The formation of tight junction proteins in epithelial cells also plays a pivotal role in the intestinal barrier in some intestinal inflammatory diseases [11,12]. In addition, apoptotic signaling is involved in the molecular mechanism of DSS-induced epithelial barrier damage, in which cytochrome C activates caspase-3 and interferes with the content of tubulin in intestinal epithelial cells [13,14].

A histone deacetylase sirtuin 1 (SIRT1) plays an important role in colitis [15]. Considerable evidence has suggested that some stibene-type compounds, such as resveratrol, show significant anti-inflammatory activity *via* regulating SIRT1 expression [16–19]. In the present study, rhapontin (3, 3′, 5-trihydroxy-4′-methoxystilbene-3-O-glucoside) is a natural stibene-type compound isolated from the Chinese medicinal herb *Rheum undulatum* L. and distributed widely in medicinal plants in the *Rheum* genus (Polygonaceae) [20,21]. Previous studies have shown that rhapontin possesses anti-allergic, anti-diabetic and anti-inflammatory activities [22,23,24]. However, the protective effect of rhapontin on colitis has not been reported.

In the present study, we used a simple chemical-induced model of acute colitis by dextran sulfate sodium (DSS) that is similar to ulcerative

* Corresponding author at: Research Department of Pharmacognosy, China Pharmaceutical University, 639 Longmian Road, Nanjing 210098, PR China.
E-mail address: zhangchaofeng@cpu.edu.cn (C. Zhang).
[1] Contributed as the first author.

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colitis [25]. The protective effects of rhapontin and its underlying mechanism were investigated *in vivo* and *in vitro*. Our results suggest that oral administration of rhapontin inhibits inflammatory responses and maintains the integrity of the intestinal barrier in mice with DSS-induced colitis partly through modulation of SIRT1 signaling.

2. Materials and methods

2.1. Chemicals and reagents

Rhapontin (purity over 98%) prepared by Chengdu Biopurity Phytochemicals Ltd. DSS (molecular weight 36,000–50,000 Da, Cat No: 160,110) was purchased from MP Biomedicals. Compound EX527 was provided by Shanghai Chembest Research Laboratories Limited (Shanghai, China). These two compounds were dissolved in dimethylsulfoxide (DMSO) for *in vitro* experiments; the final concentration of DMSO was <0.1% LPS from *Escherichia coli* 026:B6 was provided by Sigma (Nanjing, China). Phorbol myristate acetate (PMA) and adenosine triphosphate (ATP) were provided by Wanleibio Co., Ltd. (Liaoning, China). The other chemical products used were of the analytical grade available.

A myeloperoxidase (MPO) activity assay kit (A044) was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and a caspase3 activity assay kit (WLA047B) was purchased from Wanleibio Co., Ltd. (Liaoning, China). Primary antibodies against bcl-2 (WL 01556), bax (WL01637), caspase 9 (WL01551), cyt-c (WL01571), SIRT1 (WL00599), AMPKα1/2 (WL02254), COX-2 (WL01750), ZO-1 (WL00046) and occludin (WL01996) were purchased from Wanleibio Co., Ltd. (Liaoning, China), Primary antibodies against α-tubulin (BS1699), β-tubulin (BS1482), iNOS (BS1186) and β-actin (BS6007M) were purchased from Bioworld Technology Co., Ltd. (U.S.A.). HRP and diaminobenzidine were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primary antibodies against RORγt (bs-10647R) were purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China), Primary antibodies against TGF-β (Massachusetts, U.S.A.) and caspase1 (ab108362) were purchased from Abcam plc (Cambridge, U.K.). An enhanced chemiluminescent (ECL) plus reagent kit (P0018) was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Trizol reagent (15,596–026) was purchased from Invitrogen Life Technologies (Thermo Fisher Scientific, Inc., USA). A First Strand cDNA Synthesis Kit (FSK-100) was purchased from TOYOBO Biotech Co., Ltd. (Japan), and Taq DNA Polymerase (EP0404) was purchased from Thermo Scientific (U.S.A.).

2.2. Animals

Female C57BL/6 mice (6 to 8 weeks old, 18–22 g) were purchased from the comparative medicine center of Yangzhou University (Yangzhou, China). The animals were allowed access to food and tap water *ad libitum* throughout the acclimatization and experimental periods. All animal experiments were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals. This study was approved by the Animal Ethics Committee of China Pharmaceutical University.

2.3. Cell culture

Human THP-1 cells were purchased from the cell bank of Shanghai (Institute of Biochemistry and Cell Biology, China) and maintained in RPMI 1640 medium with 10% fetal bovine serum (Gibco, Grand Island, NY). THP-1 cells were stimulated by PMA (100 ng/ml) for 12 h to differentiate into macrophages (THP-Ms). THP-M cells were treated with rhapontin and/or EX527 (1 μM) for 12 h and then stimulated with LPS (100 μM) for 4 h and ATP (5 mM) for 1 h.

Human colonic epithelial cells NCM460 were purchased from Nanjing Musai Bio-Tech Co., Ltd. They were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (v/v) in a CO_2 incubator with 5% CO_2. Culture media were also supplemented with 100 IU/ml penicillin and 100 lg/ml streptomycin. The culture medium was replaced every other day. Cells at 80% confluence were used for all of the assays. For the *in vitro* experiments, NCM 460 cells were treated with TNF-α (10 ng/ml) for 6 h. Then, some of them were incubated with rhapontin at different concentrations.

2.4. Cell viability

Cell viability was assayed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded in 96-well plates at a density of 10,000 cells per well in 0.2 ml DMEM medium. After 24 h of exposure, the wells were treated with a final concentration of 5 mg/ml MTT and incubated at 37 °C for 4 h. The formazan crystals were dissolved in 0.2 ml DMSO and read at 570 nm in a microquant plate reader.

2.5. Establishment of DSS-induced acute colitis model in mice

Acute colitis in mice was induced by administration of DSS in drinking water (4%, *w*/*v*). The mice received either distilled drinking water (control) or DSS drinking water (model) for 7 days and were thereafter provided with drinking water for 1 day [26]. The mice were randomly assigned to the following groups (10 animals/group): control, DSS-treated, rhapontin-treated, and rhapontin/DSS-treated groups. Rhapontin (20, 50 or 100 mg/kg) was given by gavage from day 1 to day 8. Then, the animals were sacrificed.

2.6. Assessment of DAI score

During the test period, body weight, stool consistency and the presence of gross blood in the feces were recorded daily and scored with the disease activity index (DAI). Briefly, DAI was summarized by the following parameters [27]: a) body weight loss (0, no loss; 1, 1–5% loss; 2, 6–10% loss; 3.10–20% loss; or 4, over 20% loss); b) diarrhea (0, normal; 2, loose stools; or 4, watery diarrhea); c) hematochezia (0, no bleeding; 2, slight bleeding; or 4, gross bleeding).

2.7. Histological evaluation

Part of the colon was fixed in 10% neutral-buffered formalin solutions for 24 h and embedded in paraffin. The colon sections were stained by hematoxylin–eosin (H&E) staining for histopathological analysis according to standard protocols. The severity of inflammation was scored by a pathologist and graded as follows [28]: 0, rare inflammatory cells in the lamina propria; 1, increased numbers of granulocytes in the lamina propria; 2, confluence of inflammatory cells extending into the submucosa; or 3, transmural extension of the inflammatory infiltrate. Crypt damage was scored as follows: 0, intact crypts; 1, loss of the basal one-third; 2, loss of the basal two-thirds; or 3, entire crypt loss.

2.8. Immunohistochemical staining

Immunohistochemical stains against α- and β-tubulin were performed using an immunohistochemistry kit (Key-Gen, Nanjing, China) in the colon. The paraffin sections (5 μm) were incubated with 3% H_2O_2 to eliminate endogenous peroxidase. After antigen retrieval by heating, non-specific binding sites were blocked by 5% skim milk in PBS for 1 h. The sections were then incubated with primary antibodies and secondary antibodies conjugated with HRP. The colour was visualized by incubated sections with diaminobenzidine.

2.9. Assay of myeloperoxidase (MPO) activity

MPO activity as an indicator of neutrophil infiltration into the inflamed colon was assayed to monitor the level of inflammation. The

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