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Berberine inhibits macrophage M1 polarization via AKT1/SOCS1/NF-κB signaling pathway to protect against DSS-induced colitis☆

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ABSTRACT

Berberine has been reported to have protective effects in colitis treatment. However, the detailed mechanisms remain unclear. Herein, we demonstrated that berberine could protect against dextran sulfate sodium (DSS)-induced colitis in mice by regulating macrophage polarization. In the colitis mouse model, berberine ameliorated DSS-induced colon shortening and colon tissue injury. Moreover, berberine-treated mice showed significant reduction in the disease activity index (DAI), pro-inflammatory cytokines expression and macrophages infiltration compared with the DSS-treated mice. Notably, berberine significantly reduced the percentage of M1 macrophages. In vitro analysis also confirmed the inhibitory effects of berberine on macrophages M1 polarization in RAW267.4 cells. Further investigation showed that berberine promoted AKT1 expression in mRNA and protein level. Silence of AKT1 abolished the inhibitory effect of berberine on macrophages M1 polarization. The berberine-induced AKT1 expression promoted suppressers of cytokine signaling (SOCS1) activation, which inhibited nuclear factor-kappa B (NF-κB) phosphorylation. In addition, we also found that berberine activated AKT1/SOCS1 signaling pathway but inhibited p65 phosphorylation in macrophages in vivo. Therefore, we concluded that berberine played a regulatory role in macrophages M1 polarization in DSS-induced colitis via AKT1/SOCS1/NF-κB signaling pathway. This unexpected property of berberine may provide a potential explanation for its protective effects in colitis treatment.

1. Introduction

Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease, are chronic and incurable inflammatory disorders of the gut, most cases having an onset during young adulthood. As one of the largest healthcare challenges worldwide, the prevalence of IBDs is increasing in these decades. With higher morbidity and mortality rates than most other diseases, IBDs incurs an important risk of complications (in children 29% at diagnosis and 59% at follow-up; in adults 16% after diagnosis) [1]. Therefore, the investigation of agents for IBDs prevention is necessary for the future therapeutic strategies.

Both ulcerative colitis and Crohn's disease are characterized by continuous or discontinuous mucosal inflammation, with epithelial cell destruction, ulceration of the mucosa, and inflammatory cell infiltration of the major portion of intestine and/or colon [2]. Accumulated evidence suggests that IBD is associated with immune response which is initiated by inflammatory cell infiltration, especially macrophages. Previous studies have demonstrated that macrophages, the central mediators of innate immune homeostasis and inflammation, migrate to the inflamed colonic mucosa and release reactive metabolites of oxygen, nitrogen and proteases that degrade the extracellular matrix [3].

Generally, macrophages can be divided into M1 and M2 phenotype. M1 macrophages have strong pro-inflammatory activities while M2 responses to anti-inflammatory reactions. Classically activated M1

Abbreviations: DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; UC, ulcerative colitis; IFN-γ, interferon < gamma > ; LPS, lipopolysaccharide; IL-1β, interleukin-1 < beta > ; TNF-α, tumor necrosis factor- < alpha > ; NO, nitric oxide; SOCS, suppressers of cytokine signaling; DAI, disease activity index; H&E, hematoxylin and eosin; PBMC, peripheral blood mononuclear cells; DAPI, 4′,6-diamidino-2-phenylindole; IHC, immunohistochemistry; NF-κB, nuclear factor-kappa B; β-ACTIN, < beta > -ACTIN; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BBR, berberine; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum

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macrophage polarization can be induced by stimulation with interferon- < gamma > (IFN-γ) and lipopolysaccharide (LPS), and these cells release pro-inflammatory cytokines, such as interleukin-1 < beta > (IL-1β), IL-6, IL-12, tumor necrosis factor- < alpha > (TNF-α), and produce high levels of nitric oxide (NO) to activate inflammation [4,5]. M2 macrophages, typically activated by IL-4 and IL-10, are associated with inflammation resolution and participate in the processes of tissues repair and remodel [6]. Studies have shown that M1 macrophages are increased and M2 macrophages are decreased in colitis, imbalance of macrophage phenotype promotes colitis development. Interestingly, shifting from the M1 to M2 macrophages reduces colitis [7], suggesting that macrophages polarization regulation could be the emerging targets for IBDs therapy.

AKT is known as protein kinase B, which is a family of serine-threonine protein kinases consisting of three isoforms AKT1, AKT2 and AKT3 [8]. AKT pathway converges inflammatory and metabolic signals to regulate macrophages responses and their activation phenotype [9]. Generation of mice lacking individual AKT1 gene are more susceptible to LPS-induced M1 macrophage polarization [10]. M1 signature genes were higher expressed in macrophages from AKT1$^{-/-}$ mice, which was accomplished by regulating RelA/nuclear factor-kappa B (NF-κB) turnover and its transcription activity [11]. Suppressers of cytokine signaling (SOCS) family, the negative regulator of cytokine signal transduction, is one of the target genes of AKT pathway. SOCS1 knockdown would increase the activation of macrophage in the peripheral tissue, leading to higher expression of genes related to M1 polarization, such as IL-1, IL-6, IL-12, and inducible NO synthase (iNOS) [12].

Berberine, a natural isoquinoline alkaloid isolated from the stems and roots of several plants, is traditionally used in the treatment of Dysentery enteritis. Numerous reports have highlighted its various pharmacological activities including analgesic [13], anti-cancer [14], anti-diabetic [15], anti-hyperlipidemic [16], cardioprotective [17] and anti-inflammatory effects [18]. In recent years, berberine exhibited its therapeutic value for IBD, which contributed to the potential antibacterial and anti-diarrhoeal activity [19], the direct antioxidant effects [20], and the protective effects of the intestinal epithelial barrier. We previously found that berberine affected macrophage polarization and inhibited production of some specific cytokines (e.g. IL-6, TNF-α, IL-12) in experimental colitis. These findings suggested that macrophages polarization may be involved in the regulation of berberine in IBD.

In the present study, we examined the effect of berberine on macrophages polarization in dextran sulfate sodium (DSS)-induced chronic colitis mouse model and explored the underlying regulatory mechanism in macrophages cell lines RAW264.7. Data in vitro and in vivo experiments suggested berberine exerted its anti-inflammation activities in colitis by inhibiting macrophages M1 polarization via AKT1/SOCS1/NF-κB signaling pathway.

2. Method and materials

2.1. Animals

Male C57BL/6 mice (6–8 weeks) were purchased from the Comparative Medicine Centre of Yangzhou University. All mice were housed under the controlled room temperature (22 °C) and photoperiods (12 h light/12 h darkness cycle) in the specific pathogen-free conditioned animal care facility. This study was carried out in strict accordance with the official recommendations of the Chinese Community Guidelines and all procedures were approved by the Institutional Animal Care and Use Committee (2016–0011, 01/27/2016–01/26/2021) of China Pharmaceutical University (Nanjing, China).

2.2. Induction of chronic colitis and pharmacological treatment

Experimental colitis was initiated by first two cycles consisted of 2% DSS (molecular weight 5000; DSS; MP Biomedicals, Irvine, CA, USA) for five days followed by drinking water for 14 days and a third cycle consisting of DSS only for five days. The first day of DSS treatment were designated as day 0 and all mice were sacrificed at day 43. Mice were divided into 3 groups: control mice = with normal saline by intragastrical administration and drinking pure water instead of berberine or DSS treatment; colitis mice = with the oral administration of 2% DSS only; pharmacological mice = with the treatment of 2% DSS plus 40 mg/kg berberine (purity > 99%, Feiyu Biotech, Nantong, China). Berberine (intragastrical administration, once a day) were given 7 days prior to the treatment of DSS until to the end of the experiment.

2.3. Clinical scoring of colitis

Disease activity index (DAI) was scored according to the average of three parameters: stool consistency, fecal blood and percentage weight loss. The scoring system was as follows: percentage of body weight loss: none = 0, 1–5% = 1, 5–10% = 2, 10–20% = 3, and ≥20% = 4; stool consistency: 0 = well-formed pellets, soft but still formed = 1; very soft = 2; diarrhea = 3; and fecal blood: 0 = no blood, 1 = positive hemoccult, 2 = blood traces in stool visible, 3 = gross rectal bleeding.

2.4. Histopathological analysis

Formalin-preserved colon tissues were processed and embedded in paraffin by standard techniques. Sections of 5 mm thick were stained with hematoxylin and eosin (H&E) and assessed by a pathologist blinded to the groups of experiment. Histopathological scoring system was assigned based on the extent and severity of inflammation, ulceration, and epithelium damage, as described by Bertin J et al. [21].

2.5. Peripheral blood mononuclear cells (PBMC) isolation

Mice were anesthetized with chloral hydrate and blood was collected by cardiac paracentesis in a tube containing heparin sodium. Anti-coagulated blood was layered onto mononuclear separating medium (Haoyang, Tianjin, China) and PBMC were purified by gradient centrifugation (400 × *g*, 30 min) according to the manufacturer's recommendations, as previously described [22].

2.6. Peritoneal macrophage extraction

Peritoneal macrophages were extracted from C57BL/6 mice by intraperitoneal injection of 4 ml of RPMI1640 media supplemented with 5% FBS and 0.5 mM ethylenediaminetetraacetic acid (EDTA). On the one hand, cells were harvested for M1 macrophages detection. On the other hand, the extracted solution was centrifuged at 300 × *g* for 10 min and isolated cells were cultured at 37 °C in RPMI1640 media plus 10% fetal bovine serum (FBS) 100 U/ml penicillin and 100 μg/ml streptomycin. After 2 h incubation, unattached cells were discarded and attached macrophages were further cultured in fresh complete medium for further study.

2.7. Isolation of lamina propria cells

Colons were dissected, washed with ice-cold PBS containing antibiotics (penicillin plus streptomycin), and cut into small pieces. Colons pieces were then incubated with RPMI medium supplemented with 3% FBS, 0.5 mM dithiothreitol (Sigma-Aldrich, St. Louis, MO, USA), 5 mM EDTA (Sigma), and antibiotics at 37 °C for 30 min to remove epithelial layer. The remaining colon segments then were incubated at 37 °C with RPMI medium containing 0.5% Collagenase D (Roche Applied Science, Basel, Switzerland) and 0.05% DNase (Roche) for 30 min with gentle shaking. The cell suspensions were passed through 70 μm cell strainer and collected for cell flow cytometry analysis.