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Oroxyloside prevents dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB pathway through PPARγ activation

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DAPI (PubChem CID: 2954)
MTT formazan (PubChem CID: 16218671)
DSS sodium salt (PubChem CID: 5167273)
GW9662 (PubChem CID: 644213)
5-ASA (PubChem CID: 4075)
LPS (PubChem CID: 53481793)

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ABSTRACT

Oroxyloside, as a metabolite of oroxylin A, may harbor various beneficial bioactivities which have rarely been reported in the previous studies. Here we established the dextran sulfate sodium (DSS)-induced experimental colitis and evaluated the anti-inflammatory effect of oroxyloside *in vivo*. As a result, oroxyloside attenuated DSS-induced body weight loss, colon length shortening and colonic pathological damage. Furthermore, oroxyloside inhibited inflammatory cell infiltration and decreased myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS) activities as well. The production of pro-inflammatory cytokines in serum and colon was also significantly reduced by oroxyloside. We unraveled the underlying mechanisms that oroxyloside inhibited NF-κB pathway by activating Peroxisome Proliferator-Activated Receptor γ (PPARγ) to attenuate DSS-induced colitis. Moreover, we investigated the anti-inflammatory effect and mechanisms of oroxyloside in the mouse macrophage cell line RAW264.7 and bone marrow derived macrophages (BMDM). Oroxyloside decreased several LPS-induced inflammatory cytokines, including IL-1β, IL-6 and TNF-α in RAW264.7 and BMDM. We also found that oroxyloside inhibited LPS-induced activation of NF-κB signaling pathway via activating PPARγ in RAW 264.7 and BMDM. Docking study showed that oroxyloside could bind with PPARγ. GW9662, the inhibitor of PPARγ, and PPARγ siRNA transfection blocked the effect of oroxyloside on PPARγ activation. Our study suggested that oroxyloside prevented DSS-induced colitis by inhibiting NF-κB pathway through PPARγ activation. Therefore, oroxyloside may be a promising and effective agent for inflammatory bowel disease (IBD).

1. Introduction

Inflammation is a beneficial response activated to restore tissue injury and pathogenic agents. However, the dysregulation of inflammation could increase risk of developing other chronic diseases. Ulcerative colitis (UC) is one kind of inflammatory bowel disease (IBD), which is a chronic and relapsing inflammation in gastrointestinal tract [1]. Nowadays, IBD affects 1.4 million Americans and at a prevalence rate of 396 per hundred thousand individuals [2]. 8–12 per hundred thousand individuals worldwide suffer from UC [3]. Therefore, exploring novel preventive intervention, that could ameliorate ulcerative colitis and counteract side-effects of conventional treatment, is particularly important from a public health perspective.

Peroxisome Proliferator-Activated Receptor γ (PPARγ, also known as NR1C3), a member of the nuclear hormone receptor family, plays a central role in adipocyte differentiation and insulin sensitivity. The evidence has documented PPARγ activation leading to decreases in the production of cytokines, chemokines, reactive oxygen species, and adhesion molecules [4]. PPARγ has been well recognized as an endogenous regulator of intestinal inflammation [5] and previous studies have reported that IBD patients exhibit decreased PPARγ levels in the colon compared with normal controls [6,7]. PPARγ activation protects intestinal tissues from the damage induced by dextran sodium sulfate (DSS), 2,4,6-trinitrobenzene sulfonic acid administration, or ischemia–reperfusion injury [5,8,9].

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Moreover, activating PPARγ displays the anti-inflammatory effects through inhibiting the activation nuclear factor-κB (NF-κB), resulting in a decrease in pro-inflammatory cytokines and chemokines [10,11].

NF-κB family consists of five related transcription factors that regulate inducible gene expression in various physiological contexts. The link between the activation of NF-κB and inflammation has been shown in various human diseases and in animal models of disease. It is known to all that NF-κB plays a critical role in regulating immunological setting [12–14], especially in IBD patients. Activated NF-κB pathway promotes the expression of various pro-inflammatory genes and influences the course of mucosal inflammation [15]. Dextran sulfate sodium (DSS)-induced colitis is a commonly used model to induce experimental intestinal inflammation [16,17]. NF-κB activation induces the production of inflammatory mediators, such as TNF-α, IL-1β and so on, which are characteristics of DSS-induced colitis [18,19].

The anti-inflammatory effect of oroxyloside (Oroxylin A 7-O-glucuronide), one of the main metabolites of oroxylin A, has rarely been reported. The previous studies have demonstrated that oroxylin A prevents inflammation through down-regulation of inflammatory gene expression by inhibiting NF-κB signaling pathway [20–22]. In this study, we investigated the anti-inflammatory effect of oroxyloside on intestinal inflammation and the potential mechanisms. *In vivo*, we established the dextran sulfate sodium (DSS)-induced experimental colitis to evaluate the protective effect of oroxyloside. Oroxyloside attenuated DSS-induced body weight loss, colon length shortening and colonic pathological damage. Oroxyloside inhibited inflammatory cell infiltration and decreased myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS) activities as well. The production of pro-inflammatory cytokines in serum and colon was also significantly reduced by oroxyloside. We further found that oroxyloside protected against DSS-induced colitis by inhibiting NF-κB pathway through activating Peroxisome Proliferator-Activated Receptor γ (PPARγ) in colons. Furthermore, these findings were confirmed in the mouse macrophage cell line RAW264.7 and bone marrow derived macrophages (BMDM). Taken together, our study indicated that oroxyloside activated PPARγ which inhibited NF-κB pathway, thus eventually alleviating DSS-induced colitis.

2. Materials and methods

2.1. Reagents and cell culture

Oroxyloside ($C_{22}H_{20}O_{11}$, MW = 460.39), purchased from Ze Lang Pharmaceuticals Corporation (Nanjing, China), was made into pharmaceutical preparation by Dr. Xue Ke (China Pharmaceutical University, China), and then freshly diluted with Dulbecco's Modified Eagle Medium (DMEM, GIBCO, Carlsbad, CA) to final concentration for *in vitro* study. On the other hand, oroxyloside was prepared as intragastric administration (0.5% CMC) for *in vivo* study. The mice of DSS-treated group were administered 0.5% CMC as vehicle.

LPS (*Escherichia coli*: Serotype O55:B5) and 5-Aminosalicylic acid (5-ASA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dextran sulfate sodium (DSS, molecular weight 36–50 kDa) was obtained from MP Biomedicals Inc. (Irvine, CA, USA). Sodium carboxyl methyl cellulose (CMC) was obtained from Sinopharm Group Co. Ltd. (Shanghai, China).

Myeloperoxidase (MPO) activity assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Dimethylsulfoxide (DMSO) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Nitric Oxide Synthase (NOS) Assay Kit was purchased from Beyotime Institute of Biotechnology (Nanjing, China). Dye DAPI was purchased from Invitrogen (Carlsbad, CA, USA). Triton X-100 was purchased from Shanghai Chao Rui Biotech. Co. Ltd. (Shanghai, China). BSA was purchased from Roche Diagnosis (Shanghai) Ltd. (Shanghai, China). ELISA kits for mouse IL-1β, IL-12, IFN-γ, TNF-α and IL-6 were purchased from Boster Biotech Co. Ltd. (Wuhan, China).

Primary antibodies against PPARγ, NF-κB, β-actin and PPARγ siRNA(m) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); antibody against p-IκBα was purchased from Cell Signaling Technology (Danvers, MA, USA) and antibodies against Lamin A were purchased from Bioworld Technology (Bioworld Technology Inc, MN, USA). Anti-CD11b was purchased from eBioscience (San Diego, CA, USA).

2.1.1. Cell culture

The mouse macrophage cells RAW 264.7 were purchased from the Cell Bank of Shanghai Institute of Biochemistry & Cell Biology at the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle Medium (Gibco, Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco, Paisley, Scotland), 100 U/ml penicillin and 100 U/ml streptomycin at 37 °C with 5% CO_2. Bone marrow derived macrophages (BMDM) were isolated from C57BL/6 mice and cultured with DMEM supplemented with 10% fetal bovine serum and 20 ng/ml GM-CSF (PeproTech, USA). Cells were harvested and seeded on cell culture dishes (60 mm × 15 mm). Exchanging culture fluid every 3 days, adherent macrophages were obtained within about one week. After being cultured for 6 h without GM-CSF, the cells were used for the experiments as bone marrow derived macrophages.

2.2. DSS-induced colitis and design of drug treatment

35–40 day old female C57BL/6 mice, weighing 18–22 g, were supplied by Shanghai Laboratory Animal Center, China Academy of Sciences. Experimental protocols were in accordance with National Institutes of Health regulations and approved by the Institutional Animal Care and Use Committee. Throughout the acclimatization and study periods, all animals had access to food and water *ad libitum* and were maintained on a 12 h light/dark cycle (21 ± 2 °C with a relative humidity of 45 ± 10%).

Acute colitis was induced in C57BL/6 mice by administration of DSS in drinking water. The mice received either regular drinking water (control) or 5% (w/v) DSS drinking water (model) for 7 days and thereafter were provided with regular water for 3 days. The mice were randomly assigned to control, DSS-treated, oroxyloside (20, 40 or 80 mg/kg)-treated and 5-ASA (80 mg/kg)-treated groups. Oroxyloside and 5-ASA were given intragastrically from day 1 to day 10, respectively.

2.3. Macroscopic assessment and histological analysis of colonic lesions

Animals were inspected and weighed daily. After colitis induction animals were sacrificed and colons were removed, opened longitudinally, and washed with phosphate-buffered saline (PBS). The pieces of colonic tissue were used for *ex vivo* analysis. Histological analysis was performed as previously described [23].

2.4. Disease activity index (DAI)

The scores of DAI ranging from 0 (healthy) to 12 (severe colitis) historically have correlated well with the pathologic findings in a DSS-induced model of IBD. DAI is the combined score of body weight loss, stool consistency and rectal bleeding, calculated just as in Table 1, as described previously [24].