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Ameliorative effects of 3,4-oxo-isopropylidene-shikimic acid on experimental colitis and their mechanisms in rats

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ABSTRACT

The aim of the present study was to investigate the therapeutic effect and mechanism of 3,4-oxo-isopropylidene-shikimic acid (ISA) on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats. (50, 100, 200 mg/kg) was administered for 14 days, 1 day after the induction of colitis by TNBS. The colonic injury and inflammation were assessed by macroscopic damage scores and myeloperoxidase (MPO) activity. Malondialdehyde (MDA) and nitric oxide (NO) levels, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in plasma were measured with biochemical methods. Prostaglandin E_2 (PGE_2) level in colon was determined by radioimmunoassay. Expressions of inducible nitric oxide synthase (iNOS), cyclo-oxygenase-2 (COX-2), inhibitor kappa B-alpha (IκBα) and nuclear factor kappa B (NF-κB) p65 proteins in the colonic tissue were detected with immunohistochemistry. Enhanced colonic mucosal injury, inflammatory response and oxidative stress were observed in the animals clystered with TNBS, which was manifested as the significant increase in colon mucosal damage index, MPO activity, levels of MDA, NO and PGE_2, as well as the expressions of iNOS, COX-2 and NF-κB p65 proteins in the colonic mucosa, and the significant decrease in expressions of IκBα proteins in the colonic mucosa. However, these parameters were found to be significantly ameliorated in rats treated with ISA at given doses, especially at 100 mg/kg and 200 mg/kg. Administration of ISA may have significant therapeutic effects on experimental colitis in rats, probably due to its mechanism of antioxidation, its inhibition of arachidonic acid metabolism and its modulation of the IκBα/NF-κB p65 expression.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic relapsing inflammatory disorders of the gastrointestinal tract. Although the etiology and pathophysiology of IBD remain to be elucidated, the current literature suggests that multiple factors contribute to the disease. For instance, neutrophil infiltration and overproduction of pro-inflammatory mediators like cytokines and arachidonate metabolites have been found to be implicated in the pathogenesis of this disease [1]. The tissue injury produced by neutrophils has been attributed to their ability to liberate a variety of reactive oxygen species (ROS), nitrogen metabolites, lytic enzymes or cytokines [2]. Large numbers of neutrophils pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to the overproduction of ROS, which appears to be associated with the increased lipid peroxidation observed in the mucosa of experimental animals and humans with IBD [3,4].

Nuclear factor-κB (NF-κB) is one of the most important oxidative stress-sensitive signaling pathways that play an important role in maintaining the cellular oxidant–antioxidant balance and mediating the expression of genes involved in the inflammatory response. NF-κB is present in the cytoplasm in an inactive state, bound with the inhibitory IκB subunit proteins [5]. NF-κB is activated by a variety of external stimulants, including ROS and cytokines, via phosphorylation of IκBs by IκB kinase. When IκBs are phosphorylated, NF-κB is freed and migrates to the nucleus, triggering the transcription of genes involved in the inflammatory response, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [6].

3,4-Oxo-isopropylidene-shikimic acid (ISA) is a derivative of shikimic acid extracted from *Illicium verum* Hook. fil. Previous research has revealed that ISA has antiinflammatory and analgesic effects, inhibits platelet aggregation and blood coagulation, and antagonizes focal cerebral ischemia injury [7,8]. It has been demonstrated that ISA can diminish the peroxidation levels of the brain in rats with ischemic damage and scavenge superoxide anion and hydroxyl radicals in vitro [9,10]. Our previous study has illustrated that ISA

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exerts an antiinflammatory effect on colitis induced by TNBS in rats [11]. However, the pivotal elements of its inhibitory action on the inflammation remain unclear. The present study was designed to analyze the protective effects of ISA on markers of oxidative stress and the expressions of the pro-inflammatory mediators iNOS and COX-2 in a rat model of experimental colitis, and to investigate whether these protective effects were associated with changes in the oxidative stress-sensitive NF-κB signaling pathway.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, 200–220 g and 10–11 weeks of age, were obtained from Beijing Vital River Laboratory Animals, China. The rats were housed under a 12-h light–dark cycle at a constant ambient temperature (22–25 °C), with normal rat chow and water ad libitum. They were allowed to acclimatize for 1 week before the experiments were started. All rats were deprived of food for 48 h prior to the experimental procedure, but were allowed to have free access to tap water throughout. The rats used in this study were handled and treated in accordance with the Animal Management Rules of the Health Ministry of the People's Republic of China (document no 55, 2001) for experimental care and use of animals.

2.2. Drugs and reagents

The drugs and reagents used in the study are as follows: 2,4,6-trinitrobenzenesulfonic acid (TNBS; from Sigma, USA; 5%(*w*/*v*)), ISA (purity > 98%; provided by the Department of Phytochemistry, Beijing University of Traditional Chinese Medicine, China), salicylazosulfapyridine (SASP; from Shanghai Sunve Pharmaceutical, China; 0.25 g/tablet), polyclonal rabbit anti-rat-iNOS, COX-2, IκBα and NF-κB p65 (from Santa Cruz Biotechnology, USA), SP-Histostain-Plus kit (from Beijing Zhongshan Golden Bridge Biotechnology Co., China), prostaglandin E_2 (PGE_2) radioimmunoassay kit (from Beijing North Institute of Biological Technology, China), and myeloperoxidase (MPO), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and nitric oxide (NO) kits from Nanjing Jiancheng Biochemical Engineering, China. The chemical structure of ISA is shown in Fig. 1.

2.3. Colitis induction and experimental protocols

Colitis was induced according to the procedure described by Wang et al. [12]. Briefly, after 48 h of fasting, the rats were anesthetized with intraperitoneal pentobarbital sodium (35 mg/kg) before the induction of colitis. TNBS (30 mg) dissolved in 0.9 ml of 30% (v/v) ethanol was instilled into the colon 8 cm from the anus through a polyethylene catheter (2 mm external diameter). The rats were kept in a head-down position for 30 min to prevent leakage of the intracolonic instillation. Rats in the control group received physiological saline instead of TNBS solution. The rats were randomly divided into six groups: (1) control—no colitis induced (p.o., saline, $n = 7$), (2) TNBS (p.o., saline, $n = 7$), (3) TNBS + SASP (p.o., 500 mg/kg SASP, $n = 7$), (4) TNBS + 200 mg/kg ISA (p.o., $n = 7$), (5) TNBS + 100 mg/kg ISA (p.o., $n = 7$), and (6) TNBS + 50 mg/kg ISA (p.o., $n = 7$). Twenty four hours after induction of colitis, each group of rats received their respective treatment twice daily for 14 days. Then, all rats were sacrificed by exsanguination of the common carotid artery under light ether anesthesia and blood was collected in vials. To determine MDA, SOD, GSH-Px, and NO, the plasma was separated, frozen immediately, and stored at −70 °C until the assays. The colon was quickly excised, freed of adherent adipose tissue, washed with ice-cold saline, and used for macroscopic scoring and biochemical studies. Portions of colon specimens were kept in 4% (w/v) paraformaldehyde for immunohistochemical study. The remaining portions of colonic specimens were frozen in liquid nitrogen and stored at −70 °C until use for measurements of MPO and PGE_2.

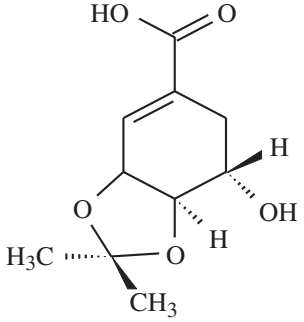

Fig. 1. Chemical structure of 3,4-oxo-isopropylidene-shikimic acid.

2.4. Evaluation of colon macroscopic damage

The entire colon of each animal was excised, cleaned of adherent adipose tissue, opened longitudinally, and rinsed with cold physiological saline to remove fecal residues. The colon was immediately examined visually and damage (colon mucosal damage index (CMDI)) was scored on a scale of 0–10 according to the criteria described by Tsune et al. [13]: grade 0 = normal appearance; grade 1 = focal hyperemia, no ulcers; grade 2 = ulcer with no significant inflammation (hyperemia and bowel wall thickening); grade 3 = ulcer with inflammation at one site; grade 4 = 2 or more sites of ulceration and/or inflammation; grade 5 = major site(s) of damage extending ≥1 cm along the length of the colon; and grades 6–8 = when an area of damage extends ≥2 cm along the colon, the score was increased by 1 for each additional cm of involvement. The adhesions were present between colon, small bowel and other organs (score 0–2) according to the criteria of Bobin-Dubigeon et al. [14]: grade 0 = no adhesion; grade 1 = minor adhesion; and grade 2 = major adhesion. Each colon was observed and evaluated by two independent observers.

2.5. Measurement of colonic MPO, plasma SOD and GSH-Px activities, and MDA and NO levels

For measurement of colonic MPO activity, the sample tissues were homogenized (50 g/l) in 50 mmol/l ice-cold potassium phosphate buffer (pH 6.0) containing 0.5% of hexadecyltrimethylammonium bromide. The homogenate was frozen and thawed thrice, then centrifuged at 4000 *g* for 20 min at 4 °C. The MPO activity in the supernatant was measured by the assay kit according to its provider's instructions. The plasma samples for determination of SOD and GSH-Px activities, and MDA and NO levels were analyzed with their corresponding assay kits according to the manufacturers' guides.

2.6. Measurement of colonic PGE_2 level

Colonic specimens used for the assay of arachidonic acid metabolites were prepared according to the protocol by Raab et al. [15], and the level of PGE_2 was measured by using the corresponding radioimmunoassay kit following the manufacturer's instructions.

2.7. Immunohistochemical localization of iNOS, COX-2, IκBα and NF-κB p65

The colonic tissues were fixed in 4% (w/v) paraformaldehyde, embedded in paraffin wax and sectioned at 5 μm. Endogenous peroxidase activity was blocked by 3% (v/v) hydrogen peroxide. Then heat rehabilitation of antigen was performed with a microwave.