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Piceatannol, a stilbene present in grapes, attenuates dextran sulfate sodium-induced colitis

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ABSTRACT

Piceatannol (3,5,3',4'-tetrahydroxy-trans-stilbene; PIC) is a polyphenol found in grapes. It is known as a protein kinase inhibitor that modifies multiple cellular targets, exerting immunosuppressive and antitumorigenic activities in several cell lines. The purpose of the present work was to evaluate the anti-inflammatory effect of PIC on dextran sulfate sodium (DSS)-induced colitis. Experimental colitis was induced in BALB/c mice by dissolving 5% DSS in their drinking water for 7 days. PIC (1, 2.5, 5, or 10 mg/kg body weight) was administrated daily per oral route for 7 days. A significant blunting of weight loss and clinical signs was observed in DSS-exposed, PIC-treated mice when compared to vehicle-treated mice. This was associated with a remarkable amelioration of the disruption of the colonic architecture, a significant reduction in colonic myeloperoxidase (MPO) activity, and a decrease in production of inflammatory mediators such as nitric oxide (NO), prostaglandin (PG) E_2, and pro-inflammatory cytokines. The present data indicate that further evaluation of the potential of PIC as an agent for the prevention and/or treatment of inflammatory bowel diseases in human clinical studies is warranted.

1. Introduction

Piceatannol (3,5,3',4'-tetrahydroxy- trans-stilbene; PIC) is a polyphenol found in grapes, *Rheum undulatum, rhubarb, and sugar cane.* It is known as a protein kinase inhibitor that modifies multiple cellular targets, exerting immunosuppressive and antitumorigenic activities in several cell lines [1–4]. PIC was originally reported to be a Syk/ZAP70-specific kinase inhibitor [2]. By inhibiting Syk/ZAP70-specific kinase activities, PIC attenuates antigen-induced anaphylactic bronchial smooth muscle contraction and suppresses the release of histamine and peptidoleukotrienes from lung fragments isolated from sensitized guinea pigs [3]. Moreover, PIC strongly inhibits TNF-induced nuclear factor (NF)-κB activation in myeloid cells, lymphocyte and epithelial cells without Syk/ZAP70-specific kinase activity [4], and selectively inhibits the tyrosine phosphorylation of signal transducer and activator of transcription (STAT)3 in human T and B cells [5]. Although PIC has been shown to exert various pharmacological effects on immune and cancer cells *in vitro*, the *in vivo* evidences are sparse.

Inflammatory bowel disease (IBD) is a group of pathologic conditions of the gastrointestinal tract in humans, of which Crohn's disease (CD) and ulcerative colitis (UC) are the most prominent [6]. Although the etiology of IBD still remains unclear, it has been suggested that inflammatory and immune responses play major roles in the pathogenesis [7,8]. A common feature of IBD is a complex interplay of cells and inflammatory mediators such as cytokines within the intestine [8,9]. Interaction of a cytokine with its specific receptor initiates signals that modify cell function in both the cytoplasm and the nucleus. STAT proteins are a family of regulatory elements participating in this process. After activation by a family of cytoplasmic tyrosine kinases termed Janus kinases (JAK), which are associated with cell surface cytokine receptors, STATs translocate into the nucleus [10,11]. Previous investigation has demonstrated that strongly activated states of STAT3 are found in patients with IBD and in animal models of colitis [12]. These findings have been confirmed in additional studies of human IBD [13]. Serum concentrations of interleukin (IL)-6, a potent mediator of STAT3 activation, have been shown to be augmented in patients with IBD [14,15]. Recent studies have demonstrated the benefit of antibodies against the IL-6 receptor in patients with Crohn's disease [16], as well as in animal models of colitis [17,18]. These data strongly suggest that activation of the IL-6/STAT3 pathway plays a key role in the development of IBD.

These observations led us to examine the effect of PIC on intestinal inflammation. It has been reported that PIC impairs activation of NF-κB and STAT-3 in various cell lines, and we were interested in determining if PIC has a therapeutic effect on inflammation by reducing NF-κB and STAT-3 activation [4,5]. In order to investigate the effects of PIC, we studied a dextran sulfate sodium (DSS)-induced mouse colitis model. This model resembles human IBD and is used for pharmacological

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analysis of potentially effective anti-inflammatory agents [19,20]. Furthermore, the DSS model faithfully reproduces many of the immunological disturbances observed in humans with IBD [21].

2. Materials and methods

2.1. Synthesis of PIC

We synthesized of PIC (Fig. 1A) in 6 steps from 3,5-dihydroxybenzoic acid by using Wittig–Horner reaction as described at Fig. 1B.

2.2. Mice and experimental protocol

The study protocol was approved by the Animal Care and Use Committee of Hallym University. Six-week-old female BALB/c mice were purchased from Japan SLC (Hamamatsu, Japan) and maintained under specific pathogen-free conditions at the animal facility of Hallym University (Chuncheon, Korea). To induce experimental colitis, the mice were treated for 7 days with 5% DSS (40,000–50,000 MW; ICN Biomedicals, Aurora, OH, USA) dissolved in filter-purified water (Millipore Corp, Bedford, MA, USA). The control mice received filtered water alone. PIC was dissolved in dimethyl sulfoxide (DMSO) and was freshly diluted in corn oil. PIC (1, 2.5, 5, or 10 mg/kg of body weight) or the vehicle (corn oil with DMSO) was administrated *by gavage* for 7 days, beginning in coordination with the start of DSS exposure. We did not observe differences in water consumption among the groups (3.5–4.0 ml/day/mouse) during the experimental period. The study protocol was approved by the Animal Care and Use Committee of Hallym University.

2.3. Assessment of DSS-induced colitis

The mice were assessed daily for the development of colitis based on body weight, gross rectal bleeding, stool consistency, and survival. Overall disease severity was assessed using a clinical scoring system with a scale of 0–4 [19].

2.4. Colon tissue culture

Tissue from the mid-colon was washed with RPMI 1640 (Hyclone, Logan, UT, USA) medium containing 2% fetal bovine serum (FBS, Hyclone) and penicillin and streptomycin (Hyclone) before being cut into smaller pieces. Then, approximately 0.5 cm of tissue was placed in 0.5 ml of 0.1% FBS containing RPMI-1460 medium, loaded in 48-well tissue culture plates, and incubated for 24 h at 37 °C in 5% CO_2.

2.5. Measurement of cytokines, NO, and PGE_2

Concentrations of various cytokines in the cell-free culture supernatants of the colon tissues were measure using a Bio-Rad Multiplex bead array instrument and cytokine kit (Bio-Rad, Irvine, CA, USA), according to the manufacturer's protocol. Nitrite and PGE_2 production were measured using the Griess reagent system (Promega, Madison, WI, USA) and an enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN, USA), respectively, according to the manufacturer's instructions.

2.6. Determination of myeloperoxidase (MPO) activity in the colon

The mouse colons (50-100 mg) were rinsed with cold PBS, blotted dry, and immediately frozen in liquid nitrogen. They were then stored at −80 °C until they were assayed for MPO activity using the *o*-dianisidine method [22].

2.7. Quantitative real time RT-PCR

The total RNA from the 100 mg of colon homogenates was isolated using an RNAeasy mini kit (Quiagen, Hilden, Germany). After RNA

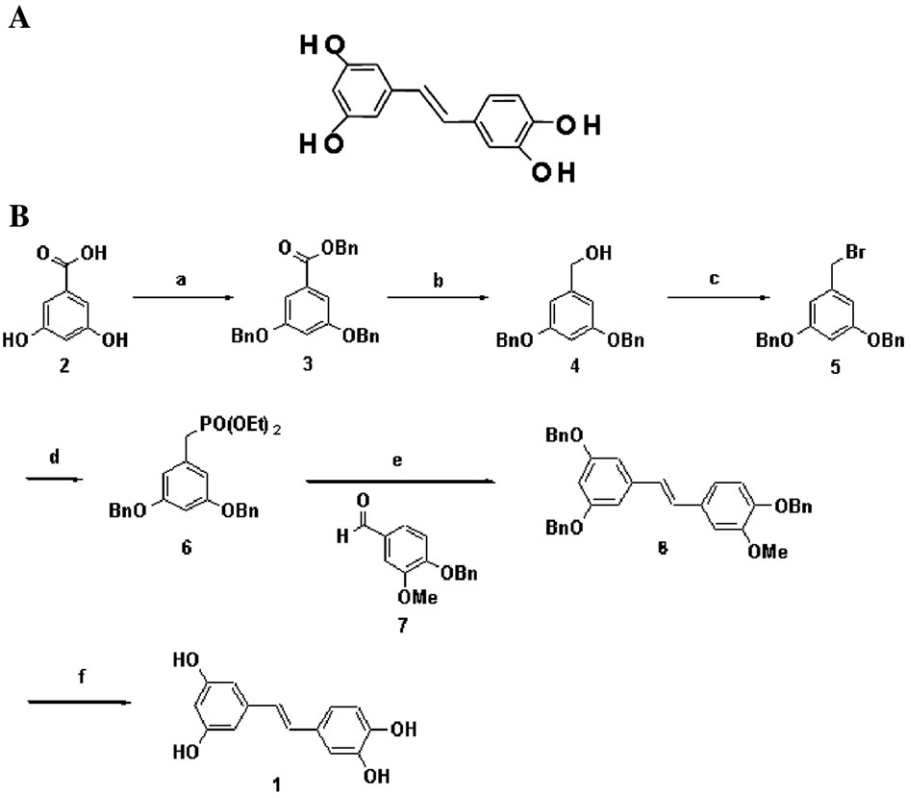

Fig. 1. (A) Chemical structure of piceatannol (PIC) and (B) scheme of PIC synthesis. Reagents and conditions are bellowed. a, BnBr(3.2eq), K2CO3(6eq), acetone, reflux, 97%; b, LAH(1.5eq), THF, at room temperature, 88%; c, PBr3(3.1eq), ACN, at room temperature, 99%; d, P(OEt)3(3.5eq), xylene, reflux, 92%; e, NaH(6eq), THF, reflux, 99%; f, BBr3(6eq), CH_2Cl_2 at room temperature, 50%.