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Research paper

In vivo and *in vitro* anti-inflammatory activities of *Persicaria chinensis* methanolic extract targeting Src/Syk/NF-κB

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ABSTRACT

Ethnopharmacologic relevance: Persicaria chinensis L. (Polygonaceae) [also synonym as *Polygonum chimnense* L.] has been used as Chinese traditional medicine to treat ulcer, eczema, stomach ache, and various inflammatory skin diseases. Due to no molecular pharmacological evidence of this anti-inflammatory herbal plant, we investigated the inhibitory mechanisms and target proteins contributing to the anti-inflammatory responses of the plant by using its methanolic extract (Pc-ME).

Materials and methods: We used lipopolysaccharide (LPS)-treated macrophages and a murine HCl/EtOH-induced gastritis model to evaluate the anti-inflammatory activity of Pc-ME. HPLC analysis was employed to identify potential active components of this extract. Molecular approaches including kinase assays, reporter gene assays, immunoprecipitation analysis, and overexpression of target enzymes were used to confirm target enzymes.

Results: Pc-ME inhibited LPS-induced nitric oxide and prostaglandin E_2 release by RAW264.7 macrophages and ameliorated HCl/EtOH-induced gastric ulcers in mice. The nuclear translocation of NF-κB (p65 and p50) was suppressed by Pc-ME. Phosphorylation of Src and Syk, their kinase activities, and formation of the signaling complex of these proteins were repressed by Pc-ME. Phosphorylation of p85 and Akt induced by Src or Syk overexpression was blocked by Pc-ME. In the mouse gastritis model, orally administered Pc-ME suppressed the increased phosphorylation of IκBα, Akt, Src, and Syk. Caffeic acid, kaempferol, and quercetin, identified as major anti-inflammatory components of Pc-ME by HPLC, displayed strong nitric oxide inhibitory activity in LPS-treated macrophages.

Conclusion: Pc-ME might play a pivotal ethnopharmacologic role as an anti-inflammatory herbal medicine by targeting Syk and Src kinases and their downstream transcription factor NF-κB.

1. Introduction

Inflammation is a component of the complex biologic response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants (Steel et al., 2013). Both acute and chronic inflammatory responses play a vital role in the natural defense mechanisms of the body's innate immune system to maintain immune homeostasis. Phagocytosis of pathogens *via* receptors such as toll-like receptors (TLRs) that recognize molecular patterns of pathogen-derived materials including lipopolysaccharide (LPS) leads to activation of immune cells such as macrophages, neutrophils, and dendritic cells, and ultimately triggers inflammatory responses (Hiraiwa and van Eeden, 2013). Activation of inflammatory cells leads to an increase in intracellular signaling through cascades involving tyrosine kinases

Abbreviations: Pc-ME, methanolic extract of *Persicaria chinensis*; HPLC, High performance liquid chromatography; PGE_2, prostaglandin E_2; NO, nitric oxide; COX, cyclooxygenase; iNOS, inducible NO synthase; TLR, Toll-like receptors (TLR); NF-κB, nuclear factor-κB; Akt, protein kinase B; IKK, IκBα kinase; MyD88, myeloid differentiation primary response protein-88; Syk, spleen tyrosine kinase; EIA, enzyme immunoassay; MTT, 3–4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LPS, lipopolysaccharide; RT-PCR, reverse transcriptase-polymerase chain reaction

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and inhibitor of κB kinase (IKK), activating the nuclear transcription factor (NF)-κB and triggering the expression of various inflammatory genes, such as inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX)-2 (Byeon et al., 2012; Lee et al., 2013; Yang et al., 2014a, 2014b). As a result, numerous inflammatory mediators, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), and proinflammatory cytokines are released to further evoke chemotactic responses of other inflammatory cells, leading to the production of hydrolytic enzymes and cytotoxic molecules (Labow et al., 2001). All of these responses help to protect the body against infection by pathogens. However, sustained inflammatory responses can result in serious diseases such as cancer, diabetes, and Alzheimer's disease (Lee, 2011; Ham and Moon, 2013). Thus, immunologists have recently turned their attention to the development of safe and effective anti-inflammatory remedies to prevent or cure chronic inflammatory conditions.

Persicaria chinensis L. [also known as *Polygonum chimnense* L.] (Polygonaceae) is a perennial, scrambling herb native to tropical and subtropical eastern Asia, and is widely distributed in Taiwan, Japan, Philippines, New Zealand, the Hawaiian Islands, and Papua New Guinea. *Persicaria chinensis* is used as a folk medicine in the Malaysian Chinese community and Tamang community of Nepal, especially for lung ailments including cancer (May, 2012; Luitel et al., 2014). Traditionally, Chinese people have used whole plants to reduce fever and eliminate toxins, and to treat dysentery, inflammatory skin disease, eczema, and corneal nebula (Wan et al., 2009). The methanolic extract of leaves has been also used as antibacterial and antifungal activities in Malaysian communities (Lai et al., 2012), leaves juice used against ulcer by Paniya, Kuruma, and Kattunaikka tribe in India (Narayanan, 2012), orally administration of leaves extract used by *chakma* tribe in Bangladesh to treat allergies and snakebites (Rahman et al., 2007).

The molecular mechanism underlying the anti-inflammatory activity of this plant extract is not understood. Therefore, in this study we focused on the immunopharmacologic mechanism of *Persicaria chinensis* using methanol extract (Pc-ME) in lipopolysaccharide (LPS)-activated macrophages and a murine HCl/EtOH-induced acute gastritis model. We report the phytochemical profiles of Pc-ME and demonstrate that its anti-inflammatory effects *in vitro* and *in vivo* are mediated through inhibition of Src and Syk tyrosine kinases and subsequent modulation of the NF-κB pathway.

2. Materials and methods

2.1. Materials

Standard compounds (Caffeic acid, kaempferol, luteolin, and quercetin) for HPLC analysis, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pam3-Cys-Ser-Lys4 (Pam3CSK4), the synthetic bacterial lipopeptide acting as the ligand for the heterodimeric TLR1/TLR2 complex, and lipopolysaccharide (LPS, *Escherichia coli* 0111:B4), a ligand of TLR4, were purchased from Sigma Chemical Co. (St. Louis, MO, USA) PP2 and piceatannol (Picea) were obtained from Calbiochem (La Jolla, CA, USA). The luciferase construct containing the promoter binding site for NF-κB was used as reported previously (Yu et al., 2011a, 2011b). Fetal bovine serum (FBS) and RPMI1640 were obtained from Gibco (Grand Island, NY, USA). RAW264.7 cells, a BALB/c-derived murine macrophage cell line (No. TIB-71), and HEK293 cells, a human embryonic kidney cell line (No. CRL-1573), were purchased from ATCC (Rockville, MD, USA. Luciferase constructs containing binding sites for NF-κB, HA-Src, and Myc-Syk were used as reported previously (Byeon et al., 2013) All other chemicals were purchased from Sigma. Phospho-specific or total antibodies used in this study were obtained from Cell Signaling Technology (Beverly, MA, USA).

2.2. Pc-ME preparation

The 95% methanol extract (Code No: PBID 110601) of the aerial parts of *Persicaria chinensis* (Pc-ME) was obtained from the Plant Extract Bank in the Plant Diversity Research Center (Daejeon, Korea; http://extract.pdrc.re.kr/extract/f.htm, e-mail: plantext@kribb.re.kr). Briefly, the dried aerial parts of *Persicaria chinensis* were pulverized to powder using a mechanical grinder. The dried powders (100 g) were then extracted with 95% methanol for 48 h in the soxhlet apparatus. The extracts were filtered and concentrated to vacuum under reduced pressure in rotary evaporator and dried in desiccators. The yield of the extract was approximately 27.8%.

2.3. Animals

Male C57BL/6 and ICR mice (6–8 weeks old, 17–21 g) were obtained from DAEHAN BIOLINK (Chungbuk, Korea) and were housed in groups of 6–8 mice under a 12-h light/dark cycle (lights on at 6 a.m.). Water and pellet diets (Samyang, Daejeon, Korea) were supplied *ad libitum*. Animals were cared for in accordance with the guidelines issued by the National Institute of Health for the Care and Use of Laboratory Animals (NIH Publication 80-23, revised in 1996). Studies were performed in accordance with guidelines established by the Institutional Animal Care and Use Committee at Sungkyunkwan University (Suwon, Korea; approval ID: SKKUBBI 12-6-1).

2.4. Preparation of peritoneal macrophages

Peritoneal exudates were obtained from C57BL/6 male mice by lavage 4 days after intraperitoneal injection of 1 ml of sterile 4% thioglycollate broth (Difco Laboratories, Detroit, MI, USA). After washing with RPMI 1640 medium containing 2% FBS, peritoneal macrophages (1×10^6 cells/ml) were plated in 100-mm tissue culture dishes for 4 h at 37 °C in a 5% CO_2 humidified atmosphere.

2.5. Cell culture

The peritoneal macrophages, and RAW264.7 cells and HEK293 cells were cultured in RPMI 1640 and DMEM medium, respectively, supplemented with 10% heat-inactivated FBS, glutamine, and antibiotics (penicillin and streptomycin) at 37 °C under 5% CO_2.

2.6. Drug treatment

For *in vitro* experiments, a stock solution of Pc-ME dissolved in 100% DMSO at a concentration of 100 mg/ml was further diluted with culture medium. For *in vivo* treatment, Pc-ME (200 mg/kg) was resuspended in 1% sodium carboxymethylcellulose (CMC) as reported previously (Yang et al., 2012).

2.7. Production of nitric oxide and prostaglandin E_2

RAW264.7 macrophage cells (1×10^6 cells/ml) were cultured for 18 h, pre-treated with Pc-ME (0–300 μg/ml) for 30 min, and further incubated with LPS (1 μg/ml) for 24 h. The inhibitory effect of Pc-ME on NO and PGE_2 production was determined by Griess assay and enzyme immunoassay as previously described (Kim and Cho, 2013b).

2.8. Cell viability test

The cytotoxic effect of Pc-ME was evaluated using a conventional MTT assay as previously reported (Jho et al., 2013; Kim and Cho, 2013a).