Isoegomaketone From *Perilla frutescens* Ameliorates Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice

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Abstract

Inflammatory bowel disease (IBD) is a worldwide health problem. An effective treatment for IBD is unavailable, and potential therapeutic agents should be developed for treating colonic inflammation. In this study, we demonstrated that isoegomaketone (IK), an essential oil component of a mutant cultivar of *Perilla frutescens* produced through radiation breeding, reduced levels of inflammatory cytokines, such as tumor necrosis factor-α, interleukin-6, interleukin-10, and interleukin-12p40, in lipopolysaccharide-stimulated mouse primary macrophages. Furthermore, when 5 mg/kg IK was administered to mice with dextran sodium sulfate-induced colitis, it ameliorated the severity of the disease as assessed by survival, body weight, and colonic damage. These results suggested that 5 mg/kg IK has a preventive effect in colitis and can be a novel alternative therapy for treating colitis.

Keywords

inflammatory bowel disease, isoegomaketone, *Perilla frutescens*, anti-inflammatory activity, proinflammatory cytokines, radiation breeding, macrophages

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*Perilla frutescens* (L.) Britton (*P. frutescens*) is an annual plant belonging to the mint family, Lamiaceae. It is widely used as food and herbal medicine in Asian countries.²⁻⁴ Perilla is a traditionally important herb considering its ethnobotanical uses.²,³ Several previous research papers have reported that *P. frutescens* has anti-inflammatory effects.⁴⁻⁶ We have developed a mutant *P. frutescens* cultivar having potent anti-inflammatory abilities due to increased phytochemicals through a gamma radiation breeding mutation.⁷ Mutant *P. frutescens* containing 10-fold isoegomaketone (IK) content exhibited enhanced anti-inflammatory and antioxidant effects in a macrophage cell line.⁸

Primary cells are excellent model systems for studying normal physiology and metabolism in vitro compared with artificial cell lines.⁹,¹⁰ Therefore, we performed this study on primary cells by isolating and maintaining bone marrow-derived macrophages (BMDMs). We investigated whether IK exhibits inhibitory effects on inflammation using enzyme-linked immunosorbent assay (ELISA) for measurement of pro-inflammatory cytokines in lipopolysaccharide (LPS)-stimulated BMDMs. IK significantly suppressed the level of tumor necrosis factor (TNF)-α, interleukin (IL)-10, IL-6, and IL-p40 in a dose-dependent manner (Figure 1). Interestingly, the TNF-α level was significantly affected by IK treatment in BMDMs, but not in RAW264.7 cells (Figure 1A). According to previous studies, substances that can modulate the inflammatory response in normal macrophages are effective in controlling intestinal inflammation.¹¹,¹² These results suggested that IK is likely to be effective for colitis.

Further, we investigated the anti-inflammatory effects of IK in a mouse model with dextran sodium sulfate (DSS)-induced colitis. To determine the therapeutic effects of IK in intestinal inflammation, we administrated DSS in either the presence or absence of IK. To evaluate the impact of IK on pathogenesis of the DSS-induced colitis,¹³ mice were orally pretreated with IK for 1 week, and further administrated with 2% DSS in drinking water for 1 week. This was followed by providing recovery time without DSS administration (Figure 2A). The main clinical feature of the model of acute DSS-induced colitis is considerable weight loss. The rates of initial body weight loss after

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treatment with 5 mg/kg IK and 150 mg/kg 5-aminosalicylic acid (5-ASA), an anti-inflammatory drug commonly used in colitis, significantly decreased after DAY 5. However, treatment of 1 mg/kg IK was not effective enough (Figure 2A). Next, we assessed disease activity index (DAI), which refers to the severity of colitis, during DSS treatment. As shown in Figure 2B, 5 mg/kg IK exhibited a reduction in the DAI scores, and a low concentration of IK also showed a slightly reduced DAI score. However, the positive control (5-ASA-treated group) exhibited the most potent protective effect against colitis. These results were consistent with a previous study indicating that IK has an anti-inflammatory effect on macrophages (both RAW264.7 and primary cells) and is effective in reducing intestinal inflammation in a dose-dependent manner. Additionally, these results indicated that the therapeutic effect of IK was similar to that of 5-ASA at low concentrations.

Another characteristic of severe colonic inflammation is colon shortening. To determine the preventive effect of IK on colitis, we measured the colon length. The colon length of the DSS-induced colitis group was shortened compared with the control group. The administration of IK prevented shortening of the colon in a dose-dependent manner in DSS, but was not statistically significant compared with the only DSS treated mice (Figure 3A). Splenic enlargement is also a marker of severe inflammation. The administration of IK and 5-ASA inhibited spleen swelling, but it was not statistically significant compared with only DSS treated mice (Figure 3B). These results suggested that IK has a protective role in colitis.

Elevated levels of proinflammatory cytokines are markers of colitis. To investigate the effects of IK on the levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in DSS-induced colitis, we assessed their levels in colon tissues. IK treatment reduced the level of proinflammatory cytokines in colon tissues, which was similar to that observed after 5-ASA treatment (Figure 4). Particularly, TNF-α level was dramatically decreased in the IK-treated group, which was consistent with BMDM results (Figures 1A and 4A).

Our study suggested that mutation using gamma radiation can produce medicinal herbs with improved properties, as seen in the case of P. frutescens, which had altered phytochemical content imparting beneficial properties. This led to the development of easy-to-use, safe, and cost-effective natural products. Thus, radiation breeding technology can be a novel strategy to develop material aimed at finding treatments for diseases which
do not have a cure. In this study, we demonstrated the anti-inflammatory effects of IK isolated from a *P. frutescens* mutant, produced via radiation breeding, on primary macrophages in vitro and intestinal inflammation in vivo. These results indicated that IK ameliorated intestinal inflammation by regulating cytokine levels related to NF-κB signaling. Further studies are

**Figure 2.** IK treatments ameliorate weight loss and disease activity index (DAI) in DSS-induced colitis in mice. (A) Timeline representation of induction of colitis and IK and 5-ASA treatments. (B) Percent body weight change (%). (C) DAI. Data represent the mean ± SEM of 3 mice per treatment (*P<.05, **P<.01, and ***P<.001 vs LPS only treated group).
required to identify more specific molecular modifications by IK treatment, such as metabolic and epigenetic changes.

Experimental

Preparation of IK

IK was isolated from the supercritical carbon dioxide extract of a radiation-induced mutant cultivar of *P. frutescens* var. *crispa*. IK was dissolved in dimethyl sulfoxide (Sigma, USA) at 1 g/mL (stock concentration). For oral administration, IK stock was dif fused in PBS containing 0.5% Tween 20 using ultrasonication.

Materials

LPS (*Escherichia coli* O111:B4) was purchased from Sigma, DSS from MP Biomedicals (USA), DMEM, penicillin–streptomycin and fetal bovine serum (FBS) from Gibco (USA), and macrophages colony stimulating factor (M-CSF) from R&D systems (USA).

Cells

BMDMs were isolated from 6-week old C57BL/6 mice and maintained in DMEM containing 10% FBS (Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA) at 37 °C in a 5% CO₂ atmosphere.

Mice

The animals were 5-week-old male BALB/c mice purchased from G-bio (Gwangju, Korea). The mice were housed under conventional conditions at 23 ± 2°C with 60 ± 5% humidity. All experiments were approved by the Animal Experimental Committee of KAERI (Approval No. KAERI-IACUC-2020-022).

ELISA

The colon tissues were homogenized and centrifuged, and the supernatant was harvested. TNF-α, IL-6, and IL-1β cytokines levels were measured using ELISA kits (R&D) according to the manufacturer’s protocol.

DSS-Induced Mice Model

Mice were randomly divided into 5 groups: (1) PBS (n = 3), (2) DSS only (n = 3), (3) DSS + IK 1 mg/kg, (4) DSS + IK 5 mg/kg, (5) DSS + 5-ASA (150 mg/kg). First, mice were orally
administered with PBS, IK, or 5-ASA for 1 week, and further drinking water was changed to supplement 2% DSS. After providing 2% DSS ad libitum, body weight and DAI (overall evaluation of colitis severity, such as change in body weight, vitality, stool consistency, and gross bleeding) was measured every day. After 1 week of DSS administration, mice were provided with normal water and monitored till day 16. At day 16, mice were sacrificed, and colon and spleen were removed for subsequent analysis.

**Statistical Analysis**

Results were presented as mean ± SD. Statistically significant differences were calculated using one-way ANOVA with Tukey’s post-hoc test using Prism 5.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical Approval**

Ethical Approval is not applicable for this article.

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**Statement of Informed Consent**

There are no human subjects in this article and informed consent is not applicable.
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