Suppression of Th2-driven, allergen-induced airway inflammation by sauchinone

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A B S T R A C T
Sauchinone, a lignan compound isolated from the root of Saururus chinensis, has been recently demonstrated to exhibit anti-inflammatory activity via the suppression of NF-κB p65 activity in vitro. In an effort to evaluate the in vivo anti-inflammatory function of sauchinone, we have evaluated the effects of sauchinone on allergen-induced airway inflammation using a murine model of allergic asthma. We observed that marked eosinophilic and lymphocyte infiltration in the BAL fluid were suppressed to a significant degree by sauchinone, and that mucus-secreting goblet cell hyperplasia and collagen deposition in the airways were also ameliorated by administration of sauchinone treatment. Moreover, gene expression of the inflammatory cytokines, IL-13, and IL-5 and eotaxin in the lung, and IL-5 in the draining lymph node were significantly decreased in sauchinone-treated mice. We demonstrated that sauchinone repressed Th2 cell development in vitro and IL-4 production by Th2 cells, and also inhibited GATA-3-mediated IL-5 promoter activity in a dose-dependent manner. Collectively, sauchinone ameliorated allergen-induced airway inflammation, in part, by repressing GATA-3 activity for Th2 cell development, indicating the possible therapeutic potential of sauchinone in airway inflammatory diseases including allergic asthma and rhinitis.

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Introduction

Sauchinone is a unique lignan isolated from Saururus chinensis (Saururaceae), which has been used traditionally as a poultice, diuretic, anti-rheumatic, and anti-inflammatory folk medicine [1,2]. The biological functions of sauchinone have been studied extensively, and the compound has been shown to exert hepatoprotective [1,2], anti-apoptotic [3], anti-osteoclastogenic [4], and anti-septic effects [5]. Additionally, sauchinone inhibits the gene expression of inducible NO synthase (iNOS), tumor-necrosis factor-α (TNFα), and cyclooxygenase-2 (COX-2) by the suppression of NF-κB activity [6,7], thus suggesting the anti-inflammatory functions of sauchinone in vitro. However, the effects and regulatory mechanisms of sauchinone on inflammatory immune responses in vivo have yet to be clearly elucidated.

The ovalbumin (OVA)-induced murine asthma model is a well-established airway inflammatory disease model, like allergic asthma in humans [8,9]. OVA sensitization and subsequent OVA challenge in a murine model induce the infiltration of immune cells including neutrophils, eosinophils, and lymphocytes in the bronchoalveolar lavage fluid (BALF), and increase airway smooth muscle proliferation, mucus-secreting goblet cell hyperplasia, and collagen deposition in the airways [10–12]. It is believed that Th2 helper 2 (Th2) cells mediate allergic airway inflammation by producing IL-4, IL-5, and IL-13, which are responsible for eosinophil recruitment, airway hyperresponsiveness, and airway remodeling [13–16]. The antigenic stimulation of CD4+ Th cells in the presence of IL-4 activates the gene transcription of Th2-specific transcription factors and drives differentiation into Th2 cells [17]. GATA-3 is a master regulator of Th2 cell development and activates IL-5 and IL-13 gene transcription via direct binding to the gene promoter [18–20]. Suppression of Th2 cell development may be crucial to the treatment of allergic airway inflammation.

In this study, we have investigated the anti-inflammatory activity of sauchinone using a murine model of allergic airway inflammation, and have determined that sauchinone substantially attenuates airway inflammation through the suppression of Th2 cell development. This finding clearly indicates that sauchinone may perform a beneficial function in the control of airway inflammatory disorders such as allergic asthma and rhinitis.
Materials and methods

Isolation and purification of sauchinone. The dried roots of S. chinensis were extracted with 80% methanol, then fractionated by extensive column chromatography and purified by semi-preparative HPLC. Sauchinone was obtained as a colorless powder, as reported previously [1,2] and the purity was confirmed as 99% by HPLC and NMR. Endotoxin-free sauchinone was used for the experiments in vitro and in vivo.

Mice. Wild-type BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, MN). All mice were housed under specific pathogen-free conditions at Ewha Womans University, and all handling of the mice and experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee.

OVA sensitization and challenge into BALB/c mice. BALB/c mice were intraperitoneally sensitized with ovalbumin (OVA, 20 mg/mouse) in aluminum hydroxide (alum, 2 mg) on day 1 and day 14, and challenged 5 times with 2% OVA in PBA by intranasal administration every 2 days beginning on day 28 [21]. Sauchinone (10 or 100 mg/kg body weight) was intraperitoneally injected into the mice immediately prior to each OVA challenge.

Ex vivo analysis of cytokine production in draining lymph node. Single cell suspensions were isolated from the draining lymph nodes of OVA-injected mice and stimulated with anti-CD3 (1 μg/ml, BD Pharmingen, San Diego, CA) for 24 h. Cell supernatants were collected and used for measuring cytokine production by ELISA.

Th cell development in the presence of sauchinone. CD4+ Th cells (>95% purity) were isolated from the lymph nodes using mouse CD4 beads, according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA) and stimulated with plate-bound anti-CD3 and anti-CD28 (1 μg/ml, BD Pharmingen, San Diego, CA). After 24 h, the cells were additionally treated with antibodies and cytokines; anti-IL-4 (5 μg/ml) and IL-12 (2 ng/ml) for Th1, anti-IFNγ (5 μg/ml), anti-IL-4 (5 μg/ml), TGFβ (5 ng/ml) and IL-6 (10 ng/ml) for Th17, and anti-IFNγ (5 μg/ml) and IL-4 (10 ng/ml) for Th2 cell development. At day 2, cells were administered with sauchinone and incubated for an additional 4–5 days until restimulation.

ELISA. Cytokines were measured with the ELISA kit in accordance with the manufacturer's recommendations (R&D Biosystems Inc.). In brief, cell supernatants or BAL fluid were collected and incubated on the capture Ab-coated ELISA plate. The plates were subsequently incubated with biotinylated anti-cytokine Abs and phosphatase-conjugated streptavidin (BD Pharmingen). Colorimet-
ric changes were evaluated using an ELISA plate reader (Molecular Devices, Paola Alto, CA). Purified and known concentrations of mouse IL-5, IL-13, and IL-17 (eBiosciences) were used for standard curves.

Intracellular cytokine staining. Th2-primed CD4+ Th cells were suspended in permeabilization buffer (10% saponin in PBS), fixed in 2% paraformaldehyde, and incubated with allophycocyanin-conjugated anti-IL-4 Ab (BD Pharmingen) for 20 min on ice. The cells were washed and analyzed using the FACS Calibur and CellQuest programs (BD Biosciences).

Real-time PCR. Total RNA was harvested from the lung using TRIzol and reverse-transcribed into cDNA using a SuperScript II RT kit (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was conducted using an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA). The primer sequences utilized were as follows: β-actin-FWD 5'-aagcaggagtatgacgagtccg-3', β-actin-REV 5'-tgggtcctgtagatggcattg-3', IL-13-FWD 5'-agaccagactcccctgtgca-3', IL-13-REV 5'-ttggctcttgtaggccttgga-3', eotaxin-FWD 5'-cagacgccccagtacctgata-3', eotaxin-REV 5'-tggctcttgtaggccttgga-3'.

Statistical analysis. Data are expressed as the means ± SDs. Statistical significance was calculated using an unpaired Student t-test. A value of *p < 0.05 was considered statistically significant. **p < 0.005; ***p < 0.0005.

Results and discussion

Sauchinone attenuates allergen-induced airway inflammation

Previous reports that sauchinone suppresses pro-inflammatory cytokine production in lipopolysaccharide-treated macrophages by inhibiting NF-kB p65 activity [5,7] prompted us to ascertain whether sauchinone can provide significant benefits in modulating inflammatory disorders. In order to evaluate the in vivo anti-inflammatory activity of sauchinone in airway inflammatory disease, we sensitized mice to OVA and injected them with vehicle or sauchinone concurrently with subsequent OVA challenges. As anticipated, immune cells such as neutrophils, macrophage, lymphocytes and eosinophils infiltrated in the BALF were markedly increased by OVA sensitization and challenge. While 10 mg/kg of sauchinone scarcely influenced on immune cell infiltration, 100 mg/kg of sauchinone significantly reduced neutrophil and lymphocyte numbers in the BALF (Fig. 1A–C). Additionally, the marked eosinophil infiltration induced by OVA was diminished at both 10 and 100 mg/kg concentrations of sauchinone (Fig. 1D). Because eosinophils have been suggested to contribute to airway remodeling, the effects of sauchinone on mucus production and collagen deposition, prominent features of airway remodeling, were evaluated by PAS and Masson’s trichrome staining. Lung tissues stained with PAS and Masson’s trichrome stain revealed that sauchinone substantially suppressed mucus-secreting goblet cell hyperplasia and collagen deposition in the airways (Fig. 1E). These results imply that sauchinone suppresses eosinophil infiltration in the BALF and attenuates airway remodeling due to mucus overproduction and collagen deposition.

OVA-mediated marked IL-5 and IL-13 production in the lung were diminished by sauchinone

Eosinophil recruitment into the airways of OVA-sensitized mice is mediated by CD4+ Th cells that generate IL-5 and IL-13 [22–26]. Because sauchinone significantly attenuated eosinophil infiltration in the lungs, we evaluated IL-5 and IL-13 expression in the lung. Although IL-5 in the BALF was not determined by ELISA, the levels...
of IL-13 were significantly increased in OVA-injected mice (Fig. 2A). However, Th2-associated IL-13 expression was markedly reduced by sauchinone injection at both protein and mRNA levels (Fig. 2A and B). Quantitative real-time PCR analysis also demonstrated that sauchinone significantly reduced IL-5 gene transcription in the lung (Fig. 2C). On the other hand, the eotaxin chemokine is also involved in eosinophil recruitment and activation and increased by IL-13 in airway epithelial cells [27,28]. Antigen-induced eotaxin expression was also attenuated by sauchinone treatment (Fig. 2D); this finding was consistent with the reduced Th2 cytokine production. Our findings collectively demonstrated that sauchinone significantly reduces Th2-driven IL-13 and IL-5 cytokines and eotaxin chemokine production in the lung.

Allergic inflammatory cytokines produced in draining lymph nodes were selectively inhibited by sauchinone

In order to confirm the inhibitory effects of sauchinone on the Th2-driven cytokines IL-5 and IL-13, we isolated single cell suspensions from draining lymph node and stimulated them for 24 h with anti-CD3. Ex vivo analyses of cytokine production showed that sauchinone induced no significant changes in IL-2 (Fig. 3A), but significantly repressed IL-5 and IL-4 production (Fig. 3B and C). In addition, pro-inflammatory cytokine IL-17, which is generated by Th17 cells and is closely associated with chronic inflammatory diseases was apparently reduced by sauchinone [29–32] (Fig. 3D). Therefore, we strongly suggest that sauchinone suppresses not only Th2 cell development but also Th2 cytokine production in differentiated Th2 cells. It is well characterized that Th2 cell differentiation and the persistent production of Th2 cytokines requires GATA-3 activity to activate cytokine gene transcription [18,33], we thus inquired the effects of sauchinone on GATA-3 expression and activity. Although the expression of GATA-3 remained unchanged by the presence of sauchinone, sauchinone treatment substantially suppressed the IL-5 promoter activity increased by enforced GATA-3 expression in a

Sauchinone inhibits Th2 cell development by decreasing GATA-3 activity

As sauchinone selectively suppresses Th2-driven cytokines and IL-17, which are responsible for allergic airway inflammation, the functions of sauchinone on Th cell development was evaluated. CD4+ Th cells stimulated with anti-CD3 and anti-CD28 were induced to differentiate into Th1, Th2, and Th17 cells and subsequently treated with various amounts of sauchinone at day 2 of Th cell differentiation. Although sauchinone is known to inhibit NF-kB p65 activity and affect NF-kB-mediated cell proliferation [6,7], Th cell proliferation and expansion upon TCR stimulation were not affected by the addition of indicated amounts of sauchinone during Th cell differentiation. Sauchinone had no significant effect on both Th1 and Th17 cell development (Fig. 4A and B), suggesting indirect function of sauchinone on the suppression of IL-17 in the inflammatory lung. However, IL-4-producing Th2 cell differentiation was significantly decreased by sauchinone in a dose-dependent fashion, and this was confirmed by intracellular cytokine staining and ELISA (Fig. 4C and D). Furthermore, sauchinone dose-dependently reduced IL-4 production in Th2 cell clone, EL4 cells triggered by TCR activation (Fig. 4E), thereby suggesting that sauchinone suppresses not only Th2 cell development but also Th2 cytokine production in differentiated Th2 cells. It is well characterized that Th2 cell differentiation and the persistent production of Th2 cytokines requires GATA-3 activity to activate cytokine gene transcription [18,33], we thus inquired the effects of sauchinone on GATA-3 expression and activity. Although the expression of GATA-3 remained unchanged by the presence of sauchinone, sauchinone treatment substantially suppressed the IL-5 promoter activity increased by enforced GATA-3 expression in a
Three independent experiments were performed. Data are expressed as means ± SDs for three independent experiments. \( p < 0.05; \) \( * \ p < 0.005; \) \( ** \ p < 0.0005. \)

dose-dependent fashion (Fig. 4E and F), suggesting the existence of an inhibitory mechanism of sauchinone on Th2 cell differentiation driven by GATA-3.

Our studies uncovered in vivo anti-inflammatory functions and the regulatory mechanism of sauchinone in a murine model of allergen-induced airway inflammation; sauchinone potently attenuates eosinophilic infiltration, goblet cell hyperplasia, and collagen deposition in the lung, and substantially suppresses allergic inflammatory cytokine production through the inhibition of GATA-3-driven Th2 cell development. In conclusion, our results strongly suggest that sauchinone may have significant therapeutic benefit in the control of allergic airway disorders.

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References


