Anti-inflammatory Effect of Columbianetin on Activated Human Mast Cells

Hyun-Ja Jeong, Ho-Jeong Na, Su-Jin Kim, Hong-Kun Rim, Noh-Yil Myung, Phil-Dong Moon, Na-Ra Han, Jae-Uk Seo, Tae-Hee Kang, Jae-Joong Kim, Youngjin Choi, In-Cheol Kang, Seung-Heon Hong, You-Ah Kim, Young-Wan Seo, Hyung-Min Kim, and Jae-Young Um

*Biochip Research Center, Hoseo University, Asan, Chungnam 336–795, Republic of Korea; a Department of Pharmacology, College of Oriental Medicine, Institute of Oriental Medicine, Kyung Hee University; b Oriental Medical Science Center, College of Oriental Medicine, Kyung Hee University; c Cancer Preventive Material Development Research Center, Department of Pharmacology, Institute of Oriental Medicine, College of Oriental Medicine, Kyung Hee University; Seoul 130–701, Republic of Korea; d College of Pharmacy, VCRC of Wonkwang University; Jeonbuk 570–749, Republic of Korea; and e Division of Marine Environment & Bioscience, Korea Maritime University; Busan 606–791, Republic of Korea. Received August 6, 2008; accepted February 2, 2009

In the present study, we extracted *Corydalis heterocarpa* with various solvents in order to find the bioactive constituents that demonstrated anti-inflammatory effects. We isolated the active compound, Columbianetin. Anti-inflammatory effect of Columbianetin has been reported but the precise effects of Columbianetin in experimental models have remained unknown. In the present study, we investigate the effect of Columbianetin on the production of histamine, interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF-α) and expression of cyclooxygenase-2 (COX-2) by using the human mast cell line (HMC-1). Various concentrations of Columbianetin were treated before the activation of HMC-1 cells with phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore, A23187. PMA plus A23187 significantly increased IL-1β, IL-6, IL-8, and TNF-α production compared with media control (p<0.05). We also show that the increased cytokines IL-1β, IL-6, IL-8, and TNF-α level was significantly inhibited by Columbianetin in a dose-dependent manner (p<0.05). Maximal inhibition rates of IL-1β, IL-6, IL-8, and TNF-α production by Columbianetin were about 102.6%, 101.1%, 95.8%, and 103.9%, respectively. Columbianetin inhibited expression of COX-2. In addition, the effect of Columbianetin was investigated on the histamine release from HMC-1 stimulated by substance P, which promotes histamine release. Columbianetin also inhibited the histamine release by substance P. In conclusion, these results indicate that Columbianetin may be helpful in regulating mast cell-mediated allergic inflammatory responses.

Key words: Columbianetin; inflammation; mast cell; interleukin; tumor necrosis factor-α

The inflammatory processes are orchestrated by inflammatory cells through a complex set of chemical signals and can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic, allergic, or auto-immune injury. In chronic inflammatory diseases, the injury persists and leads to tissue damage. During inflammation, the inflammatory region is infiltrated with mononuclear cells, producing a range of inflammatory mediators, including inflammatory cytokines.

Mast cells are widely distributed in the connective tissues of mammals and other vertebrates, where they are frequently located in close proximity to blood vessels. Mast cells are important effector cells in allergic reactions as well as in inflammatory processes due to their ability to secrete numerous cytokines. In view of such findings, the versatile role played by mast cells is now recognized, especially in allergic inflammation. Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several proinflammatory and chemotactic cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-4, IL-6, IL-8, IL-13, and transforming growth factor (TGF)-β. Notably, modulation of the production of cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease.

In recent years, cyclooxygenase (COX)-2 plays important roles in various tumors and inflammatory diseases. COX-2, one of the major mediators of the inflammatory reactions, is also strongly induced in activated monocytes and macrophages. Several recent studies demonstrated that prostaglandin D2 (PGD2), the COX-2 metabolite released from activated mast cells, is also essential for the pathogenesis of eosinophilic airway inflammations. Previously, it has been reported that COX-2 inhibitors abolished the PGD2 synthesis and attenuated eosinophil accumulation in the airway’s inflammations.

*Corydalis heterocarpa* has been used traditionally to treat the boil or dysentery as well as a poison in Korea. In this study, Columbianetin was isolated from *Corydalis heterocarpa* but its molecular mechanisms are not well understood. Ng et al., reported that Columbianetin inhibited lipid peroxidation in brain and kidney homogenates. It has been reported that one of the active compounds from roots of *Angelica pubescens*, Columbianetin acetate, significantly demonstrated anti-inflammatory and analgesic activities. Kang and Kim, reported that columbianetin-O-beta-D-glucopyranoside exhibited significant neuroprotective activities against glutamate-induced toxicity.

To investigate the effect of Columbianetin on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore-induced cytokine production in the present study, we analyzed the production of IL-1β, IL-6, IL-8, and TNF-α on the Columbianetin treated human mast cell line, HMC-1. We also investigated the COX-2 expression by Columbianetin.

Our results revealed that Columbianetin, the anti-inflammatory constituent from *Corydalis heterocarpa* was related to inhibition of inflammatory substances on activated mast cells.© 2009 Pharmaceutical Society of Japan
cell.

MATERIALS AND METHODS

**Reagents** Cell culture medium, Iscove's modified Dulbecco's medium (IMDM) was purchased from Gibco BRL (Grand Island, NY, U.S.A.). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187, avdin-horseradish peroxidase (HRP), 2,2-azio-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), and other reagents were obtained from Sigma (St. Louis, MO, U.S.A.). Anti-human IL-1β and TNF-α antibodies (Ab), biotinylated anti-human IL-1β and TNF-α Ab, and recombinant human IL-1β and TNF-α were purchased from R&D Systems (Minneapolis, MN, U.S.A.). Anti-human IL-6 and IL-8 Ab, biotinylated anti-human IL-6 and IL-8 Ab, and recombinant IL-6 and IL-8 were obtained from PharMingen (Sandiego, CA, U.S.A.). Anti-COX-2 Ab was purchased from Santa Cruz Biotecology (Santa Cruz, CA, U.S.A.).

**Cells Culture and Stimulation** Human leukemic cell line, HMC-1(17) cells were grown in IMDM medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μM monothioglycerol, and 10% heat-inactivated fetal bovine serum (FBS) at 37 °C, in 5% CO₂ and 95% humidity. Cells were treated with Columbianetin for 30 min prior to stimulation with 20 nM of PMA plus 100 μg/ml of A23187 and in-

**Extraction, Fractionation, and Isolation** Whole plants of Corydalis heterocarpa were collected in heunri jeol-namdo, Korea in July, 2006. Shade-dried whole plants of Corydalis heterocarpa were extracted twice overnight with CH₃Cl and MeOH at room temperature, respectively. The combined crude extracts were concentrated in vacuo at 40°C to leave a dark brown gum (41.1 g) and then partitioned between CH₂Cl₂ and H₂O. Each layer was further partitioned with n-hexane/85% aq. MeOH and n-BuOH/H₂O, respectively, to afford the n-hexane (7.3 g), 85% aq. MeOH (12.0 g), n-BuOH (4.3 g) and H₂O (20.0 g) fractions. A half of 85% aq. MeOH fraction (6.0 g) was subjected to C₁₈ reversed-phase vacuum flash chromatography with stepwise gradient mixtures of MeOH–H₂O (50%, 60%, 70%, 80%, 90% aq. MeOH, and 100% MeOH). Further purification of 60% aq. MeOH fraction (1.0 g) by silica gel preparative-TLC with 10% MeOH in CH₂Cl₂ as eluent gave 329.1 mg of Columbianetin.

**Structure** Columbianetin (Fig. 1): amorphous white solid, mp 160—163 °C; [α]₁₀° +264° (c=1.1, MeOH); HR-ES-MS *m/z*: 246.0892 (Calcd for C₁₄H₁₄O₄, 246.0892); ¹H-NMR (300 MHz, CD₃OD) δ: 7.82 (1H, d, J=9.4 Hz, H-4), 7.36 (1H, d, J=8.3 Hz, H-5), 6.76 (1H, d, J=8.3 Hz, H-6), 6.15 (1H, d, J=9.4 Hz, H-3), 4.77 (1H, t, J=9.0 Hz, H-2'), 3.30 (2H, d, J=9.0 Hz, H-1'), 1.37 (3H, s, H-4'/-5'), 1.25 (3H, s, H-4'/-5'), 13C-NMR (75 MHz, CD₃OD) δ: 165.5 (C-7), 163.0 (C-2), 152.3 (C-9), 146.1 (C-4), 130.2 (C-5), 115.0 (C-10), 114.2 (C-8), 112.1 (C-3), 107.8 (C-6), 92.5 (C-2'), 72.3 (C-3'), 28.1 (C-1'), 25.4 (C-4'/-5'), 25.3 (C-4'/-5').

**MTT Assay** Cell viability was determined by MTT assay. Briefly, 500 μl of HMC-1 cells suspension (3×10⁵ cells) was cultured in 4-well plates for 8 h after treatment by each concentration of Columbianetin. Twenty microliters of MTT solution (5 mg/ml) was added and the cells were incubated at 37 °C for an additional 4 h. After washing the supernatant out, the insoluble formazan product was dissolved in DMSO. Then, optical density of 96-well culture plates was measured using an ELISA reader at 540 nm.

**ELISA Assay** Secreted cytokine levels in culture supernatants of HMC-1 cells were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s protocol (for IL-1β and TNF-α assay, R&D Systems; for IL-6 and IL-8 assay, PharMingen). Absorption of the avidin-horseradish peroxidase color reaction was measured at 405 nm and compared with serial dilutions of human recombinants as a standard.

% inhibition=\((a-b)\times100/a\)

where ‘a’ is cytokine secretion without Columbianetin and ‘b’ is cytokine secretion with Columbianetin.

**Histamine Assay** Histamine was measured from cells according to the manufacturer’s specification using histamine assay kit (R & D System Inc., Minneapolis, MN, U.S.A.).

% inhibition=\((a-b)\times100/a\)

where ‘a’ is histamine release without Columbianetin and ‘b’ is histamine release with Columbianetin.

**Statistical Analysis** The experiments shown are a summary of the data from at least-three experiments and are presented, as the mean±S.D. Statistical significance of the data was determined using the ANOVA with Tukey post hoc test; a value of \(p<0.05\) was accepted as statistically significant.

**RESULTS**

**Effect of Columbianetin on the Viability of HMC-1 Cells** To assess the effect of Columbianetin on the viability of HMC-1 cell, we performed MTT assay. As a result, Columbianetin did not affect the cell viability (Fig. 2).

Fig. 1. Structure of Columbianetin

Columbianetin was isolated from Corydalis heterocarpa.
**Effect of Columbianetin on PMA Plus A23187-Induced IL-1β Production in HMC-1 Cells**

To assess the regulatory effect of Columbianetin on IL-1β production, the HMC-1 cells were treated with PMA (20 nM) plus A23187 (1 μM) for 8 h. The supernatants were analyzed using an ELISA for IL-1β. PMA plus A23187 significantly increased cytokine production compared with media control in the HMC-1 cells (0.04±0.01 ng/ml for media control; 0.12±0.02 ng/ml for PMA plus A23187, p<0.05). We also tested the effect of Columbianetin (0.01, 0.1, 1, 10, 100 μg/ml) on PMA plus A23187-induced IL-1β production. IL-1β increased by PMA plus A23187 was significantly inhibited by Columbianetin, 0.1, 1, 10, and 100 μg/ml (Fig. 3a, p<0.05). The inhibition rate was about 102.6% at 100 μg/ml.

**Effect of Columbianetin on PMA Plus A23187-Induced IL-6 Production in HMC-1 Cells**

To assess the regulatory effect of Columbianetin on IL-6 production, the HMC-1 cells were treated with PMA (20 nM) plus A23187 (1 μM) for 8 h. The supernatants were analyzed using an ELISA for IL-6. PMA plus A23187 significantly increased cytokine production compared with media control in the HMC-1 cells (0.09±0.01 ng/ml for media control; 0.48±0.13 ng/ml for PMA plus A23187, p<0.05). We also tested the effect of Columbianetin on PMA plus A23187-induced IL-6 production. IL-6 increased by PMA plus A23187 was significantly inhibited by Columbianetin (Fig. 3b, p<0.05). The inhibition rate was about 101.1% at 100 μg/ml.

**Effect of Columbianetin on PMA Plus A23187-Induced IL-8 Production in HMC-1 Cells**

To assess the regulatory effect of Columbianetin on IL-8 production, the HMC-1 cells were treated with PMA (20 nM) plus A23187 (1 μM) for 8 h. The supernatants were analyzed using an ELISA for IL-8. PMA plus A23187 significantly increased cytokine production compared with media control in the HMC-1 cells (0.08±0.01 ng/ml for media control; 4.35±1.21 ng/ml for PMA plus A23187, p<0.05). We also tested the effect of Columbianetin on PMA plus A23187-induced IL-8 production. IL-8 increased by PMA plus A23187 was significantly inhibited by Columbianetin (Fig. 3c, p<0.05 at 100 μg/ml). The inhibition rate was about 95.8%.

**Effect of Columbianetin on PMA Plus A23187-Induced TNF-α Production in HMC-1 Cells**

To assess the regulatory effect of Columbianetin on TNF-α production, the HMC-1 cells were treated with PMA (20 nM) plus A23187 (1 μM) for 8 h. The supernatants were analyzed using an ELISA for TNF-α. PMA plus A23187 significantly increased cytokine production compared with media control in the HMC-1 cells (0.26±0.07 ng/ml for media control; 0.80±0.18 ng/ml for PMA plus A23187, p<0.05). We also tested the effect of Columbianetin on PMA plus A23187-induced TNF-α production. Columbianetin (10, 100 μg/ml) significantly inhibited the PMA plus A23187-induced TNF-α production (Fig. 3d, p<0.05). The inhibition rate was about 103.9% at 100 μg/ml.

**Effect of Columbianetin on PMA Plus A23187-Induced COX-2 Expression in HMC-1 Cells**

To determine the effect of Columbianetin on the COX-2 expression induced by PMA plus A23187, Western blot analyses were performed. As shown in Fig. 4, the treatment with PMA plus A23187 for 12 h increased the COX-2 expression, but pre-treatment with Columbianetin decreased the COX-2 expression level.
significantly enhanced histamine release compared with the control ($p<0.05$). Histamine release was significantly decreased by pretreatment of Columbianetin.

**DISCUSSION**

In the present study, we reported that Columbianetin inhibited the PMA plus A23187-induced proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) production in HMC-1. Following activation, mast cells release inflammatory mediators such as histamine, heparin, tryptase, prostaglandins, and leukotrienes. In addition, mast cells also express transcripts or proteins for TNF-α, IL-1β, IL-4, IL-5, IL-6 IL-8, and IL-13. These cytokines are released as prestored cytokines but are also newly synthesized upon mast cell activation. IL-1β is a pro-inflammatory cytokine and a central mediator in the cytokine network, and is known to control important functions both in the immune system and inflammation. TNF-α and IL-6 play major role in triggering and sustaining the inflammatory allergic response in mast cells. TNF-α promotes inflammation, leukocyte infiltration, and tissue fibrosis and is thought to be an initiator of cytokine related inflammatory states via stimulation of cytokine production. IL-6 is also produced from mast cells and its local accumulation is associated with a skin allergic reaction. The human chemokine IL-8 released from mast cells might act on surrounding cells such as neutrophils, T and B cells, and eosinophils and induce migration and activation on inflammatory effector cells. Activation of mast cells with substance P also causes transcription and translation of several different cytokines/chemokines such as TNF-α, macrophage inflammatory protein-1 and GM-CSF, RANTES, MCP-1, CXCL8, along with other proinflammatory compounds, proteases (chymase and tryptase), histamine, leukotrienes and PGD2.

Recent reports have described the anti-inflammatory effects of novel compounds by the management of mast cell activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation. Histamine-1 receptor antagonist was shown to inhibit mast cell granule-stored mediators, as well as cytokine activation.

In conclusion, we have shown that Columbianetin can regulate the production of IL-1β, IL-6, IL-8, and TNF-α and expression of COX-2 in PMA plus A23187-stimulated HMC-1 cells. Columbianetin also inhibited the histamine release. Given these findings, the regulation of the inflammatory cytokine production pathway by Columbianetin in HMC-1 cells could form the basis of a new strategy for the treatment of mast cell-mediated inflammatory allergic diseases.

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