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Original Article

The Anti-inflammatory Mechanism of Xanthoangelol E is Through the Suppression of NF-κB/Caspase-1 Activation in LPS-stimulated Mouse Peritoneal Macrophage

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Angelica keiskei has exhibited numerous pharmacological effects including antitumor, antimetastatic, and antidiabetic effects. However, the anti-inflammatory effects and mechanisms employed by xanthoangelol E isolated from *Angelica keiskei* are incompletely understood. In this study, we attempted to determine the effects of Xanthoangelol E on the lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophage. The findings of this study demonstrated that xanthoangelol E inhibited the production of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and prostaglandin E_2 (PGE_2). Xanthoangelol E inhibited the enhanced levels of cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) caused by LPS. Additionally, we showed that the anti-inflammatory effect of xanthoangelol E is through the regulation of the activation of nuclear factor (NF)-κB and caspase-1. These results provide novel insights into the pharmacological actions of xanthoangelol E as a potential candidate for the development of new drugs to treat inflammatory diseases.

Key Words: Xanthoangelol; Inflammation; Macrophage; Nuclear factor-kappa B; Caspase-1

INTRODUCTION

Inflammation is a process that involves the action of multiple factors within a complex network. The movement of leukocytes to the inflammation site is important for the pathogenesis of inflammatory conditions. In the presence of infection, bacteria or bacterial products may activate resident macrophages and lymphocytes. Macrophage activation has been determined to perform an important role in the inflammatory process (Adamson and Leitinger, 2011; Beutler, 2000) and to generate potent pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, and interleukin (IL)-6, which induce inflammation and recruit other immune cells (Bunikowski et al., 2001). Although TNF-α and IL-6 are beneficial host defenses, they can also trigger pathological conditions when expressed in excess quantities (Moudgil and Choubey, 2011). For example, massive stimulation of macrophages after a severe Gram-negative bacterial infection can result in excessive production of inflammatory cytokines and the development of fatal septic shock syndrome, as well as multiple organ failure (Tamayo et al., 2011). Additionally, higher levels of inflammatory cytokines have also been implicated in a variety of chronic inflammatory diseases including rheumatoid arthritis, psoriasis, and Crohn's disease (Guerreiro et al., 2009; Mircic and Kavanaugh, 2011). Hence, there is currently a strong interest in agents that can block the generation or activities of inflammatory cytokines.

Cyclooxygenases (COX) generate a variety of prostaglandins (PGs), which have been implicated in a number of physiological events, including the progression of inflammation, immunomodulation, and the transmission of pain (Ulmann et al., 2010). Two COX isoenzymes were identified:

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COX-1, the constitutive enzyme, generates PGs that protect the stomach and kidney against damage; and COX-2, the inducible enzyme induced by inflammatory stimuli such as cytokines, generates PGs that contribute to the inflammatory pain and swelling (Kamei et al., 2004). Although COX-2 is normally expressed at very low levels, it is rapidly induced by a variety of stimuli, such as cytokines, growth factors, hormones, and carcinogens, and is believed to be responsible for producing the prostaglandins associated with the mediation of inflammation (Li et al., 2011; Ghoshal et al., 2011).

Nitric oxide (NO) generated by the inducible NO synthase (iNOS) isoform is an essential component of hosts' innate immune and inflammatory responses to a variety of pathogens, including intracellular bacteria, viruses, fungi, and parasites. However, the overproduction of NO can prove harmful and can result in septic shock, rheumatoid arthritis, and autoimmune diseases (Leiro *et al*., 2004). Therefore, therapeutic agents that inhibit iNOS may effectively ameliorate these inflammatory conditions.

Nuclear factor-kappa B (NF-κB) performs a crucial function in the expression of many genes involved in immune and inflammatory responses (Tak and Firestein, 2001; Stark et al., 2001). NF-κB is a member of the Rel family of transcription factors and typically occurs as a heterodimer composed of a p50 and p65 subunit. After a variety of stimuli, the IκB proteins are phosphorylated, ubiquinated, and degraded, allowing for NF-κB to translocate into the nucleus where it can bind specific DNA sequences located in the promoter regions of target genes and activate gene transcription (Jobin and Sartor, 2000). Recently, many studies have reported the role of NF-κB in inflammatory disorders (Ma et al., 2004; Ishiguro et al., 2006). Therefore, NF-κB is currently regarded as an ideal target for molecular therapies to treat inflammation.

Caspase-1 is a member of a family of caspases with large prodomains, and its activation is involved in apoptosis and inflammation (Lee et al., 2001; Wang et al., 2005). Activation of caspase-1 induces an inflammatory response via the production of pro-inflammatory cytokines and the recruitment of neutrophils (Faubel et al., 2007). It has been reported that caspase-1 deficiency reduced intestinal inflammation (Siegmund et al., 2001). The results of these studies demonstrated that caspase-1 activation is an attractive target for the treatment of inflammatory diseases.

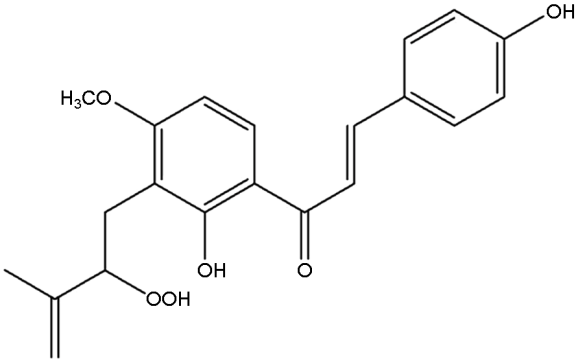

Fig. 1. Chemical structure of xanthoangelol E

Angelica keiskei has traditionally been used as a diuretic, laxative, analeptic and galactagogue. Xanthoangelol E isolated from *Angelica keiskei* is a chalcone constituent (Fig. 1). However, the precise molecular mechanisms of xanthoangelol E on the inflammatory response have yet to be clearly elucidated. In an effort to elucidate the mechanism responsible for xanthoangelol E's anti-inflammatory effect, we evaluated the effects of xanthoangelol E in lipopolysaccharide (LPS)-induced the production of inflammatory cytokine and PGE_2, and expression of COX-2 and iNOS proteins. Additionally, we evaluated the effects of xanthoangelol E on the activation of NF-κB and caspase-1 in mouse peritoneal macrophages.

MATERIALS AND METHODS

Reagents

3-(4,5-dimethylthiazol-2-yl)-diphenyl-tetrazoliumbromide (MTT) bicinchoninic acid protein (BCA), and LPS were purchased from Sigma (St. Louis, MO). Rabbit polyclonal antibody (Ab) to iNOS was obtained from Transduction Laboratories (Lexington, KY). Anti-mouse TNF-α, biotinylated anti-mouse TNF-α, recombinant mouse TNF-α and caspase-1 assay kit were purchased from R&D Systems (Minneapolis, MN). Anti-mouse IL-6, biotinylated anti-mouse IL-6 and recombinant mouse IL-6 were purchased from Pharmingen (Sandiego, CA). Dulbeccos Modified

Eagles Medium (DMEM) and thioglycollate (TG) were purchased from Difco Laboratories (Detroit, MI). The specific Ab against COX-2, NF-κB and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Animals

The Male C57BL/6 (6 weeks old) mice were purchased from the Dae-Han Experimental Animal Center (Eumsung, South Korea), and the animals were maintained in the College of Pharmacy, Wonkwang University. The mouse were housed five to ten per cage in a laminar air-flow room maintained at a temperature of 22 ± 1℃ and relative humidity of 55 ± 10% throughout the study. Animal experimental procedures were approved by the ethics committee of Daegu Haany University.

Isolation and identification of xanthoangelol E

The aerial parts of *Angelica keiskei* (4.2 kg) were extracted with 70% aqueous ethanol (EtOH, 8 L × 3) at room temperature for 24 h and subsequently filtered a filter paper. The filtrate was concentrated *in vacuo*. The EtOH extracts was dissolved in water (1 L) and then successively partitioned with ethyl acetate (EtOAc, 1 L × 3) and *n*-buthanol (*n*-BuOH, 1 L × 3). They were concentrated to afford the residues of EtOAc (43.5 g, AKE), *n*-BuOH (108 g, AKB) and the water fractions.

The EtOAc fraction (43 g) was applied to a SiO_2 c.c. (Ø 10 × 25 cm), which was eluted successively with solvents of increasing polarity [*n*-hexane-EtOAc (10:1 → 8:1 → 5:1 → 3:1); $CHCl_3$-MeOH (15:1 → 10:1 → 8:1 → 5:1 → 3:1); $CHCl_3$-MeOH-H_2O (7:3:1 → 65:35:10 → 6:4:1)] to afford 31 fractions (ASE-1~ASE-31). Fraction ASE-15 (792 mg) was purified using a silica gel column chromatography (SiO_2 c.c) (Ø 5 × 10 cm) and eluted with *n*-hexane-EtOAc (50:1) to produce xanthoangelol E. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA).

Peritoneal macrophage cultures

Mice were i.p. injected with 2.5 ml TG, and TG-elicited macrophages were harvested after 3~4 days, as reported previously (Jeong et al., 2004). Using 8 ml of HBSS containing 10 U/ml heparin, peritoneal lavage was performed. Then, the cells were distributed in DMEM, which was supplemented with 10% heat-inactivated FBS, in 4-well tissue culture plates (3×10^5 cells/well) incubated for 4 h at 37℃ in an atmosphere of 5% CO_2, washed three times with HBSS to remove non-adherent cells, and equilibrated with DMEM that contained 10% FBS before treatment.

MTT assay

Cell viability was determined using MTT assay. Briefly, 500 μl of peritoneal macrophage cells suspension (3×10^5 cells) was cultured in 4-well plates for 24 h after treatment by each concentration of xanthoangelol E. 50 μl of MTT solution (5 mg/ml) was added and then cells were incubated for 4 h at 37℃. After washing the supernatant out, the insoluble formazan product was dissolved in DMSO. Then, optical density of 96-well culture plates was measured using enzyme-linked immunosorbent assay (ELISA) reader at 540 nm. The optical density of formazan formed in untreated control cells was taken as 100% of viability.

Cytokines assay

Cytokine (TNF-α and IL-6) assay was performed by a modified ELISA. In this method, the wells of 96-well plates were coated with mouse monoclonal Abs specific for TNF-α and IL-6. The coated plates were washed with PBS containing 0.05% Tween 20 prior to subsequent steps in the assay. All reagents used in this assay were incubated for 2 h at 37℃. Recombinant TNF-α and IL-6 were diluted and used as standards. Serial dilutions that started from 10 ng/ml were used to establish the standard curve. The assay plates were sequentially exposed to biotinylated mouse TNF-α, IL-6, avidin peroxidase, and ABTS substrate solution containing 30% H_2O_2. The absorbance values of the plates were recorded at 405 nm.

Prostaglandins E_2 (PGE_2) assay

The PGE_2 concentration in colon tissue was measured by enzyme-linked immunosorbent assay (ELISA) using a

PGE_2 assay kit (Stressgen Biotechnologies, USA) according to the manufacturer's directions. Duplicate aliquots of supernatant were measured for each sample.

Measurement of nitrite (NO) concentration

Peritoneal macrophages (3×10^5 cells/well) were pretreated with xanthoangelol E for 1 h, and then treated with rIFN-γ (10 U/ml) and LPS (1 μg/ml). To measure the nitrite content, 100 μl aliquots were removed from conditioned medium and incubated with an equal volume of Griess reagent (1% sulfanilamide/0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloridey/2.5% H_3PO_4) at room temperature for 10 min. The absorbance at 540 nm was determined in a plate reader. The NO_2^- level was determined using sodium nitrite as the standard. This value was determined in each experiment and subtracted from the value obtained from the medium with peritoneal macrophages.

Preparation of cytoplasmic and nuclear extract

Nuclear and cytoplasmic extracts were prepared as described previously (Schoonbroodt et al., 1997). Briefly, after the cells were activated with LPS and then washed with ice-cold phosphate-buffered saline (PBS). These cells were resuspended in 60 μl of buffer A (10 mM Hepes/KOH, 2 mM $MgCl_2$, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, and 0.5 mM PMSF, pH 7.9). The cells were allowed to swell on ice for 15 min, lysed gently with 2.5 μl of 10% Nonide P (NP)-40, and centrifuged at 2,000 g for 10 min at 4℃. The supernatant was collected and used as the cytoplasmic extracts. The nuclei pellet was resuspended in 40 μl of buffer B (50 mM HEPES/KOH, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, and 0.5 mM PMSF, pH 7.9), left on ice for 20 min, inverted and the nuclear debris was spun down at 15,000 g for 15 min to remove nuclear debris. The supernatant (nuclear extract) was collected, frozen in liquid nitrogen and stored at -70℃ until ready for analysis.

Western blot analysis

For analysis of the level of indicated proteins in the text, stimulated cells were rinsed twice with ice-cold PBS and then lysed in ice-cold lysis buffer (PBS containing 0.1% SDS, 1% triton and 1% deoxycholate). Cell lysates were centrifuged at 15,000 ×g for 5 min at 4℃; the supernatant was then mixed with an equal volume of 2 × SDS sample buffer, boiled for 5 min and then separated through a 10% denaturing protein gel. After electrophoresis, the protein was transferred to nylon membranes by electrophoretic transfer. The membranes were blocked in 5% skim milk for 2 h, rinsed and incubated overnight at 4℃ with primary antibodies. After three washes in PBST/0.1% Tween 20, the membranes were incubated for 1 h with HRP-conjugated secondary antibodies. After three washes in PBST/0.1% Tween 20, the antibody-specific proteins were visualized using an enhanced chemiluminesence detection system according to the recommended procedure (Amersham Corp. Newark, NJ, USA).

Caspase-1 activity assay

The enzymatic activity of caspase-1 was assayed using a caspase colorimetric assay kit according to the manufacturer's protocol (R & D systems, Minneapolis, USA). The lysed cells were centrifuged at 14,000 rpm for 5 min. The protein supernatant was incubated with 50 μl reaction buffer and 5 μl caspase substrate at 37℃ for 2 h. The absorbance was measured was measured using a plate reader at a wavelength of 405 nm. Equal amounts of the total protein from each lysate were quantified using a BCA quantification kit.

Statistical analysis

The results are presented as the mean ± S.E.M. of at least three experiments. The results were examined using an independent *t*-tests and ANOVA with a Tukey *post hoc* test. A P value < 0.05 of was considered significant.

RESULTS

The effects of xanthoangelol E on production of TNF-α and IL-6 in LPS-stimulated mouse peritoneal macrophage

The effects of xanthoangelol E on TNF-α and IL-6 production from LPS-stimulated mouse peritoneal macrophages were evaluated. As shown in Fig. 2, the production

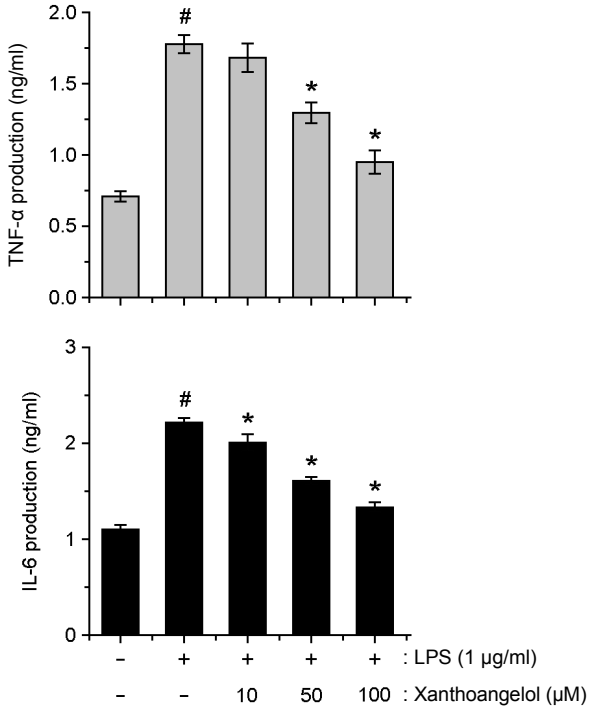

Fig. 2. Effect of xanthoangelol E on TNF-α and IL-6 production in LPS-stimulated mouse peritoneal macrophages. Cells (3×10^5 cells/ml) were pretreated for 1 h with xanthoangelol E (10~100 μM), and then stimulated for 24 h with LPS (1 μg/ml). The levels of TNF-α and IL-6 in the supernatant were measured via ELISA. All data are expressed as the means ± S.E.M of three independent experiments ($^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. LPS alone).

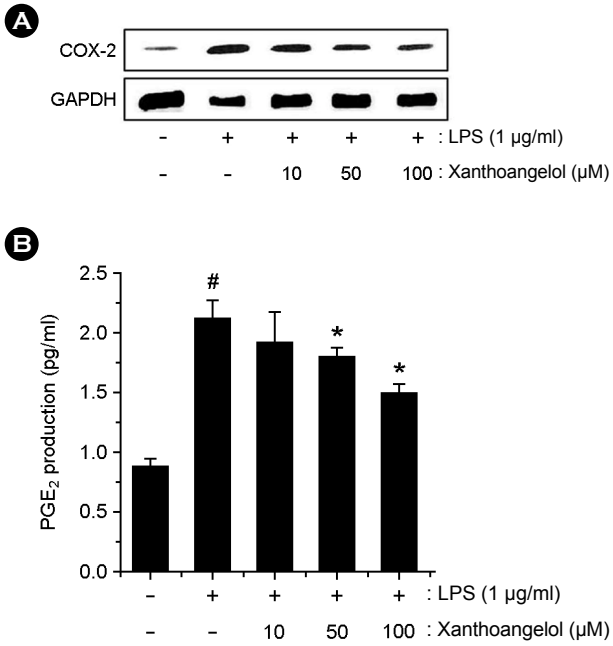

Fig. 3. Effect of xanthoangelol E on COX-2 expression and PGE_2 production in LPS-stimulated mouse peritoneal macrophages. (A) Cells (5×10^6 cells/ml) were pretreated for 1 h with xanthoangelol E (10~100 μM), and then stimulated for 24 h with LPS (1 μg/ml). The protein extracts were assayed via Western blot analysis for COX-2. (B) Cells (3×10^5 cells/ml) were pretreated for 1 h with xanthoangelol E (10~100 μM), and then stimulated for 24 h with LPS (1 μg/ml). The amount of PGE_2 production was measured with immunoassay kits. All data represent the means ± S.E.M of three independent experiments ($^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. LPS alone).

of TNF-α and IL-6 in response to LPS was inhibited via pre-treatment with xanthoangelol E (10~100 μM). The maximal inhibition percentage of TNF-α and IL-6 production by xanthoangelol E (100 μM) were approximately 46.7% ($P < 0.05$) and 40.2% ($P < 0.05$), respectively. No cell cytotoxicity by xanthoangelol E was observed (data not shown).

The effects of xanthoangelol E on the production of PGE_2 and the expression of COX-2 in LPS-stimulated mouse peritoneal macrophage

Western blot analysis was conducted to determine the effects of xanthoangelol E on LPS-induced COX-2 expression. The cells were pretreated for 1 h with xanthoangelol E and then treated for 24 h with LPS. As shown in Fig. 3A, LPS enhanced the levels of COX-2 expression relative to that observed in un-stimulated cells. However, xanthoangelol E inhibited the enhanced COX-2 levels.

COX-2 catalyzes the biosynthesis of PGE_2; therefore, we evaluated xanthoangelol E to determine whether or not it exerted an effect on PGE_2 production. As shown in Fig. 3B, PGE_2 production was enhanced in response to LPS treatment; however, this increase was inhibited significantly by xanthoangelol E pretreatment in a dose-dependent manner. The maximal inhibition percentage of PGE_2 production by xanthoangelol E (100 μM) was approximately 33.4% ($P < 0.05$).

The effects of xanthoangelol E on the production of NO and expression of iNOS expression in LPS-stimulated mouse peritoneal macrophage

In order to investigate the effects of xanthoangelol E on LPS-induced NO production, cells were pretreated for 1 h with xanthoangelol E and then cultured for 6 h with rIFN-γ

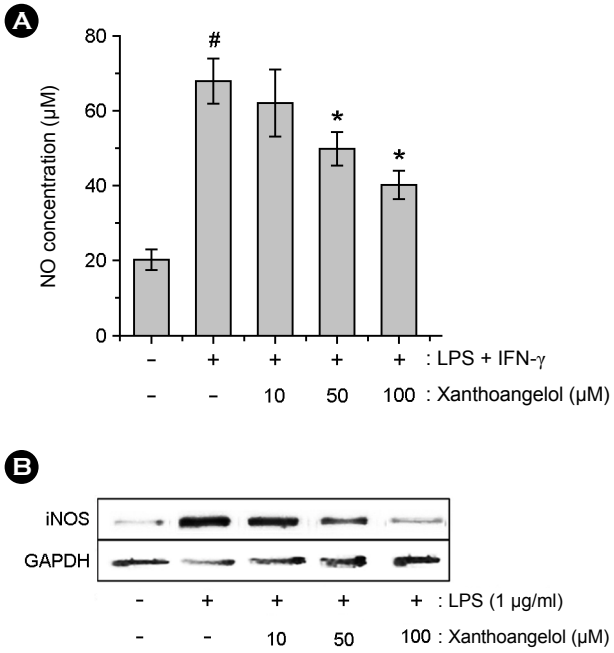

Fig. 4. Effect of xanthoangelol E on NO production and iNOS expression in LPS-stimulated mouse peritoneal macrophages. (A) Cells were pretreated for 1 h with xanthoangelol E (10~100 μM), followed by 6 h of treatment with rIFN-γ (10 U/ml), and 24 h of LPS stimulation (1 μg/ml). NO production in the medium was measured via the Griess reaction. The amount of NO production was quantitatively assessed using $NaNO_2$ as a standard. (B) The protein extracts were assayed by Western blot analysis for iNOS. All data are expressed as the means ± S.E.M of three independent experiments ($^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. LPS + rIFN-γ).

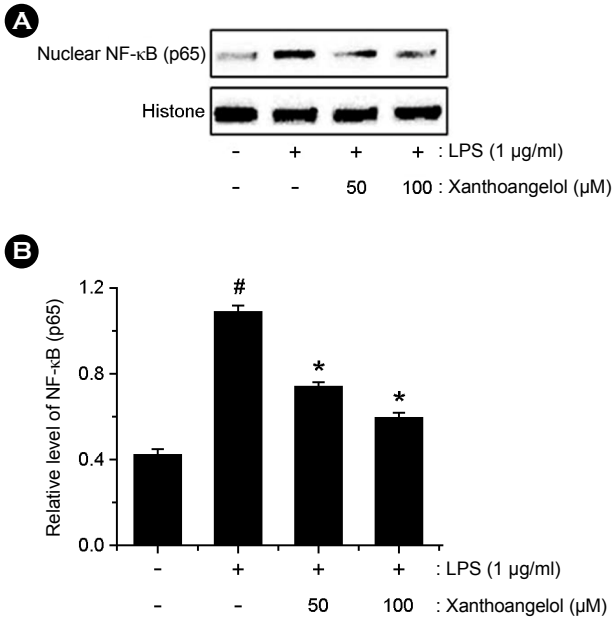

Fig. 5. Effect of xanthoangelol E on NF-κB activation in LPS-stimulated mouse peritoneal macrophages. (A) Cells (5×10^6) were pretreated for 1 h with xanthoangelol E (50~100 μM) and then treated for 1 h with LPS (1 μg/ml). Nuclear extracts were prepared as described in the *Materials and Methods* section and evaluated for RelA/p65 via Western blot analysis. (B) The relative expression level of RelA/p65 was measured using an image analyzer. All data are expressed as the means ± S.E.M of three independent experiments ($^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. LPS alone).

(10 U/ml), after which they were stimulated for 24 h with LPS (1 μg/ml). The resultant NO production was then determined by measuring the nitrite concentrations in the cell supernatants. Xanthoangelol E was shown to induce a reduction in NO production in a dose-dependent manner (Fig. 4A), with a maximal inhibition rate of NO production by xanthoangelol E (100 μM) being measured as 41.1% ($P < 0.05$). Next, the Western blot analysis was conducted to determine the effect of xanthoangelol E on LPS-induced iNOS expression in murine peritoneal macrophages. As shown in Fig. 4B, LPS treatment induced a significant increase in iNOS expression; however, xanthoangelol E treatment inhibited this increase in iNOS expression.

The effects of xanthoangelol E on NF-κB activation in LPS-stimulated mouse peritoneal macrophage

As NF-κB activation requires nuclear translocation of the RelA/p65 subunit of NF-κB, we assessed the effects of xanthoangelol E on the nuclear pool of RelA/p65 protein levels via Western blot analysis. In LPS-stimulated cells, the levels of Rel/p65 were increased in the nucleus, but xanthoangelol E reduced these enhanced levels of Rel/p65 (Fig. 5A). The relative level of Rel/p65 was represented in Fig. 5B.

The effects of xanthoangelol E on caspase-1 activation in LPS-stimulated mouse peritoneal macrophage

We investigated whether xanthoangelol E could suppress activation of caspase-1 in LPS-stimulated cells. The cells were pretreated for 1 h with xanthoangelol E and then treated for an additional 12 h with LPS. We measured the effects of xanthoangelol E on caspase-1 activation using a caspase-1 assay kit. As shown in Fig. 6, LPS treatment induced caspase-1 activation. However, the enhanced caspase-1 activity was significantly reduced by xanthoangelol E

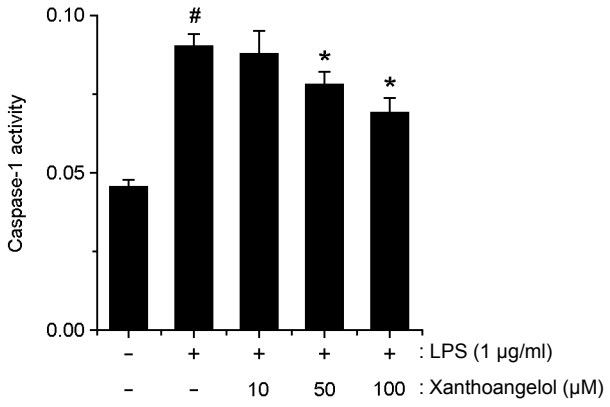

Fig. 6. Effect of xanthoangelol E on caspase-1 activation in LPS-stimulated mouse peritoneal macrophages. The cells were pretreated with xanthoangelol E (10~100 μM) for 1 h prior to LPS stimulation for 12 h. The enzymatic activity of caspase-1 was tested by a caspase colorimetric assay. All data are expressed as the means ± S.E.M of three independent experiments ($^{\#}P < 0.05$ vs. control, $^{*}P < 0.05$ vs. LPS alone).

treatment in a dose-dependent manner.

DISCUSSION

The findings of this study show that xanthoangelol E inhibits the production of TNF-α, IL-6, and PGE_2 and the expression of COX-2 and iNOS. In addition, this anti-inflammatory effect of xanthoangelol E is through the regulation of NF-κB and caspase-1 activation in mouse peritoneal macrophages. These results suggest an important molecular mechanism by which xanthoangelol E reduces inflammation.

Macrophages play key roles in inflammation. During the onset of the inflammatory process, these phagocytic cells become activated and have destructive effects. At the inflammation site, a macrophage is activated to release a host of inflammatory mediators such as TNF-α and IL-6. These mediators may contribute to the initiation and progression of the distributive inflammatory process. TNF-α has an important amplifying effect in asthmatic inflammation and stimulates airway epithelial cells to produce cytokines (Woolley and Tetlow, 2000). IL-6 is a multifunctional cytokine that is rapidly elevated in response to inflammatory triggers. Recently in Japan, humanized anti-human IL-6 receptor monoclonal Ab has been clinically developed as a therapeutic agent for some autoimmune inflammatory diseases, such as rheumatoid arthritis and Crohn's disease (Nishimoto, 2005). It was reported that cyclosporin A has been used to treat atopic dermatitis, owing to the suppression of IL-6 and IL-8 production in cases of severe pediatric atopic dermatitis (Bunikowski et al., 2004). Therefore, the development of new biological therapies for inflammatory disease has generally focused on the blockage of members of the inflammatory cascade, such as cytokines. In this study, we focused on how xanthoangelol E regulates the TNF-α and IL-6 levels in LPS-simulated mouse peritoneal macrophage. We showed that xanthoangelol E inhibited the secretion of TNF-α and IL-6 induced by LPS. The results suggest that xanthoangelol E may have a potential effect on the anti-inflammatory response through the regulation of cytokine production. Although xanthoangelol E inhibited the TNF-α and IL-6 level, the effect of xanthoangelol E on other cytokine levels is not elucidated in the present study. Thus, further study is necessary to clarify the effect of xanthoangelol E on various cytokines levels in a LPS-stimulated macrophage.

COX-2, one of the major mediators of inflammatory reactions, is also strongly induced in activated macrophages and performs a crucial function in some physiological processes. COX-2 is an inducible enzyme that is detected at low concentrations in healthy tissues. During inflammation, COX-2 levels are up-regulated (Crofford *et al*., 2000). Several recent studies have shown that PGs, which are a metabolite of COX-2, contribute to the pain and swelling associated with inflammation (Jones and Lamdin, 2010; Eisenach et al., 2010). It is reported that COX-2 expression is increased in the inflamed mucosa of patients with inflammatory bowel disease and PGs are also essential for the pathogenesis of eosinophilic airway inflammation (Roberts et al., 2001; Bochenek et al., 2004). COX-2 inhibitors are widely prescribed to reduce inflammation and alleviate pain (Mastbergen et al., 2006). NO is also critical for numerous biological processes including inflammation. Although NO has been shown to perform a crucial role in host defenses against a variety of pathogens, overproduction of NO has been proven to be harmful and may result in septic shock, rheumatoid arthritis, and autoimmune diseases

(Nagy et al., 2010). Therefore, therapeutic agents that inhibit iNOS may be useful in relieving such inflammatory conditions. In this study, we attempted to investigate whether the anti-inflammatory effect of xanthoangelol E is through the suppression of inflammatory mediators such as COX-2, PGs and NO in mouse peritoneal macrophage. The results showed that xanthoangelol E inhibited the expression of COX-2 and iNOS and production of PGE_2 and NO in a dose-dependent manner. From this, we propose that the anti-inflammatory activity of xanthoangelol E may be associated with reductions of inflammatory mediators.

Accumulating evidence demonstrated that the increase of inflammatory mediators (e. g., TNF-α, IL-6, and COX-2) are associated with NF-κB activation. In its inactive state, NF-κB is sequestered in the cytoplasm bound to its inhibitory protein, IκB-α, which, when stimulated, is degraded --thus allowing NF-κB translocate into the nucleus and activate pro-inflammatory genes expression. The degradation of IκB-α is a key step in the NF-κB-induced transcription of certain inflammatory genes, including inducible COX-2 and iNOS. Therefore, NF-κB has been recognized as an ideal target for molecular therapies employed to treat inflammatory diseases. For this reason, extensive efforts have been made to develop new treatments that target NF-κB. In this study, the results showed that xanthoangelol E inhibited the NF-κB translocation in murine peritoneal macrophages. Therefore, we hypothesize that xanthoangelol E might exert anti-inflammatory effects via NF-κB activation. Although xanthoangelol E attenuated the activation of NF-κB, the effect of xanthoangelol E on the pathways involving NF-κB (phosphorylation of IκB-α, IKK activation, and TLR) was not determined. Therefore, further studies will be necessary in order to precisely clarify the role of xanthoangelol E on the NF-κB pathway in inflammatory response.

The increase of inflammatory mediators is associated with increased activation of caspase-1 (Wang et al., 2005). It was reported that caspase-$1^{-/-}$ mice demonstrated a reduced IL-6 production (Humke et al., 2000; Druilhe et al., 2001). In another study, it was revealed that the activation of caspasse-1 induced NF-κB and MAPK-signaling pathways leading to the activation of p38 and ERK (Bauernfeind et al., 2009; Taxman et al., 2011). Previously, we demonstrated that caspase-1 inhibitor reduced the production of TNF-α and IL-6 in LPS-stimulated mouse peritoneal macrophage (Kim et al., 2011). Correctly, these studies suggested that the activation of caspase-1 is an attractive target for therapies for the treatment of inflammatory diseases. Therefore, we postulated that the anti-inflammatory effects of xanthoangelol E are mediated, at least in part, via the suppression of caspase-1 activation. In this study, we noted that xanthoangelol E suppressed the LPS-induced activation of caspase-1. This finding demonstrated that the inhibitory effects of xanthoangelol E on inflammation be derived from the regulation of caspase-1 activation. Although xanthoangelol E attenuated caspase-1 activation, the effect of xanthoangelol E on other pathways-involved caspase-1 upstream/downstream is not elucidated in the present study. Thus, further investigation is necessary to clarify the role of xanthoangelol E on caspase-1 associated pathway in a LPS-stimulated mouse peritoneal macrophage.

In conclusion, the anti-inflammatory activities of xanthoangelol E could be attributed, at least in part, to the regulation of inflammatory mediators. These anti-inflammatory effects of xanthoangelol E are caused by the inhibition of LPS-induced NF-κB activation and caspase-1 activation. These results provide experimental evidence showing that xanthoangelol E might be potential candidate in the treatment of inflammatory diseases.

Acknowledgements

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REFERENCES

Adamson S, Leitinger N. Phenotypic modulation of macrophages in response to plaque lipids. Curr Opin Lipidol. 2011. 22: 335-342.

Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V, Latz E. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol. 2009. 183: 787-791.

Beutler B. Tlr4: central component of the sole mammalian LPS sensor. Curr Opin Immunol. 2000. 12: 20-26.

Bunikowski R, Gerhold K, Bräutigam M, Hamelmann E, Renz H, Wahn U. Effect of low-dose cyclosporin a microemulsion on disease severity, interleukin-6, interleukin-8 and tumor necrosis factor alpha production in severe pediatric atopic dermatitis. Int Arch Allergy Immunol. 2001. 125: 344-348.

Bochenek G, Nizankowska E, Gielicz A, Swierczyńska MM, Szczeklik A. Plasma 9alpha, 11beta-PGF_2, a PGD2 metabolite, as a sensitive marker of mast cell activation by allergen in bronchial asthma. Thorax. 2004. 59: 459-464.

Crofford LJ, Oates JC, McCune WJ, Gupta S, Kaplan MJ, Catella-Lawson F, Morrow JD, McDonagh KT, Schmaier AH. Thrombosis in patients with connective tissue diseases treated with specific cyclooxygenase-2 inhibitors. A report of four cases. Arthritis Rheum. 2000. 43: 1891-1896.

Druilhe A, Srinivasula SM, Razmara M, Ahmad M, Alnemri ES. Alnemri. Regulation of IL-1beta generation by Pseudo-ICE and ICEBERG, two dominant negative caspase recruitment domain proteins. Cell Death Differ. 2001. 8: 649-657.

Eisenach JC, Curry R, Rauck R, Pan P, Yaksh TL. Role of spinal cyclooxygenase in human postoperative and chronic pain. Anesthesiology 2010. 112: 1225-1233.

Faubel S, Lewis EC, Reznikov L, Ljubanovic D, Hoke TS, Somerset H, Oh DJ, Lu L, Klein CL, Dinarello CA, Edelstein CL. Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney. J Pharmacol Exp Ther. 2007. 322: 8-15.

Ghoshal S, Trivedi DB, Graf GA, Loftin CD. Cyclooxygenase-2 deficiency attenuates adipose tissue differentiation and inflammation in mice. J Biol Chem. 2011. 286: 889-898.

Guerreiro CS, Ferreira P, Tavares L, Santos PM, Neves M, Brito M, Cravo M. Fatty acids, IL6, and TNF-alpha polymorphisms: an example of nutrigenetics in Crohn's disease. Am J Gastroenterol. 2009. 104: 2241-2249.

Humke EW, Shriver SK, Starovasnik MA, Fairbrother WJ, Dixit VM. ICEBERG: a novel inhibitor of interleukin-1beta generation. Cell. 2000. 103: 99-111.

Ishiguro K, Ando T, Maeda O, Hasegawa M, Kadomatsu K, Ohmiya N, Niwa Y, Xavier R, Goto H. Paeonolattenuates TNBS-induced colitis by inhibiting NF-κB and STAT1 transactivation. Toxicol Appl Pharmacol. 2006. 217: 35-42.

Jeong HJ, Chung HS, An HJ, Seo SW, Kim TG, Won JH, Shin JY, Ahn KS, Kim HM. The immune-enhancing effect of the herbal combination Bouum-Myunyuk-Dan. Biol Pharm Bull. 2004. 27: 29-33.

Jobin C, Sartor RB. The IκB/NF-κB system: a key determinant of mucosal inflammation and protection. Am J Physiol-Cell Physiol. 2000. 278: C451-62.

Jones P, Lamdin R. Oral cyclo-oxygenase 2 inhibitors versus other oral analgesics for acute soft tissue injury: systematic review and meta-analysis. Clin Drug Investig. 2010. 30: 419-437.

Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K, Kawaguchi H, Ishikawa Y, Ishii T, Uematsu S, Akira S, Murakami M, Kudo I. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. J Biol Chem. 2004. 279: 33684-33695.

Kim SJ, Chung WS, Kim SS, Ko SG, Um JY. Antiinflammatory Effect of Oldenlandia diffusa and its Constituent, Hentriacontane, through Suppression of Caspase-1 Activation in Mouse Peritoneal Macrophages. Phytother Res. 2011. 25: 1537-1546.

Lee SH, Stehlik C, Reed JC. Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing. J Biol Chem. 2001. 276: 34495-34500.

Leiro JM, Alvarez E, Arranz JA, Cano E, Orallo F. Antioxidant activity and inhibitory effects of hydralazine on inducible NOS/COX-2 gene and protein expression in rat peritoneal macrophages. Int Immunopharmacol. 2004. 4: 163-177.

Li H, Bradbury JA, Dackor RT, Edin ML, Graves JP, DeGraff LM, Wang PM, Bortner CD, Maruoka S, Lih FB, Cook DN, Tomer KB, Jetten AM, Zeldin DC. Cyclooxygenase-2 regulates Th17 cell differentiation during allergic lung inflammation. Am J Respir Crit Care Med. 2011. 184: 37-49.

Ma TY, Iwamoto Akotia V, Pedram A, Boivin MA, Said HM. TNF-α-induced increase in intestinal epithelial tight junction permeability requires NF-κB activation. Am J Physiol Gastrointest Liver Physiol. 2004. 286: G367-376.

Mastbergen SC, Marijnissen AC, Vianen ME, Zoer B, van Roermund PM, Bijlsma JW, Lafeber FP. Inhibition of COX-2 by celecoxib in the canine groove model of osteoarthritis. Rheumatol. 2006. 45: 405-413.

Mircic M, Kavanaugh A. Inhibition of IL6 in rheumatoid arthritis and juvenile idiopathic arthritis. Exp Cell Res. 2011. 317: 1286-1292.

Moudgil KD, Choubey D. Cytokines in autoimmunity: role in induction, regulation, and treatment. J Interferon Cytokine Res. 2011. 31: 695-703.

Nagy G, Koncz A, Telarico T, Fernandez D, Ersek B, Buzás E, Perl A. Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus. Arthritis Res Ther. 2010. 12: 210.

Nishimoto N. Cytokine signal regulation and autoimmune disorders. Autoimmunity. 2005. 38: 359-367.

Roberts PJ, Morgan K, Miller R, Hunter JO, Middleton SJ. Neuronal COX-2 expression in human myenteric plexus in active inflammatory bowel disease. Gut. 2001. 48: 468-472.

Schoonbroodt S, Legrand-Poels S, Best-Belpomme M, Piette J. Activation of the NF-kappaB transcription factor in a T-lymphocytic cell line by hypochlorous acid. Biochem J. 1997. 321(Pt 3): 777-785.

Siegmund B, Lehr HA, Fantuzzi G, Dinarello CA. IL-1 beta-converting enzyme (caspase-1) in intestinal inflammation. Proc Natl Acad Sci USA. 2001. 98: 13249-13254.

Stark LA, Din FV, Zwacka RM, Dunlop MG. Aspirin-induced activation of the NF-kappaB signaling pathway: a novel mechanism for aspirin-mediated apoptosis in colon cancer cells. FASEB J. 2001. 15: 1273-1275.

Tak PP, Firestein GS. NF-κB: a key role in inflammatory disease. J Clin Invest. 2001. 107: 7-11.

Tamayo E, Fernández A, Almansa R, Carrasco E, Heredia M, Lajo C, Goncalves L, Gómez-Herreras JL, de Lejarazu RO, Bermejo-Martin JF. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw. 2011. 22: 82-87.

Taxman DJ, Holley-Guthrie EA, Huang MT, Moore CB, Bergstralh DT, Allen IC, Lei Y, Gris D, Ting JP. The NLR adaptor ASC/PYCARD regulates DUSP10, mitogen-activated protein kinase (MAPK), and chemokine induction independent of the inflammasome. J Biol Chem. 2011. 286: 19605-19616.

Ulmann L, Hirbec H, Rassendren F. P2X4 receptors mediate PGE_2 release by tissue-resident macrophages and initiate inflammatory pain. EMBO J. 2010. 29: 2290-2300.

Wang X, Wang HY, Bryan EF, Zhang WH, Huo CF, Guan YJ, Zhang Y, Bruey JM, Reed JC, Friedlander RM. Dysregulation of receptor interacting protein-2 and caspase recruitment domain only protein mediates aberrant caspase-1 activation in Huntington's disease. J Neurosci. 2005. 25: 11645-11654.

Woolley DE, Tetlow LC. Mast cell activation and its relation to proinflammatory cytokine production in the rheumatoid lesion. Arthritis Res. 2000. 2: 65-74.