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Byakangelicol, isolated from *Angelica dahurica*, inhibits both the activity and induction of cyclooxygenase-2 in human pulmonary epithelial cells

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Abstract

We examined the inhibitory mechanism of byakangelicol, isolated from *Angelica dahurica*, on interleukin-1β (IL-1β)-induced cyclooxygenase-2 (COX-2) expression and prostaglandin E_2 (PGE_2) release in human pulmonary epithelial cell line (A549). Byakangelicol (10–50 μM) concentration-dependently attenuated IL-1β-induced COX-2 expression and PGE_2 release. The selective COX-2 inhibitor, NS-398 (0.01–1 μM), and byakangelicol (10–50 μM) both concentration-dependently inhibited the activity of the COX-2 enzyme. Byakangelicol, at a concentration up to 200 μM, did not affect the activity and expression of COX-1 enzyme. IL-1β-induced p44/42 mitogen-activated protein kinase (MAPK) activation was inhibited by the MAPK/extracellular signal-regulated protein kinase (MEK) inhibitor, PD 98059 (30 μM), while byakangelicol (50 μM) had no effect. Treatment of cells with byakangelicol (50 μM) or pyrrolidine dithiocarbamate (PDTC; 50 μM) partially inhibited IL-1β-induced degradation of IκB-α in the cytosol, translocation of p65 NF-κB from the cytosol to the nucleus and the NF-κB-specific DNA–protein complex formation. Taken together, we have demonstrated that byakangelicol inhibits IL-1β-induced PGE_2 release in A549 cells; this inhibition may be mediated by suppression of COX-2 expression and the activity of COX-2 enzyme. The inhibitory mechanism of byakangelicol on IL-1β-induced COX-2 expression may be, at least in part, through suppression of NF-κB activity. Therefore, byakangelicol may have therapeutic potential as an anti-inflammatory drug on airway inflammation.

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Introduction

Prostaglandins are lipid mediators that are involved in many normal physiological processes, and are implicated in many pathophysiological processes such as inflammation, oedema, fever, hyperalgesia, colonic cancer and Alzheimer's disease (Mitchell et al 1995). Cyclooxygenase is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the precursor of a wide group of biologically active mediators such as PGE_2, prostacyclin and thromboxane A_2 (Vane et al 1998). There are two cyclooxygenase isozymes that have been identified in man and which bear 60% homology : COX-1 and COX-2 (Xie et al 1991 ; Mitchell et al 1995). COX-1 is generally thought to produce prostaglandins which serve to maintain cellular homoeostasis and is known to be expressed constitutively in many cell types, including endothelial cells, platelets and gastric mucosa (Vane 1994). COX-2, on the other hand, is induced by many pro-inflammatory stimuli, including cytokines (Maier et al 1990) and bacterial lipopolysaccharide (Mitchell et al 1993) in cells in-vitro and at the site of inflammation in-vivo (Vane et al 1994). COX-2 is thought to be the isoform responsible for the production of pro-inflammatory prostaglandins in various models of inflammation (Chan et al 1995). As COX is a target for non-steroidal anti-inflammatory drugs (NSAIDs), this pathway is pharmacologically important (Mitchell et al 1995). Furthermore, the use of isoform-selective COX inhibitors has revealed that many anti-inflammatory benefits of NSAIDs are derived from COX-2 inhibition while many undesirable side effects result from COX-1 inhibition (Mitchell et al 1995; DeWitt 1999). Despite the clinical usefulness of NSAIDs, currently the most effective drugs in the treatment of chronic inflammatory diseases, such as asthma, are corticosteroids

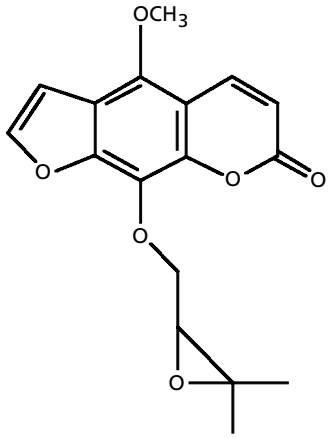

Figure 1 Chemical structure of byakangelicol.

(Barnes 1999). These down-regulate various inflammatory processes, including prostaglandin synthesis, via repression of pro-inflammatory genes such as COX-2 (Newton et al 1997b; Barnes 1999).

The airway epithelium has an active role in airway inflammation by producing multiple mediators. It has been demonstrated that pro-inflammatory cytokines, such as interleukin-1β (IL-1β), increased COX-2 expression and PGE_2 release in human pulmonary epithelial cells (A549) (Mitchell et al 1994; Newton et al 1997b). Medicinal plants have been used as traditional remedies in oriental countries over hundreds of years. *Angelica dahurica* is a well-known oriental crude drug employed as a diaphoretic, analgesic and antipyretic agent (Yen 1992). Recently, under large-scale screening experiments, we found that byakangelicol (Figure 1), a furanocoumarin derivative isolated from *A. dahurica* (Hata et al 1963), possessed an inhibitory effect on IL-1β-induced COX-2 expression and PGE_2 release in human pulmonary epithelial cells (A549). In this study, we investigated the inhibitory mechanism of byakangelicol on IL-1β-induced COX-2 expression and PGE_2 release in A549 cells.

Materials and Methods

Materials

Byakangelicol was isolated from the dried root of *Angelica dahurica* (Hata et al 1963). It was provided by Prof. K. Y. Yen (Graduate Institute of Pharmacognosy Science, Taipei Medical University, Taipei, Taiwan). Pyrrolidine dithiocarbamate (PDTC), Trizma base, dithiothreitol (DTT), glycerol, phenylmethylsulfonyl fluoride (PMSF), pepstatin A, leupeptin, sodium dodecyl sulfate (SDS) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT) were purchased from Sigma Chem. Co. (St Louis, MO). NS-398 and PD 98059 were purchased from Calbiochem-Novabiochem (San Diego, CA). Penicillin/streptomycin, fetal calf serum (FCS) and Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 were purchased from Life Technologies Inc. (Gaithersburg, MD). A PGE_2 enzyme immunoassay kit was obtained from Cayman Chem. Co. (Ann Arbor, MI). Antibodies specific for COX-2 and p65 NF-κB were purchased from Transduction Laboratories (Lexington, KY). Antibodies specific for phospho-p44/42 mitogen-activated protein kinase (MAPK), p44/42 MAPK, IκB-α and α-tubulin were purchased from Santa Cruz Biochemicals (Santz Cruz, CA). Anti-mouse-IgG-conjugated alkaline phosphatase was purchased from Jackson Immuno Research Laboratories (West Grove, PA). A digoxigenin (DIG) gel shift kit, 4-nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) were purchased from Boehringer Mannheim (Mannheim, Germany). Protein assay reagents were purchased from Bio-Rad (Hercules, CA).

Cell culture

A549 cells, a human pulmonary epithelial carcinoma cell line with type II alveolar epithelial cell differentiation, were obtained from American Type Culture Collection and grown at 37°C in DMEM/Ham's F-12 nutrient mixture containing 10% FCS and penicillin/streptomycin (50 U mL^{-1}) in a humidified incubator. When confluent, cells were disaggregated in trypsin solution, washed in DMEM/Ham's F-12 supplemented with 10% FCS, centrifuged at 125 *g* for 5 min, then resuspended and subcultured according to standard protocols.

Measurements of PGE_2 release and the activity of the COX-2 and COX-1 enzymes

A549 cells, which under serum-starved conditions have been reported to constitutively express COX-1 and upon stimulation with IL-1β to express COX-2, were selected for these experiments (Netwon et al 1997b; Patel et al 1999). A549 cells were cultured in 12-well culture plates. For experiments designed to examine the effects of byakangelicol on the release of PGE_2 due to endogeneous arachidonic acid, cells were treated with vehicle (0.05% DMSO, control), IL-1β (0.1 ng mL^{-1}), or pretreated with byakangelicol (10–50 μM) or PDTC (50 μM) followed by IL-1β, and incubated in a humidified incubator at 37°C for 24 h. After incubation, the medium was removed and stored at −80°C until assay. PGE_2 in the medium was assayed by using the PGE_2 enzyme immunoassay kit according to the procedures described by the manufacturer. In experiments designed to measure the effects of byakangelicol on the activity of the COX-2 enzyme, cells were treated with IL-1β (0.1 ng mL^{-1}) for 24 h, after which cells were washed with phosphate-buffered saline (PBS, pH 7.4), and fresh medium was added. Cells were then treated with vehicle (0.05% DMSO, control), NS-398 (0.01–1 μM) or byakangelicol (10–50 μM) for 30 min followed by arachidonic acid (30 μM), and incubated in a humidified incubator at 37°C for 30 min. After incubation, the medium was removed for PGE_2 enzyme immunoassay. In experiments designed to measure the effects of byakangelicol on the activity of the

COX-1 enzyme, cells were treated with vehicle (0.05% DMSO, control) or byakangelicol (50–200 μM) for 30 min followed by arachidonic acid (30 μM), and incubated in a humidified incubator at 37°C for 30 min. After incubation, the medium was removed for PGE_2 enzyme immunoassay.

Measurement of cell viability

The viability of A549 cells treated with byakangelicol was determined by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium (MTT) assay as previously described (Mossman 1983). A549 cells were cultured in 12-well culture plates and treated with vehicle (0.05% DMSO, control) or byakangelicol (10–50 μM) for 24 h. After incubation, the cells were washed with PBS, resuspended in medium with MTT (5 mg mL^{-1}) and incubated in a humidified incubator at 37°C for 4 h. The medium was then removed for measurement of the absorbance of the converted dye at 550 nm using a microplate reader.

Protein preparation and Western blotting

For determination of the expression of COX-1, COX-2, α-tubulin, phosphorylated p44/42 MAPK and non-phosphorylated p44/42 MAPK in A549 cells, proteins were extracted, and Western blotting analysis was performed as described previously (Lin et al 2001). Briefly, A549 cells were cultured in 10-cm Petri dishes. After reaching confluence, cells were treated with vehicle (0.05% DMSO, control), IL-1β (0.1 ng mL^{-1}), or pretreated with byakangelicol (10–50 μM) followed by IL-1β, and incubated in a humidified incubator at 37°C for 24 h. After incubation, cells were washed with PBS (pH 7.4), incubated with extraction buffer (10 mM Tris (pH 7.0), 140 mM NaCl, 2 mM PMSF, 5 mM DTT, 0.5% NP-40, 0.05 mM pepstatin A and 0.2 mM leupeptin) with gentle shaking, and then centrifuged at 12 500 *g* for 30 min. The cell extract was then boiled in a ratio of 1:1 with sample buffer (100 mM Tris (pH 6.8), 20% glycerol, 4% SDS and 0.2% bromophenol blue). Electrophoresis was performed using 10% SDS-polyacrylamide gels (2 h, 110 V, 40 mA, 30 μg of protein per lane). Separated proteins were transferred to PVDF membranes (2 h, 40 V); nonspecific IgGs were blocked with 5% fat-free milk powder, and incubated for 2 h with specific antibodies for COX-2, α-tubulin, phosphorylated p44/42 MAPK or nonphosphorylated p44/42 MAPK. The blot was then incubated with anti-mouse or anti-rabbit IgG linked to alkaline phosphatase (1:1000) for 2 h. Subsequently, the membrane was developed with NBT/BCIP as a substrate. In experiments designed to measure the effects of byakangelicol on the COX-1 expression, cells were treated with vehicle (0.05% DMSO, control) or byakangelicol (50–200 μM) in the absence of IL-1β for 24 h in a humidified incubator at 37°C. After incubation, cells were prepared for immunodetection using COX-1-specific antibody. Quantitative data were obtained using a computing densitometer with Image-Pro plus software (Media Cybernetics, MD).

Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)

A549 cells were cultured in 10-cm culture Petri dishes. After reaching confluence, cells were incubated with vehicle (0.05% DMSO, control), IL-1β (0.1 ng mL^{-1}) or pretreated with byakangelicol (50 μM) or PDTC (50 μM) followed by IL-1β, and then incubated in a humidified incubator at 37°C for 30 min. The cytosolic and nuclear protein fractions were then separated as described previously (Chen et al 1998). Briefly, cells were washed with ice-cold PBS and then pelleted. The cell pellet was resuspended in hypotonic buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.5 mM DTT, 10 mM aprotinin, 10 mM leupeptin and 20 mM PMSF) for 15 min on ice and then vortexed for 10 s. Nuclei were pelleted by centrifugation at 15 000 *g* for 1 min. Supernatants containing cytosolic proteins were collected. A pellet containing nuclei was resuspended in hypertonic buffer (20 mM HEPES (pH 7.6), 25% glycerol, 1.5 mM $MgCl_2$, 4 mM EDTA, 0.05 mM DTT, 10 mM aprotinin, 10 mM leupeptin and 20 mM PMSF) for 30 min on ice. Supernatants containing nuclear proteins were collected by centrifugation at 15 000 *g* for 2 min and then stored at −70°C. In studies of p65 NF-κB translocation, the nuclear extracts were used; only cytosolic extracts were used to study IκB-α degradation. The extracts were subjected to SDS-PAGE using a 10% running gel and Western blotting analysis was performed as described above.

An electrophoretic mobility shift assay (EMSA) was performed using a DIG gel shift kit. Briefly, a double-stranded oligonucleotide probe containing NF-κB sequences (5′-AGTTGAGGGGACTTTCCCAGGC-3′; Promega) was purchased and end-labelled with DIG using terminal transferase. The nuclear extract (10–15 μg) was incubated with 4 ng of a DIG-labelled NF-κB probe in 10 μL binding buffer containing 10 μg poly(dI-dc), 1 μg poly L-lysine, 100 mM HEPES (pH 7.6), 5 mM EDTA, 50 mM $(NH_4)_2SO_4$, 5 mM DTT, 1% w/v Tween 20 and 150 mM KCl at 25°C for 15 min. DNA/nuclear protein complexes were separated from the DNA probe by electrophoresis on a 6% polyacrylamide gel. The gel was then transferred to a nylon membrane. The gel was incubated with 0.1% milk in Tris-base saline buffer containing 0.1% Tween-20 (TBST) at room temperature for 30 min, and then with anti-DIG linked to alkaline phosphatase for 30 min. The immunoreactive band was finally detected with CSPD (disodium 3-[4-methoxyspiro-{1,2-dioxetane-3,2′-[5-chloro]tricylco[3.3.1.1 ^{3,7}]decan}-4-yl] phenyl phosphate) detecting reagents and exposed to X-ray film. Quantitative data were obtained using a computing densitometer with Image-Pro plus software (Media Cybernetics, MD).

Statistical analysis

Results shown are the mean ± s.e.m. of three or four independent experiments. One-way analysis of variance followed by, when appropriate, Bonferroni's multiple range test was used to determine the statistical significance of the

difference between means. A P value of less than 0.05 was taken to be statistically significant.

Results

Effects of byakangelicol on IL-1β-induced PGE_2 release and COX-2 expression

Basal levels of PGE_2 released from A549 cells was low (3.8 ± 0.2 ng mL^{-1}, n = 4). However, incubation of A549 cells with IL-1β (0.1 ng mL^{-1}) for 24 h resulted in a marked release of PGE_2 (29.2 ± 3.1 ng mL^{-1}, n = 4). Pretreatment of cells with byakangelicol (10–50 μM) for 30 min attenuated IL-1β-induced PGE_2 release in a concentration-dependent manner (Figure 2A). The IC50 of byakangelicol (concentration producing 50% inhibition) on the inhibition of IL-1β-induced PGE_2 release was 15.2 ± 0.8 μM. The NF-κB inhibitor, PDTC, also almost completely inhibited the IL-1β-induced PGE_2 release (Figure 2A). Stimulation of A549 cells with IL-1β (0.1 ng mL^{-1}) for 24 h caused the expression of COX-2 protein. When cells were pretreated for 30 min with byakangelicol (10–50 μM), the IL-1β-induced COX-2 expression was concentration-dependently inhibited by byakangelicol (Figure 2B, C). The IC50 of byakangelicol on the inhibition of IL-1β-induced COX-2 expression was 20.5 ± 1.4 μM. PDTC (50 μM) also markedly attenuated the IL-1β-induced COX-2 expression (Figure 2B, C). Treatment of A549 cells with byakangelicol (10, 25 and 50 μM) for 24 h did not affect the cell viability (98.8 ± 0.6%, 98.4 ± 0.3% or 96.4 ± 0.4%, respectively, of the control, n = 4).

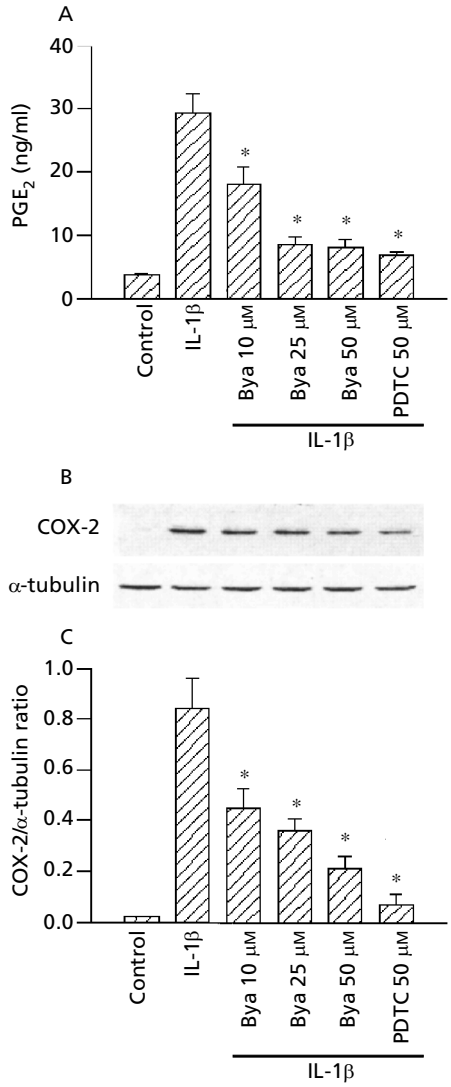

Figure 2 Effect of byakangelicol and PDTC on IL-1β-induced PGE_2 release and COX-2 expression in A549 cells. A. Cells were pretreated with various concentrations of byakangelicol or PDTC (50 μM) for 30 min before incubation with IL-1β (0.1 ng mL^{-1}) for 24 h. The medium was then removed, and the release of PGE_2 was measured. Results are expressed as the mean$\pm$s.e.m. (n = 4). *$P<0.05$ compared with the IL-1β-treated group. B. Cells were pretreated with various concentrations of byakangelicol or PDTC (50 μM) for 30 min before incubation with IL-1β (0.1 ng mL^{-1}) for 24 h. Cells were then prepared for immunodetection using COX-2- or α-tubulin-specific antibody as described in Methods. Equal loading in each lane is demonstrated by similar intensities of α-tubulin. C. The extent of COX-2 and α-tubulin protein expression were quantitated using a densitometer with Image-Pro plus software. The relative level was calculated as the ratio of COX-2 to α-tubulin protein levels. Results are expressed as the mean$\pm$s.e.m. (n = 4). *$P<0.05$ compared with the IL-1β-treated group. Bya, byakangelicol.

Effects of byakangelicol on the activity of the COX-2 enzyme

The activity of the COX-2 enzyme was quantified by providing cells with exogeneous arachidonic acid, the substrate for COX, and measuring its conversion into PGE_2. Incubation of A549 cells with IL-1β (0.1 ng mL^{-1}) for 24 h resulted in a marked increase in the activity of the COX-2 enzyme (measured in the presence of 30 μM exogeneous arachidonic acid for 30 min). Treatment of cells with the selective COX-2 inhibitor NS-398 (0.01–1 μM) or byakangelicol (10–50 μM) concentration-dependently inhibited the increase in activity of the COX-2 enzyme caused by IL-1β (Figure 3). The IC50 values of byakangelicol and NS-398 on the inhibition of COX-2 activity were 25.5 ± 1.6 and 0.07 ± 0.03 μM, respectively.

Effects of byakangelicol on the activity of the COX-1 enzyme and COX-1 expression

A549 cells are reported to constitutively express COX-1 under serum-starved conditions (Netwon et al 1997b; Patel et al 1999). Treatment of serum-starved cells with byakangelicol (50–200 μM) for 30 min did not affect the constitutive activity of the COX-1 enzyme (measured in the presence of 30 μM exogeneous arachidonic acid for 30 min) (Figure 4A). The constitutive COX-1 expression was also not affected by byakangelicol (50–200 μM) (Figure 4B, C).

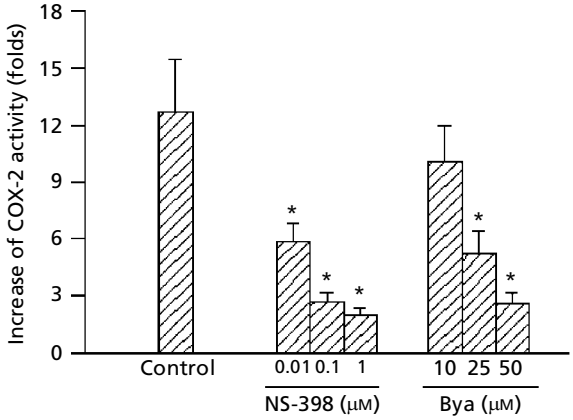

Figure 3 Effect of NS-398 and byakangelicol on increases in activity of the COX-2 enzyme caused by IL-1β in A549 cells. Cells were treated with vehicle or IL-1β (0.1 ng mL^{-1}) for 24 h, after which cells were washed and fresh medium was added. Cells were then treated with various concentrations of NS-398 or byakangelicol for 30 min followed by arachidonic acid (30 μM), and incubated at 37°C for 30 min. After incubation, the medium was removed for PGE_2 enzyme immunoassay. Results are expressed as the mean±s.e.m. (n = 3). *$P < 0.05$ compared with the control group. Bya, byakangelicol.

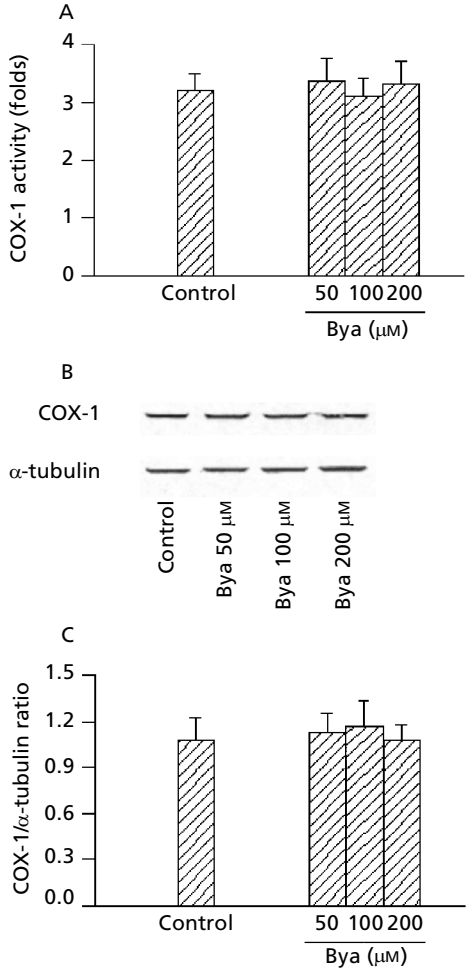

Figure 4 Effect of byakangelicol on the activity of COX-1 enzyme and COX-1 expression in A549 cells. A. Cells were treated with various concentrations of byakangelicol for 30 min followed by arachidonic acid (30 μM), and incubated at 37°C for 30 min. After incubation, the medium was removed for PGE_2 enzyme immunoassay. Results are expressed as the mean±s.e.m. (n = 3). B. Cells were treated with various concentrations of byakangelicol for 24 h at 37°C. After incubation, cells were prepared for immunodetection using COX-1- or α-tubulin-specific antibodies as described in Methods. Equal loading in each lane was demonstrated by similar intensities of α-tubulin. C. The extents of COX-1 and α-tubulin protein expression were quantitated using a densitometer with Image-Pro plus software. The relative level of COX-1 expression was calculated as the ratio of COX-1 to α-tubulin protein levels. Results are expressed as the mean±s.e.m. (n = 3). Bya, byakangelicol.

Effects of byakangelicol on IL-1β-induced p44/42 MAPK activation

The activation of p44/42 MAPK has been demonstrated to be involved in IL-1β-induced COX-2 expression and PGE_2 release in pulmonary epithelial cells (Newton et al 2000). Since activation of MAPKs requires phosphorylation at the threonine and tyrosine residues, immunoblot analysis was performed to examine the MAPKs phosphorylation using anti-phospho-p44/42 MAPK-specific antibodies. Treatment of A549 cells with IL-1β (0.1 ng mL^{-1}) for 10 min resulted in a marked activation of p44/42 MAPK. When cells were pretreated for 30 min with byakangelicol (50 μM) or the MAPK/extracellular signal-regulated protein kinase (MEK) inhibitor, PD 98059 (30 μM), the IL-1β-induced activation of p44/42 MAPK was completely inhibited by PD 98059, while byakangelicol had no effect (Figure 5A, B). None of these treatments had any effect on the protein level of p44/42 MAPK (Figure 5A).

Effects of byakangelicol on IL-1β-induced NF-κB activation

Previous studies have demonstrated that treatment of A549 cells with IL-1β results in marked translocation of p65 NF-κB from cytosol to the nucleus as well as the degradation of IκB-α in cytosol (Lin et al 2000). To determine whether the inhibitory action of byakangelicol is due to its effect on degradation of IκB-α and translocation of p65 NF-κB, immunoblot analysis of IκB-α and p65 NF-κB in the cytosol and nucleus, respectively, were performed. Stimulation of cells with IL-1β (0.1 ng mL^{-1}) for 30 min resulted in marked translocation of p65 NF-κB from cytosol to the nucleus as well as degradation of IκB-α in the cytosol. After pretreatment of cells for 30 min with byakangelicol (50 μM) or PDTC (50 μM), IL-1β-induced degradation of IκB-α and translocation of p65 NF-κB were partially inhibited by byakangelicol or PDTC (Figure 6A). To investigate whether byakangelicol inhibits the activation of NF-κB, analysis of NF-κB-specific DNA–protein binding activity by EMSA was performed. In nuclear extracts of unstimulated cells, a slight intensity in NF-κB-specific DNA–protein complex formation was observed. Stimulation of cells with IL-1β (0.1 ng mL^{-1}) for 30 min resulted in marked activation of NF-κB-specific DNA–protein complex

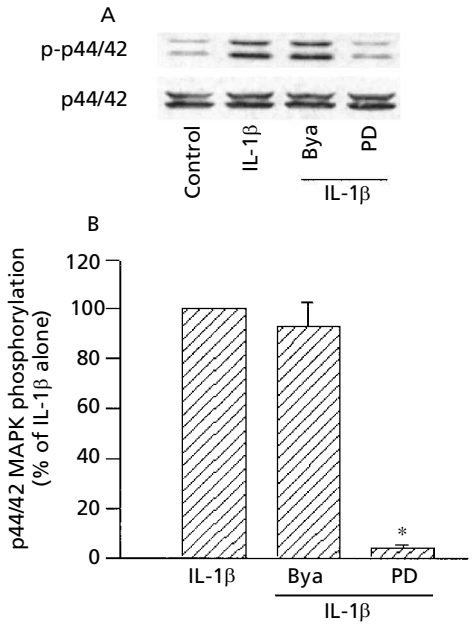

Figure 5 Effect of byakangelicol and PD 98059 on IL-1β-induced activation of p44/42 MAPK in A549 cells. A. Cells were pretreated with byakangelicol (50 μM) or PD 98059 (30 μM) for 30 min before incubation with IL-1β (0.1 ng mL^{-1}) for 10 min. Whole-cell lysates were prepared and subjected to Western blotting using antibodies specific for phospho-p44/42 MAPK (p-p44/42) or p44/42 MAPK (p44/42) as described in Methods. B. The extent of p44/42 MAPK activation was quantitated using a densitometer with Image-Pro plus software. Results are expressed as the mean$\pm$s.e.m. (n = 4). *$P < 0.05$ compared with the IL-1β-treated group. Bya, byakangelicol; PD, PD 98059.

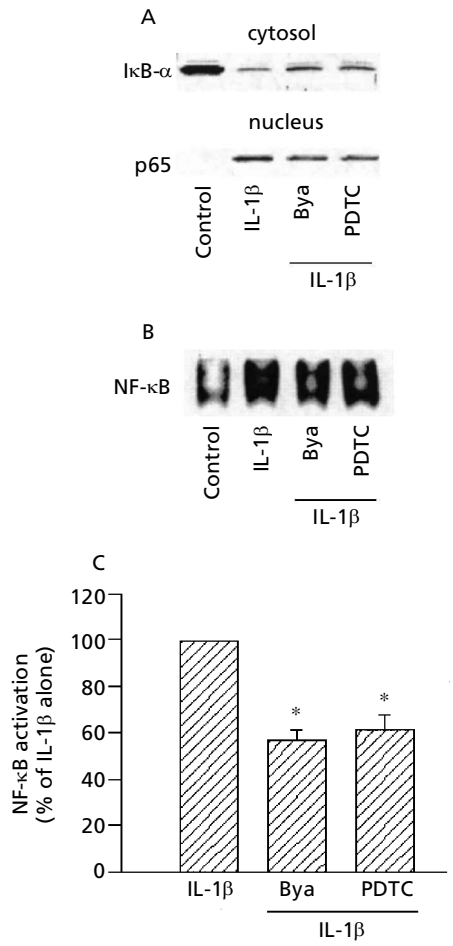

Figure 6 Effect of byakangelicol and PDTC on IL-1β-induced degradation of IκB-α, translocation of p65 NF-κB and NF-κB-specific DNA–protein complex formation in A549 cells. Cells were pretreated with byakangelicol (50 μM) or PDTC (50 μM) for 30 min before incubation with IL-1β (0.1 ng mL^{-1}) for 30 min, then the subcellular (cytosol and nucleus) fractions were prepared. A. Cytosolic levels of IκB-α and nuclear levels of p65 NF-κB were immunodetected with IκB-α- or p65 NF-κB-specific antibody, respectively, as described in Methods. Data are representative of three independent experiments, which gave essentially identical results. B. NF-κB-specific DNA-protein binding activity in nuclear extracts was determined by electrophoretic mobility shift assay (EMSA) as described in Methods. C. The extent of NF-κB activation was quantitated using a densitometer with Image-Pro plus software. Results are expressed as the mean$\pm$s.e.m. (n = 3). *$P < 0.05$ as compared with the IL-1β-treated group. Bya, byakangelicol.

formation. When cells were pretreated for 30 min with byakangelicol (50 μM) or PDTC (50 μM), the IL-1β-induced activation of NF-κB-specific DNA–protein complex formation was partially inhibited by byakangelicol or PDTC (Figure 6B, C).

Discussion

In this study, we found that byakangelicol suppressed IL-1β-induced COX-2 expression and PGE_2 release. Furthermore, byakangelicol concentration-dependently inhibited the activity of the COX-2 enzyme. These results suggest that the inhibitory effects of byakangelicol on IL-1β-induced PGE_2 release may be through suppression of COX-2 expression, as well as the activity of the COX-2 enzyme. This is the first study showing that byakangelicol, a furanocoumarin derivative, can inhibit the activity and expression of the COX-2 enzyme. The IC50 of byakangeliciol on the inhibition of COX-2 activity is about 25 μM. On the other hand, byakangelicol at a concentration up to 200 μM did not affect the activity of the COX-1 enzyme. The selectivity ratio of byakangelicol on the inhibition of the activity between COX-1 and COX-2 enzyme was more than 8 fold. Activation of p44/42 MAPK has been demonstrated to be involved in the IL-1β-induced COX-2 expression in human pulmonary epithelial cells (Newton et al 2000). In this study, we found that byakangelicol did not affect IL-1β-induced activation of p44/42 MAPK, suggesting that the inhibitory mechanism of byakangelicol on IL-1β-induced COX-2 expression might not be mediated through suppression of p44/42 MAPK activation.

The COX-2 gene in man has two putative NF-κB binding sites in the promoter region (Appleby et al 1994; Tazawa et al 1994). Previous studies have also demonstrated that transcription factor NF-κB is involved in IL-1β-induced

COX-2 expression in A549 cells (Newton et al 1997a; Lin et al 2000). Therefore, it is possible that suppression of IL-1β-mediated COX-2 expression by byakangelicol may be mediated by suppression of NF-κB activity. The transcription factor NF-κB is constitutively present in cells as a heterodimer, consisting of a p50 DNA-binding subunit and a p65 trans-activating subunit. NF-κB is normally held in cytoplasm in an inactivated state by the inhibitor protein IκB-α. After activation, the cytosolic NF-κB/IκB-α complex dissociates, and free NF-κB is translocated to the nucleus where it activates the responsive gene (Baeuerle & Henkel 1994; Barnes & Karin 1997). Our study demonstrates that IL-1β-induced degradation of IκB-α and translocation of p65 NF-κB are partially inhibited by byakangelicol. Furthermore, we found that the IL-1β-induced activation of NF-κB-specific DNA–protein complex formation is partially inhibited by byakangelicol. These results indicate that the inhibitory action of byakangelicol on IL-1β-mediated COX-2 expression may be mediated, at least in part, by suppression of the activity of this transcription factor. This agrees with more complex regulation of COX-2 expression in which other transcription factors, such as nuclear factor-interleukin-6 or the cAMP response element, may be involved (Inoue et al 1995).

Conclusions

This study has demonstrated that byakangelicol may inhibit IL-1β-induced PGE_2 release in A549 cells. The inhibitory effects of byakangelicol on IL-1β-induced PGE_2 release may occur through suppression of COX-2 expression, as well as the activity of the COX-2 enzyme. The selectivity ratio of byakangelicol on the inhibition of the activity between COX-1 and COX-2 enzyme was more than 8 fold. The inhibitory mechanism of byakangelicol on IL-1β-induced COX-2 expression may be, at least in part, through inhibition of NF-κB activity. Thus, byakangelicol may have therapeutic potential as an anti-inflammatory drug in airway inflammation.

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