

Prevention and relaxation effects of *Liriope platyphylla* on bronchial asthma *in vitro* model by suppressing the activities of MAPK/NF- κ B pathway

In-Seung Lee^{1,2,*}, Dong-Hyun Kim^{3,4,*}, Kang-Hoon Kim^{1,2}, Jiyoung Park^{1,2}, Yumi Kim^{1,2}, Eun-Jin An^{1,2}, Kwanil Kim^{2,3,4}, Hee-Jae Jung^{2,3,4} & Hyeung-Jin Jang^{1,2}

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Abstract

Backgrounds: Bronchial asthma (BA) is a common type of asthma, defined as a chronic inflammation, hyperplasia, and hypercontraction of the airway. In this study, we investigated whether *Liriope platyphylla* extract (LPP) can help prevent inflammation, suppress hyperplasia, and support relaxation via regulation of MAPK/NF- κ B activity.

Methods: To investigate the inflammation prevention effects, A549 cells, a human airway epithelial cell line, were pretreated with various concentration of LPP and then treated with EGF recombinant (10 ng/mL). Anti-hyperplasia and muscle relaxation experiments were performed with asthma condition human bronchial smooth muscle (hBSM) cells. The effects of LPP on MAPK/NF- κ B activity and MAPK/NF- κ B-related signaling, such as COX-2 and iNOS expression, was analyzed through western blot analysis. The mRNA expression levels of Th-2-cell-related cytokines, including *IL-6* and *TNF- α* , and contraction-related factors, such as *PLC β* , *IP $_3$ R*, and *MLCK*, were determined by real-time PCR.

Results: LPP pretreatment significantly reduced MAPK/

NF- κ B activity in EGF-induced asthma condition A549 cells. Therefore, there was significant suppression of both COX-2 and iNOS expression. In addition, the mRNA expression of *IL-6* and *TNF- α* was dose dependently reduced. Although there was no statistical significance, factors related to contraction showed a tendency to decrease.

Conclusion: Based on our results, we believe that LPP shows good potential for application as a therapeutic agent for asthma through inflammation prevention and bronchial smooth muscle relaxation.

Keywords: *Liriope platyphylla*, LPP, Asthmatic human bronchial smooth muscle cell, MAPK, NF- κ B, Preventing inflammation, Relaxation, Anti-hyperplasia

Introduction

Asthma, a common disease in modern society, is suffered by 4 to 5 percent of the population¹. The number of asthma cases has steadily increased since the 1970s, and there are no signs of it slowing². Therefore, the number of patients with asthma in Korea is expected to increase as well. Despite many studies on asthma, the exact mechanisms of asthma have not been clearly identified. The causes of asthma are varied and complex. However, recent studies have suggested that the basic mechanisms of asthma are airflow obstruction and airway hyper-responsiveness due to chronic inflammation of the airways^{3,4}. In airway epithelial cells, inflammatory mediators such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)- β are secreted to induce airway smooth muscle proliferation

¹College of Korean Medicine, Kyung Hee University, 26, Kyungheedaero-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea

²Department of Science in Korean Medicine, Graduate School, Kyung Hee University

³Department of Clinical Korean Medicine, Graduate School, Kyung Hee University

⁴Division of Allergy, Immune and Respiratory System, Department of Internal Medicine, College of Korean Medicine, Kyung Hee University, 23 Kyungheedaero-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea

*These authors contributed equally to this work.

Correspondence and requests for materials should be addressed to H. J. Jang (✉hjjang@khu.ac.kr)

as well as the proliferation of myofibroblasts, or collagen of the basement membrane to release growth factors and participate in airway remodeling^{5,6}.

Inflammation and structural changes take place over a large component of the bronchial wall; thus, the epithelial layer, fibroblast layer, and the extracellular matrix, vascular endothelium, and airway smooth muscle (ASM) are involved^{7,8}. In particular, the typical features of asthma include inflammatory cell activation and recruitment, shedding of bronchial epithelium, mucous metaplasia, subepithelial fibrosis, myofibroblast hyperplasia, angiogenesis, and increased ASM mass^{9–11}. All these pathological events are triggered by auto-oids (e.g., leukotrienes), cytokines, chemokines, and growth factors whose production and biological effects depend at least in part on the activation of mitogen-activated protein kinases (MAPKs)¹². MAPKs, which include several subgroups, such as c-Jun amino-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs) and p38 proteins, all share the common property of being activated via a three-module phosphorylation cascade, and they represent an important point of convergence for multiple signaling pathways that are activated in inflammation, immunity, cell death, and proliferation¹³. Therefore, these enzymes may influence many cellular events relevant to asthma pathophysiology, including type 2 helper (Th2) cell differentiation, inflammatory cell infiltration, epithelial and endothelial functions, as well as myofibroblast hyperplasia and ASM activity^{12,14}. Indeed, MAPK activation may be detected in both immune/inflammatory and bronchial resident cells.

Nuclear factor kappa-light chain-enhancer of activated B cell (NF- κ B) family members are believed to play a central role in a variety of acute and chronic inflammatory diseases. For this reason, the NF- κ B signaling pathway has been the focus of extensive research over the last 20 years^{15,16}. NF- κ B is activated in response to many stimuli, including physical and chemical stress, lipopolysaccharide (LPS), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), T and B cell mitosis, and pro-inflammatory cytokines. Its protein complexes play an important role in regulating the immune response to infection^{17–19}. Because it regulates many genes involved in inflammation, several studies have demonstrated that NF- κ B is highly activated in a variety of human diseases, such as rheumatoid arthritis, atherosclerosis, multiple sclerosis, and asthma; it has also been active in inflammatory sites in mouse models^{18,20,21}. When NF- κ B is activated, p50 and p65 bind to the nucleus, and the p50 and p65 subunits bind to DNA, thus resulting in a variety of gene expressions, such as nitric oxide (NO), cyclooxygenase-2 (COX-2), and tumor necrosis factor- α (TNF- α)²².

The crucial role of Th2 cell immune pathway elements in airway hyper-responsiveness (AHR) has been identified. The effect of Th2 cells in the inflammatory reaction of allergic asthma has been pointed out in several studies^{23–25}. When an antigen in the atmosphere enters the antigen-presenting dendritic cells, Th0 cells in the lymph are differentiated into Th2 cells and secrete interleukin (IL)-4, 5, 9, 13, and tumor necrosis factor (TNF)- α . They stimulate various cells to secrete immunoglobulin E (IgE) and inflammatory mediators, which cause AHR and airway obstruction, leading to asthma symptoms^{26,27}. Therefore, the main aim in the treatment of allergic asthma is to inhibit Th2-cell-associated cytokines or to promote Th1-cell-associated cytokines.

Asthma medications are classified into two classes: bronchodilators, such as β 2-agonists that alleviate symptoms within a short period of time, and disease modifiers, such as corticosteroids that suppress allergic inflammation of the bronchial tubes²⁸. These treatments are effective when used in the treatment of asthma, since bronchodilators have an immediate effect, and disease controllers also have a modulating effect on inflammation^{29,30}. However, due to side effects, such as adrenal suppression, decreased bone density, growth disturbance, as well as skin and eye complications, prescribing caution is needed^{31,32}.

The root part of *Liriope platyphylla* has been used for the treatment of respiratory diseases in traditional Korean medicine. *Liriope platyphylla* extract (LPP) has been shown to exert anti-inflammatory effects on a murine asthma model and to improve airway mucus clearance³³, and it shows potential as a disease controller. Also, our previous study showed the potential for inhalation of LPP as a bronchodilator³⁴. However, the purpose of this study was to determine whether LPP has potential for use as a therapeutic agent to protect against and prevent asthma. In this work, we examined how LPP affects the inflammatory reaction in epidermal growth factor (EGF)-induced inflamed human epithelial cells and the contract-relax reaction in asthmatic human airway smooth muscle cells.

Materials & Methods

Materials

Human EGF recombinant was obtained from R&D System Inc. (Minneapolis, MN, USA). Rabbit anti-phospho-NF- κ B p65, anti-NF- κ B p65, anti-COX-2, anti-iNOS, anti-phospho-ERK1/2, anti-ERK1/2, anti-phospho-JNK, anti-JNK and anti-Lamin B1 were purchased from Cell Signaling Tech (CST, Beverly, MA, USA). Mouse anti- β -actin, goat anti-rabbit IgG-HRP, and goat anti-mouse IgG-HRP were purchased from Santa Cruz

Table 1. Primer sequence for real-time quantitative PCR (qPCR) analysis.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
<i>GAPDH</i>	GCCACATCGCTCAGACACC	CCCAATACGACCAAATCCGT
<i>IL-6</i>	AGGGCTCTTCGGCAAATGTA	GAAGGAATGCCCATTAACAACAA
<i>TNF-α</i>	GCAGGTCTACTTTGGGTCATTG	GCGTTTGGGAAGGTTGGA
<i>PLCβ</i>	AACAAAATGCAGCTTAGCAGGATA	AGAGCTGAGGCATATAGTTGGATGA
<i>IP3R</i>	TGTGTTGGCTGAAGACACTATCACT	GCCGCGGTAATGTGTTTTTC
<i>MLCK</i>	TGGTCCTGGAGATCGTGCA	ACGCTCCGTCAGCTCAAAGT

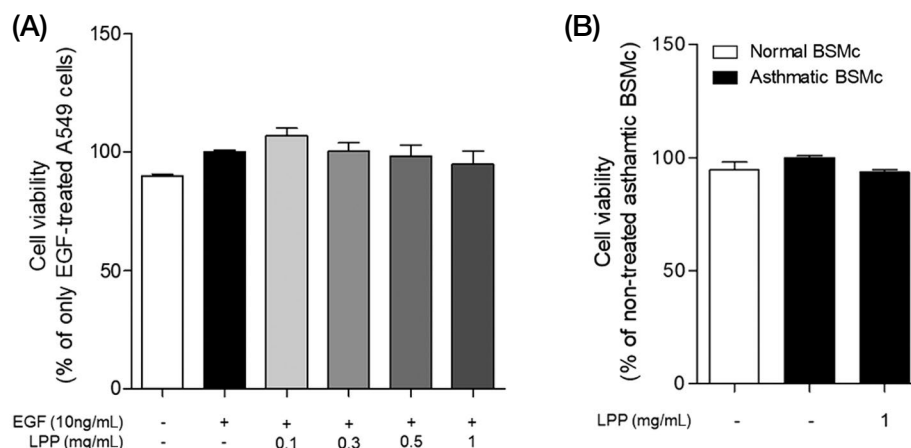


Figure 1. Cell viability assay of LPP on EGF-induced asthma condition A549 cells and hBSM cells. (A) Cell viability of the LPP pretreatment on A549 cells. The white bar graph is non-treated group and black bar graph is only EGF-treated group. (B) The cytotoxicity of the LPP on asthmatic human bronchial smooth muscle cells. The white bar graph is normal BSM cells and the black bar graph is BSM cells from asthma patients. Statistical significance was determined by unpaired t test (one-tailed) compared to only EGF-treated group. There is no cytotoxicity of the LPP in both cells; A549 and hBSM cells.

Biotechnology (Dallas, TX, USA).

Sample preparation

LPP was prepared as described our previous papers^{35,36}. Briefly, the root part of the herb was extracted with distilled water (DW), and filtration, evaporation, and freeze drying were performed to acquire the LPP.

Cell lines and culture condition

A549 cells (American Type Culture Collection, Rockville, MD, USA) and human bronchial smooth muscle (hBSM) cells (Lonza, Basel, Switzerland) were used in all *in vitro* studies. A549 cells were cultured in D-MEM high-glucose medium (Corning Inc., NY, USA) containing 10% FBS and 1% penicillin and streptomycin (P/S, Gibco, NY, USA). hBSM cells were cultured in SmGMTM-2 Smooth Muscle Growth Medium-2 (Lonza) containing 5% FBS and provided growth factors. All cells were incubated at 37°C in 5% CO₂.

Cell viability assay

MTT assays were performed to evaluate the cytotoxicity

of LPP on A549 and hBSM cells. Ten thousand A549 cells were cultured in each well of a 96-well plate and incubated at 37°C in 5% CO₂ overnight. The next day, the cells were pretreated with various concentrations of LPP (0.1 to 1 mg/mL) in RPMI containing 1% P/S at 37°C in 5% CO₂ for 1 h. After LPP treatment, the medium was changed to RPMI containing human EGF recombinant (10 ng/mL), and the samples were incubated at 37°C in 5% CO₂ for 1 h. Thirty microliters of MTT solution (2 mg/mL) was added to each well, and the cells were incubated for 2 h. The medium of each well was removed, and 50 μ L of dimethylsulfoxide (DMSO; Sigma-Aldrich, MO, USA) was added. Using an automated spectrophotometric plate reader at 570 nm, the optical density of formazan was measured. The cytotoxicity of LPP (1 mg/mL) on hBSM cells was investigated by the above-mentioned method.

Western blot analysis

Twenty micrograms of whole protein or 6 μ g of nucleus was separated by SDS page (8, 10, or 12%) electrophoresis, and then transferred onto a nitrocellulose

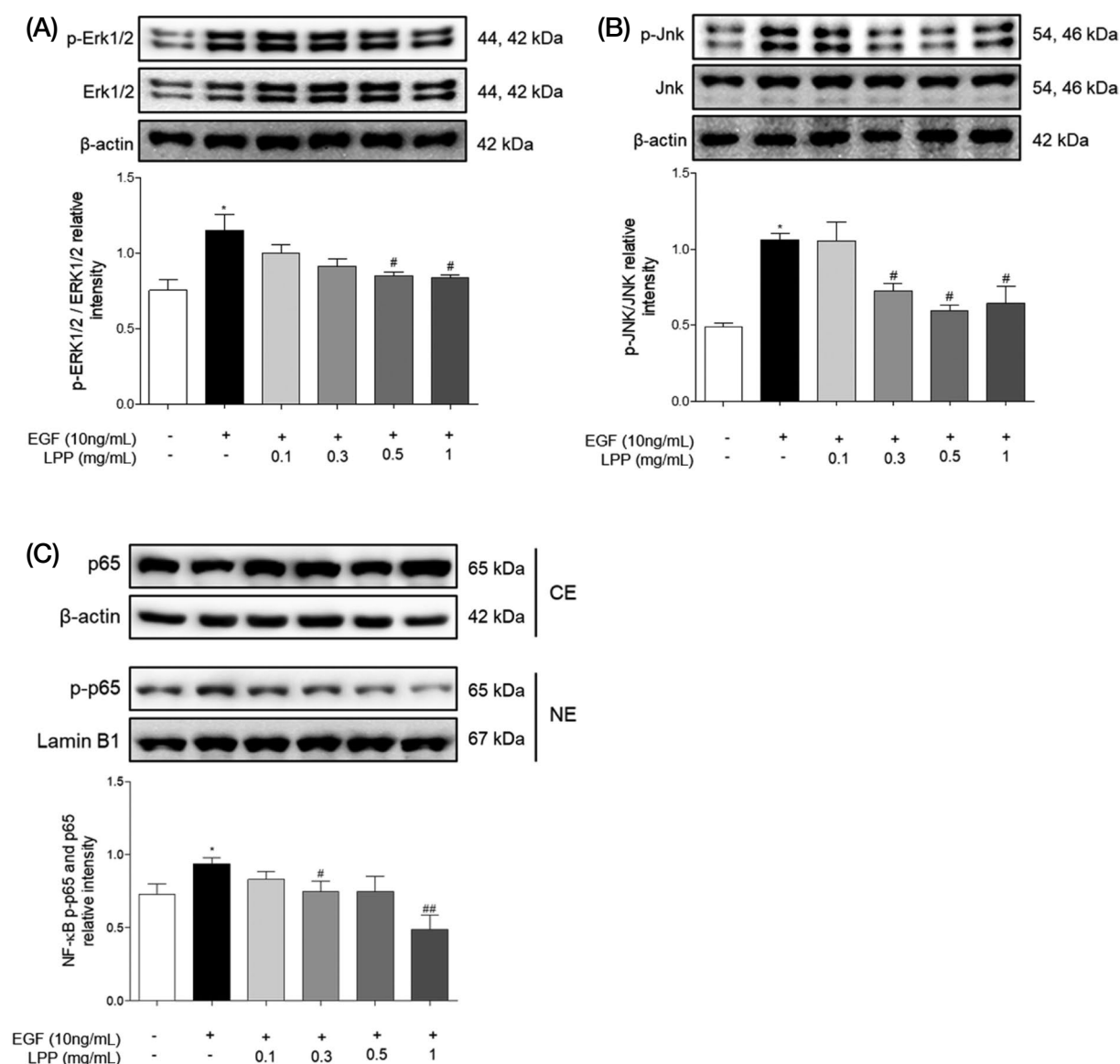


Figure 2. Preventing inflammation effect of LPP in EGF-induced inflamed A549 cells via inhibiting MAPK/NF-κB activity. Cells were pretreated with LPP (0.1 to 1 mg/mL) for 1 h and stimulated inflammation with EGF (10 ng/mL) for 1 h. (A) The protein expression of the MAPK signaling pathway using at treated doses (0.1 to 1 mg/mL) and (B) the protein expression of NF-κB activity in cytosol and nucleus. Ratio of each protein expression was measured by ImageJ. Statistical significance was determined by unpaired t test (one-tail) compared to EGF-induced inflamed A549 cells. Values are means ± S.E.M. *, $P < 0.05$ compared to non-EGF treated A549 cells. #, $P < 0.05$; ##, $P < 0.01$ compared to only EGF-treated A549 cells.

membrane (Bio-Rad, Hercules, CA, USA)³⁷. The membranes were incubated with various antibodies against p-ERK1/2, ERK1/2, p-JNK, JNK, NF-κB p-p65, NF-κB p65, COX-2, iNOS, Lamin B1 (diluted 1 : 1,000) and β-actin (diluted 1 : 3,000) at 4°C overnight. Then the membranes were extensively washed by Tris-buffered saline with 0.1% Tween 20 and 0.04% NaN₃ (TBST) and then incubated with the following secondary antibodies: goat anti-rabbit IgG-HRP (diluted 1 : 10,000)

and goat anti-mouse IgG-HRP (diluted 1 : 10,000). Bands were visualized with an enhanced chemiluminescence (ECL) kit (EMD Millipore Co., Billerica, MA, USA) and were quantified with the Image J program (National Institutes of Health, MD, USA).

Isolation of the total RNA and real-time PCR

The total RNA was isolated from each cell line using

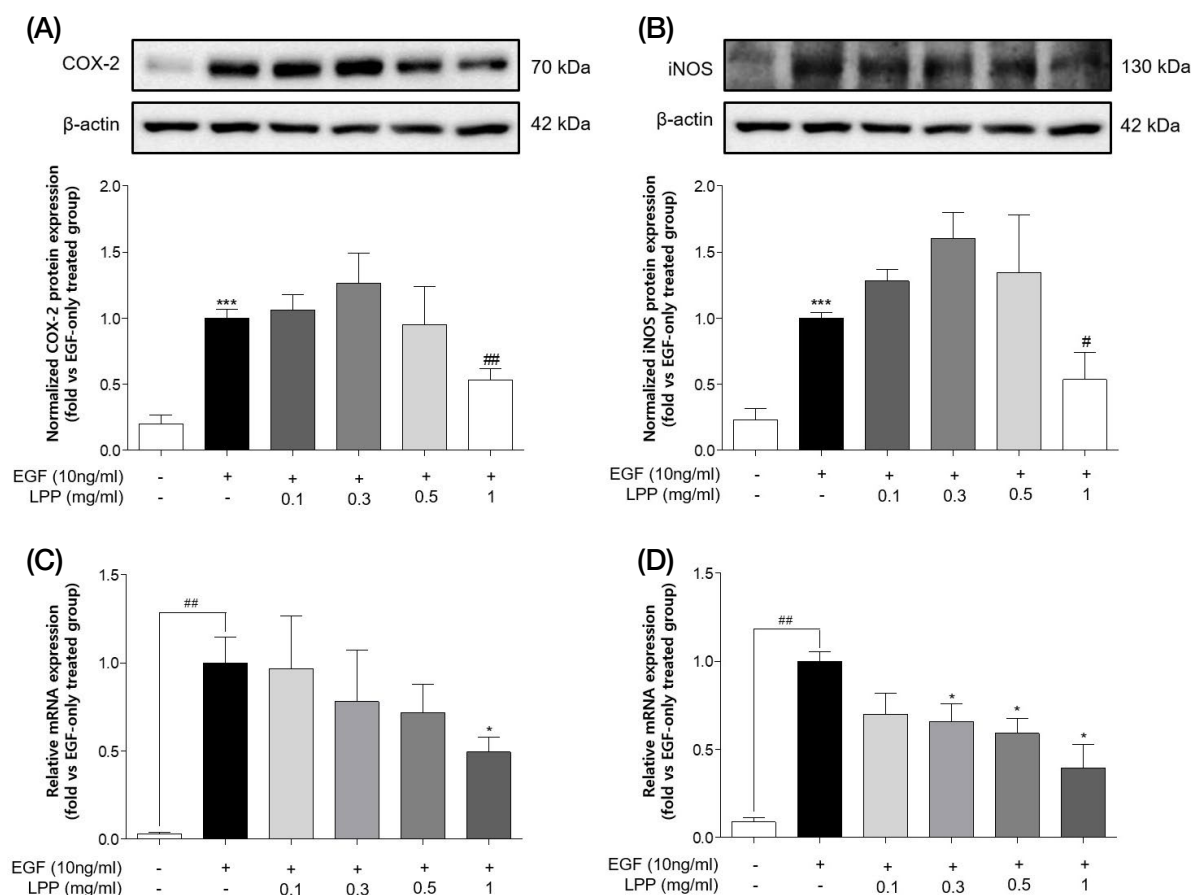


Figure 3. Regulation effect of LPP on Immune reaction cytokines in EGF-induced inflamed A549 cells. The western blot results representative the COX-2 and iNOS expression by LPP treatment. COX-2 and iNOS expression levels were normalized with β -actin was measured by densitometry analysis and represented as bar charts (A and B). The real-time PCR results showed the mRNA expression of cytokines study (C and D). Statistical significance was determined by unpaired *t* test (one-tail) compared to EGF-induced inflamed A549 cells. Values are means $\pm$ S.E.M. **, $P < 0.01$; ***, $P < 0.001$ compared to non-EGF treated A549 cells. #, $P < 0.05$; ##, $P < 0.01$ compared to only EGF-treated A549 cells.

Hybrid-RTM (GeneAll, South Korea)^{38,39}. Real-time PCR was used to determine the effects of LPP on EGF-induced bronchial asthma condition in A549 cells and hBSM cells. The total RNA (500 ng) was converted to cDNA using oligo (dT). It was then held at 55°C for 60 min followed by 85°C for 5 min and then kept at 4°C until used. The primer sequences used for the real-time PCR analysis are listed in Table 1. Real-time quantitative PCR was performed using the Universal SYBR Green Master Mix (Applied Bio systems, USA). Amplification of the cDNA was performed by heating the samples as follows: 95°C for 15 min followed by 40 cycles at 95°C for 30 s, at 59°C for 30 s, and at 72°C for 30 s⁴⁰. Real-time PCR analysis was performed on an Applied Bio-systems StepOne system (Applied Bio-systems, USA). In this study, quantification based on the relative expression of a target gene versus GAPDH gene ($2^{-\Delta\Delta Ct}$) was utilized to determine the level of mRNA

expression.

Statistical analysis

Data were represented with the means $\pm$ SEM. The GraphPad Prism 5 software package (GraphPad Software, San Diego, CA, USA) was used to conduct the statistical analysis of the results. An unpaired student's *t* test (two-tailed) was conducted to analyze the western blot band and real-time PCR results. A *P* value less than 0.05 was considered to be statistically significant.

Results

The cytotoxicity of LPP on EGF-induced asthmatic A549 cells and hBSM cells

To examine the cytotoxicity of LPP on A549 cells, the cells were first treated with various concentrations of

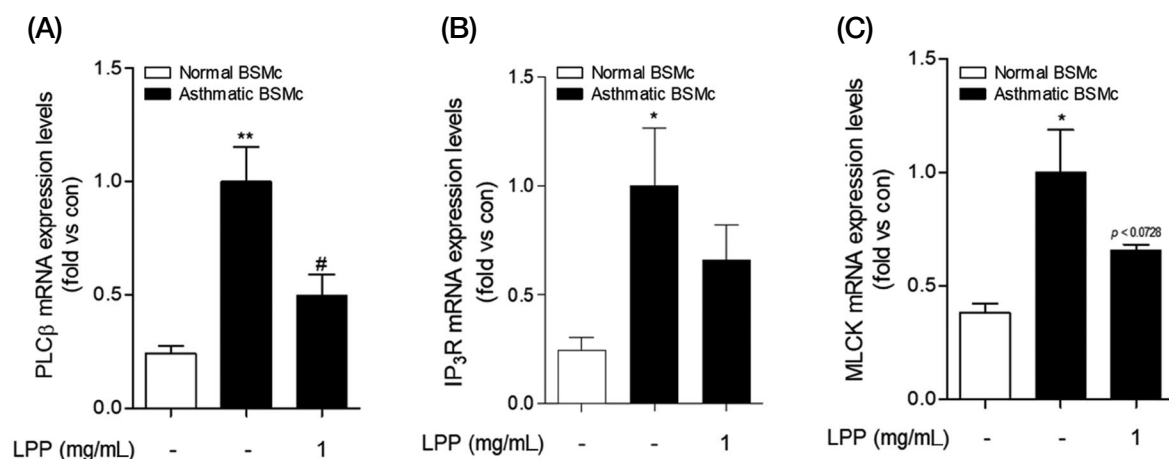


Figure 4. Relaxation effects of LPP on asthmatic hBSM cells. The real-time PCR results showed mRNA expression levels of contraction-related factors (A) *PLCβ* (B) *IP₃R* and (C) *MLCK*. Statistical significance was determined by unpaired t test (one-tail) compared to non-treated asthmatic hBSM cells. The empty bar graph demonstrates normal hBSM cells, and the black bar graph demonstrate asthmatic hBSM cells. Values are means $\pm$ S.E.M. *, $P < 0.05$; **, $P < 0.01$ compared to normal hBSM cells. #, $P < 0.05$ compared to non-treated asthmatic hBSM cells.

LPP (0.1 to 1 mg/mL) in RPMI medium without fetal bovine serum (FBS) for 1 h and treated with EGF recombinant (10 ng/mL) for 1 h. There was no observed cytotoxicity of LPP in the EGF-induced bronchial asthma condition A549 cells compared to only EGF-treated cells. Also, 1,000 μ g of LPP has no cytotoxicity on asthmatic hBSM cells.

Inflammation prevention effects of LPP on asthmatic A549 cells via MAPK/NF- κ B pathway

To observe the inflammation prevention effects of LPP on inflammation induced human lung epithelial cells, we pretreated cell samples with LPP (100 to 1,000 μ g/mL) and then induced inflammation by applying EGF (10 ng/mL). MAPK/NF- κ B activation, which plays a central role in immune and inflammatory responses was analyzed by western blot analysis to evaluate the effects of LPP treatment on A549 cells. The results showed that LPP pretreatment dose dependently inhibited phosphorylation of ERK1/2 and JNK and significantly reduced the expression of NF- κ B phosphorylation of p65 in the A549-cell activated MAPK/NF- κ B pathway by EGF recombinant. By reducing the ratio of p-ERK1/2/ERK1/2, p-JNK/JNK and NF- κ B p-p65/ NF- κ B p65, LPP pretreatment is associated with the MAPK/NF- κ B pathway; thereby LPP would prevent inflammation.

Cytokines and immune reaction reduction effects of LPP

Th2-cell-associated cytokines and NF- κ B-associated immune reaction levels in EGF-induced asthmatic con-

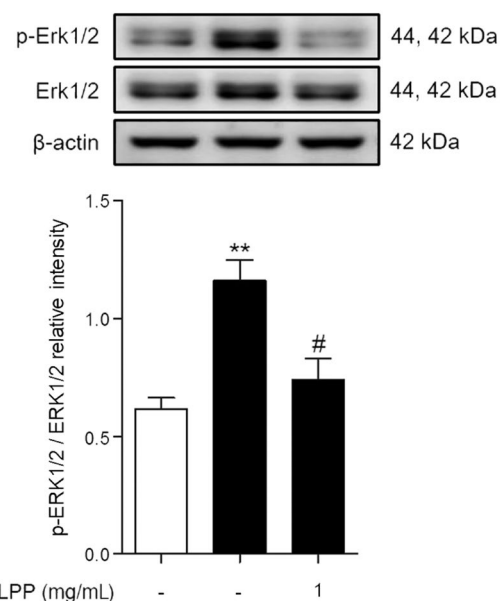


Figure 5. Anti-hyperplasia effects of LPP on asthmatic hBSM cells via ERK pathway. The protein expression of the ERK pathway using LPP treatment (1 mg/mL) on asthmatic hBSM cells. Ratio of each protein expression was measured by ImageJ. Statistical significance was determined by unpaired t test (one-tail) compared to non-treated asthmatic hBSM cells. The empty bar graph demonstrates normal hBSM cells, and the black bar graph demonstrate asthmatic hBSM cells. Values are means $\pm$ S.E.M. **, $P < 0.01$ compared to normal hBSM cells. #, $P < 0.05$ compared to non-treated asthmatic hBSM cells.

dition A549 cells were determined by real-time PCR and western blot analysis, respectively. The results

showed that EGF treatment not only remarkably stimulated cytokines, such as *IL-6*, and *TNF- α* , but also increased the expression levels of NF- κ B-associated immune reaction, including COX-2 and iNOS expression. As the concentration of LPP was increased, the cytokines and the proteins expression levels were significantly reduced.

Relaxation effects of LPP on asthmatic hBSM cells

From the inflammation inhibitory effects of LPP in EGF-induced asthmatic condition A549 cells via regulating the MAPK/NF- κ B pathway, we anticipated that LPP may have a relaxation effect on asthmatic contractile hBSM cells. To determine the proper concentration of LPP against EGF-induced asthmatic condition A549 cells, we studied the cytotoxic effect on asthmatic hBSM cells. The relaxation effect of LPP was examined on asthmatic hBSM cells by real-time PCR in sequence. We found that LPP (1 mg/mL) significantly inhibited a contraction-related factor, *phospholipase C (PLC) β* . Although there were no significant differences, the decreased tendencies of *inositol trisphosphate 3 receptor (IP₃R)* and *myosin light-chain kinase (MLCK)* mRNA expression levels were determined in LPP-treated asthmatic hBSM cells in comparison to non-treated asthmatic hBSM cells.

Cell proliferation inhibitory effects of LPP on asthmatic hBSM cells

Asthmatic hBSM cells show both contraction and hyperplasia. From the relaxation effect of LPP on asthmatic hBSM cells, we also examined the regulation effects of LPP on hyper-proliferated hBSM cells by western blot analysis. The result showed that the ratio of p-ERK 1/2/ERK1/2 was significantly reduced by LPP treatment.

Discussion

Bronchial asthma (BA), one of the most serious health problems in the world, affects about 5% to 10% of patients of all ages⁴¹. It causes periodic attacks of coughing, wheezing, breathlessness, and even chest congestion. BA is characterized by an inflammatory reaction in the bronchial epithelium as well as contraction and hyperplasia in bronchial smooth muscle cells^{41–44}. Our previous study demonstrated LPP-attenuated airway hyper-responsiveness and the inflammatory response in an ovalbumin (OVA)-induced asthmatic animal model. In the present study, we found that LPP treatment significantly regulated inflammatory reaction in EGF-induced asthmatic condition A549 cells by inhibiting

MAPK/NF- κ B activation; also, the effects of muscle relaxation and inhibition of hyper-proliferation were observed in asthmatic BSM cells.

MAPKs represent an important point of convergence for multiple signaling pathways that are activated in inflammation, immunity, cell death, and proliferation^{12,13,45,46}. Therefore, these enzymes may influence many cellular events relevant to asthma pathophysiology, including Th2 cell differentiation, inflammatory cell infiltration, epithelial and endothelial functions, as well as myofibroblast hyperplasia and BSM activity^{14,47,48}. Indeed, MAPK activation may be detected in both immune/inflammatory and bronchial resident cells. ERK mediates asthmatic airway inflammation by regulating the biosynthesis of several cytokines and chemokines⁴⁹. In addition, the ERK pathway is believed to play a central role in the signal transduction system that allows cells to pass through the limits of the cell cycle⁵⁰. JNK, also known as stress-activated protein kinase, has been implicated in a multitude of diseases ranging from cancer to immunological/inflammation conditions^{51,52}. The present study confirmed that LPP pretreatment significantly suppressed ERK1/2 and JNK phosphorylation, increased by EGF-treatment in asthmatic condition A549 cells. Moreover, we also investigated whether LPP has a regulatory effect on hyperplasia via the ERK pathway, and we confirmed that there was a significance reduction of ERK1/2 in LPP-treated asthmatic hBSM cell. The significant reduction of ERK by LPP in both asthmatic epithelium cells and bronchial smooth muscle cells suggests the possible therapeutic effects of LPP against inflammation and hyperplasia of BSM cells due to asthma. The next step was to determine whether LPP pretreatment also blocked the transcription of NF- κ B in asthmatic condition A549 cells. Therefore, we investigated the significance of NF- κ B activity inhibition by conducting a western blot analysis.

NF- κ B is a transcription factor that acts as a major player in the pathogenesis of inflammation, and it regulates the expressions of multiple genes, including inducible nitric oxide (iNOS), COX-2, and cytokines^{53,54}. Because up-regulated NF- κ B activity is related to asthmatic inflammation, agents that directly reduce NF- κ B activity have been investigated^{55,56}. In our study, LPP pretreatment was demonstrated to significantly reduce the transcribed NF- κ B, up-regulated by EGF treatment. This reduced expression of NF- κ B by the application of LPP in EGF-induced asthmatic condition A549 cells confirmed that LPP affects COX-2 and iNOS expression, known as downstream effects of NF- κ B activation.

COX-2 is an enzyme that produces prostaglandins E₂ (PGE₂), and the presence of this molecule lead to inflammation and pain^{57–59}. We found that highly ex-

pressed COX-2 levels by 10 ng/mL of EGF treatment and increased COX-2 levels were dose-dependently reduced by LPP pretreatment in asthmatic condition human bronchial epithelial cells. The reduction of COX-2 expression by LPP pretreatment through the inhibition of NF- κ B activation may lead to inhibition of PGE₂ production. NO, endogenously synthesized by iNOS, might serve as an inflammometer for conditions such as asthma because it is abundant in the air exhaled by asthmatic patients^{60,61}. Excessive iNOS expression is closely related to the production of NO, and it is considered a biomarker of asthma. iNOS promotes airway inflammation via the up-regulation of chemokine expression⁶². NF- κ B activation can stimulate the expression of iNOS due to the presence of the NF- κ B binding region on the iNOS promoter⁶³. We confirmed that iNOS expression was significantly decreased by pretreatment with a high concentration of LPP. Reduced iNOS expression levels indicate the inhibition of nitrosative stress, an important factor in asthma treatment. Therefore, these results indicate that LPP has regulatory effects on PGE₂ and NO production at the transcription level via inhibited NF- κ B activation.

Various studies have reported that Th2-cell-related cytokines are involved in the pathophysiology of asthma^{64,65}. Cytokines such as IL-6 and TNF- α are considered to be involved in various inflammatory diseases, including asthma and chronic pulmonary disease (COPD). IL-4 induces IgE synthesis, which leads to eosinophil infiltration into the airway tissues and mucus hypersecretion. A key inflammatory cytokine, IL-6, plays a role in inducing inflammatory responses and can regulate various aspects of the CD4 T-cell-mediated response, including cytokine production and suppressive activity⁶⁶. TNF- α , which is related to systemic inflammation, causes PGE₂ production through COX-2 activation. The up-regulated TNF- α level enhances the coagulation-promoting action of vascular endothelial cells, activates macrophages, increases adhesion molecule expression, and increases neutrophil infiltration⁶⁷. The reduction of cytokine secretion observed in this study demonstrates that PGE₂ production may be suppressed by the blocking of TNF- α secretion; thus, the inflammation, fever, and pain related to PGE₂ production may be reduced.

BSM plays an important role in bronchial contraction as well as structural remodeling of the bronchial wall. Thus, BSM contraction and proliferation are significantly associated with asthma onset and progression⁵⁰. We confirmed inhibition of hyperproliferation by confirming that the LPP reduces the expression level of ERK1/2. In sequence, factors associated with muscle contraction, such as PLC β , IP₃R, and MLCK, which are highly expressed in asthmatic patients, were

identified. In the present study, we confirmed that the mRNA expression of contraction-related factors, such as PLC β , IP₃R, and MLCK, were significantly increased in asthmatic hBSM cells compared to normal hBSM cells. After confirmation, mRNA expression of PLC β was greatly reduced by LPP treatment. Even though there was not a statistically significant reduction, the decrease tendency was demonstrated in the mRNA expression of IP₃R and MLCK. It can be concluded that muscular relaxation occurs since we confirmed that muscle-contraction-related factors were decreased by LPP treatment.

Conclusion

Our results demonstrate that LPP could be useful in preventing inflammation by inhibiting MAPK/NF- κ B activation, which plays an important role in inflammation in human airway epithelial cells under EGF-induced asthma conditions. Moreover, it inhibits cellular hyperplasia and muscle contraction in asthmatic hBSM cells. Therefore, we are confident of the potential of LPP as a therapeutic agent for the treatment of asthma through inflammation prevention and bronchial smooth muscle relaxation.

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Conflict of Interest In-Seung Lee, Dong-Hyun Kim, Kang-Hoon Kim, Jiyoung Park, Yumi Kim, Eun-Jin An, Kwanil Kim, Hee-Jae Jung & Hyeung-Jin Jang declare that they have no conflict of interest.

Human and animal rights The article does not contain any studies with human and animal. This study was performed following institutional and national guidelines.

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