

ORIGINAL ARTICLE

Inhibitory effects of albuterol and fenoterol on RANTES and IP-10 expression in bronchial epithelial cells

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Keywords

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Abstract

Short-acting β_2 -adrenoreceptor agonist (SABA) is the major asthma reliever as indicated in the GINA guidelines. Regulated on activation, normal T expressed and secreted (RANTES) is a chemokine that attracts eosinophils, mast cells, and basophils toward site of allergic inflammation. Interferon γ -inducible protein (IP)-10 is a Th1-related chemokine that is also important in asthmatic inflammation and also involved in our immune defense against pathogens. Bronchial epithelial cells are first-line barrier against invasive pathogen and also have immunomodulatory function. However, whether albuterol and fenoterol (two SABAs) have modulatory effects on RANTES and IP-10 expression in bronchial epithelial cells is unknown. The human bronchial epithelial cell lines, BEAS-2B cells, were pre-treated with different concentrations of albuterol, fenoterol or dibutyryl-cAMP (a cyclic AMP analog) before polyinosinic-polycytidylic acid (poly I:C) stimulation. In some condition, BEAS-2B cells were pre-treated with ICI-118551, a selective β_2 -adrenoreceptor antagonist, 30 min before albuterol or fenoterol treatment. The levels of RANTES and IP-10 were measured by ELISA. Intracellular signaling was investigated using cAMP assay, mitogen-activated protein kinase (MAPK) inhibitor, nuclear factor (NF)- κ B inhibitor, and western blot. Albuterol and fenoterol suppressed poly I:C-induced RANTES and IP-10 expression of BEAS-2B cells. ICI-118551 could partly reverse the suppressive effects of albuterol and fenoterol on RANTES and IP-10 expression. Albuterol and fenoterol increased intracellular cAMP levels. Dibutyryl-cAMP conferred the similar effects of albuterol and fenoterol. Western blot revealed that albuterol suppressed p-ERK, p-JNK and pp38, and also their associated kinase expression. Albuterol had no effect on pp65 expression. Albuterol and fenoterol could suppress poly I:C-induced RANTES and IP-10 expression in human bronchial epithelial cells via at least partly the β_2 -adrenoreceptor-cAMP and the MAPK pathways, implicating that albuterol and fenoterol could exert anti-inflammatory effect and benefit asthmatic patients by suppressing RANTES and IP-10 expression. However, these suppressive effects of albuterol and fenoterol may inhibit the defense against viral infection.

Short-acting β_2 -adrenoreceptor agonist (SABA) is the major reliever in asthma management. As indicated in the Global Initiative for Asthma (GINA) guidelines, they are as-needed reliever medications in each treatment step. Clinically, SABAs are the medications of choice for quick relief of bronchospasm during asthma exacerbations and for the pre-treat-

ment of exercise-induced bronchoconstriction (1). Apart from asthma, SABAs are also widely used as prescribed medications in infants and children with common respiratory tract infection and, more often, virus-induced wheezing in acute bronchiolitis (2). Treatment recommendations of respiratory syncytial virus (RSV)-induced bronchiolitis from the Agency

for Healthcare Research and Quality (AHQR) state that nebulized albuterol (one of SABAs) is possible effective when combined with ipratropium bromide use (3). However, some meta-analyses showed that the efficacy of SABAs in the treatment of RSV-induced acute bronchiolitis is controversial (4). The effectiveness of routine use of SABAs for acute cough in adults and children without underlying pulmonary disease is inconclusive (5).

Asthma is a chronic inflammatory disorder of the airways with the characteristics of inflammatory cells infiltration (including eosinophils, monocytes, and neutrophils), epithelial cells shedding, and airway hyper-responsiveness, with skewed T helper type 1 (Th1) and Th2 differentiation toward Th2 cells response (6). There are two main phases, early and late, in the asthmatic inflammation. The early phase is associated with allergen-activated tissue resident mast cells and basophils, and the late phase is associated with blood-borne inflammatory cells. Cross-linking of the IgE-FcεRI complexes by allergen can lead to the releasing of proinflammatory mediators by mast cells or basophils, such as histamine, prostaglandins, cysteinyl leukotrienes, and chemokines. As the inflammatory response progresses, the recruitment of inflammatory cells (such as eosinophils, neutrophils) toward the inflammation site needs the involvement of chemokine (7). In asthmatic children, the serum Th2-related chemokine levels are increased and could be decreased after medical intervention (8).

Bronchial epithelial cells are the first-line defense in our respiratory system. They act as a physical barrier that protects us from external exposure and also modulate immune response by producing cytokines and chemokines, recruiting and activating inflammatory cells (9). In response to allergen challenge, bronchial epithelial cells can produce Regulated on activation, normal T expressed and secreted (RANTES)/CCL 5 chemokine, resulting in recruitment and activation of eosinophils, mast cells, and basophils (10). Virus infection of airway epithelial cells is one of the important triggers of airway inflammation process and asthma exacerbation (11). After viral infection, bronchial epithelial cells can secrete interferon γ -inducible protein 10 (IP-10)/CXCL 10, which recruit Th1 cells via CXCR3 to eliminate intracellular pathogen (12). In asthma, IP-10 contributes to airway hyper-responsiveness and inflammation (13), and the plasma level of IP-10 elevated remarkably during asthmatic exacerbation (14). High level of serum IP-10 can be used as an index to predict a virus-induced asthma and is strongly associated with the severity of airflow obstruction (15).

Polyinosinic-polycytidylic acid (Poly I:C) is an analog of viral double-stranded RNA, which can stimulate bronchial epithelial cells through toll-like receptor (TLR)-3, leading to inflammatory responses. Animal study implicated that TLR-3 activation by poly I:C plays the critical role in lung inflammation and function impairment and is regarded as one of the mechanisms by which viral infection contributes to asthma exacerbation (16).

In this study, we investigated the *in vitro* effects of albuterol and fenoterol, two of the SABAs, on RANTES and IP-10 expression in poly I:C-stimulated bronchial epithelial cells and further explored the detailed intracellular mechanisms.

Methods

Cell preparation

BEAS-2B cells (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μ g/ml of streptomycin at 37°C and 5% CO₂ in a humidified incubator. Cells were centrifuged and resuspended in fresh media in 24-well plates at a concentration of 2×10^5 /ml 24 h before experimental use. The cells were pre-treated with albuterol or fenoterol at different concentrations (0.01–1 μ M) 1 h before poly I:C (10 μ g/ml) stimulation. Cell supernatants were collected 12 and 24 h after poly I:C stimulation. In some cases, the cells were pre-treated with a selective β 2-adrenoreceptor antagonist, ICI-118551 (Imperial Chemical Industries, Wilmslow, Cheshire, UK), 30 min before treatment of the cells with albuterol or fenoterol, and then stimulated with poly I:C 1 h after albuterol treatment. In some cases, BEAS-2B cells were pre-treated with a cyclic AMP analog, dibutyryl-cAMP (Sigma Chemical Co., St. Louis, MO, USA), at different concentrations (0.1–10 μ M) 30 min before poly I:C stimulation. It has been reported that Poly I:C can activate mitogen-activated protein kinase (MAPK) and nuclear factor- κ B (NF κ B) pathways in human airway epithelial cells (17). To investigate the intracellular signals, some cells were pre-treated with MAPK-p38 inhibitor (SB203580), MAPK-JNK inhibitor (SP600125), MAPK-ERK inhibitor (PD98059) or a NF κ B inhibitor (BAY117082) 30 min before stimulated with poly I:C (10 μ g/ml). Supernatants were collected 24 h after poly I:C stimulation.

Enzyme-linked immunosorbent assay

RANTES/CCL 5 and IP-10/CXCL 10 concentrations of the cell supernatants were determined using commercially available enzyme-linked immunosorbent assay (ELISA)-based assay systems (R&D Systems, Minneapolis, MN, USA). Assays were performed using the protocol recommended by the manufacturer.

cAMP assays

cAMP assay was performed using a commercial cAMP enzyme immunoassay kit (Sigma Chemical Co., St. Louis, MO, USA). BEAS-2B cells (1×10^6) were incubated with albuterol or fenoterol (1–10 μ M), or vehicle for 30 min, and were lysed by adding lysis buffer. After 10 min, the total cell lysates were centrifuged at 600 *g* for 10 min. The supernatants were used for detecting intracellular cAMP. cAMP was measured according to the manufacturer's instruction.

Western blotting

After pre-treatment with albuterol (0.01–1 μ M) for 1 h, the cells were stimulated with poly I:C (10 μ g/ml) and were lysed

1 h later with an equal volume (300 μ l) of ice-cold lysis buffer. Samples were centrifugated at $13000 \times g$ for 15 min, and equal amounts of cell lysates from each experimental condition were analyzed by western blot with anti-MAPK (p38, ERK and JNK) and anti-phospho-MAPK (pp38, p-ERK and p-JNK), anti-NF κ B-p65 and anti-NF κ B-phospho-p65 (SantaCruz Biotechnology Inc., Santa Cruz, CA, USA). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibodies and the enhanced chemiluminescence system (Amersham Pharmacia Biotech, Boston, MA, USA).

MAPK activity assay

After treatment for 1 h with or without albuterol (0.1–1 μ M), the cells were stimulated with poly I:C (10 μ g/ml) and were lysed with equal volumes of ice-cold 150- μ l lysis buffer 1 h later. ERK, JNK, and p38 MAP kinase activities in cells were measured by non-radioactive ERK, JNK, and p38 MAPK assay kits (Cell Signaling Technology, Danvers, MA, USA). Assays were performed using the protocols recommended by the manufacturer. ELK-1, c-JUN, and ATF-2 were used as substrates for ERK, JNK, and p38 MAPK assay *in vitro*, and phosphorylated ELK-1, phosphorylated c-JUN, and phosphorylated ATF-2 were detected by western blot using anti-phospho-ELK-1 (pELK-1), anti-phospho-c-JUN (p-c-JUN), and anti-phospho-ATF-2 (pATF-2) antibodies to determine ERK, JNK, and p38 MAPK activity, respectively.

Statistical analysis

All data are presented as mean $\pm$ standard deviation. Differences between experimental and control groups were analyzed using the Mann–Whitney *U*-test. *p*-value < 0.05 was considered statistically significant.

Results

Albuterol and fenoterol suppressed poly I:C-induced RANTES and IP-10 expression in BEAS-2B cells

To examine the effect of SABAs on the expression of chemokines in the human bronchial epithelial cell lines, BEAS-2B cells were pre-treated with different concentrations of albuterol (0.01–1 μ M) for 1 h and were stimulated with poly I:C (10 μ g/ml). As shown in Fig. 1, albuterol could suppress poly I:C-induced RANTES by approximately 35%, 45%, and 54% at the concentration of 0.01, 0.1, and 1 μ M, respectively, 24 h after poly I:C stimulation (Fig. 1a). Albuterol could suppress poly I:C-induced IP-10 by approximately 37%, 48%, and 54% at the concentration of 0.01, 0.1, and 1 μ M, respectively, 24 h after poly I:C stimulation (Fig. 1b). Similarly, fenoterol significantly suppressed RANTES by approximately 34%, 49%, and 45% at the concentration of 0.01, 0.1, and 1 μ M, respectively, 24 h after poly I:C stimulation (Fig. 1c) and also suppressed IP-10 by approximately 49%, 49%, and 61% at the concentration of

0.01, 0.1, and 1 μ M, respectively, 24 h after poly I:C stimulation (Fig. 1d). However, albuterol and fenoterol (0.1 μ M) alone had no effect on RANTES or IP-10 expression in BEAS-2B cells.

Albuterol and fenoterol suppressed poly I:C-induced RANTES and IP-10 expression in BEAS-2B cells via the β 2-adrenoreceptor and cAMP pathway

It is known that SABAs exert their functions through the β 2-adrenoreceptor, leading to increased levels of intracellular cAMP (18). To examine whether the effect of albuterol on poly I:C-induced RANTES and IP-10 expression was mediated through the β 2-adrenoreceptor, BEAS-2B cells were pre-treated with a selective β 2-adrenoreceptor antagonist, ICI-118551 (1 and 10 μ M). As shown in Fig. 2, ICI-118551 was able to partially reverse the suppressive effects of 0.1 μ M albuterol to 84% of RANTES (Fig. 2a) and 72% of IP-10 (Fig. 2b) expression induced by poly I:C. ICI-118551 almost completely reversed the suppressive effect of 0.1 μ M fenoterol on poly I:C-induced RANTES (Fig. 2c) expression and partially reversed the suppressive effect of 0.1 μ M fenoterol to 78% of IP-10 (Fig. 2d) expression induced by poly I:C. These data implicated that these suppressive effects were β 2-adrenoreceptor dependent.

Because activation of β 2-adrenoreceptor can increase intracellular cAMP levels, we next investigated whether albuterol and fenoterol could elevate intracellular cAMP in BEAS-2B cells. As shown in Fig. 3a,b, both albuterol and fenoterol elevated intracellular cAMP. Fenoterol, a full agonist for β 2-adrenoreceptor, was more potent in increasing cAMP than albuterol, which is a partial agonist for β 2-adrenoreceptor. We next examined whether increasing intracellular cAMP could confer the modulatory effect of albuterol and fenoterol using a cAMP analog, dibutyryl-cAMP (0.1–10 μ M). As shown in Fig. 3c,d, dibutyryl-cAMP also suppressed poly I:C-induced RANTES and IP-10 expression, similar to the effects of albuterol on the expression of poly I:C-induced RANTES and IP-10 in BEAS-2B cells. Taken together, these data suggested that the inhibitory effects of albuterol and fenoterol on the expression of RANTES and IP-10 in BEAS-2B cells were via, at least partly, the β 2-adrenoreceptor-cAMP pathway.

Albuterol suppressed poly I:C-induced RANTES and IP-10 expression via MAPK-ERK-ELK-1, JNK-c-JUN, p38-ATF-2 but not NF κ B-p65 pathways

Poly I:C, a ligand for toll-like receptor 3, can activate MAPK and NF κ B pathways in human airway epithelial cells (17). To investigate whether albuterol suppressed the expression of RANTES and IP-10 via these pathways, the cells were pre-treated with MAPK and NF κ B inhibitors 30 min before poly I:C treatment. As shown in Fig. 4a,b, PD98059 (MAPK-ERK inhibitor), SP600125 (MAPK-JNK inhibitor), SB203580 (MAPK-p38 inhibitor), and BAY117082 (NF κ B inhibitor) suppressed poly I:C-induced RANTES and IP-10 expression, suggested that MAPK-ERK, JNK, p38, and

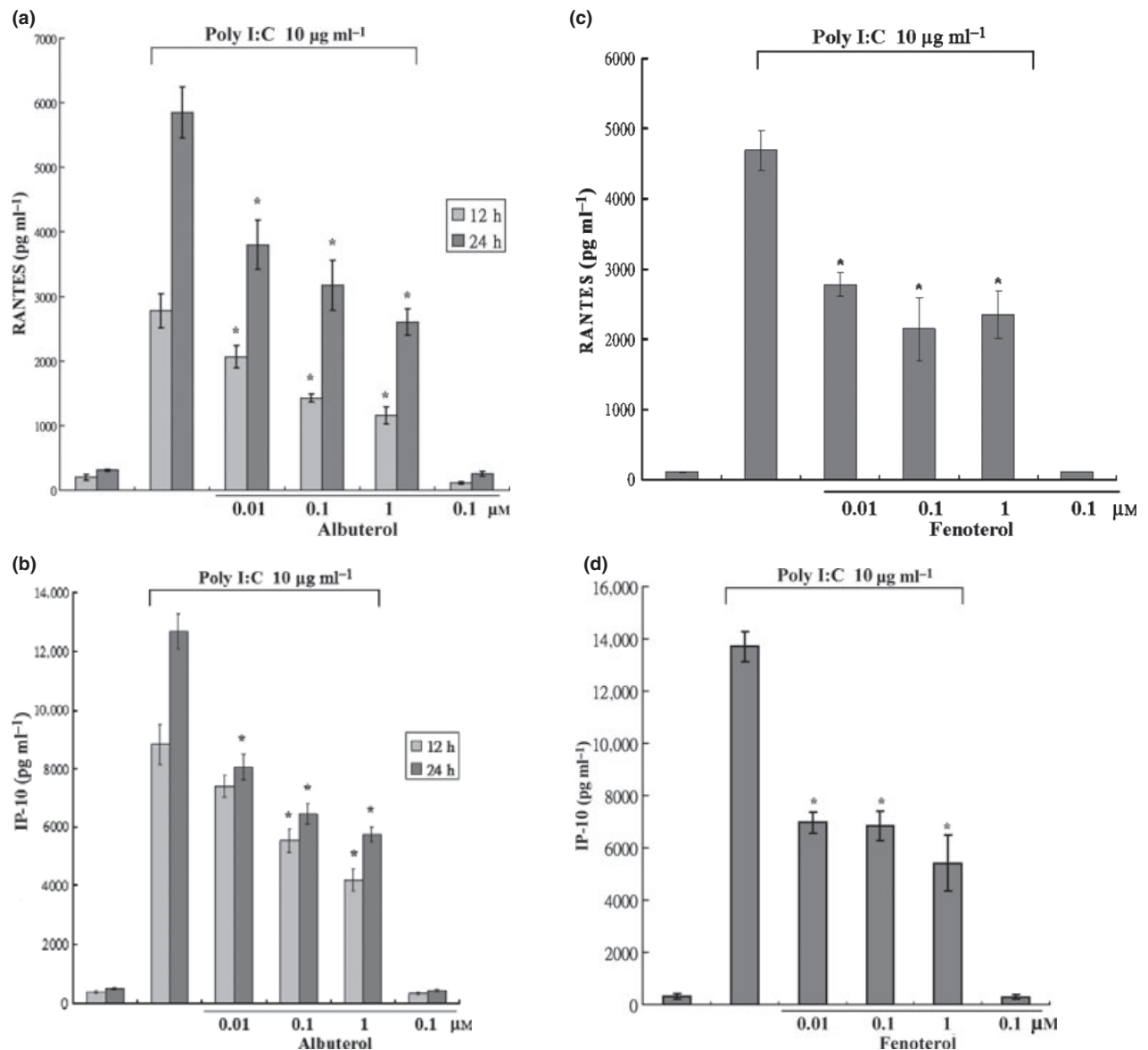


Figure 1 Albuterol (0.01–1 μM) significantly suppressed poly I:C-induced (a) RANTES and (b) IP-10 expression in BEAS-2B cells in a dose-dependent manner. Fenoterol (0.01–1 μM) also significantly

suppressed poly I:C-induced (c) RANTES and (d) IP-10 expression. Results represent six experiments. (* $p < 0.05$ between poly I:C only and poly I:C plus SABA treatment).

NF κ B-p65 signaling are important pathways of poly I:C-induced RANTES and IP-10 expression in BEAS-2B cells after poly I:C stimulation. The data of western blot showed that albuterol suppressed p-ERK, p-JNK, and pp38 expression (Fig. 5a), but no effect on pp65 expression (Fig. 5b) on poly I:C-stimulated BEAS-2B cells. We further investigated the downstream kinase activity of MAPKs, including ELK-1, c-JUN, and ATF-2. As shown in Fig. 6, albuterol suppressed p-ELK-1, p-c-JUN, and p-ATF-2 expression. These data suggested that albuterol may suppress poly I:C-induced RANTES and IP-10 via suppressing, at least in

part, MAPK-ERK-ELK-1, JNK-c-JUN, and p38-ATF-2 pathways in BEAS-2B cells.

Discussion

As the preferred as-needed reliever medications in the GINA guidelines, SABA is no doubt effective in relieving symptoms of acute asthma attack (1). However, the immunomodulatory function of SABAs on bronchial epithelial cells is still unclear. In the present study, we clearly demonstrated that SABAs, such as albuterol and fenoterol, could suppress poly

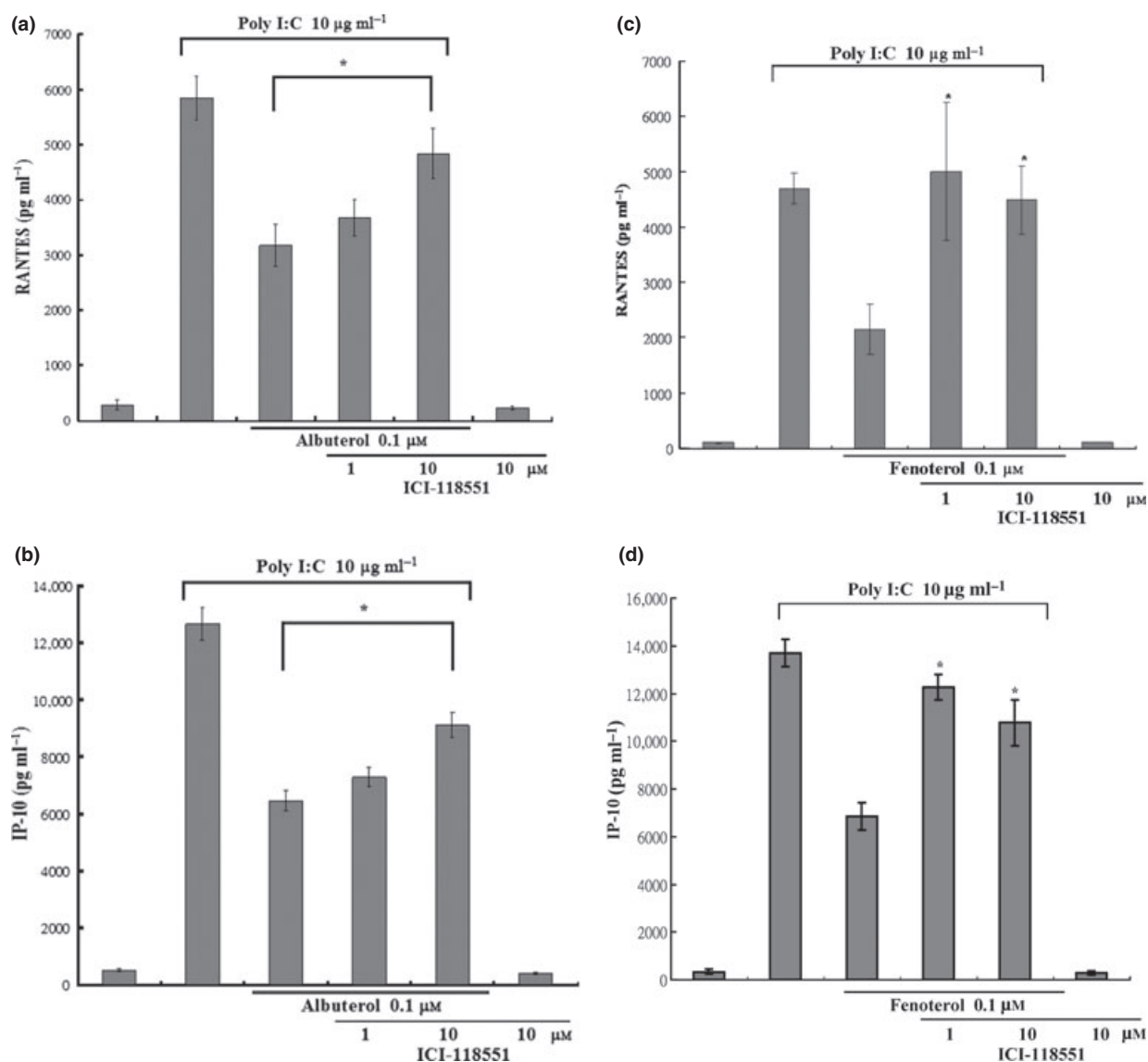


Figure 2 ICI-118551 (1–10 μM) reversed the suppressive effects of albuterol on poly I:C-induced (a) Regulated on activation, normal T expressed and secreted (RANTES) and (b) IP-10 expression and also reversed the suppressive effects of fenoterol on poly I:C-

induced (c) RANTES and (d) IP-10 expression in BEAS-2B cells after 24 h of poly I:C stimulation. Results represent six experiments. (* $p < 0.05$ between poly I:C plus SABA treatment with and without ICI-118551).

I:C-induced RANTES and IP-10 expression in BEAS-2B cells. These suppressive effects were β_2 -adrenoreceptor dependent. Albuterol and fenoterol increased the generation of intracellular cAMP, and a cAMP analog conferred similar suppressive effects on RANTES and IP-10 expression. Taken together, albuterol and fenoterol suppressed poly I:C-induced RANTES and IP-10 via the β_2 -adrenoreceptor-cAMP pathway. By using MAPK-inhibitors and western blot analysis, we also demonstrated that these suppressive effects may be, at least partly, via the MAPK-ERK-ELK-1, JNK-c-JUN, and p38-ATF-2 pathways. These findings illustrated that SABAs, apart from potent bronchodilation that we have ever

known, have immunomodulatory effects on chemokines expression in human bronchial epithelial cells.

RANTES has been shown to recruit and activate monocytes, eosinophils, basophils, and several lymphocyte subpopulations and is associated with acute viral respiratory tract infection in children (19). RANTES is significant in the pathogenesis of asthma with involvement in early- and late-phase allergic responses, hyper-responsiveness and remodeling of the airways (7). IP-10 is also associated with airway inflammation and hyper-reactivity (13). Plasma level of IP-10 in asthma patients is significantly higher during an acute exacerbation than a stable convalescent period (14). In virus-

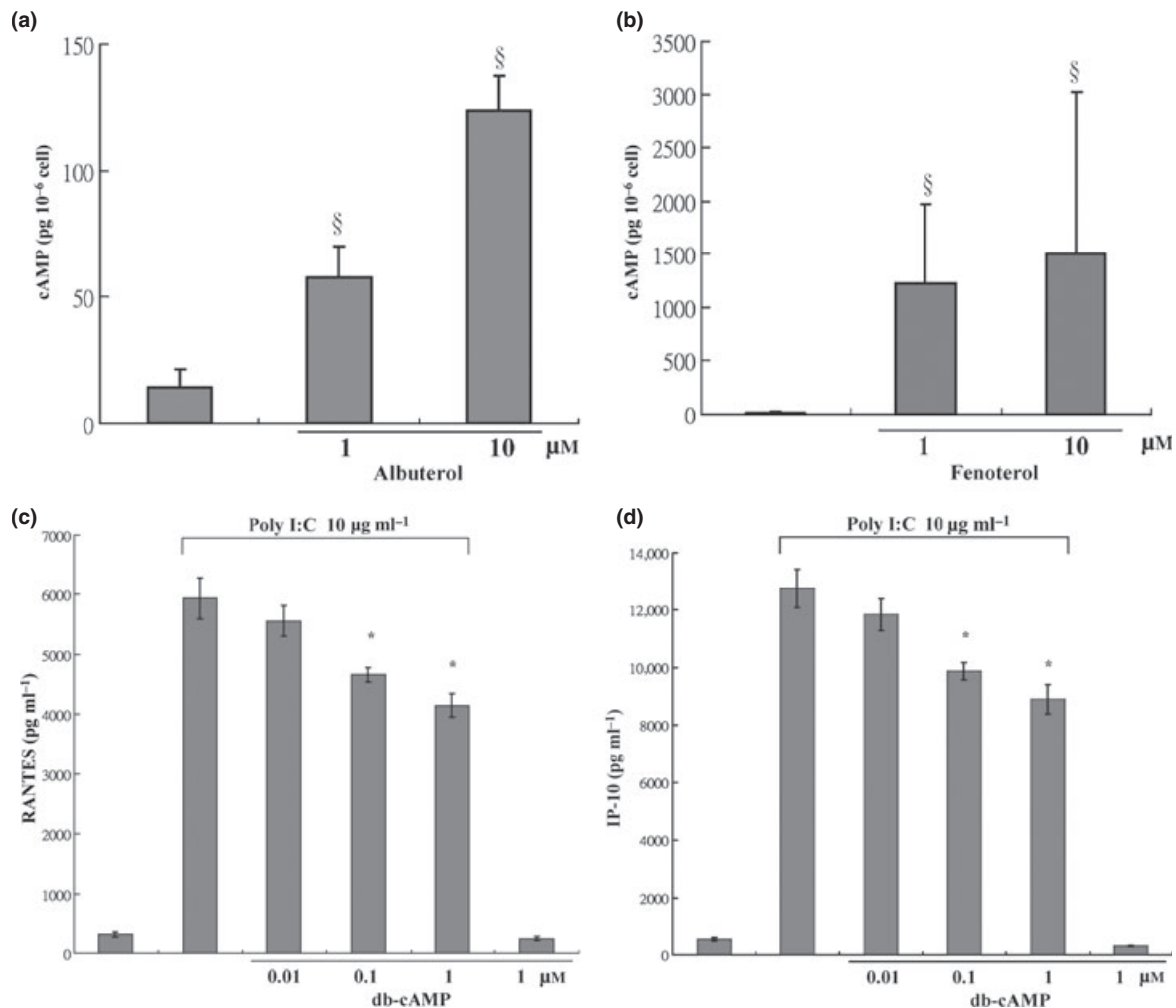


Figure 3 (a) Albuterol and (b) Fenoterol could significantly increase intracellular cAMP levels in BEAS-2B cells. Results represent three experiments in cAMP assay, Dibutyl-cAMP (0.1–10 μM) significantly suppressed (c) Regulated on activation, normal T expressed and secreted and (d) IP-10 expression in BEAS-2B cells after 24 h of

poly I:C stimulation in a dose-dependent manner. Results represent six experiments. (§ $p \leq 0.05$ between groups treated with or without SABA; * $p < 0.05$ between poly I:C only and poly I:C plus dibutyl-cAMP treatment).

induced asthma, IP-10 level is elevated remarkably and is associated with the degree of airflow obstruction and therefore is regarded as a predictive index for both asthma exacerbation and virus-induced asthma (15). In the present study, albuterol and fenoterol could suppress poly I:C-induced RANTES and IP-10, suggested that SABAs may decrease asthmatic airway inflammation, hyper-responsiveness, and remodeling via suppressing chemokine expression by bronchial epithelial cells.

Mitogen-activated protein kinase and NF κ B pathways are two important signaling pathways that play essential regulatory roles in both innate and adaptive immune responses. There are three major families of MAPKs: ERK, JNK, and p38. MAPK pathways are activated through a triple kinase cascade: MKKK (MAPK kinase kinase)-MKK (MAPK kinase)-MAPK. MAPK pathways respond to a wide range of stimulations and transduce into alternations in gene expres-

sion and regulation of cell function. ERK pathway is majorly stimulated by growth factors and mitogens. JNK and p38 are mostly stimulated by inflammatory cytokines, chemicals, and stress. All of the three pathways are associated with growth, differentiation, and apoptosis. In addition, ERK is associated with development, and JNK and p38 are associated with inflammation (20). In asthmatic patients, phosphorylation of ERK1/2 and p38 is correlated with disease severity (21). NF κ B pathway is another important pathway in regulation of immune and inflammatory responses, which can be activated by many stimuli, including proinflammatory cytokines, T- and B-cell mitogens, bacteria, lipopolysaccharide, viruses, viral proteins, double-stranded RNA, and cellular stresses, followed by induction of the expression of diverse target genes expression (22). NF κ B activation is responsible for many inflammatory genes expression in airway cells from patients with asthma (23). In our study, we demonstrated

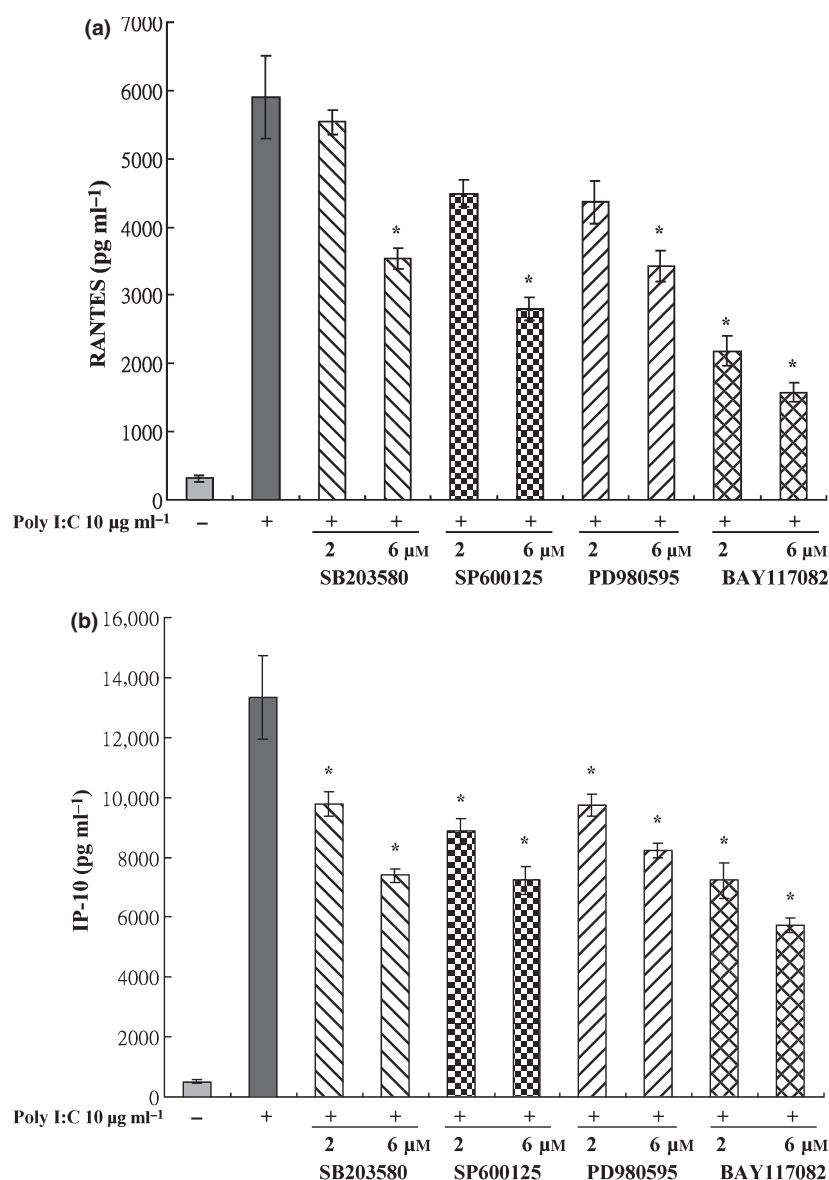


Figure 4 SB203580, SP600125, PD980595, and BAY117082 suppressed poly I:C-induced (a) RANTES expression and (b) IP-10 expression in BEAS-2B cells. Results represent six experiments. (* $p < 0.05$ between poly I:C only and poly I:C plus inhibitor treatment).

that albuterol could significantly suppress RANTES and IP-10 expression via suppressing the MAPK-ERK-ELK-1, JNK-c-JUN, and p38-ATF-2 pathways, providing further understanding for intracellular mechanism by which SABAs could modulate immune reaction.

The immunomodulatory effects of SABAs are also reported by other studies. In human airway smooth muscle cells, albuterol can suppress the release of eosinophil-activating cytokines (granulocyte macrophage-colony-stimulating factor, RANTES, and eotaxin) via activation of β_2 -adrenoreceptors and generation of cAMP (24). Procaterol, one of SABAs, can inhibit eosinophils migration directly and further suppress the release of eosinophil chemotactic activity from BEAS-2B cells via a cAMP-dependent mechanism (25). In an animal study, albuterol can inhibit allergen-induced bronchoalveolar lavage eosinophils, serum total IgE, and inflam-

matory cytokines (interleukin (IL)-2 and IL-13) secretion by T-cells (26). A clinical pharmacological study reported that both albuterol and procaterol can suppress both immediate and late airway responses in asthma (27). From the aspect of chemokine regulation, our data further supported the current notion that SABAs have certain degree of anti-allergic inflammatory effects.

During respiratory tract infection, virus replicates in bronchial epithelial cells resulting in the production of cytokines, which are important in the development of antiviral Th1-type immune responses. Virus infection results in the production of chemokines (such as IP-10, RANTES, IL-8, monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage inflammatory protein (MIP)-1 α), proinflammatory (such as IL-1 β , IL-6, IL-18, TNF- α) and antiviral cytokines (such as interferon (IFN)- α , IFN- β) (28). As a strong Th1-related

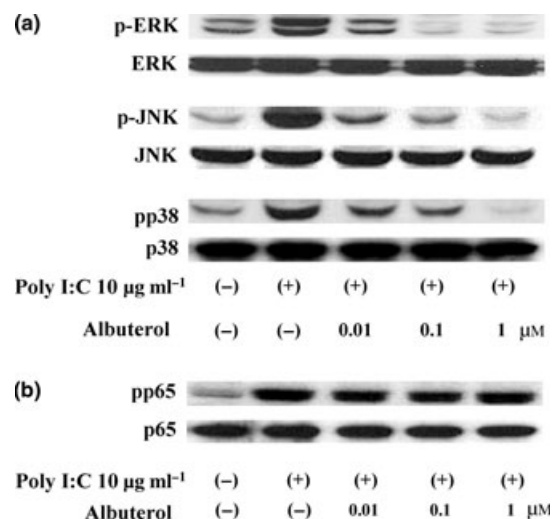


Figure 5 Albuterol suppressed poly I:C-induced (a) p-ERK, p-JNK, and pp38, but not (b) pp65 expression in BEAS-2B cells. One experiment representative of three is shown.

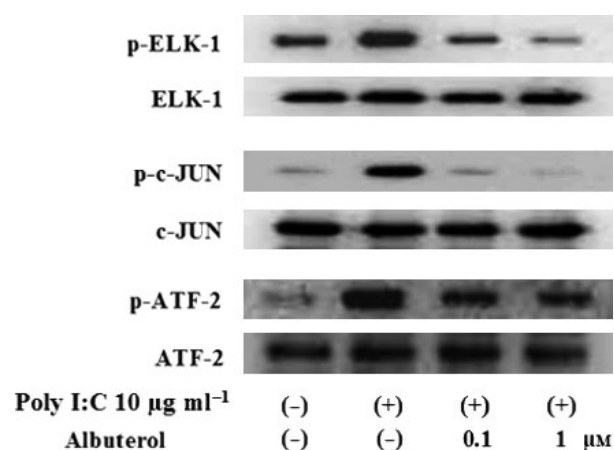


Figure 6 Albuterol suppressed poly I:C-induced nuclear p-ELK-1, p-c-JUN, and p-ATF-2 expression in BEAS-2B cells. One experiment representative of three is shown.

chemokine, IP-10 is also involved in our adaptive immune defense against viral infection by recruiting Th1 cells toward infection site through activation of CXCR3 (12). Other chemokines, such as RANTES, MIP-1 α , monokine induced by IFN- γ (MIG/CXCL9), and IFN-inducible T cell alpha

chemoattractant (I-TAC/CXCL11), are also important in the recruitment of Th1 cells (30). Th1 cells can release cytokines such as IFN- γ and IL-2 that can induce activation of neutrophils, macrophages, and cytotoxic T-cells, and also class-switching for IgG production in B-cells, which are essential in our adaptive immunity against intracellular pathogens. The suppressive effect of SABAs on IP-10 expression may weaken our protection against viral invasion and retard pathogens elimination and may therefore prolong the course and increase the severity of viral infection.

Using SABAs in patients with virus-induced wheezing or common respiratory infections seems to be a nowadays convention in hospital or outpatient medical treatment (3). Although there is no doubt SABAs are effective and beneficial in relieving bronchospasm in acute asthma exacerbation, the benefits of routinely use of SABAs in adults with acute cough or in children without obvious airflow obstruction are still lack of supportive evidences (5). Albuterol could not significantly reduce symptoms in children with acute bronchiolitis (29). The regular use of nebulized SABA in acute severe asthma may increase adverse effect (e.g., tremor and palpitation) and even length of hospital stay (30). Therefore, the benefits of prolonged or routine use of SABAs should be weight against the accompanying adverse effects, and the possibility of weakening acquired immunity caused by suppression of chemokine related to pathogen elimination.

In conclusion, albuterol and fenoterol could suppress RANTES and IP-10 expression in poly I:C-stimulated human bronchial epithelial cells via the β 2-adrenoreceptor-cAMP and the MAPK-ERK-ELK-1, JNK-c-JUN, p38-ATF-2 pathways. SABAs could exert immunomodulatory effect by inhibiting chemokine expressions and could benefit asthmatic patients by suppressing airway inflammation, especially for the late-phase inflammation induced by allergen challenging. However, the suppression of chemokine production by SABAs may weaken the acquired immunity for pathogen elimination and therefore should be cautiously used in common respiratory infection and virus-induced asthma exacerbation.

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