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RESEARCH PAPER

Effects of combination therapy with montelukast and carbocysteine in allergen-induced airway hyperresponsiveness and airway inflammation

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Background and purpose: Montelukast and S-carbocysteine have been used in asthmatic patients as an anti-inflammatory or mucolytic agent respectively. S-carbocysteine also exhibits anti-inflammatory properties.
Experimental approach: Ovalbumin (OVA) sensitized BALB/c mice were challenged with OVA for 3 days followed by single OVA re-challenge (secondary challenge) 2 weeks later. Forty-eight hours after secondary challenge, mice were assessed for airway hyperresponsiveness (AHR) and cell composition in bronchoalveolar lavage (BAL) fluid. Suboptimal doses of 10 mg·kg^{-1} of S-carbocysteine by intraperitoneal injection (ip), 20 mg·kg^{-1} of montelukast by gavage, the combination of S-carbocysteine and montelukast or 3 mg·kg^{-1} of dexamethasone as a control were administered from 1 day before the secondary challenge to the last experimental day. Isolated lung cells were cultured with OVA and montelukast to determine the effects on cytokine production.
Key results: Treatment with S-carbocysteine or montelukast reduced both AHR and the numbers of eosinophils in BAL fluid. Neutralizing IFN-γ abolished the effects of S-carbocysteine on these airway responses. Combination of the two drugs showed further decreases in both AHR and eosinophils in the BAL fluid. Goblet cell metaplasia and Th2-type cytokines, interleukin (IL)-4, IL-5 and IL-13, in BAL fluid were decreased with montelukast treatment. Conversely, S-carbocysteine increased Th1-type cytokines, IFN-γ and IL-12 in BAL fluid.
Conclusions and inplications: The combination of two agents, montelukast and S-carbocysteine, demonstrated additive effects on AHR and airway inflammation in a secondary allergen model most likely through independent mechanisms of action.
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Keywords: carbocysteine; montelukast; airway hyperresponsiveness; neutrophils; eosinophils

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; H&E, haematoxylin-eosin; IL, interleukin; LT, leukotriene; OVA, ovalbumin; PAS, periodic acid-Schiff; RL, lung resistance

Introduction

Asthma represents the most prevalent chronic pulmonary disease, and the number of cases with acute exacerbation and mortality are still a worldwide concern (Eder *et al.*, 2006; Milgrom, 2007). The pathogenesis of asthma is thought to be due to chronic allergic inflammation accompanied by progressive airway dysfunction. A number of inflammatory cell types, chemical mediators, cytokines and reactive oxygen species may play important roles (Effros and Nagaraj, 2007). Regardless of intensive treatment to control the disease, such as inhaled corticosteroid therapy, acute and severe exacerbations and in some cases, progressive pulmonary dysfunction remain (Tamesis and Covar, 2008). New therapeutic approaches have been sought, however, to date very few remain in clinical use (Effros and Nagaraj, 2007).

The cysteinyl leukotrienes, leukotriene (LT) C_4, E_4 and D_4, are potent inducers of airway smooth muscle contraction and development of airway hyperresponsiveness (AHR), vascular permeability and mucus production (Busse and Kraft, 2005). In targeting this class of lipid mediators, the selective cysteinyl LT receptor 1 antagonist, montelukast, has been widely used in the treatment of asthma and has been shown to be effective through the suppression of Th2 cytokine production and airway inflammation (Frieri *et al.*, 2003). In the subset of

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asthmatics associated with aspirin-induced asthma or children with mild asthma, montelukast has been shown to be more effective than an inhaled corticosteroid (Kemp, 2003; Szefler *et al*., 2005).

S-carbocysteine was originally introduced as a mucoregulator to decrease mucus viscosity and improve mucus clearance, and has been used in the treatment of mucus-associated respiratory diseases such as chronic obstructive pulmonary disease (COPD) for more than 30 years (Tatsumi *et al*., 2007). Further, S-carbocysteine has been shown to have an antioxidant effect and inhibitory activity on neutrophil chemotaxis (Ishii *et al*. 2002; Sade *et al*., 2003). We previously showed that S-carbocysteine treatment reduced AHR and inflammatory cell infiltration into the airways through increasing levels of Th1 cytokines (Takeda *et al*., 2005).

Thus, S-carbocysteine and montelukast have distinct activities in reducing allergen-induced airway inflammation and airway dysfunction. Nonetheless, there are limitations with the use of either drug alone. Montelukast given alone is not enough to reduce use of inhaled corticosteroids in childhood asthma patients (Strunk *et al*., 2008) and S-carbocysteine does not have clear evidence of efficacy in asthma. Therefore, in this study, the potency of a combination of S-carbocysteine and montelukast in allergen-induced AHR and airway inflammation was examined in a secondary allergen challenge model where airway allergic inflammation was established before drug treatment was initiated in an attempt to more closely model the clinical situation.

Methods

Animals

All animal care and experimental procedures were conducted under a protocol approved by the Institutional Animal Care and Use Committee of National Jewish Health. Female BALB/c mice from 6 to 8 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The animals were maintained on an ovalbumin (OVA)-free diet.

Experimental protocol

The experimental protocol for sensitization and secondary challenge to allergen was previously described (Takeda *et al*., 2005). Briefly, mice were sensitized by intraperitoneal (ip) injection of 10 μg of OVA (Grade V, Sigma Chemical Co., St. Louis, MO, USA) emulsified in 2.0 mg of alum (AlumImuject: Pierce, Rockford, IL, USA) in a total volume of 100 μL on days 1 and 7 followed by aerosolized OVA challenge (primary, 0.2% in saline) for 20 min on days 14, 15 and 16 by ultrasonic nebulizer (model NE-U07, Omron Healthcare, Kyoto, Japan). On day 30, mice received a single secondary challenge via the airways with 1% OVA for 20 min. Control mice received saline as the secondary challenge.

To determine the effects of S-carbocysteine and montelukast, alone or together, on airway allergic inflammation and AHR, 10 $mg \cdot kg^{-1}$ per day of S-carbocysteine daily in 100 μL distilled water was given by intraperitoneal injection. Montelukast, the selective $CysLT_1$ antagonist (nomenclature follows Alexander *et al*., 2009), was given at 5, 20 or 50 $mg \cdot kg^{-1}$ per day in 100 μL distilled water orally by gavage. These suboptimal doses were set based on a previous study with S-carbocysteine (Takeda *et al*., 2005) and in preliminary studies with montelukast to optimize the opportunity to detect additive effects on lung allergic responses. All drug treatments were carried out from 2 days before the secondary allergen challenge through the last day of the experiment. As a positive control treatment, dexamethasone sodium phosphate (Sigma) (3 $mg \cdot kg^{-1}$ per day per animal) was administered by ip injection in the same schedule as S-carbocysteine and montelukast. Control groups of mice received saline challenge as the secondary challenge and treated with the drugs in the same manner. S-carbocysteine was provided by Kyorin Pharmaceutical (Tokyo, Japan) and montelukast was obtained from Merck & Co. (Whitehouse Station, NJ, USA).

To investigate the mechanism of S-carbocysteine effects on airway responses, anti-mouse interferon (IFN)-γ (Clone 37895, R&D Systems, Minneapolis, MN, USA) (100 μg per mouse) was administered intravenously 2 h before secondary OVA challenge. Control mice received a similar dose of rat IgG.

Determination of airway responsiveness

Airway responsiveness was assessed as changes in airway function after challenge with aerosolized methacholine (Sigma). Mice were anesthetized, tracheostomized, mechanically ventilated, and lung function was assessed as described previously (Takeda *et al*., 2005). Ventilation was achieved at 160 breaths per minute at a tidal volume of 0.16 mL with a positive end-expiratory pressure of 2–4 cm H_2O (SN-480, Shinano, Tokyo, Japan). Lung resistance (RL) was continuously computed (Labview, National Instruments, TX, USA) by fitting flow, volume and pressure to an equation of motion, using a recessive least squares algorithm.

Aerosolized methacholine was administered through bypass tubing via an ultrasonic nebulizer (model 5500D, DeVilbiss, Somerset, PA, USA) placed between the expiratory port of the ventilator and the four-way connector. Aerosolized methacholine was administered for 8 s with a tidal volume of 0.45 mL and frequency of 60 BPM using another ventilator. The data for RL were continuously collected for up to 3 min and maximum values were taken. The data were also expressed as the provocative concentration 200 (PC_{200}; i.e. the concentration at which airway pressure was 200% of its baseline value) to compare responses in multiple groups.

Bronchoalveolar lavage (BAL)

Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with Hank's balanced solution (HBSS, 1 × 1 mL 37°C) and numbers of leukocytes were counted in each sample (Coulter Counter, Coulter Corporation, Hialeah, FL, USA). Differential cell counts were performed by counting 200 cells on cytocentrifuged preparations (Cytospin 3, Thermo Fisher Scientific, Inc., Waltham, MA, USA) followed by Wright-Giemsa stain. BAL fluid supernatants were collected and stored at −70°C until cytokine measurements.

Cell preparation for in vitro *cytokine production*

To determine the effect of montelukast on cytokine production, cells were isolated and cultured with OVA and mon-

telukast. Briefly, lungs were removed 48 h after secondary OVA challenge. Lung cells were obtained with collagenase digestion followed by 35% Percoll (Sigma) gradient centrifugation to remove epithelial cells. Lung cells (2×10^{-5}) were cultured with or without 100 μg·mL^{-1} of OVA. Montelukast was added to these conditions at concentrations of 0, 0.01, 0.1, 1 and 10 μM. After 24 h incubation at 37°C, supernatants were collected and stored at –80°C until assay.

Measurement of cytokine levels

Cytokine levels in the BAL fluid were measured as previously described (Takeda *et al*., 2005). Briefly, measurements of interleukin (IL)-4, IL-5, IL-10, IFN-γ, and IL-12 were performed by ELISA (BD-PharMingen, San Diego, CA, USA) with 96-well plates (Immulon 2; Dynatech, Chantilly, VA, USA); IL-13 measurements were performed using an ELISA kit (QuantikineM; R&D Systems), all following the manufacturers' protocols. The limits of detection were 1.5 pg·mL^{-1} for IL-13, 4 pg·mL^{-1} for IL-4 and IL-5, 16 pg·mL^{-1} for IL-12, and 10 pg·mL^{-1} for IL-10 and IFN-γ.

Histological studies

Lungs were inflated through the trachea with 1 mL of 10% formalin and fixed in 10% formalin by immersion. Blocks of lung tissue were cut around the main bronchus and embedded in paraffin. Sections (6 μm) were cut and stained with haematoxylin-eosin (H&E) to analyse inflammatory cell infiltration. For detection of mucus-containing cells, formalin-fixed airway tissue-sections were stained with periodic acid-Schiff (PAS). The slides were taken to pictures with a microscope (BX40, Olympus America, Melville, NY, USA) equipped with a digital camera (Q-color 3; Olympus America Inc.) and images were stored on a Macintosh computer. Goblet cell metaplasia was quantified as the number of pixels on the computer converted from PAS+ areas along the airway epithelium. Results were quantified with NIH Image J software (version 1.38), available on the internet at http://rsbweb.nih.gov/ij/download.html. Four different fields per slide in 4–6 samples from each group of mice were examined without knowledge of the treatments.

Statistical analysis

All results were expressed as the mean ± standard error of the mean (SEM). The Tukey-Kramer test was used for comparisons between multiple groups. As measured values may not be normally distributed due to the small sample sizes, non-parametric analysis, Mann–Whitney *U*-test, was also used to confirm that the statistical differences remained significant. The *P*-values for significance were set to 0.05 for all tests.

Results

Effects of S-carbocysteine and montelukast on OVA-induced airway inflammation and AHR after secondary challenge

Forty-eight hours after secondary challenge, mice developed increasing AHR in response to increasing doses of inhaled methacholine (Figure 1A,B) and an eosinophil-dominant inflammatory response in the BAL fluid was demonstrated compared with saline-challenged mice (40% of total BAL fluid cells) (Figure 1C). When mice were treated with 10 mg·kg^{-1} of S-carbocysteine, airway responsiveness to methacholine was decreased significantly and the numbers of eosinophils were decreased by approximately 50% compared with saline-treated mice following secondary allergen challenge. Montelukast was administered at doses of 5, 20 and 50 mg·kg^{-1} and the corresponding PC_{200} values were 4.8 ± 0.6, 7.6 ± 1.8 and 9.9 ± 1.3 mg methacholine·mL^{-1} respectively. A montelukast dose of 20 mg·kg^{-1} was selected for all subsequent experiments, which in addition to decreasing AHR, also reduced eosinophilic airway inflammation compared with control mice. The combination of S-carbocysteine and montelukast demonstrated additive effects, further decreasing both AHR and airway inflammation compared with either drug alone. Dexamethasone treatment, the positive treatment control, prevented the development of AHR and airway inflammation in secondary OVA challenged mice. None of the drug treatments showed effects in mice which received saline in the secondary challenge phase.

Histological analysis

Histological analyses of H&E- and PAS-stained tissue sections were performed on the lungs removed from the same animals as described above (Figure 2A). In OVA sensitized and challenged mice, cell infiltration into the peribronchial regions was observed after secondary challenge (Figure 2A, panel b), whereas few cells were observed in the peribronchial regions after saline challenge (Figure 2A, panel a). On the slides, no obvious differences between S-carbocysteine-, montelukast- and saline-treated mice were observed (Figure 2A, panels b–d). However, the combination of S-carbocysteine and montelukast decreased levels of cell infiltration in the peribronchial area (Figure 2A, panel e). Dexamethasone treatment reduced these levels as well. Quantitative analysis of the PAS+ areas revealed goblet cell metaplasia and mucus hyperproduction after secondary OVA challenge (Figure 2B). Following S-carbocysteine or montelukast treatment, PAS+ cells were reduced significantly compared with saline-treated mice, and these reductions were even greater following combination treatment. Dexamethasone treatment also decreased PAS+ cells in the airways.

Cytokine levels in BAL fluid

To examine the effects of S-carbocysteine and montelukast on Th1 or Th2 cytokine production following secondary allergen challenge, levels of different cytokines in the BAL fluid were measured. As shown in Figure 3, levels of all Th2 cytokines, IL-4, IL-5 and IL-13, as well as IL-10 were significantly increased in the BAL fluid following secondary allergen challenge compared with saline challenge. Montelukast treatment alone decreased IL-4, IL-5 and IL-13, whereas IL-10 levels were increased. Conversely, S-carbocysteine treatment did not alter any of Th2 cytokine levels or IL-10. However, the levels of Th1-type cytokines, IFN-γ and IL-12, were increased after S-carbocysteine treatment. The combination

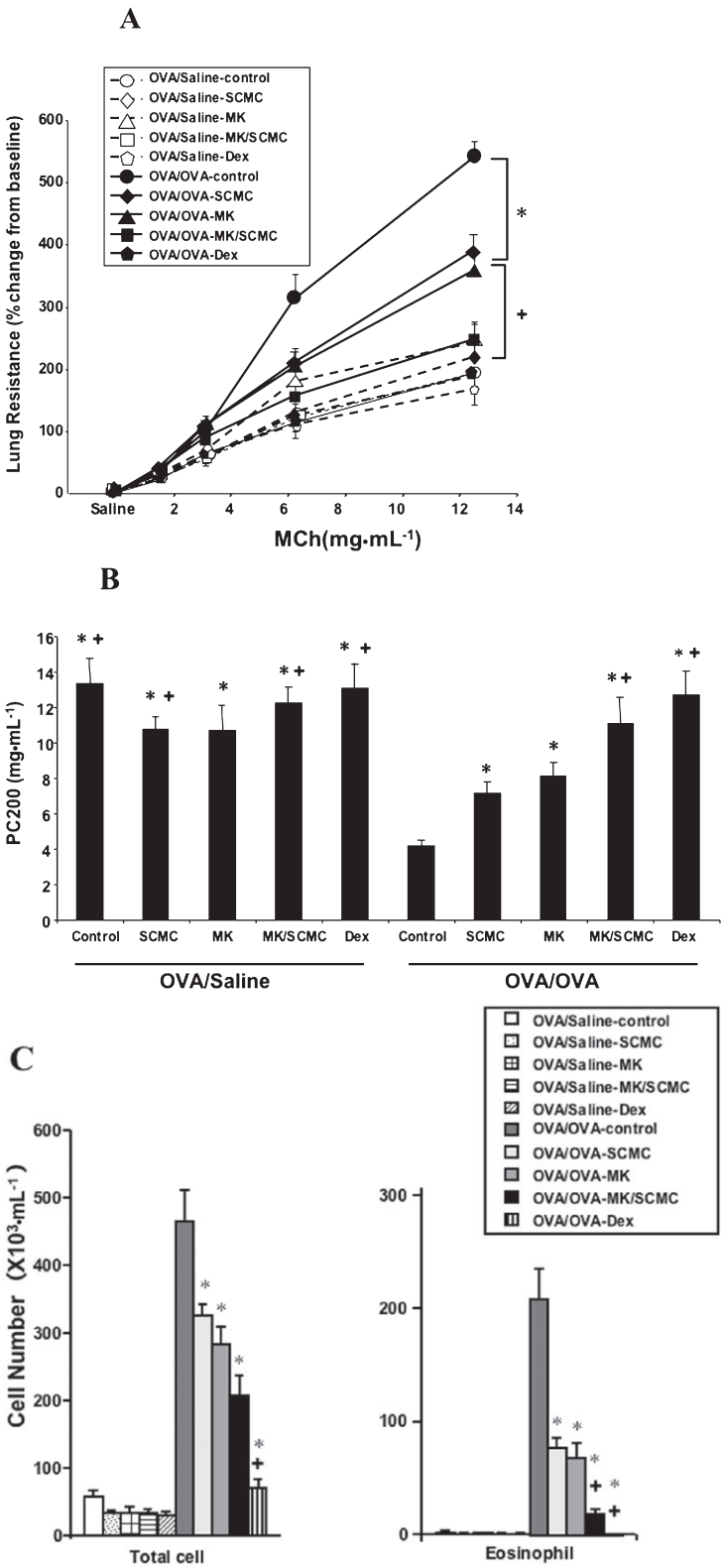

Figure 1 Effects of montelukast (MK) and/or S-carbocysteine (SCMC) on airway function and inflammation. (A) Changes in lung resistance (RL) 48 h after secondary challenge. RL values were obtained in response to increasing concentrations of inhaled methacholine (MCh). (B) Airway responsiveness expressed as provocative concentration (PC_{200}) values for each treatment. (C) Total and eosinophil cell numbers in BAL fluid obtained 48 h after secondary challenge. Values are expressed as mean ± SEM ($n = 8$). Mice were treated with S-carbocysteine (OVA/OVA-SCMC), montelukast (OVA/OVA-MK), the combination (OVA/OVA-SCMC/MK) or dexamethasone (Dex). Control groups were exposed to saline in the secondary challenge following sensitization and primary challenge to OVA (OVA/SAL) and also received treatment in the same manner as the secondary OVA challenge groups. Significant differences (*$P < 0.05$) compared with OVA/OVA and (+$P < 0.05$) compared with OVA/OVA-SCMC or OVA/OVA-MK. BAL, bronchoalveolar lavage; OVA, ovalbumin.

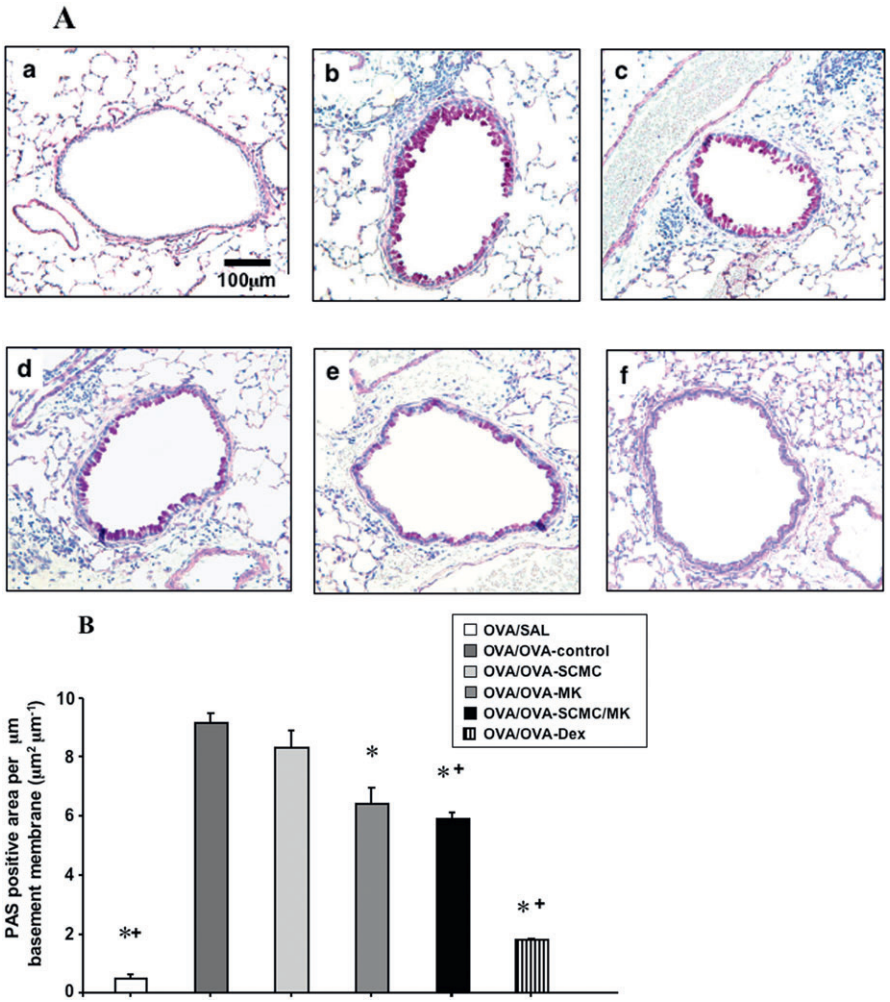

Figure 2 Effects of S-carbocysteine (SCMC) and/or montelukast (MK) on lung histology. (A) Periodic acid-Schiff (PAS) and H&E as a counter stain. Sections of lung tissue were prepared 48 h after secondary challenge. Saline challenge at secondary challenge (panel a). Secondary allergen challenge with control treatment (panel b), S-carbocysteine treatment (panel c), montelukast treatment (panel d), combination of S-carbocysteine and montelukast (panel e) or dexamethasone (Dex) treatment (panel f). (B) Goblet cell metaplasia 48 h after secondary allergen challenge was quantified in PAS-stained sections and expressed per μm of basement membrane. Mice were treated with S-carbocysteine (OVA/OVA-SCMC), montelukast (OVA/OVA-MK), the combination (OVA/OVA-SCMC/MK) or dexamethasone (OVA/OVA-Dex) treatment at secondary allergen challenge following sensitization and primary allergen challenge. Control groups were exposed to saline at secondary challenge following sensitization and primary challenge to OVA (OVA/SAL). Values are expressed as mean ± SEM ($n = 8$). Significant differences (*$P < 0.05$) compared with OVA/OVA and (+$P < 0.05$) compared with OVA/OVA-SCMC. H&E, haematoxylin-eosin; OVA, ovalbumin.

of S-carbocysteine and montelukast did not show further changes in BAL cytokine levels compared with S-carbocysteine or montelukast treatment alone. Dexamethasone treatment reduced IL-4, IL-5 and IL-13 levels and increased IL-10, IFN-γ and IL-12 in secondary OVA challenged mice. None of the drug treatments affected responses in saline challenged mice compared with controls (data not shown).

Anti-inflammatory mechanism of S-carbocysteine is through increasing production of IFN-γ

To determine whether the increases in IFN-γ associated with S-carbocysteine treatment was the major pathway for S-carbocysteine-mediated inhibition of allergen-induced AHR and airway inflammation, anti-IFN-γ was administered prior to secondary allergen challenge in mice treated with S-carbocysteine. As shown in Figure 4, S-carbocysteine-treated mice which received anti-IFN-γ developed AHR and eosinophilic airway inflammation comparable to mice which were not treated with S-carbocysteine.

Cytokine levels in in vitro *cultured cells*

As S-carbocysteine was shown to modulate cytokine levels *in vitro* in a previous study (Takeda *et al.*, 2005), the effects of montelukast on cytokine production in lung cells cultured in the presence of allergen were determined. The increased levels of IL-5 and IL-13 from OVA-stimulated lung cells were not altered by montelukast treatment *in vitro* (Figure 5).

Discussion

Bronchial asthma is a complex syndrome in which many factors play a role in its pathogenesis (Effros and Nagaraj,

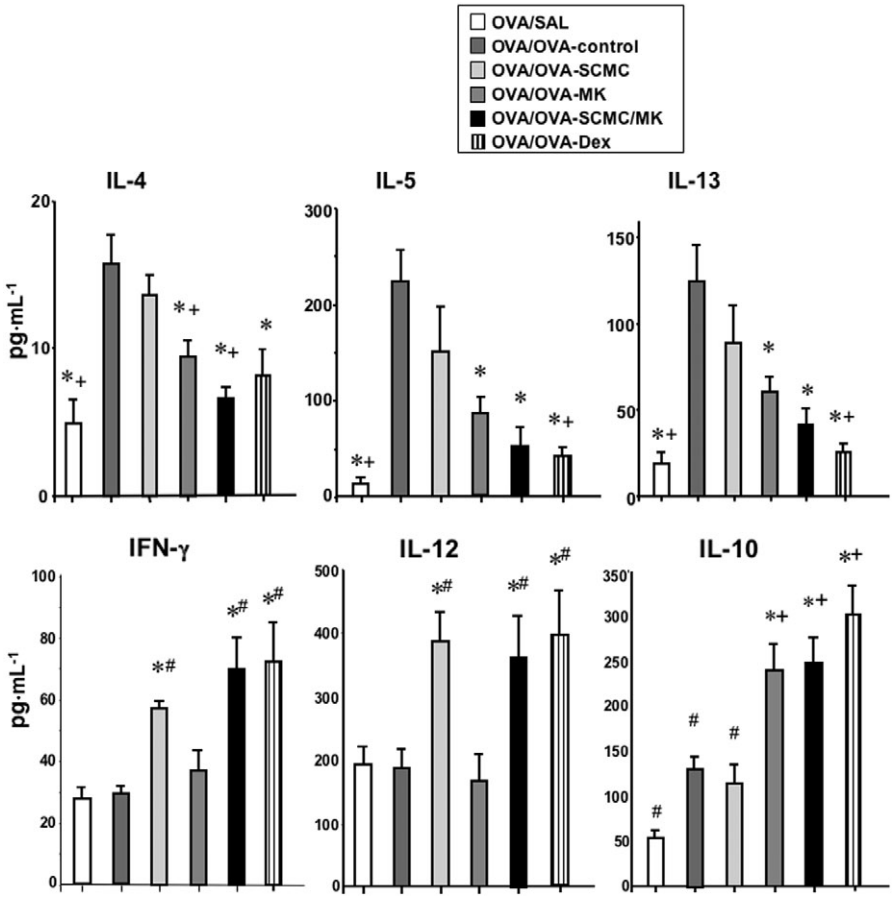

Figure 3 Cytokine levels in BAL fluid in mice receiving saline challenge or secondary allergen challenge following S-carbocysteine (SCMC), montelukast (MK) or saline treatment. Mice were treated with S-carbocysteine (OVA/OVA-SCMC), montelukast (OVA/OVA-MK), combination (OVA/OVA-SCMC/MK) or dexamethasone (OVA/OVA-Dex) treatment at secondary allergen challenge following sensitization and primary allergen challenge. Control groups were exposed to saline at secondary challenge following sensitization and primary challenge to OVA (OVA/SAL). Values for each group are expressed as mean ± SEM ($n = 8$ in each group). Significant differences ($+P < 0.05$) compared with OVA/OVA-SCMC group ($*P < 0.05$) compared with OVA/OVA group and ($\#P < 0.05$) compared with OVA/OVA-MK. BAL, bronchoalveolar lavage; OVA, ovalbumin.

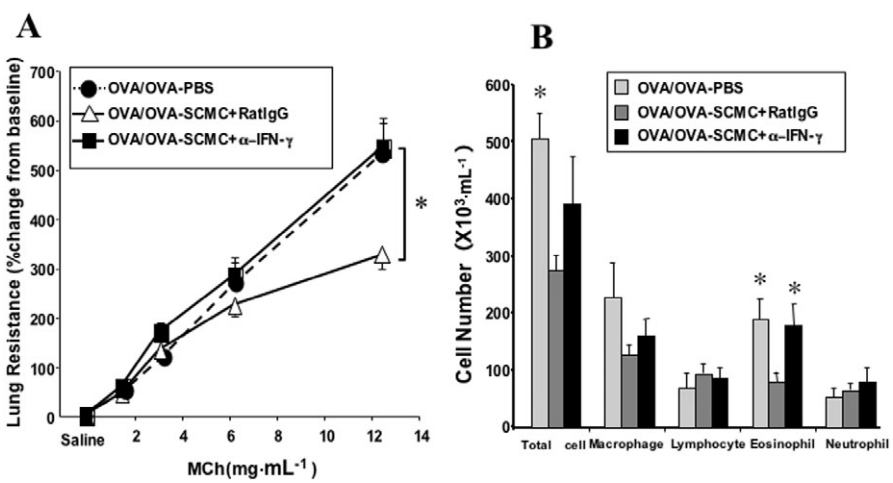

Figure 4 Effects of anti-IFN-γ in S-carbocysteine (SCMC)-treated mice following secondary allergen challenge. (A) Changes in lung resistance (RL) 48 h after secondary challenge. RL values were obtained in response to increasing concentrations of inhaled methacholine (MCh). (B) Cell composition in BAL fluid obtained 48 h after secondary challenge. Values are expressed as mean ± SEM ($n = 8$). Mice were treated with S-carbocysteine (OVA/OVA-SCMC) and received 100 μg of anti-IFN-γ (α–IFN-γ) or rat IgG (RatIgG). Control groups received PBS in the secondary challenge phase. Significant differences ($*P < 0.05$) compared with OVA/OVA-SCMC + RatIgG. BAL, bronchoalveolar lavage; OVA, ovalbumin.

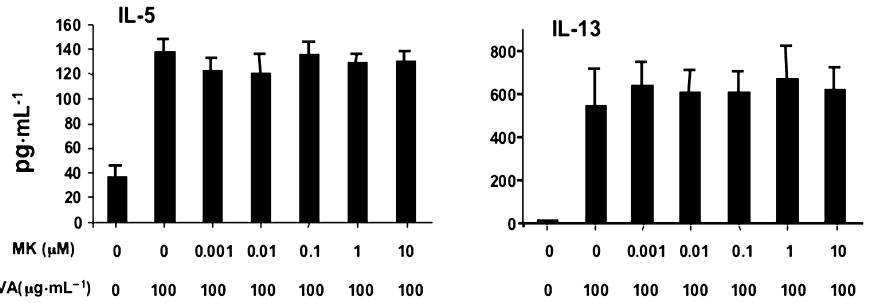

Figure 5 Cytokine levels (IL-5 and IL-13) in supernatants from cultured lung cells. Isolated lung cells were cultured for 24 h with or without 100 μg·mL^{-1} of OVA in the presence of various concentrations of montelukast (MK; 0, 0.001, 0.01, 0.1, 1 and 10 μM). N.S. no significant difference were detected. IL, interleukin; OVA, ovalbumin.

2007). Montelukast, a selective $CysLT_1$ antagonist, has shown efficacy as an anti-inflammatory and anti-allergy agent in many allergic disease and is widely used to treat asthmatics (Kemp, 2003). Based on the National Asthma Education and Prevention Program, Expert Panel Report (http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm), montelukast is recommended as monotherapy in mild asthma and in combination with inhaled corticosteroids in more severe disease. Subsets of children with asthma have shown benefits with montelukast treatment (Wahn and Dass, 2008). However, the limitations of montelukast monotherapy have been extensively discussed particularly when switching treatment from an inhaled corticosteroid to montelukast, which resulted in increased treatment failures in adult asthmatics (American Lung Association Asthma Clinical Research Centers, 2007). S-carbocysteine has been used as a mucoregulator and has also shown other therapeutic effects on airway inflammation through anti-oxidant activity which is induced through glutathione secretion from cells (Brandolini *et al*., 2003; Guizzardi *et al*., 2006; Garavaglia *et al*., 2008). In animal models, S-carbocysteine improved SO_2 gas-induced lung inflammation and normalized levels of fucose and sialic acid content in mucin glycoprotein, and prevented the increase in expression levels of mucin 5AC (MUC5AC) protein in the airway epithelium (Ishibashi *et al*., 2004). Furthermore, S-carbocysteine has shown some efficacy on mucociliary transport and suppression of the cough reflex in asthmatics (Solopov *et al*., 1988; Ishiura *et al*., 2003). Recently, we showed that S-carbocysteine down-regulated allergen-induced AHR and airway inflammation through immunomodulatory effects (Takeda *et al*., 2005).

In the present study, OVA sensitized and challenged mice developed eosinophilic airway inflammation and AHR to inhaled methacholine after a single provocative (secondary) OVA challenge. As allergic airway inflammation was previously established in this model, the airway conditions are likely more similar to the clinical situation than in a primary challenge model. Indeed, anti-IL-5 treatment did not attenuate lung allergic responses following secondary allergen challenge nearly as effectively as following primary challenge (Kanehiro *et al*. 2001) and anti-IL-5 has shown limited benefit in the clinic (Broide, 2009). Following montelukast or S-carbocysteine treatment alone, mice developed decreased levels of AHR and reduced numbers of eosinophils in the airways. The combination of montelukast and S-carbocysteine further decreased the levels of airway responsiveness, eosinophilic airway inflammation and goblet cell metaplasia. In asthmatics as well as in animal models, montelukast has been shown to reduce allergic airway inflammation and AHR (Eum *et al*., 2003; Jayaram *et al*., 2005), and Wu *et al*. (2003) demonstrated that montelukast treatment reduced the levels of Th2 type cytokines in OVA-sensitized and challenged mice (Wu *et al*., 2003). We previously showed that S-carbocysteine treatment modulated AHR and allergic airway inflammation most likely through the up-regulation of Th1-type cytokine production from monocytes in the airways (Takeda *et al*., 2005).

To address the underlying mechanisms of the additive effects of the two treatments, S-carbocysteine and montelukast, in allergen-induced airway inflammation and AHR, BAL cytokine levels were assayed. Th1-type cytokines, IFN-γ and IL-12, were up-regulated following S-carbocysteine treatment, similar to the results demonstrated in the previous study with higher doses of S-carbocysteine (Takeda *et al*., 2005). In the present study, neutralization of IFN-γ with specific antibody during S-carbocysteine treatment eliminated the inhibitory effects of S-carbocysteine on lung allergic responses, indicating that the increases in IFN-γ with S-carbocysteine treatment were critical. Montelukast treatment did not alter these BAL Th1 cytokine levels but, conversely, the levels of BAL Th2-type cytokines, IL-4, IL-5 and IL-13, were reduced with montelukast treatment, as others have reported (Ishibashi *et al*., 2004). IL-10 levels in BAL were increased with montelukast but not with S-carbocysteine. Our previous study also demonstrated that high-dose (100 mg·kg^{-1}) S-carbocysteine treatment reduced Th2-type cytokines and increased IL-10 levels in BAL. The dose of S-carbocysteine (10 mg·kg^{-1}) administered in this study was much lower and S-carbocysteine showed effects only on Th1-type responses but no effects on Th2 cytokine or IL-10 levels.

Montelukast exhibits anti-inflammatory effects in human asthmatics as well as in mouse models of allergen-induced airway inflammation, and also inhibits airway smooth muscle contraction (Aharony, 1998, Henderson *et al*., 2002; Eum *et al*., 2003). The mechanisms underlying the anti-inflammatory properties of montelukast may be related to impairment of the recruitment of inflammatory cells, eosinophils and lymphocytes to the airways (Prinz *et al*., 2005; Meliton *et al*., 2007). Unlike S-carbocysteine, montelukast did not alter cytokine production in cultured lung mononuclear

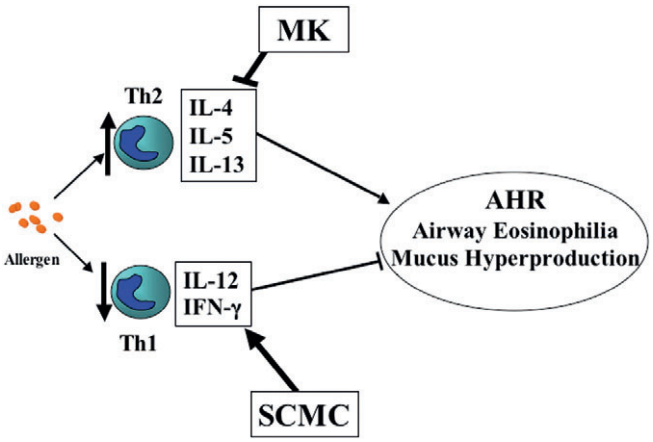

Figure 6 Diagram of the roles of S-carbocysteine (SCMC) and montelukast (MK) in reducing allergic airway inflammation and AHR. S-carbocysteine primarily up-regulates Th1-type cytokine production from monocytes and prevented the differentiation of Th0 cells into Th2 cells, consequently decreasing the development of Th2 responses. Montelukast down-regulates production of the Th2 cytokines, IL-4, IL-5 and IL-13. Together the drugs inhibit the development of AHR, eosinophilia and goblet cell metaplasia through two distinct and complementary pathways. AHR, airway hyperresponsiveness. IL, interleukin.

cells, but did reduce Th2 cytokine levels in the BAL fluid. As this was associated with reduced inflammatory cell accumulation in the BAL fluid, the effects of montelukast appear to be primarily on blocking inflammatory cell recruitment than direct effects on cytokine production. Taken together, our data identify distinct roles for both S-carbocysteine and montelukast in the modulation of allergen-induced airway inflammation and AHR with montelukast predominantly down-regulating Th2-type cytokine production and S-carbocysteine up-regulating Th1-type cytokine production in the airways (Figure 6). IL-10, a known anti-inflammatory cytokine (Mosser and Zhang, 2008), was also up-regulated following the combination of S-carbocysteine and montelukast treatment. Thus, the combination of S-carbocysteine and montelukast demonstrated additive effects in the prevention of allergen-induced airway inflammation and AHR through complementary activities and as such, this combination may be beneficial in the treatment of asthmatics, especially those refractory to treatment with either drug alone and where the use of corticosteroids must be reduced.

Acknowledgements

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Conflict of interest

None to declare.

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