Clinical, Physiological and Anti-Inflammatory Effect of Montelukast in Patients with Cough Variant Asthma

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Key Words
Cough variant asthma · Leukotriene receptor antagonist · Montelukast · Induced sputum · Cysteinyl leukotrienes · Eosinophils · Cough receptor sensitivity

Abstract

Background: Cough variant asthma (CVA) is a phenotype of asthma presenting solely with coughing, characterized by airway hyperresponsiveness, eosinophilic inflammation and a cough response to bronchodilators. Leukotriene receptor antagonists (LTRAs) are antiasthma medications with anti-inflammatory and bronchodilatory properties. Although LTRAs exert antitussive effects in CVA, the mechanisms involved are unknown. Objectives: This study aimed to clarify the antitussive mechanisms of LTRAs in CVA patients.

Methods: We prospectively observed the effect of montelukast (10 mg) daily for 4 weeks in 23 consecutive nonsmoking adults with anti-inflammatory treatment-naive CVA. We evaluated, before and after treatment, the cough visual analogue scale (VAS), pulmonary function (spirometry and impulse oscillation), methacholine airway responsiveness, cough receptor sensitivity, expressed by the concentration of capsaicin inducing 2 or more (C2) and 5 or more (C5) coughs, sputum eosinophil counts and levels of inflammatory mediators, including cysteinyl leukotrienes, leukotriene B4, prostaglandin (PG) D2, PGE2, PGF2α and thromboxane B2. We compared the baseline characteristics of the patients based on the symptomatic response to montelukast, defined as a decrease in the cough VAS of >25% (n = 15) or ≤25% (n = 8). Results: Montelukast significantly decreased the cough VAS (p = 0.0008), sputum eosinophil count (p = 0.013) and cough sensitivity (C2: p = 0.007; C5: p = 0.039), whereas pulmonary function, airway responsiveness and sputum mediator levels remained unchanged. Multivariate analysis showed that a better response to montelukast was associated solely with younger age (p = 0.032).

Conclusion: The antitussive effect of montelukast in CVA may be attributed to the attenuation of eosinophilic inflammation rather than its bronchodilatory properties.

Introduction

Cough variant asthma (CVA) is a variant form of asthma that presents solely with cough [1] and is one of the most common causes of chronic cough worldwide [2]. Pa-
Patients with CVA have mild airway hyperresponsiveness (AHR), as demonstrated by methacholine challenge, and bronchodilators such as inhaled β₂-agonists and/or oral sustained-release theophyllines are effective against the cough [1]. Modest but variable airflow limitation indicates that bronchial constriction is involved in the mechanism of cough in CVA [3].

Findings in induced sputum, bronchoalveolar lavage and bronchial biopsies have shown that eosinophilic inflammation is involved in CVA as well as in classic asthma with wheezing [4, 5]. Several inflammatory mediators are also implicated in CVA [6, 7]. Prostanoids such as prostaglandin (PG) E₂, PGF₂α and thromboxane (TX) A₂ modulate airway caliber [8, 9] and enhance the cough response to capsaicin [10, 11], which is produced by various cells including eosinophils [12]. Metabolites of lipoxygenase products have recently been identified as ligands of the transient receptor potential vanilloid 1 receptor, also known as the capsaicin receptor [13, 14].

Cysteinyl leukotriene (cys-LT) receptor antagonists (LTRAs) are antiasthma medications that reduce clinical symptoms and improve pulmonary function as well as airway inflammation in patients with classic asthma [15, 16]. An uncontrolled observational study has shown that the LTRA montelukast reduces airway levels of cys-LTs in association with improvements in the quality of life of patients with classic asthma already under treatment with inhaled corticosteroids (ICS) [17]. Although LTRAs exert antitussive effects in CVA [18–20], details of the mechanisms underlying these effects, including anti-inflammatory effects, remain unknown.

The present study examined the effect of montelukast on cough symptoms, pulmonary function, capsaicin cough receptor sensitivity, AHR and sputum inflammatory indices including numbers of eosinophils and levels of inflammatory mediators [cys-LTs, leukotriene B₄ (LTB₄), PGD₂, PGE₂, PGF₂α and TXB₂] in patients with CVA, in order to investigate its antitussive mechanisms.

**Patients and Methods**

**Study Design**

This was a prospective observational study. To elucidate the antitussive mechanism of montelukast, we measured, before and after treatment, cough symptoms, pulmonary function, capsaicin cough receptor sensitivity, AHR and sputum inflammatory indices including eosinophil counts and levels of mediators such as cys-LTs, LTB₄, PGD₂, PGE₂, PGF₂α and TXB₂ in patients with CVA. The study was approved by the Institutional Review Board of Kyoto University Hospital, and written informed consent was obtained from all participants.

**Measurement of Sputum Levels of Inflammatory Mediators**

Concentrations of cys-LTs, LTB₄, PGD₂-methoxime, PGE₂, PGF₂α and TXB₂ in the supernatant of induced sputum were measured with sandwich enzyme immunoassay kits (cys-LTs and PGE₂: Amersham Biosciences Corp., N.J., USA; LTB₄, PGD₂-methoxime, PGF₂α and TXB₂: Cayman Chemical, Ann Arbor, Mich., USA) according to the manufacturers’ instructions. Duplicate measurements were averaged for analysis. Because PGD₂ and TXA₂ are both relatively unstable, we measured PGD₂-methox-
Pulmonary Function

We conducted IOS using an MS-IOS (Erich Jaeger, Hoechberg, Germany) as described elsewhere [25, 27–29] and according to standard recommendations [30]. We measured R5 and R20 as indices of total and proximal airway resistance, respectively, and considered the difference between them (R5 – R20) a surrogate marker of small airway resistance. Reactance at 5 Hz (X5) was also measured as an index of peripheral airway abnormalities [29, 25, 27–29]. IOS is a simple and noninvasive method of assessing pulmonary function without forced maneuvers, and the indices respond to therapeutic intervention or correlate with pathological indices more sensitively than those of spirometry [25, 27–29, 31].

The spirometric indices forced expiratory volume in 1 s (FEV1) and mid-forced expiratory flow (FEF25–75%), as well as the reversibility of FEV1 with a β-agonist, were measured according to recent recommendations [32].

Capsaicin Cough Challenge

Capsaicin cough receptor sensitivity was measured as described previously with slight modifications [33]. Briefly, 10 doubling concentrations of capsaicin (0.61–312.5 μM) were inhaled during tidal breathing until 2 or more coughs and 5 or more coughs were induced (cough thresholds C2 and C5, respectively). The patients inhaled a saline control followed by progressively increasing concentrations of capsaicin. Each 15-second capsaicin inhalation was interspersed with saline inhalation for 45 s as a blind.

Methacholine AHR

AHR was examined by continuous methacholine inhalation with simultaneous measurement of respiratory resistance (Rrs; cm H2O/l/s) (Astograph; Chest, Tokyo, Japan) [34]. Briefly, two- fold increasing concentrations of methacholine solution in 10 dose steps (49–25,000 μg/ml) were prepared. They were inhaled during tidal breathing from nebulizers with an output of 0.15 ml/min. After recording the baseline Rrs during inhalation of physiological saline for 1 min, methacholine was inhaled sequentially, starting from the lowest concentration, at 1-min intervals. The cumulative dose of inhaled methacholine at the inflection point at which Rrs begins to increase (Dmin) was used as the index of airway sensitivity. This variable was measured in terms of a unit defined as 1-min inhalation of 1 mg/ml methacholine. Inhalation of methacholine was continued until Rrs reached twice the baseline value. The slope of the methacholine-Rrs dose-response curve (SRrs) was used as the measure of airway reactivity. The total cumulative dose of methacholine after inhalation of the highest dose was 50 units.

This method was developed and established by Takishima et al. [34] and further validated by our group [35].

Response to Montelukast

Patients were assigned to groups according to their response to montelukast. A better symptomatic response to montelukast was arbitrarily defined as a ≥25% decrease in VAS (ΔVAS ≥25%).

Table 1. Changes in 23 patients treated with montelukast for 4 weeks

<table>
<thead>
<tr>
<th>Montelukast treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Cough VAS, mm</td>
<td>40.3 (22.1)</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>103 (13)</td>
</tr>
<tr>
<td>FEF25–75%, % predicted</td>
<td>96 (30)</td>
</tr>
<tr>
<td>R5, kPa/l/s</td>
<td>0.31 (0.10)</td>
</tr>
<tr>
<td>R20, kPa/l/s</td>
<td>0.27 (0.07)</td>
</tr>
<tr>
<td>R5 – R20, kPa/l/s</td>
<td>0.04 (0.04)</td>
</tr>
<tr>
<td>X5, kPa/l/s</td>
<td>−0.12 (0.06)</td>
</tr>
<tr>
<td>log C2, μM</td>
<td>0.49 (0.72)</td>
</tr>
<tr>
<td>log C5, μM</td>
<td>0.79 (0.75)</td>
</tr>
<tr>
<td>log Dmin, units</td>
<td>0.32 (1.08)</td>
</tr>
<tr>
<td>SRrs, cm H2O/l/s/min</td>
<td>2.08 (1.64)</td>
</tr>
</tbody>
</table>

Sputum indices

<table>
<thead>
<tr>
<th></th>
<th>Montelukast treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>after</td>
<td></td>
</tr>
<tr>
<td>Eosinophils, × 10⁵/g</td>
<td>1.23 (3.36)</td>
<td>0.55 (1.25)</td>
</tr>
<tr>
<td>cys-LTs, ng/ml</td>
<td>18.4 (15.8)</td>
<td>12.8 (14.7)</td>
</tr>
<tr>
<td>LTB4, ng/ml</td>
<td>5.24 (6.37)</td>
<td>4.86 (9.64)</td>
</tr>
<tr>
<td>PGD2, ng/ml</td>
<td>0.14 (0.29)</td>
<td>0.05 (0.05)</td>
</tr>
<tr>
<td>PGE2, ng/ml</td>
<td>1.25 (1.11)</td>
<td>1.11 (0.91)</td>
</tr>
<tr>
<td>PGF2α, ng/ml</td>
<td>0.83 (0.62)</td>
<td>0.74 (0.49)</td>
</tr>
<tr>
<td>TXB2, ng/ml</td>
<td>1.94 (1.79)</td>
<td>1.58 (1.41)</td>
</tr>
</tbody>
</table>

Data are means (SD).

Baseline characteristics, including physiological and inflammatory indices, of these patients were compared with those of the remaining patients (ΔVAS ≤25%).

Statistical Analysis

Values are expressed as means (SD). Changes in symptoms after treatment with montelukast were analyzed using the Wilcoxon signed-rank test. Subgroups classified according to the response to montelukast were compared by univariate analysis using the Mann-Whitney U test or Fisher’s exact probability test. Independent factors associated with the response to treatment were tested using logistic regression analysis. p values of <0.05 were considered statistically significant.

Results

Effect of Montelukast

Table 1 and figure 1 show changes in symptoms, physiological indices and inflammatory parameters of sputum before and after treatment with montelukast. The cough VAS improved from 40.3 (22.1) to 19.3 (14.8) mm after 4 weeks (p = 0.008), with a mean (SD) improvement rate of 52.2% (33.0). With respect to cough receptor sensitivity, C2 increased in 13 of the 23 patients (57%) after
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Baseline Clinical Characteristics according to Response to Montelukast

The symptomatic response to montelukast was better (ΔVAS >25%) in 15 of the 23 patients (65%). The results of the univariate analysis showed that the better respondents were significantly younger (p = 0.026) and more sensitive to methacholine (p = 0.030) than those with ΔVAS ≤25% (table 2). Other indices, including IOS and spirometry, log C5, airway reactivity, sputum eosinophil count and sputum mediator levels including that of cys-LTs, did not differ between the subgroups. Montelukast responses, assessed as the percentage change in physiological indices (FEV1, FEV25-75%, R5, R20, R5–R20, X5, log C2, log C5, log Dmin or SRrs) and the number of sputum eosinophils, also did not differ between subgroups (data not shown). Multiple linear regression analysis including age and log Dmin identified younger age as the sole predictor of a better responsiveness to montelukast (table 3).

An analysis of 17 patients who had adequate pairs of sputum samples before and after treatment revealed no
Airway caliber changes that may be
log Dmin 0.26 (0.063–1.08) 0.064
Age 0.89 (0.78–0.99) 0.032

Values in parentheses represent 95% confidence intervals.

significant correlations among percentage changes in VAS, sputum eosinophils, log C2 and log C5 (data not shown). In addition, percentage changes in the sputum eosinophil count were unrelated to those in sputum mediator levels (data not shown). In all of the 23 subjects, percentage changes in VAS were also unrelated to those in C2 or C5 (data not shown).

Discussion

We investigated the clinical, physiological and anti-inflammatory effects of montelukast in patients with CVA. Effects on the cough VAS and cough receptor sensitivity were confirmed as in other studies [18, 19]. We also discovered a significant decrease in sputum eosinophil count, although IOS and spirometry indices remained unchanged.

Ample evidence supports the effectiveness of LTRAs against classic asthma [15, 16, 36], and their value against CVA has also been reported [18–20, 37]. A placebo-controlled crossover study found that 2 weeks of treatment with zafirlukast significantly improved cough scores and capsaicin cough receptor sensitivity in 8 patients with CVA, while FEV1 values remained unchanged [18]. Four weeks of treatment with montelukast improved subjective and objective measures of cough frequency and scores on the Asthma Quality of Life questionnaire without a concomitant change in FEV1 in 8 patients with CVA, as compared with 6 who received a placebo [19]. However, the antitussive mechanisms of the LTRAs were not examined in these small studies, except for one analysis of bronchial tissue from a subset of patients (n = 13), which showed that responders to montelukast had increased numbers of CD63-positive cells (activated mast cells) compared with nonresponders [37].

Airflow limitation is usually minimal in CVA, as it was in our patients, while being involved in the pathogenesis of cough in CVA that is attenuated by bronchodilation [1, 3, 4, 21]. Airway caliber changes that may be missed by spirometry can be sensitively detected by IOS [22, 25, 27–29, 31], but we found no significant changes in indices of IOS, nor in indices of spirometry, after treatment with montelukast, which was consistent with previous findings [18, 19]. The antitussive effect of montelukast in CVA may thus be attributable to its anti-inflammatory properties that attenuate eosinophilic inflammation rather than to bronchodilation.

Tussive inflammatory mediators, which may activate afferent sensory nerve endings, have been implicated in the pathogenesis of cough [7, 38, 39]. Inhalation studies in healthy individuals [9, 10] and inhibition studies of the specific antagonists of inflammatory mediators in asthmatic patients [11] have shown that prostanoids such as PGE2, PGF2α, and TXA2 are associated with the cough reflex. A recent in vitro study revealed that the metabolites of lipoxygenase products such as 12- and 15-(S)-hydroperoxyeicosatetraenoic acids, 5- and 15-(S)-hydroxyeicosatetraenoic acids and LTB4 might be ligands for transient receptor potential vanilloid 1 receptors [13]. The expression of such receptors is increased in the airways of patients with chronic cough, including those with CVA [40], indicating their involvement in cough hypersensitivity [14]. However, evidence supporting direct tussive effects of cys-LTs on ‘cough receptors’ is scarce.

Montelukast decreases cys-LT levels in the exhaled breath of patients with classic asthma [17, 41]. To our knowledge, this is the first study to investigate the detailed anti-inflammatory effect of an LTRA in CVA, and we revealed that montelukast significantly decreased sputum eosinophils as well as the cough VAS and capsaicin sensitivity but did not affect sputum levels of cys-LTs, LTB4, PGE2, PGE2α, PGD2, PGF2α and TXB2. Since eosinophils are a source of various prostanoids [12] and cys-LTs [42], we postulated that montelukast exerts antitussive effects by attenuating eosinophilic inflammation with a resultant fall in some prostanoid and cys-LT levels in CVA. However, our results disproved this notion. Percentage changes in sputum eosinophils were unrelated to those of sputum levels of mediators, including cys-LTs.

With respect to cys-LTs, the discrepancy of the results between the previous breath condensate studies conducted in classic asthma patients presenting with wheezing [17, 41] and our sputum study of CVA patients may have derived from possible pathophysiological differences between CVA and classic asthma. In CVA and the related condition eosinophilic bronchitis, activation of
mast cells has been implicated in the increased sputum levels of the mast cell-derived tussive mediators histamine and PGD$_2$ [7], while this is not a feature of classic asthma [43]. A recent bronchial biopsy study has also implicated the activation of mast cells in the pathophysiology of CVA [37]. Such evidence may suggest that mast cells rather than eosinophils are a relatively more important source of cys-LTs in patients with CVA as compared with those with classic asthma. As for other mediators measured in this study, major cellular sources are cells other than eosinophils (e.g., mast cells for PGD$_2$, alveolar macrophages for PGE$_2$ and neutrophils for LTB$_4$) [44] which are poorly responsive to LTRAs, unlike eosinophils. This may explain why our mechanistic hypothesis was not verified. Since ICS, which are potent inhibitors of eosinophilic inflammation, do not alter cough sensitivity in CVA [45], whereas LTRAs do, as we and others [18] have shown, LTRAs might exert a different antitussive mechanism from ICS.

In a study by Birring et al. [7], sputum mediator levels were measured in 18 healthy subjects and in three subgroups of chronic cough patients (CVA or eosinophilic bronchitis, n = 20; various causes of nonasthmatic cough, n = 20; idiopathic cough, n = 22). Levels of PGD$_2$ and PGE$_2$ were significantly higher in all cough subgroups compared with controls, and histamine levels were also increased in the CVA and eosinophilic bronchitis and idiopathic cough subgroups, whereas cys-LT levels were increased only in the CVA and eosinophilic bronchitis subgroup. These results indicate that cys-LTs are specifically involved in cough associated with eosinophilic airway disorders, whereas PGD$_2$, PGE$_2$ and histamine are universally involved in the common mechanism of cough.

One possible mechanism for the association of cys-LTs with cough in CVA is via the tussive mediator substance P. The expression of substance P in the airway epithelium is increased in CVA patients compared with classic asthma patients and healthy subjects, both of whom express similar amounts of substance P [46]. We have also reported that plasma levels of substance P are elevated in patients with CVA and cough-predominant asthma as compared with healthy subjects [47]. cys-LTs induce the release of substance P through the stimulation of airway afferent nerve fibers [48], and LTRAs inhibit such release [48, 49]. Evidence that LTRAs attenuate cough receptor sensitivity in CVA [18] but not classic asthma [50] might be consistent with this hypothesis, but the details remain to be clarified.

The effect of LTRAs in classic asthma is variable. This has been attributed to the effects of genetics [51], smoking [52] and also age. Symptoms and lung function respond better to zafirlukast in patients aged <65 years than in older patients [53]. The effect of zafirlukast is better among patients aged <50 years [54, 55]. Our results are consistent with these findings, but the mechanisms involved are unknown. Sputum levels of mediators mainly derived from mast cells, such as PGD$_2$, were not increased in our patients with better responses to montelukast, which contradicts the suggested hypothesis [37].

Some limitations of our study should be noted. We arbitrarily defined a better response to montelukast as a >25% decrease of VAS based on our clinical experience, not on evidence. The European Respiratory Society guidelines on the assessment of cough recommend using the cough VAS for assessment of chronic cough severity [23]. Actually, there is evidence that the cough VAS score is highly reproducible [56] and responsive to intervention [57] when used as an outcome measure in clinical studies of chronic cough. However, the minimal change in VAS needed to judge the efficacy of intervention remains to be determined. The British Thoracic Society guidelines of cough have defined the minimal significant improvement of cough as a change in VAS of 15 mm [58]. However, this is not even based on evidence. We readily admit that future validation studies are essential for this issue. The lack of a control group is another limitation of our study. This might have affected the precise evaluation of treatment effects. However, a placebo effect of montelukast upon sputum eosinophils and coughing that had persisted for an average of 3.5 years seems unlikely. We believe that the observed efficacy of montelukast on the cough VAS, cough sensitivity and sputum eosinophilia but not on pulmonary function measures is relevant in the absence of controls. Moreover, this short-term study could not investigate the effect of treatment on the long-term consequences of disease such as the development of irreversible airflow obstruction [1], airway remodeling [5, 59] and progression to classic asthma, which might be preventable by ICS [60].

In conclusion, we found that the cough VAS, sputum eosinophil counts and cough sensitivity significantly improved in CVA patients treated with montelukast for 4 weeks, while pulmonary function did not change. The antitussive effect of montelukast in CVA might be attributable to its anti-inflammatory ability rather than bronchodilation. However, the detailed mechanisms remain to be clarified by future controlled studies.
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