

ORIGINAL ARTICLE

AIRWAY DISEASES

A dual CysLT_{1/2} antagonist attenuates allergen-induced airway responses in subjects with mild allergic asthma

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Abstract

Background: The cysteinyl leukotrienes (cysLTs) play a key role in the pathophysiology of asthma. In addition to functioning as potent bronchoconstrictors, cysLTs contribute to airway inflammation through eosinophil and neutrophil chemotaxis, plasma exudation, and mucus secretion. We tested the activity of the dual cysLT_{1/2} antagonist, ONO-6950, against allergen-induced airway responses.

Methods: Subjects with documented allergen-induced early (EAR) and late asthmatic response (LAR) were randomized in a three-way crossover study to receive ONO-6950 (200 mg) or montelukast (10 mg) or placebo q.d. on days 1–8 of the three treatment periods. Allergen was inhaled on day 7 two hours postdose, and forced expiratory volume in 1 s (FEV₁) was measured for 7 h following challenge. Sputum eosinophils and airway hyperresponsiveness were measured before and after allergen challenge. The primary outcome was the effect of ONO-6950 *vs* placebo on the EAR and LAR.

Results: Twenty-five nonsmoking subjects with mild allergic asthma were enrolled and 20 subjects completed all three treatment periods per protocol. ONO-6950 was well tolerated. Compared to placebo, ONO-6950 significantly attenuated the maximum % fall in FEV₁ and area under the %FEV₁/time curve during the EAR and LAR asthmatic responses (*P* < 0.05) and allergen-induced sputum eosinophils. There were no significant differences between ONO-6950 and montelukast.

Conclusions: Attenuation of EAR, LAR, and airway inflammation is consistent with cysLT₁ blockade. Whether dual cysLT_{1/2} antagonism offers additional benefit for treatment of asthma requires further study.

Cysteinyl leukotrienes (cysLTs) have long been implicated in the pathogenesis of allergic asthma, functioning as potent constrictors of airway smooth muscle and mediators of inflammation (1). CysLTs exert their effect through cysLT receptors 1 (cysLT₁) and 2 (cysLT₂) (2) and newly identified receptors responsive to LTE₄ (3, 4). Antileukotrienes inhibit either LT synthesis or cysLT₁ binding, and both approaches attenuate the important hallmarks of asthma pathophysiology (5, 6).

CysLT receptors are expressed on a variety of structural and hematopoietic cells involved in asthma pathophysiology (7–9) and overexpressed in allergic tissue (10–12). In asthma, cysLT₁ blockade works in part by inhibiting the recruitment of eosinophils (13); clinical trials evaluating montelukast,

pranlukast, and zafirlukast have reported a reduction of eosinophil levels in airways and blood of asthmatic patients following treatment (5, 6, 14, 15). The effect of cysLT₂ blockade in asthma has not been examined. Patients suffering from chronic persistent asthma demonstrated greater improvement in lung function when treated with a cysLT synthesis inhibitor *vs* a cysLT₁ antagonist (16). Examination of isolated human lung tissue has shown that antagonism of cysLT₂ receptors suppressed antigen-induced contraction, while dual antagonism of cysLT₁ and cysLT₂ receptors resulted in an additive inhibitory effect on anaphylactic contractions (10). Together, these support combined cysLT_{1/2} inhibition for treatment of asthma.

ONO-6950 (gemilukast) is a selective cysLT_{1/2} receptor antagonist (17). In guinea pigs, ONO-6950 inhibited LTC₄-induced bronchoconstriction (a cysLT₁/LT₂-dependent response) and S-hexylglutathione-induced airway vascular hyperpermeability (a cysLT₂-dependent response) more potently than montelukast (18, 19). In this study, we compared the effects of ONO-6950 to placebo and montelukast on allergen-induced asthmatic responses in subjects with mild allergic asthma.

Methods

Study design

This was a multicenter, double-blind, randomized, placebo-controlled, three-way crossover study, comparing treatment with 200 mg ONO-6950, 10 mg montelukast, or placebo (Fig. 1). The primary outcome was the effect of ONO-6950 *vs* placebo on allergen-induced early (EAR) and late asthmatic response (LAR) asthmatic responses to inhaled aeroallergen. Early asthmatic response was defined by an acute fall in a forced expiratory volume in 1-s (FEV₁) ≥ 20% within 2 h following allergen challenge, and LAR was defined by a fall in FEV₁ ≥ 15% between 3 and 7 h following allergen challenge. Secondary outcomes compared ONO-6950 and montelukast on EAR and LAR and compared the effect of ONO-6950 *vs* placebo and montelukast on airway hyperresponsiveness (AHR), sputum eosinophils, and exhaled nitric oxide levels (FeNO). The study was registered with clinicaltrials.gov NCT01551147 and approved by the research ethics board of each institution. Signed informed consent was obtained from all subjects, and the study was conducted from August 2012 to April 2013.

Subjects were screened over a period of 14 days. Those meeting eligibility criteria were randomized to three treatment periods of 8 days each, separated by 13–20 day washouts. Pulmonary function and AHR were required to be at baseline to enter each treatment period. Dosing was carried

out on days 1–8. Allergen challenges were conducted 2 h postdosing on day 7. Methacholine challenge and sputum induction were performed on day 1 (before dosing), day 6 (24 h preallergen challenge), and day 8 (24 h postallergen challenge). Sputum was also induced 7 h postallergen challenge.

Eligibility criteria

Nonsmoking subjects between 18 and 60 years old with mild allergic asthma were recruited. Subjects were steroid-naïve, used infrequent inhaled beta₂-agonist for treatment of asthma, had FEV₁ ≥ 70% of predicted and methacholine PC₂₀ ≤ 16 mg/ml, and developed allergen-induced EAR and LAR asthmatic responses. Subjects had no other lung disease, no lower respiratory tract infection, or worsening of asthma for 6 weeks before screening and avoided exposure to sensitizing allergens apart from house dust mite during the study.

Study medication

Administration of 200 mg ONO-6950, 10 mg montelukast, or placebo utilized a double-dummy approach; subjects received two oral tablets and one oral capsule each morning after breakfast.

Laboratory procedures

Methacholine inhalation challenge

Methacholine PC₂₀ was measured using tidal breathing from a Wright nebulizer (20). The test was terminated when a fall in FEV₁ of at least 20% of the baseline value occurred.

Allergen inhalation challenge

Allergen inhalation was performed during a screening period (21, 22) and the same dose of allergen was inhaled for each of the three treatment periods. The EAR and LAR area under

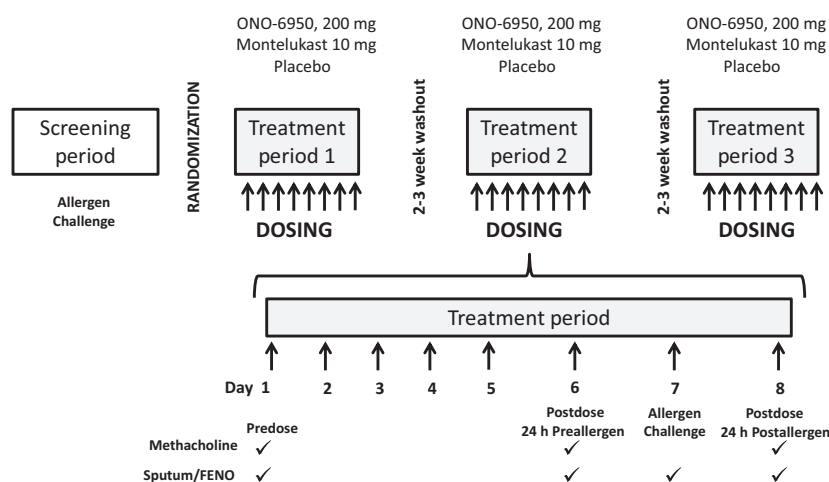


Figure 1 Study schematic.

Table 1 Mean (SD) subject demographics and baseline characteristics

Age (years)	29.2 (9.3)
Sex	12 male/13 female
Race	22 Caucasian/3 Asian
Ethnicity	2 Hispanic or Latino/23 non-Hispanic or Latino
Predicted FEV ₁ (%)	92.7 (12.2)
EAR (maximum % fall)	33.3 (8.7)
LAR (maximum % fall)	26.8 (8.8)
Methacholine PC ₂₀ (mg/ml)	Geometric mean 2.0 (min/max 0.17/14.0)

FEV₁, forced expiratory volume in 1 s; EAR, early asthmatic response; LAR, late asthmatic response; PC₂₀, provocative concentration causing a 20% fall in FEV₁.

the curve (AUC) were calculated from 0 to 2 h and 3 to 7 h, respectively (AUC 0–2 h, AUC 3–7 h) using graphics software that calculated the area of the FEV₁–time response curve.

Sputum analysis

Sputum was induced and processed using the method described by Pizzichini and co-workers (23). Two slides were stained with Diff Quik (American Scientific Products, McGaw Park, IL, USA), and differential counts were performed.

Statistical analysis

Methacholine PC₂₀ values were log-transformed prior to statistical analysis. All efficacy outcomes were analyzed using the per-protocol dataset from 20 subjects, to accommodate the crossover design. The EAR and LAR were analyzed

using a general linear model including its extension of generalized estimating equation. Methacholine PC₂₀ and % sputum cells were assessed by the change pre/post-treatment and pre/postallergen challenge. Statistical significance was set at $P < 0.05$.

Results

Twenty-five subjects were randomized into one of three treatment sequences (Table 1) with compliance of 100%, 96.2%, and 99.5%. Five subjects were excluded from per-protocol analyses because they did not complete all three dosing evaluations: one withdrew consent, two had respiratory tract infections, one had worsening asthma, and one had missing assessments.

Allergen-induced early and late asthmatic responses

The mean (SD) maximum % fall in FEV₁ for EAR and LAR was 28.2 (12.0)% and 20.5 (12.8)%, respectively, for placebo treatment. Both ONO-6950 and montelukast significantly inhibited the maximum % fall in FEV₁ and AUC in FEV₁ (%) for the EAR and LAR compared to placebo (all $P < 0.01$) (Fig. 2). The mean (SD) maximum % fall in FEV₁ for EAR and LAR was 15.5 (8.5)% and 11.6 (8.0)%, respectively, for ONO-6950 and 11.9 (7.5)% and 12.8 (9.3)%, respectively, for montelukast treatment. The AUC showed the same differences as the maximum % fall in FEV₁ the AUC for EAR and LAR were 33.7 (19.1) and 55.7 (37.4), respectively, for placebo, 13.4 (12.4) and 28.1 (24.6), respectively, for ONO-6950, and 11.5 (12.2) and 31.1 (27.7), respectively, for montelukast. There was no difference in EAR or LAR outcomes between ONO-6950 and montelukast.

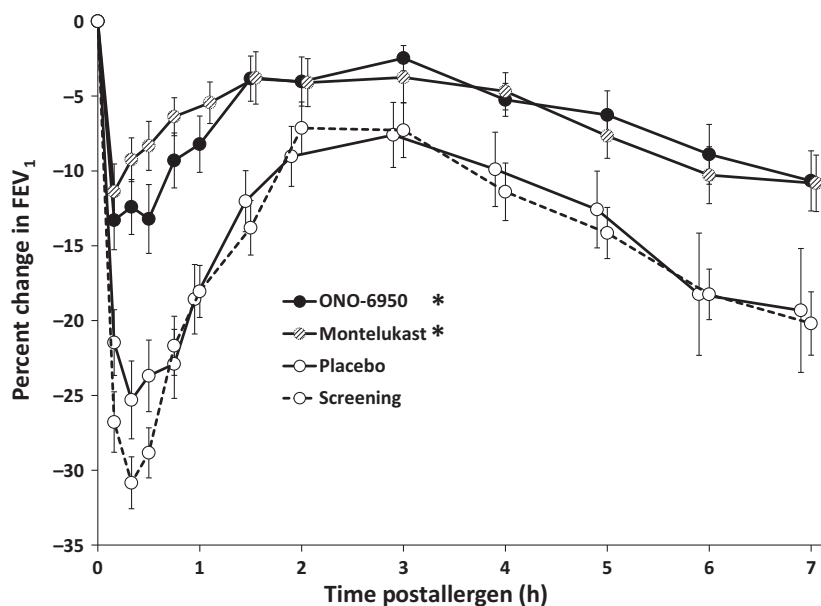


Figure 2 Mean ($\pm$ SEM) % fall in FEV₁ during the early and late asthmatic responses. * $P < 0.01$ Significant effect of ONO-6950 and

montelukast vs placebo on the early and late asthmatic responses. FEV₁, forced expiratory volume in 1 s.

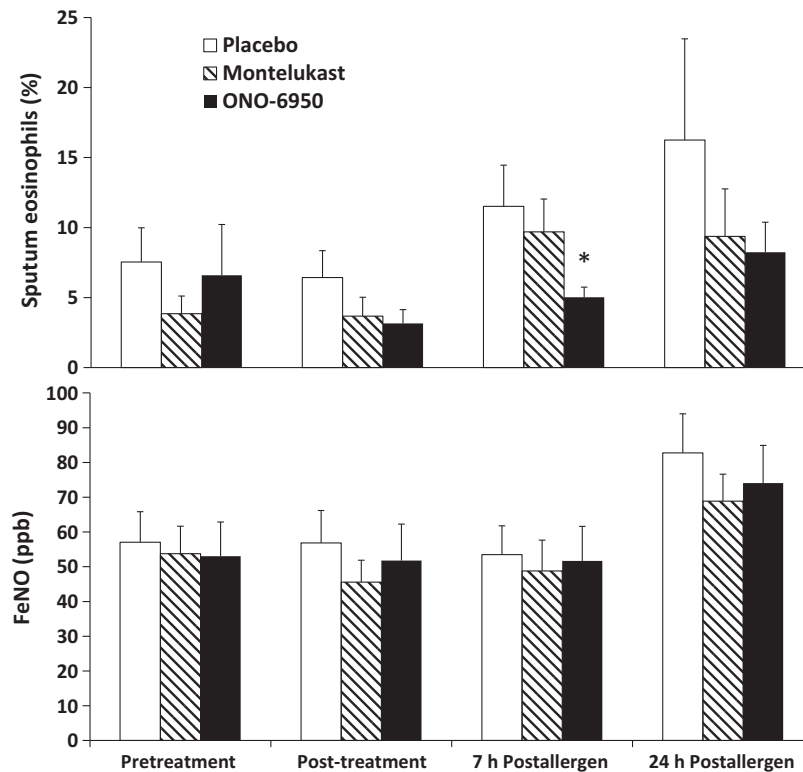


Figure 3 Mean ($\pm$ SEM) sputum eosinophils and fractional exhaled nitric oxide (FeNO) at baseline pretreatment, post-treatment at day 6, and 7 and 24 h after allergen challenge with

ONO-6950 (solid bars), montelukast (hatched bars), or placebo (open bars). * $P < 0.05$ Significant effect vs placebo allergen-induced change.

Sputum inflammatory cells

ONO-6950 reduced the total number of inflammatory cells/mg sputum at 7 h postchallenge ($P = 0.048$), but there was no effect of ONO-6950 or montelukast on the change from prechallenge baseline. With placebo treatment, the sputum eosinophil level significantly increased after allergen challenge from a mean (SD) of 2.2 (1.6)% prechallenge, to 11.0 (7.4)% at 7 h and 15.9 (23.7)% at 24 h postchallenge. With ONO-6950, the sputum eosinophils increased from 2.4 (3.2)% prechallenge to 6.4 (3.7)% at 7 h and 9.3 (10.4)% at 24 h postchallenge, and with montelukast, the sputum eosinophils increased from 2.5 (3.4)% prechallenge to 8.8 (5.9)% at 7 h and 9.4 (13.2)% at 24 h postchallenge. Compared to placebo, the allergen-induced change in eosinophils at 7 h postallergen was significantly inhibited by ONO-6950 ($P = 0.026$) but not by montelukast ($P = 0.20$) (Fig. 3). At 24 h postallergen, there was a 50% reduction in eosinophils with montelukast and ONO-6950 treatment, but this was not statistically different than placebo. There was no difference in eosinophil levels between ONO-6950 and montelukast at any time.

AHR and fractional exhaled nitric oxide

There was no effect of ONO-6950 or montelukast on methacholine PC₂₀ (Table 2) or FeNO (Fig. 3) following 6 days of treatment, or on the allergen-induced shift.

Table 2 Mean (min, max) methacholine PC₂₀ values (mg/ml)

Study period	Placebo	Montelukast	ONO-6950
Day 1	3.6 (0.01, 11.9)	3.0 (0.01, 9.4)	4.1 (0.02, 15.8)
Day 6	4.6 (0.03, 29.0)	4.5 (0.02, 20.9)	4.8 (0.02, 16.4)
Day 8	4.1 (0.02, 19.8)	2.7 (0.02, 9.5)	4.7 (0.02, 25.5)

Safety

No serious adverse events (AEs) were reported in this study. The 45 AEs reported (Table 3) were mostly mild, with moderate events occurring approximately equally across the treatment groups during the dosing and evaluation periods. The highest incidence of AEs occurred in the ONO-6950 group; dry mouth and headache occurred in two subjects; and the subject who experienced dry mouth reported other AEs (nausea and vomiting). These AEs were considered to be possibly related to study drug.

Discussion

ONO-6950 is the first dual cysLT_{1/2} receptor antagonist to be tested in humans. Eight days of oral treatment with 200 mg ONO-6950 in subjects with mild allergic asthma was found to be safe and significantly attenuated the allergen-induced EAR, LAR, and sputum eosinophils. The comparator

Table 3 Adverse events (AEs)

Study period	Placebo	Montelukast	ONO-6950	Washout
Number of subjects	23	22	23	25
All AEs	9/7 (30.4)	4/4 (18.2)	22/9 (39.1)	10/8 (32)
Serious AEs (SAEs)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Mild AEs	7/5 (21.7)	3/3 (13.6)	18/5 (21.7)	7/5 (20.0)
Moderate AEs	2/2 (8.7)	1/1 (4.5)	4/4 (17.4)	3/3 (12.0)
Severe AEs	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)
Drug-related AEs	0/0 (0.0)	0/0 (0.0)	13/2 (8.7)	0/0 (0.0)
Drug unrelated AEs	9/7 (30.4)	4/4 (18.2)	9/8 (34.8)	10/8 (32.0)
AEs leading to discontinuation	1/1 (4.3)	0/0 (0.0)	1/1 (4.3)	1/1 (4.0)

Number of events/number of subjects (percentage of subjects).

control, 10 mg montelukast, also attenuated the EAR and LAR, but not the allergen-induced increase in sputum eosinophils. The suppressive effects of ONO-6950 on sputum eosinophils, although not significantly different, trended toward being greater than those of montelukast.

We recruited subjects with mild, allergic asthma who, following inhalation of a relevant allergen, experience EAR, LAR, and AHR. Mast cell activation in the airways and subsequent release of the spasmogenic mediators, histamine and cysLTs, is largely responsible for the development of the EAR (24), whereas the LAR is associated with increases in AHR and airway inflammatory cells, most notably eosinophils (25).

ONO-6950 and montelukast each attenuated both the EAR and LAR by approximately 50%, and this effect is consistent with previous studies of cysLT₁ receptor antagonists and 5-lipoxygenase-activating protein (FLAP) inhibitors (26–32) while 5-lipoxygenase inhibitors and LTB₄ inhibitors have been ineffective (33–36). In the current study, the difference in sputum eosinophils reached significance at 7 h for ONO-6950 vs placebo, but there was no difference compared to montelukast (6.4% vs 8.8%). Furthermore, the change in sputum eosinophils was not different at 24 h for ONO-6950 compared to placebo or montelukast. While signaling through cysLT₁ receptor is important for the development of allergen-induced bronchoconstriction, there is insufficient power in the current study to draw conclusions on whether cysLT₂ may be involved in driving eosinophil responses.

Addition of a cysLT₁ receptor antagonist to inhaled corticosteroid (ICS) offers no additional benefit on allergen-induced responses (27), and in a meta-analysis, it significantly increased FEV₁ but had no effect on exacerbations or asthma quality of life (37). This suggests that addition of cysLT₁ receptor antagonist to ICS is largely ineffective in ICS-sensitive patients. Whether adding dual cysLT_{1/2} receptor antagonist would be effective is unknown.

The function of cysLT₂ receptor in human asthma remains poorly understood. LTC₄ and LTD₄ bind cysLT₂ receptors with lower affinity than cysLT₁ receptors, and LTE₄ does not appear to signal through either receptor (38, 39). There is compelling evidence that LTE₄ signals through P2Y₁₂ receptor and GPR99 (3, 4). The cysLT receptors network is quite complex and complicated by cross-regulation (40). Furthermore, the partially overlapping distribution of cysLT₁ and cysLT₂ receptors suggests that they can have complementary

and distinct functions; inhibition of cysLT₁ or cysLT₂ receptor on primary mast cells has the opposite effects on LTD₄-mediated signaling (41).

This study demonstrates that dual cysLT_{1/2} receptor antagonism with ONO-6950 significantly inhibits allergen-induced airway eosinophilia at 7 h postchallenge. LTE₄, but not LTD₄ inhalation, has been reported to increase eosinophil numbers in the airways (42, 43). However, *in vitro* experiments evaluating the direct effects of LTE₄ on eosinophil migration (44) or differentiation of eosinophil progenitors (45) have not been compelling. Furthermore, eosinophils expressing P2Y₁₂ and GPR99 were not found to be activated by LTE₄ (46), suggesting that eosinophils themselves are not the direct target of LTE₄. To this end, LTD₄ has been found to potentiate IL-13-induced eotaxin-3 release from human primary bronchial epithelial cells through the upregulation of cysLT₁ (13); however, a mechanism whereby cysLT₂ blockade inhibits eosinophil accumulation in the airways has not been identified. Emerging literature has demonstrated a direct function of cysLTs on newly identified effector cells, such as innate lymphoid cells (47), thereby contributing to Th2 immunity in unexpected ways.

Conclusions

Although the role of cysLT₂ is not well understood, this study demonstrates that the dual cysLT_{1/2} antagonist ONO-6950 may provide additional regulation of airway eosinophilia in patients with asthma.

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Author contributions

All authors made substantial contributions to the conception and design of the study, study management, data collection, interpretation of the results, and revision of the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Conflicts of interest

This study was funded by ONO Pharmaceutical Co., Ltd. (Osaka, Japan) with administrative support provided by the Allergen Network of Centres of Excellence. MT, JF, MT,

and TN were employees of ONO Pharmaceutical Co., Ltd., during the study. The remaining authors have no conflict of interest to declare.

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