Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects

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Background: The cysteinyl leukotrienes (cysLTs) have been implicated in the pathogenesis of allergen-induced airway responses. The purpose of this study was to evaluate the effects of pretreatment with the cysLT receptor antagonist pranlukast on allergen-induced early asthmatic responses (EARs) and late asthmatic responses (LARs) and on allergen-induced airway hyperresponsiveness (AHR).

Methods: Ten atopic, nonsmoking patients with mild asthma and previously demonstrated early- and late-phase allergen-induced asthmatic responses participated in a double-blind, placebo-controlled, cross-over study, comparing treatment with either 450 mg pranlukast given twice daily or placebo for 5.5 days. A methacholine challenge was performed before administration of medication, and the result was expressed as the PC_{20}. An allergen inhalation challenge was performed on the morning of the fifth day of treatment 2 hours after administration of medication. Methacholine challenges were also performed 2 hours after medication on days 4 and 6 (24 hours before and 24 hours after allergen administration) to examine allergen-induced AHR.

Results: Pranlukast attenuated allergen-induced early responses, late responses, and AHR. The mean (SEM) maximal percent fall in FEV_1 from baseline during the early response was 30.0% (5.1%) during placebo treatment and 15.5% (3.5%) during pranlukast treatment (mean difference, 14.5%; 95% confidence interval [CI], 5.3 to 23.7; *P* = .007), with a mean protection afforded by pranlukast of 48.3%. The mean maximal percent fall in FEV_1 during the late response was 34.7% (5.3%) during placebo treatment and 24.0% (4.4%) during pranlukast treatment (mean difference, 10.7%; 95% CI, 4.1 to 17.3; *P* = .006), with a mean protection afforded by pranlukast of 30.8%. The mean allergen-induced shift in PC_{20} was –1.76 (0.32) doubling doses during placebo treatment and –0.38 (0.31) doubling doses during pranlukast treatment (mean difference, –1.38 doubling doses; 95% CI, 0.44 to 2.32; *P* = .012), with a mean protection afforded by pranlukast of 78.4%.

Conclusion: These results demonstrate that pranlukast can attenuate allergen-induced early and late airways responses and AHR and adds further support for an important role for the cysLTs in mediating allergen-induced asthmatic responses. (J Allergy Clin Immunol 1998;102:177-83.)

Key words: *Asthma, pranlukast, cysteinyl leukotriene receptor antagonist, allergens*

Inhalation of environmental allergens is an important cause of asthma. In the laboratory, inhalation of allergen by sensitized subjects causes an early asthmatic response (EAR) within 15 minutes of inhalation, which is manifested by airway narrowing that usually resolves within 2 to 3 hours. In more than 50% of adult asthmatic subjects, a more prolonged late asthmatic response (LAR) also develops, beginning 2 to 4 hours after inhalation.[1] The LAR is often accompanied by airway hyperresponsiveness (AHR) to bronchoconstrictor stimuli, such as histamine or methacholine, which can last for days or weeks after allergen exposure.[3] LARs and AHR are associated with the influx of inflammatory cells, such as eosinophils,[4] into the airways.

Considerable evidence is now available supporting a role for cysteinyl leukotrienes (cysLTs) (eg, LTC_4, LTD_4, and LTE_4) in the pathogenesis of asthma. The cysLTs contract airway smooth muscle in vitro,[5,6] and inhalation of cysLTs causes airway narrowing in both normal and asthmatic subjects in vivo.[7-9] CysLTs are released from human lungs after allergen challenge in vitro,[10] whereas increases in cysLTs in bronchoalveolar lavage fluid[11] and increases in urinary LTE_4[12,13] have been observed after allergen challenge in vivo. CysLTs also increase microvascular permeability[14] and stimulate secretion of mucus,[15] suggesting a possible involvement in the inflammatory process associated with the LAR.

First generation cysLT receptor antagonists (LY171,883, L649,923, and L648,051) showed modest efficacy in attenuating the allergen-induced EAR but had little or no effect on the LAR. However, in more recent

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TABLE I. Subject characteristics

Subject no.	Age (yrs)	Sex	Height (cm)	Weight (kg)	FEV_1	FEV1 (% predicted)	PC_{20} (mg/mL)*	Allergen	EAR (% fall)†	LAR (% fall)†	Treatment Sequence
1	18	F	174	63	3.47	89	0.27	*Dermatophagoides farinae*	33.3	33.3	Pr/P
2	20	F	165	54.5	3.2	90	2.99	*D farinae*	32.2	29.1	Pr/P
3	23	M	174	144	3.71	98	26.97	Grass	41.1	19.2	Pr/P
4	20	M	170.5	53	4.23	97	2.29	*D farinae*	16.5	29.2	P/Pr
5	27	M	195	90	4.93	94	2.2	Grass	45.2	21.3	Pr/P
6	18	F	163	49	3.00	83	17.67	Grass	27.0	18.1	P/Pr
7	44	M	178	71	3.49	85	12.33	*D farinae*	51.2	42.0	Pr/P
8	44	F	168	62	3.15	103	3.4	*D farinae*	28.5	36.1	P/Pr
9	19	F	166	66	3.25	90	0.3	*D farinae*	41.1	24.5	P/Pr
10	19	F	174	63	3.85	99	1.5	*D farinae*	22.3	17.9	Pr/P
Mean (SEM)	25 (3.2)		172.8 (2.9)	71.6 (8.8)	3.63 (0.19)	92.8 (2.0)	2.86 (1.63)		33.8 (3.4)	27.1 (2.6)	

Pr, Pranlukast; *P,* placebo.
*Geometric mean and %SEM.
†During screening (period 1, day 2).

Abbreviations used
AHR: Airway hyperresponsiveness
AUC: Area under the curve
cysLT: Cysteinyl leukotriene
EAR: Early asthmatic response
LAR: Late asthmatic response

studies, pretreatment with second generation potent and specific cysLT receptor antagonists, such as ICI 204,219[16] and MK-571,[17] and LT biosynthesis inhibitors, such as MK-886,[18] MK-0591,[19] and BAY x1005,[20] have partially attenuated both the EAR and LAR, providing further direct evidence for a role of cysLTs in the development of both the EAR and LAR. The role of cysLTs in the pathogenesis of allergen-induced AHR is less certain. Although Taylor et al.[16] showed that ICI 204,219 significantly attenuated AHR 6 hours after allergen inhalation, others have not been able to confirm such findings at 24 and 30 hours after allergen administration following pretreatment with the 5-lipoxygenase activating protein (FLAP) antagonists MK-886[18] and MK-0591.[19]

Pranlukast is a potent and selective cysLT receptor antagonist, which markedly attenuates LTC_4- and LTD_4-induced contractions in vitro and in vivo in guinea pigs[21] and human subjects.[22,23] Pranlukast has also been shown to inhibit antigen-induced bronchoconstriction in ovalbumin-sensitized guinea pigs[21] and to inhibit the early bronchoconstrictor response in asthmatic subjects after house dust inhalation.[24] Recently, pranlukast has been shown to antagonize LTD_4-induced microvascular leakage and eosinophil influx into the airways in guinea pigs, suggesting that pranlukast may possess antiinflammatory properties.[25] Pranlukast has shown clinical activity in patients with asthma, with improvements in pulmonary function, symptom scores,[26,27] and reductions in bronchial hyperresponsiveness.[28,29] The compound was well tolerated and no drug-related changes in hematology or biochemical variables were observed.

The purpose of this study was to evaluate the effects of pretreatment with pranlukast (SB-205312, ONO-1078) on the allergen-induced EAR and LAR and subsequent increase in AHR to methacholine in atopic subjects with mild asthma.

METHODS

Subjects

Ten subjects (4 men and 6 women) (Table I) were entered into the study and included in the statistical analysis. The study was approved by the Hospital Ethics Committees, and each subject gave written informed consent before beginning the study. All subjects were studied in the asthma research laboratory at McMaster University Medical Centre. All subjects had a history of mild, stable asthma as defined by the American Thoracic Society and documentation of asthma exacerbations induced by environmental allergen or allergens. Subjects were only using inhaled β_2-agonists to treat their asthma on an intermittent basis. Subjects were not included in the study if they had been treated chronically with oral glucocorticosteroids in the past year or inhaled corticosteroids or cromones in the past 2 months. Other than a clinical diagnosis of asthma, all subjects were determined to be healthy on the basis of medical history, physical examination, electrocardiogram, chest radiograph, and laboratory screening (ie, hematology, blood chemistry, and urinalysis). With the exception of house dust mite allergen, subjects were not currently exposed to allergens to which they were sensitized. Subjects had had no exacerbations of asthma and no respiratory infections for at least 6 weeks before the start of the investigation. Baseline FEV_1 was greater than 70% of predicted normal value[30] in all subjects on all study days. All subjects were lifetime nonsmokers. Women of childbearing potential were accepted for participation in the study only if using effective contraceptive measures for at least 1 month before screening (pregnancy tests were performed before treatment).

Study design

The study was performed in a double-blind, placebo-controlled manner with a cross-over design. The study was divided into 3 periods: a screening period (period 1) and two treatment periods (periods 2 and 3). On all study days, subjects came to the laboratory having refrained from use of inhaled β_2-agonists and ingestion of caf-

feine-containing products for at least 8 hours and having refrained from vigorous exercise on the morning of each visit. Study periods were separated by at least 2 weeks.

Screening. On day 1 of period 1, spirometric tests were performed to ensure subjects had an FEV_1 greater than 70% of predicted value. An allergen skin prick test was performed to determine atopic status, followed by a skin prick titration with doubling dilutions of the allergen that produced the greatest wheal on the skin test. A methacholine inhalation challenge was performed to determine the methacholine PC_{20}. The end point of the allergen skin prick titration and methacholine PC_{20} were used to estimate the PC_{20} of the inhaled allergen extract by using the formula described by Cockcroft et al.[31] Study day 2 took place 1 to 3 days after day 1. An allergen inhalation challenge was performed to document the presence of an EAR and LAR to the inhaled allergen; the starting concentration of allergen extract for inhalation was 2 doubling concentrations below the estimated allergen PC_{20}.

To be eligible for entry into the treatment period of the study, subjects were required to exhibit both a methacholine PC_{20} less than 32 mg/mL on day 1 of period 1 and an allergen-induced EAR (defined as >15% fall in FEV_1 between 0 and 3 hours after allergen inhalation) and allergen-induced LAR (defined as >15% fall in FEV_1 between 3 and 7 hours after allergen inhalation).

Treatment periods. Subjects completed 2 treatment periods, 1 with administration of pranlukast and 1 with administration of matching placebo. Subjects were entered into the treatment sequence according to a randomized schedule. Each period consisted of 6 consecutive days. On day 1, a methacholine inhalation challenge was performed. Because the airways response to inhaled allergen is determined, in part, by the level of airway responsiveness, each treatment period was started only when the methacholine PC_{20} had returned to within a single doubling concentration of the value determined at day 1 of period 1.

Subjects began taking study medication in the clinic on day 1. Pranlukast (3×150 mg) or matching placebo was taken orally twice daily after breakfast and an evening meal on days 1 to 5 and on the morning of day 6. On day 4, a methacholine inhalation challenge was performed 2 hours after administration of the morning dose of medication. On day 5, an allergen inhalation challenge was performed 2 hours after administration of the morning dose of medication. On Day 6, a methacholine challenge was performed 2 hours after administration of the morning dose of medication (approximately 24 hours after inhalation of allergen). A follow-up visit was scheduled for 7 to 10 days after the last visit day, in which pulmonary function tests and a respiratory exam were performed.

Challenge procedures

Methacholine inhalation challenges were performed according to the method of Cockcroft et al.[32] Subjects inhaled saline followed by increasing doubling concentrations of methacholine chloride using a Wright nebulizer (output = 0.13 to 0.15 mL/min; mass median aerodynamic diameter of particles = 1.3 μ). The nose was clipped and aerosols were inhaled through a mouth piece during tidal breathing for 2 minutes. FEV_1 was measured by using a water-sealed spirometer (Warren E. Collins, Inc). The test was continued until a 20% fall in FEV_1 from postsaline baseline was obtained. The PC_{20} was interpolated from individual dose-response curves drawn on a semilogarithmic noncumulative scale. Solutions of methacholine chloride were stored at 4° C and administered at room temperature.

Allergen inhalation challenges were performed as previously described[1] by using a Wright nebulizer operated by oxygen at 50 psi and at a flow rate that gave an output of 0.13 mL/min. FEV_1 was measured by using a water-sealed spirometer, with triplicate FEV_1 measurements at baseline and single FEV_1 measurements after allergen inhalation; volumes were recorded at body temperature, atmospheric pressure, and saturated with water vapor. For the screening allergen challenge in period 1, doubling concentrations of allergen were inhaled by tidal breathing (nose clipped) for 2 minutes, with FEV_1 measured 10 minutes after each inhalation. Inhalations were stopped when the FEV_1 had fallen by at least 15% from baseline. FEV_1 was subsequently measured at 20, 30, 45, 60, 90, and 120 minutes and at hourly intervals up to 7 hours after allergen inhalation. During the treatment periods (periods 2 and 3), only the 3 highest concentrations of allergen used in period 1 were inhaled. House dust extracts were obtained from Miles/Hollister-Stier (Mississagua, Ontario), and grass extracts were obtained from Dr Jerry Dolovich (Hamilton, Ontario). For each subject, allergen extracts from the same batch were used during the screening and both treatment periods. Allergen extracts were stored at –70° C and diluted in PBS with 1.5% benzyl alcohol for skin tests and allergen inhalation on the day of use.

Measurement of plasma levels of pranlukast. On day 5 of periods 2 and 3 (allergen inhalation challenge days), blood samples were obtained before and at 2, 5, and 8 hours after administration of medication for measurement of plasma levels of pranlukast (SB-205312) and its metabolite (SB-240103).

Analysis

Airways responses to inhaled allergen were expressed as the percent fall in FEV_1 from preallergen baseline and plotted against time. In addition, for each subject, the maximal percent decrease in FEV_1 from baseline during the EAR and LAR was recorded, and the trapezoidal area under the percent change in FEV_1-time curve for the EAR ($AUC_{0\text{-}3h}$) and the LAR ($AUC_{3\text{-}7h}$) was calculated.

The method of Hills and Armitage[33] for analysis of a 2-period cross-over study was used to compare (1) the maximal percent decrease in FEV_1 from baseline during the EAR, (2) the maximal percent decrease in FEV_1 from baseline during the LAR, (3) $AUC_{0\text{-}3h}$, and (4) $AUC_{3\text{-}7h}$ between the 2 treatment periods (pranlukast and placebo, $n = 10$). Summary statistics are expressed as arithmetic means and SEM.

All analyses of methacholine PC_{20} were performed on log-transformed values, with summary statistics expressed as geometric mean and percent SEM. The effect of pranlukast and placebo on allergen-induced increases in airway responsiveness was assessed by comparing the difference (log PC_{20} [24 hours after allergen inhalation] – log PC_{20} (24 hours before allergen inhalation]) by using the method of Hills and Armitage[33] for analysis of a 2-period cross-over study ($n = 9$); a negative difference signified an increase in airway responsiveness. One subject was excluded from this analysis because the postallergen methacholine challenge was delayed for 24 hours because of insufficient recovery of the baseline FEV_1 (<60% of predicted value) on day 6.

Probability values less than 0.05 (2-tailed) were considered statistically significant. In all analyses, tests for period and carry-over effects were performed, and no significant period or carry-over effects were observed.

RESULTS

Treatment with pranlukast attenuated allergen-induced EARs and LARs (Fig. 1). The maximal percent fall in FEV_1 during the EAR was 30.0% (SEM, 5.1%) during placebo treatment and 15.5% (SEM, 3.5%) during pranlukast treatment (mean treatment difference, 14.5; 95% CI, 5.3 to 23.7; $P = .007$) (Table II). The $AUC_{0\text{-}3h}$ during the EAR was 55.7%.h (SEM, 10.3%.h) during placebo treatment and 21.5%.h (SEM, 5.7%.h) during pranlukast

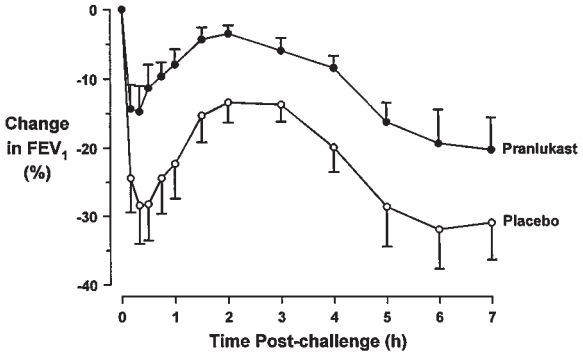

FIG. 1. Mean (± SEM) percent change in FEV_1 from baseline during the EAR and LAR to allergen inhalation after pranlukast *(filled circles)* and placebo *(open circles)* pretreatment.

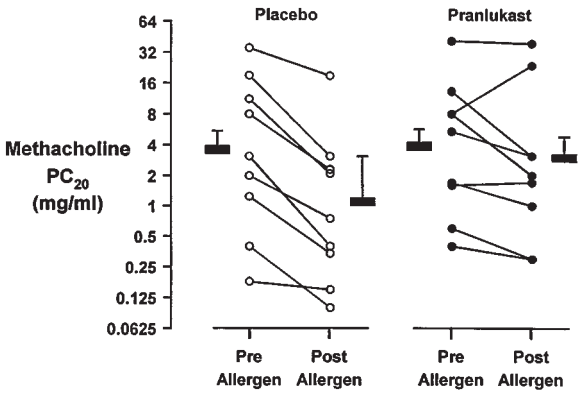

FIG. 2. Methacholine PC_{20} at 24 hours before and 24 hours after allergen inhalation following pranlukast *(filled circles)* and placebo *(open circles)* pretreatment.

treatment (mean treatment difference, 34.2; 95% CI, 14.6 to 53.8; $P = .004$). The maximal percent fall in FEV_1 during the LAR was 34.7% (SEM, 5.3%) during placebo treatment and 24.0% (SEM, 4.4%) during pranlukast treatment (mean treatment difference, 10.7; 95% CI, 4.1 to 17.3; $P = .006$). The $AUC_{3\text{-}7h}$ during the LAR was 99.4%.h (16.1%.h) during placebo treatment and 57.0%.h (10.6%.h) during pranlukast treatment (mean treatment difference, 46.7; 95% CI, 24.8 to 68.6; $P = .002$). The mean protection afforded by pranlukast for the allergen-induced EAR was 48.3%, and that for the LAR was 30.8% (Table II).

Treatment with pranlukast also attenuated allergen-induced AHR (Fig. 2). The baseline methacholine PC_{20} was not significantly different between treatments, with the mean values being 2.28 mg/mL (%SEM, 1.65) during placebo treatment and 2.46 mg/mL (%SEM, 1.63) during pranlukast treatment ($n = 10$). Twenty-four hours after allergen inhalation, the mean values were 3.55 mg/mL (%SEM, 1.92) during placebo treatment and 3.94 mg/mL (%SEM, 1.77) during pranlukast treatment ($n = 9$). Allergen inhalation resulted in a reduction in the methacholine PC_{20} to 1.05 mg/mL (%SEM, 1.95) during placebo treatment and to 3.03 mg/mL (%SEM, 1.78) during pranlukast treatment ($n = 9$) (Fig. 2). The mean allergen-induced shift in PC_{20} was –1.76 (SEM, 0.32) doubling doses during placebo treatment and –0.38 (SEM, 0.31) doubling doses during pranlukast treatment (mean difference, –1.38 doubling doses; 95% CI, 0.44 to 2.32; $P = .012$). The baseline FEV_1 immediately before the allergen inhalation was not significantly different during the 2 treatment periods, being 3.27 L (0.15 L) during placebo treatment and 3.30 L (0.15 L) during pranlukast treatment.

On the day of the allergen challenge, after 4 days of pranlukast treatment, the mean area under the concentration-time curve ($AUC_{0\text{-}8h}$) for the plasma concentrations of pranlukast was 4400 ng.h/mL (SD, 2077 ng.h/mL) and 409 ng.h/mL for its metabolite (SB-204103) (SD, 270 ng.h/mL). Mean plasma concentrations before challenge were 679 ng/mL (SD, 436 ng/mL) for pranlukast and 44 ng/mL (SD, 36 ng/mL) for SB-240103, whereas the maximal measured plasma concentrations were 1050 ng/mL (SD, 445 ng/mL) for pranlukast and 94 ng/mL (SD, 59 ng/mL) for SB-240103. After placebo administration, neither pranlukast nor SB-240103 were measurable in any plasma samples.

DISCUSSION

This study has demonstrated that the cysLT receptor antagonist pranlukast produces a partial reduction in EAR, LAR, and AHR after allergen inhalation challenge in subjects with mild asthma. These results confirm previous studies with regard to EAR and LAR and add support to the hypothesis that cysLTs are important mediators during both EARs and LARs. In addition, this study has shown, for the first time, that the AHR seen at 24 hours after allergen inhalation is attenuated by a $cysLT_1$-receptor antagonist, indicating that the release of the cysLTs are important in causing this allergen-induced response. This effect was not a direct effect of pranlukast on methacholine airway responsiveness because treatment with pranlukast for 3 days had no effect on methacholine airway responsiveness measured before allergen inhalation.

Studies in both animals and human subjects have demonstrated that pranlukast (SB-205312, ONO-1078) is a potent cysLT receptor antagonist. Pranlukast inhibits LTD_4-induced contractions of guinea pig ileum and trachea in vitro[21] and produces concentration-dependent shifts in the LTC_4 and LTD_4 concentration-response curves in human bronchi in vitro with a potency that is 100 times greater than FPL-55712, the first cysLT receptor antagonist.[22] Orally administered pranlukast produces a dose dependent attenuation of intravenous LTC_4- and LTD_4-induced contractions in guinea pig airways in vivo and significantly inhibits ovalbumin-induced bronchoconstriction in sensitized guinea pigs.[21] In human subjects pranlukast produced a 26-fold increase in the PC_{35} sGaw for LTD_4 in normal subjects.[23] By comparison, first generation cysLT antagonists, such as L649,923[34] and LY1718833,[35] produced 3- to 5-fold shifts in the LTD_4 dose-response curve.

TABLE II. Early and late airway responses and methacholine responsiveness after allergen inhalation during pranlukast and placebo treatment*

	Placebo	Pranlukast	Percent inhibition	*P* value
Baseline FEV_1	3.27 (0.15)	3.30 (0.15)	—	NS
EAR (0 to 3 hrs)				
Maximum percent fall in FEV_1	30.0 (5.1)	15.5 (3.5)	48.3	.007
AUC FEV_1	55.7 (10.3)	21.5 (5.7)	61.4	.004
LAR (3 to 7 hrs)				
Maximum percent fall in FEV_1	34.7 (5.3)	24.0 (4.4)	30.8	.006
AUC FEV_1	99.4 (16.1)	57.0 (10.6)	42.7	.002
log difference PC_{20}	–1.76 (0.32)	–0.38 (0.31)	78.4	.012

log difference PC_{20} = log PC_{20} after allergen inhalation – log PC_{20} after allergen inhalation.
*Values are means ± SEM.

Pranlukast is also a selective inhibitor of the $cysLT_1$-receptor. In guinea pigs pranlukast failed to inhibit bronchoconstriction induced by LTB_4, arachidonic acid, $PGF_{2\alpha}$, and acetylcholine,[21] whereas in an in vitro study on human tracheal and bronchiolar smooth muscle, pranlukast was unable to antagonize contractions induced by $PGF_{2\alpha}$, histamine, carbachol, or KCl.[36] Although another study found that pranlukast slightly but significantly inhibited contractions induced by histamine and $PGF_{2\alpha}$, this may be explained by $PGF_{2\alpha}$- and histamine-induced release of small amounts of LTs from human bronchial tissues, rather than by a lack of selectivity per se.[22]

The attenuation of the maximum percent fall in FEV_1 by 48% during the EAR and by 31% during the LAR after treatment with pranlukast is consistent with results of other studies that have examined the effect of cysLT antagonists and LT biosynthesis inhibitors on allergen-induced airways responses. Pretreatment with the orally available cysLT antagonist ICI 204,219 resulted in an 81% attenuation of the maximum percent fall in FEV_1 during the EAR and a 54% attenuation during the LAR.[16] Intravenous administration of the cysLT antagonist MK-571 resulted in an attenuation of the maximum percent fall in FEV_1 of 61% during the EAR and 60% during the LAR,[17] whereas administration of the orally available MK-0476 resulted in an attenuation of 52% and 36% during the EAR and LAR, respectively.[37] The FLAP antagonists MK-0591, MK-886, and BAY x1005 have shown similar degrees of inhibition, with attenuation of the maximum percent fall in FEV_1 ranging from 29% to 57% during the EAR and from 58% to 87% during the LAR.[18-20]

The mechanism by which pranlukast attenuates the EAR is probably through modulation of the cysLT constrictor effects on bronchial smooth muscle. In vitro studies on antigen-induced contraction of human bronchial strips have suggested that the action of cysLTs occurs later than the action of histamine during the EAR.[38] The incremental allergen-dosing regimen used in this study does not allow us to examine the time dependency of the effects of pranlukast on the early allergen-induced bronchoconstrictor response. However, in a previous study, 1 week of treatment with 150 mg twice daily pranlukast resulted in significant attenuation of the percent fall in FEV_1 from 20 to 60 minutes but not in the first 10 minutes after a single dose of allergen inhalation.[24] There does appear to be some degree of overlap between the timing of the cysLT- and histamine-induced bronchoconstriction because the maximal percent fall in FEV_1 during the EAR is inhibited to a greater extent after combined pretreatment with a cysLT antagonist and an H_1 receptor antagonist compared with treatment with either the cysLT or H_1 antagonist alone.[39]

It is interesting to speculate whether the attenuation of the LAR by pranlukast is a result of antagonism of the action of cysLTs released by inflammatory cells (eg, eosinophils) in the airways during the LAR or a reduction in the influx of inflammatory cells into the airways by antagonizing the proinflammatory effects of cysLTs. Evidence is available in support of proinflammatory properties of cysLTs. In allergic sheep, inhalation of LTD_4 results in the development of an early and a late increase in pulmonary resistance,[40] although inhaled LTD_4 did not produce a similar LAR in atopic asthmatic subjects who had demonstrated a late response to allergen.[41] Laitinen et al.[42] have shown that inhalation of LTE_4 by asthmatic subjects resulted in a selective increase in the number of eosinophils and neutrophils in the lamina propria, and Diamant et al.[43] have shown that LTD_4 given to asthmatic subjects resulted in marked eosinophilia in induced sputum 4 hours later. Recent animal and human experiments have shown that cysLT receptor antagonists can inhibit cysLT- and allergen-induced inflammatory responses in the airways. In guinea pigs administration of pranlukast attenuated the LTD_4-induced pulmonary microvascular leakage and eosinophil influx into the airways and also antagonized antigen-induced eosinophil influx in ovalbumin-sensitized guinea pigs.[25] In a recent study by Calhoun et al.,[44] the potent cysLT receptor antagonist ICI 204,219 reduced the number of eosinophils and basophils recovered in bronchoalveolar lavage fluid in asthmatic patients challenged with allergen compared with placebo 48 hours after a segmental allergen challenge.

Although consistent results from several recent studies have established a role for cysLTs in the pathogenesis of the EAR and LAR, a role of cysLTs in the development

of allergen-induced AHR is less certain. Taylor et al.[16] observed a significant attenuation of AHR 6 hours after allergen inhalation following pretreatment with the cysLT receptor antagonist ICI 204,219, but Friedman et al.[18] and Diamant et al.[19] were unable to confirm this finding at 24 and 30 hours after allergen inhalation following treatment with the FLAP antagonists MK-886 and MK-0591, respectively. This study is the first to demonstrate significant attenuation of allergen-induced AHR in asthmatic subjects 24 hours after allergen inhalation. This may be because all of the previously reported studies were likely underpowered to be able to demonstrate such an effect.[45]

Increased airway responsiveness has been shown after inhalation of cysLTs. In normal subjects some studies have shown cysLT-induced increases in airway responsiveness to histamine,[46,47] but this has not been a consistent finding.[48,49] In asthmatic subjects a single dose of LTE_4 that produced a mean reduction in specific conductance (sGaw) of 41% resulted in an increase in responsiveness to histamine at 1 hour after administration that lasted up to 1 week.[50]

Given the proinflammatory effects of cysLTs and the strong association between the allergen-induced influx of inflammatory cells into the airways, the development of a LAR, and increases in airway responsiveness,[2,3,51] it is tempting to ascribe the reduction in AHR seen in this study to a pranlukast-mediated attenuation of the inflammatory influx into the airways. CysLTs may also contribute to allergen-induced AHR through other mechanisms. Lee et al.[52] demonstrated that pretreatment with LTE_4, but not LTC_4 and LTD_4, enhances the contractile response to histamine in guinea pig tracheal spirals in vitro. Furthermore, pretreatment with the cyclooxygenase inhibitor indomethacin did not suppress contractions elicited by LTE_4 but completely inhibited the augmentation of the histamine response. It has also been shown that indomethacin has no effect on the allergen-induced EAR or LAR but significantly attenuates allergen-induced AHR in asthmatic subjects.[53] Thus it may be speculated that a component of the allergen-induced AHR is mediated by means of cysLT-induced generation of cyclooxygenase products independent of any mechanisms associated with cysLT-mediated inflammatory responses.

In summary, the results of this study have shown that the cysLT receptor antagonist pranlukast partially attenuates allergen-induced EARs, LARs, and AHR, adding further support to the hypothesis that cysLTs are important mediators in both the allergen-induced EAR and LAR in asthmatic subjects and implicating cysLTs as mediators in the AHR seen 24 hours after allergen inhalation.

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