Montelukast causes prolonged, potent leukotriene D_4–receptor antagonism in the airways of patients with asthma

Montelukast, a new specific oral cysteinyl LT_1-receptor antagonist was evaluated for its activity in attenuating inhaled leukotriene D_4 (LTD_4) bronchoconstriction in patients with asthma. In two double-blind, placebo-controlled, randomized crossover studies, patients with mild asthma (forced expiratory volume in 1 second [FEV_1] ≥70%) were studied. In trial A, LTD_4 challenge began 4 hours (peak plasma concentration) after a single dose of placebo or 5, 20, 100, and 250 mg montelukast. In trial B, an LTD_4 challenge was started 20 hours after administration of placebo, 40 mg montelukast, or 200 mg montelukast. During each challenge, twofold increasing concentrations of LTD_4 were inhaled until specific airways conductance (sGaw) decreased by at least 50% (PC_{50}) or the highest concentration of LTD_4 was inhaled. In trial A with all doses and in trial B with the 200 mg dose, bronchoconstriction was attenuated (50% fall in sGaw was not observed) up to the highest dose of LTD_4 administered. In trial B, during the 40 mg period, only two of six patients exhibited a 50% fall in sGaw; PC_{50} ratios (montelukast 40 mg/placebo) were 18 and 45 in these two patients. These results indicate that montelukast is a highly potent and long-lasting antagonist of LTD_4-induced bronchoconstriction in patients with asthma. (Clin Pharmacol Ther 1997;61:83-92.)

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There is accumulating evidence that the cysteinyl leukotrienes C_4, D_4, and E_4 (LTC_4, LTD_4, and LTE_4), components of the slow-reacting substance of anaphylaxis[1] play an important role in bronchial asthma. These leukotrienes are potent bronchoconstrictors, being up to 1000 times more potent than histamine or methacholine in both normal and asthmatic subjects.[1-5] In in vitro and in vivo animal models, these substances cause mucus production, decreased mucociliary clearance, and increases in vascular permeability.[6,7]

Clinical trials using potent cysteinyl leukotriene (Cys LT_1) receptor antagonists have shown the importance of these leukotrienes in bronchial asthma.[8-12] Montelukast, a competitive and selective Cys LT_1-receptor antagonist, is one of the most potent compounds yet described. Montelukast competes against ^{3}H-LTD_4 for the receptor in human u937 cells in the presence of 1% human plasma and has a 50% receptor inhibiting concentration (IC_{50}) of approximately 0.7 nmol/L.[13]

The studies described in this report were designed to determine the potency of montelukast as a Cys LT_1-receptor antagonist in the airways of patients with asthma. To accomplish this objective, the degree and duration of receptor blockade were determined by performing inhalational LTD_4 challenges after oral administration of montelukast. These effects were investigated between 4 and 8 hours (peak plasma concentrations) and between 20 and 24 hours (at the end of a once daily dosing interval) after administration.

METHODS

Patients

Nine nonsmoking male patients, between 21 and 47 years of age, participated in these trials

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Table I. Demographic data

Patient No.	*Age (yr)*	*Height (cm)*	*Weight (kg)*	*Prestudy FEV_1 (% predicted)*	*Asthma medication*
Trial A					
1	27	174	62	97.1	
2	35	174	85	81.9	Fenoterol
3	27	174	60	70.5	Albuterol (salbutamol)
4*	40	182	70	70.2	
5*	46	176	75	77.2	
6*	22	186	79	91.7	
7*	21	178	70	94	
8*	21	186	75	82.7	
Trial B					
4	40	182	70	71.2	
5	47	176	75	79	
6	23	186	79	84.2	
7	21	178	70	96	
8	21	186	75	75.3	
9	21	179	67	99.3	
Median	25	178.5	72.5	82.3	
Range	21-47	174-186	60-85	70.2-99.3	

FEV_1, Forced expiratory volume in 1 second.
*Patient participated in both trial A and trial B.

(eight in trial A, six in trial B; five patients in trial A also participated in trial B). Each participant had mild asthma as shown by clinical history and baseline forced expiratory volume in 1 second (FEV_1) ≥70% of the predicted value after inhaled bronchodilators were withheld for at least 6 hours. Patients also demonstrated reversibility of airway obstruction (a ≥15% increase in absolute FEV_1 after β-agonist inhalation) or airway hyperreactivity to histamine or methacholine bronchoprovocation within the previous 12 months. In addition, patients had been free of respiratory infection symptoms for at least 4 weeks before the start of the study and had been nonsmokers for at least 1 year. Patients did not require long-term asthma therapy; all participants used inhaled β-agonist as needed. Patients were excluded if they had concomitant medical disorders as determined by history and by physical, hematologic, chemistry, urinalysis, and electrocardiographic examinations.

Patient demographic data are summarized in Table I. The protocol of both studies was approved by the Ethical Committee of the University Hospitals Leuven, and written informed consent was obtained from all participants.

Study design

General. The inhibition of LTD_4-induced bronchoconstriction by oral montelukast 4 to 8 hours after dosing (at peak plasma concentration)[14] and 20 to 24 hours (at the end of a once-daily dosing interval) after administration was assessed in separate studies. In each trial, two clinic visits preceded double-blind, randomized, placebo-controlled crossover periods. The number of treatment periods (there were at least 3 days between each treatment) and the duration of dosing (montelukast or placebo) depended on the individual trial.

On the day of inhalational challenge, each patient had an FEV_1 ≥70% of the predicted value after β-agonist was withheld for at least 6 hours. For an individual patient, all LTD_4 inhalational challenges were performed at the same time of the day.

LTD_4-induced bronchoconstriction was measured by the change in specific airways conductance (sGaw) as determined by body plethysmography. The concentration of LTD_4 causing a decrease in sGaw of 50% (PC_{50}) was considered the end point in all treatment periods. The ratio ("fold shift'") of the PC_{50} values (montelukast/placebo) was used as a measure of montelukast

potency as an antagonist of airway LTD_4-receptors.

To qualify for the double-blind portion of either trial, patients were required to demonstrate reproducible bronchoconstriction to inhaled LTD_4 (within two doubling doses) during the two prestudy "baseline'" visits.

Trial A: 4 to 8 hours after administration (peak plasma concentration). To determine the level of inhibition present at the peak plasma concentration (approximately 4 to 6 hours after oral administration), the effect of four single oral doses of montelukast (5, 20, 100, and 250 mg) and placebo were evaluated. This trial was separated into two parts. Part 1 consisted of two panels (A and B) of three patients each. Treatment in panel A was 100 and 250 mg montelukast and placebo, whereas patients in panel B received 20 and 100 mg montelukast and placebo. In part 2, patients received lower doses: 5 and 20 mg montelukast and placebo. In each part, patients received their study medications in a randomized crossover manner. Inhalational LTD_4 challenge was started approximately 4 hours after administration of drug or placebo and was completed by 8 hours during each treatment period.

Trial B: 20 to 24 hours after administration. To determine the level of inhibition at the end of a once-daily dosing interval, (approximately 20 to 24 hours after dosing) six patients with asthma received, during three separate study periods, either 40 mg montelukast, 200 mg montelukast, or placebo, in a randomized crossover manner. In this trial, patients received their doses with the evening meal on 2 consecutive days, sufficient to achieve steady-state concentrations (plasma half-life, 4 hours).[14] During the third day of each period, inhalational LTD_4 challenge was started approximately 20 hours after and completed by 24 hours after the second daily dose.

Prestudy challenges

A set of at least six sGaw values was obtained before and after the inhalation of LTD_4 diluent. Each patient was required to demonstrate consistency of the prediluent and postdiluent mean sGaw values (the mean postdiluent sGaw value was within 20% of prediluent mean value). The postdiluent mean sGaw value was used as the baseline for subsequent PC_{50} calculations.

Inhaled LTD_4 was administered in serial doubling concentrations at 10- to 15-minute intervals. A set of at least six sGaw measurements was made at 3, 5, and 10 minutes after each LTD_4 concentration; the mean was calculated at each time point. If the mean at 5 minutes was greater than the mean at 3 minutes by 10% or more, the 10-minute sGaw measurements were not performed. The 10-minute sGaw measurements were also omitted if the mean value at 5 minutes was greater than the mean value at 3 minutes by less than 10%, but within 20% of the patient's baseline (postdiluent) mean value. The lowest of the 3-, 5-, or 10-minute mean values obtained after each LTD_4 concentration was used as the true value for that concentration.

The challenge was stopped if there was a mean fall in sGaw of more than 50% or if the maximal concentration of LTD_4 were reached. sGaw was measured every 10 minutes after LTD_4 challenge was completed until a value within 20% of the postdiluent mean value was observed. A patient was entered into the study only if the two prestudy PC_{50} sGaw values were within two doubling concentrations.

Study challenges

During each patients treatment periods, the initial LTD_4 concentration was approximately two doubling concentrations below the lower of the two PC_{50} values obtained during the prestudy challenges. After inhalation of diluent, twofold increasing concentrations of LTD_4 were inhaled at 10- to 15-minute intervals. At least six measurements of sGaw were taken at 3, 5, and 10 minutes after each inhalation. The mean value was calculated at each time point. The minimum mean value was used in the calculation of the concentration of LTD_4 that caused a 50% decrease in sGaw (PC_{50} sGaw). The LTD_4 challenge was continued until sGaw decreased by at least 50% or the maximum LTD_4 concentration (approximately 100-fold above the mean of the two PC_{50} values obtained on the 2 prestudy days) was reached.

Pulmonary function measurements

Airway resistance was measured with a constant-volume body plethysmograph (Medical Graphics Inc., St. Paul, Minn.). sGaw (the inverse of airway resistance measured at functional residual capacity) was calculated with standard methods.[15] The FEV_1 obtained before inhalational challenge was measured in triplicate with a Microspirometer (Micro Medical Ltd., Kent, England).

Table II. Placebo PC_{50} values compared with prestudy estimates*

Patient No.	*Prestudy test No. 1*	*Prestudy test No. 2*	*Placebo period*	*Placebo/prestudy ratio†*
Trial A				
1	0.10	0.05	0.0354	0.53
2	0.012	0.012	0.0114	0.95
3	0.025	0.025	0.0060	0.24
4‡	0.05	0.10	0.1536	2.30
5‡	0.025	0.012	0.0068	0.42
6‡	0.003	0.006	0.0138	3.45
7‡	0.80	0.80	0.6600	0.83
8‡	0.024	0.10	0.0738	1.91
Trial B				
4	0.05	0.10	0.0862	1.29
5	0.024	0.012	0.0353	2.21
6	0.003	0.006	0.0104	2.60
7	0.80	0.80	0.7592	0.95
8	0.024	0.10	0.0852	2.20
9	0.10	0.20	0.0953	0.71

PC_{50}, Provocative concentration of leukotriene D_4 (μg/ml) that caused a 50% fall in specific airway conductance.
*Values represent the first LTD_4 doubling dose concentration causing a 50% or greater fall in sGaw.
†Average of two prestudy values.
‡Patient participated in both trial A and trial B.

Aerosolization

Aerosol generation was performed by an efficient jet nebulizer (Mallinckrodt Diagnostica, Petten, Holland).[16] The nebulizer was filled with 0.5 ml of each concentration of LTD_4 (or diluent), all of which was sprayed by compressed nitrogen during 1 minute into a drying chamber (a 30 L collapsible plastic bag). Subsequently, all aerosol was inhaled by tidal breathing over 3 to 4 minutes while oxygen was supplied into the mouthpiece at a flow of 4 L/min, with the patient wearing a nose clip.

LTD_4 inhalation challenges

Aliquots of the stock LTD_4 solution (Cascade Biochem Ltd., Reading, Berkshire, England) were stored under nitrogen at −70° C in vials containing 200 mg/ml 1:4 ethanol–phosphate buffered saline buffer at pH 7.2 (diluent). Immediately before use, twofold serial dilutions were prepared and kept on ice under nitrogen.

Montelukast

Study medication (Merck Research Laboratories, Rahway, N.J.) was supplied as hard gelatin capsules. All active drug and placebo capsules were identical in appearance.

Montelukast drug assay

Plasma for montelukast concentration was obtained immediately before dosing (as a negative control) and then at the completion of the LTD_4 challenge. Samples were protected from exposure to light and were maintained frozen at −70° C until analysis. Montelukast plasma concentration was determined to a limit of 30 ng/ml with use of HPLC.[17]

Safety measurements

Tolerability was determined by physical examination (including vital signs), electrocardiogram, blood chemistry and hematology, and urinalysis. Spontaneously reported adverse events were recorded during each treatment period.

Data analysis

The LTD_4 concentration-response curves were obtained by plotting the percentage change in sGaw (using the postdiluent mean as the baseline value) against the LTD_4 (log_2) concentration. In those periods in which at least a 50% fall in sGaw was achieved, the PC_{50} value was determined by linear interpolation. In those periods in which a 50% decrease in sGaw was not achieved, the PC_{50} value was recorded as the highest concentration of LTD_4 administered to the airway preceded by a "greater than'" sign (the true PC_{50} value was at least this large).

Potency was determined as the ratio of the PC_{50} value for an individual montelukast dose compared with the placebo value (PC_{50} montelukast dose/PC_{50} placebo). Because each patient served as his own

Table III. Trial A: Individual PC_{50} sGaw values and fold shifts*

	Part 1, panel A				
	PC_{50} sGaw (μg/ml)			*Fold shift vs placebo*	
Patient No.	*250 mg*	*100 mg*	*Placebo*	*250 mg*	*100 mg*
1	>6.4	>6.4	0.0354	>181	>181
3	>3.2	>6.4	0.0052	>613	>1225
4	>6.4	>3.2	0.1536	>42	>21
	Part 1, panel B				
	PC_{50} sGaw (μg/ml)			*Fold shift vs placebo*	
	20 mg	*100 mg*	*Placebo*	*20 mg*	*100 mg*
2	>1.6	>1.6	0.0114	>141	>141
5	>1.6	>1.6	0.0068	>237	>237
6	>0.4	>0.4	0.0138	>29	>29
	Part 2				
	PC_{50} sGaw (μg/ml)			*Fold shift vs placebo*	
	20 mg	*5 mg*	*Placebo*	*20 mg*	*5 mg*
3	>1.6	>1.6	0.0067	>240	>240
7	>50	>50	0.6600	>76	>76
8	>6.3	>6.3	0.0738	>85	>85

sGaw, Specific airway conductance.
*Median fold shift for the 5, 20, 100, and 250 mg groups were at least 85, 113, 161, and 181 folds, respectively.

placebo control in these crossover trials, ratios were determined within patients. When appropriate, individual median PC_{50} or ratio (PC_{50} montelukast/PC_{50} placebo) values were determined within treatment groups. In addition, the consistency of the bronchial response to inhaled LTD_4 was determined as the ratio of the placebo value (double blind) compared with the average of the two prestudy (open) values. All other data are presented as mean ± SD unless otherwise noted.

RESULTS

LTD_4 challenge reproducibility

All patients demonstrated reproducible prestudy LTD_4 challenges; in each patient, the two prestudy values were within two doubling concentrations. In addition, there was little difference between the prestudy estimates and placebo period PC_{50} values for each patient (Table II).

Trial A: 4 to 8 hours after administration (peak plasma concentration)

There were no important differences in postdiluent (baseline) sGaw values among treatment groups and periods (mean ± SD: 0.87 ± 0.40, 0.96 ± 0.43, 0.98 ± 0.46, 0.97 ± 0.25, and 0.78 ± 0.09 for the placebo and the 5, 20, 100, and 250 mg groups, respectively). PC_{50} values for all periods are summarized in Table III. Individual responses are presented graphically in Fig. 1. Mean montelukast plasma concentrations for all dose levels obtained at the completion of the LTD_4 challenge are given in Table IV (predosing concentrations were undetectable). A ≥50% fall in sGaw (up to the maximum concentration of LTD_4 administered) was not observed during any montelukast treatment (5 to 250 mg).

Trial B: 20 to 24 hours after administration

Postdiluent (baseline) sGaw measurements did not differ significantly among the different treatment groups or periods (mean ± SD: 0.93 ± 0.44, 1.14 ± 0.41, 1.12 ± 0.55 for the placebo, 40 mg, and 200 mg groups, respectively). PC_{50} values for all treatment periods are summarized in Table V. Individual responses are represented graphically in Fig. 2. All montelukast plasma concentrations at the completion of the LTD_4 challenge are shown in Table V (all predosing concentrations were undetectable.)

During the 200 mg periods, a ≥50% fall in sGaw (up to the maximum concentration of LTD_4

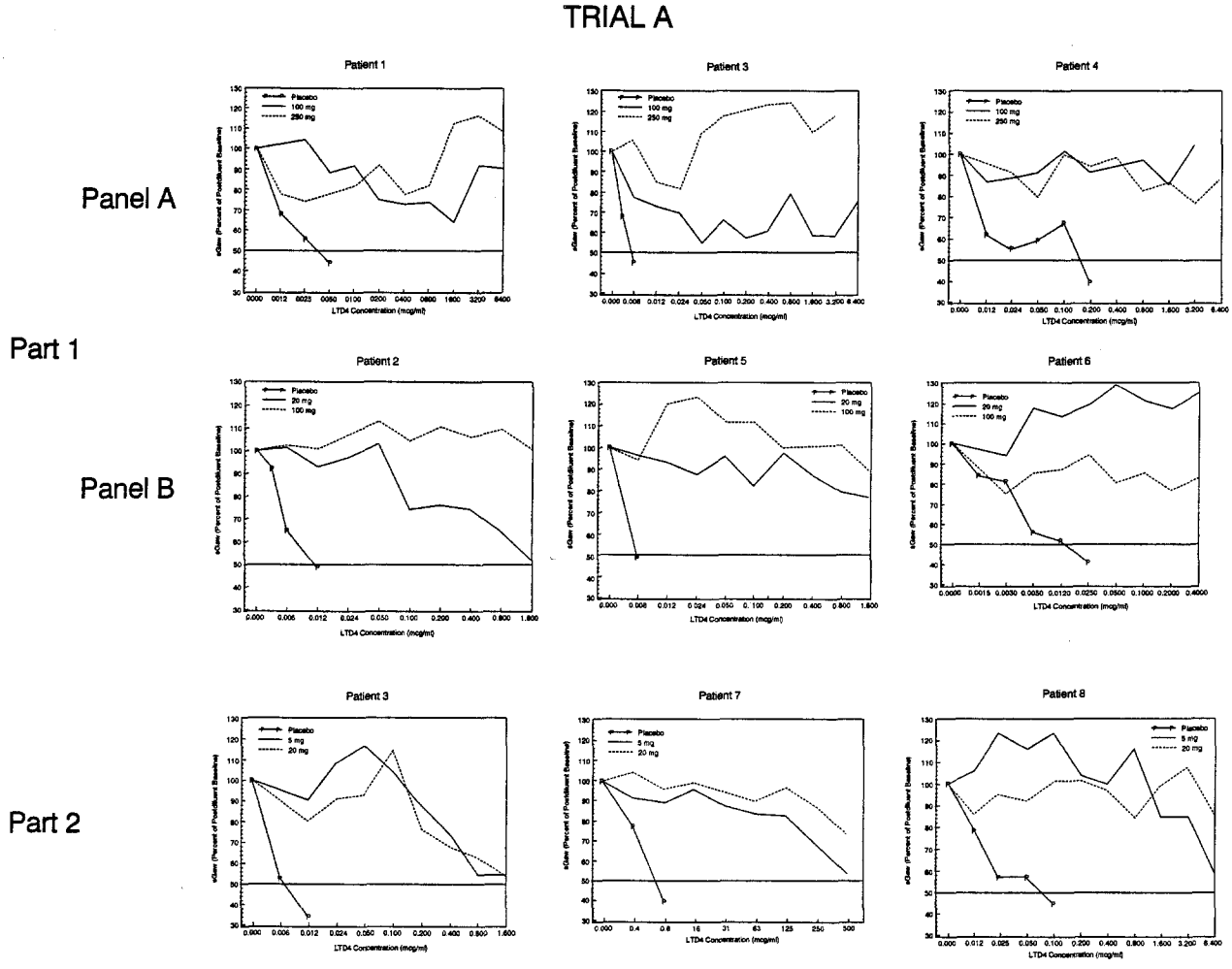

Fig. 1. Trial A: Specific airway conductance (sGaw) value (as a percentage of postdiluent baseline) plotted against dose of inhaled leukotriene D_4 (LTD_4; in micrograms per milliliter) by patient for each treatment period. One patient (patient 3) was used in two different parts of the study.

Table IV. Trial A: Postchallenge montelukast plasma concentration (μg/ml)

Treatment	*Mean ± SD*
5 mg	0.12 ± 0.07
20 mg	0.44 ± 0.20
100 mg	1.40 ± 0.64
250 mg	4.63 ± 2.51

administered) was not observed. During the 40 mg period, only two of six patients demonstrated a 50% decrease in sGaw; ratios of the PC_{50} values compared with placebo for these two patients were 18 and 45. These patients were observed to have the lowest postchallenge plasma concentrations—0.039 and <0.029 μg/ml, respectively.

Tolerability

Montelukast was well tolerated in this study. Clinically important changes in electrocardiograms, physical examinations, vital signs, or laboratory safety tests were not observed. Two adverse experiences, considered possibly related to study drug, were reported in one subject participating in trial A. This subject reported sleepiness after the 20 mg and 100 mg doses of montelukast. LTD_4 challenges were well tolerated by all patients; not only was rescue medication not required, but sGaw values returned to baseline within 40 minutes after challenge.

Table V. Trial B: Individual PC_{50} values (in micrograms per milliliter LTD_4) and fold shifts compared with placebo

	PC_{50}			*Fold shift versus placebo (montelukast plasma concentration)**	
Patient No.	*40 mg*	*200 mg*	*Placebo*	*40 mg*	*200 mg*
4	1.56	>6.3	0.0862	18 (0.039)	>73 (0.580)
5	>1.6	>1.6	0.0353	>45 (0.180)	>45 (1.410)
6	>0.8	>0.4	0.0104	>77 (0.046)	>38 (0.495)
7	>50	>50	0.7592	>66 (0.327)	>66 (2.017)
8	>6.3	>6.4	0.0852	>74 (0.050)	>75 (0.752)
9	4.33	>12.5	0.0953	45 (<0.029) >56†	>131 (0.301) >70†

LTD_4, Leukotriene D_4.
*Parentheses indicate postchallenge montelukast plasma concentration in micrograms per milliliter.
†Median fold shift.

DISCUSSION

These studies show that montelukast, a new specific Cys LT_1-receptor antagonist,[13] provides potent long-lasting inhibition of LTD_4-induced receptor activation in the airways of patients with asthma. This conclusion is supported by the observation that a single oral dose of ≥5 mg montelukast, 4 to 8 hours after dosing, prevented a ≥50% fall in sGaw due to inhaled LTD_4; 20 to 24 hours after dosing, montelukast substantially inhibited the fall in sGaw due to inhaled LTD_4 (median fold shift was at least 56 with 40 mg, the lowest dose tested). The reproducibility of inhaled LTD_4-induced bronchoconstriction shows the validity of this challenge test for defining Cys LT_1-receptor potency in the airways of patients with asthma.

Bronchoconstriction measurements in inhalational challenge studies have not been universally standardized. In our study, airway constriction was measured by sGaw and defined as a 50% decrease. Other studies have used the end point of a 20% reduction in FEV_1[18] or a 35% decrease in sGaw.[19] Specific airway conductance (using a 50% fall) may be a more sensitive measurement of bronchoconstriction than the FEV_1.[20] Moreover, measurements of specific airway conductance do not require deep inspirations, which by themselves may affect bronchomotor tone.[21]

Montelukast provides a greater duration of inhibition than a recently described Cys LT_1-receptor antagonist, ICI 204-219 (zafirlukast). In normal volunteers (using a 35% fall in sGaw as the end point), 40 mg zafirlukast caused substantial inhibition of LTD_4-induced bronchoconstriction (approximately 100-fold from the placebo response) 2 hours after oral administration[19]; however, 12 hours after this 40 mg dose, the PC_{35} sGaw was only tenfold above the placebo response. In patients with asthma, the 40 mg dose of zafirlukast (using a 20% fall in FEV_1 as the end point) also caused only a tenfold shift after 12 hours compared with placebo.[18]

Our data suggest the possibility of a relationship between the montelukast dose (and plasma concentrations) and the ability to inhibit LTD_4-induced bronchoconstriction, both at the time of the montelukast peak plasma levels (4 hours after administration) and 20 to 24 hours after administration. In general, at peak plasma concentration, the 5 mg dose resulted in greater falls in sGaw values after LTD_4 challenge compared with the higher doses (although none reached a 50% fall); 20 to 24 hours after dosing, the 40 mg periods with the lowest postchallenge montelukast plasma concentrations (<0.029 and 0.039 μg/ml) were associated with the greatest falls in sGaw—fold shifts of 45 and 18, respectively. However, a definitive, sufficiently powered study of the inhibitory effect of lower doses would be required to determine whether a true dose-concentration response relationship exists. In previous studies, using a different Cys LT_1-receptor antagonist,[18,19] a weak relationship between dose and the magnitude of the shift of the LTD_4 response curve was observed.

An assessment of the in vivo potency and duration of activity of a new receptor antagonist provides important information to determine a range of doses and dosing intervals to be used in clinical studies (the objective of these studies is clinical dose

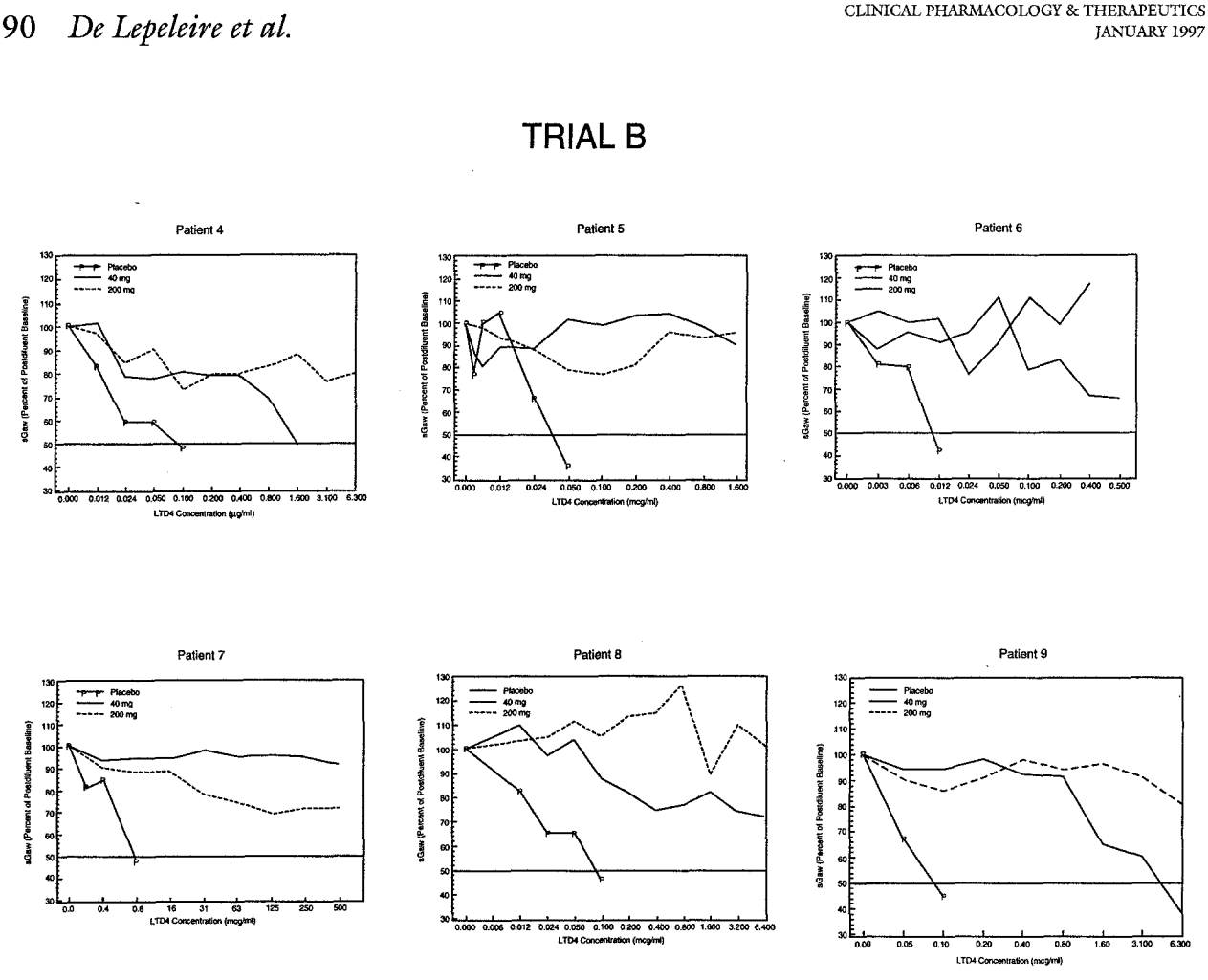

Fig. 2. Trial B: Specific airway conductance (sGaw) value (as a percentage of the postdiluent baseline) plotted against dose of inhaled leukotriene D_4 (LTD_4; in micrograms per milliliter) by patient for each treatment period. Five patients (patients 4, 5, 6, 7, and 8) from trial A also participated in trial B.

selection). The determination of this range of doses would be greatly simplified if an understanding of the relationship between potency and clinical activity were available. (The most appropriate time to assess potency during the dosing interval will be dependent on the pharmacology of an individual compound). In the case of Cys LT_1-receptor antagonists, it would be useful to know whether there was a threshold inhibition of LTD_4-induced bronchoconstriction (for example, at the end of the dosing interval) that must be achieved before clinical benefit will be observed. This issue has been addressed with β-blockers and H_1-receptor antagonists; studies with these compounds suggest that a twentyfold shift of the dose-response curve results in clinical activity.[22,23]

Recently, clinical end point data for zafirlukast have become available; 40 mg twice daily, but not lower doses, were thought to be clinically effective. These data, coupled with the LTD_4 challenge data generated in previous studies,[18,21] suggested that a fold shift of 10 at trough of the twice-daily dosing interval might be sufficient to result in a clinical response.[12] The dose of montelukast that causes a tenfold shift at trough of the once-daily dosing interval is not known; however, it can be anticipated from the data in this study that a montelukast dose <40 mg once daily will demonstrate this level of receptor blockade (which may then translate into clinical efficacy).

The evidence supporting the importance of the cysteinyl leukotrienes as mediators of human asthma has been rapidly accumulating. These leukotrienes have been recovered from the lavage fluid of patients with asthma[24-26] and were recovered in this fluid after aspirin[27] and plicatic acid[28] challenge

in patients with asthma who were sensitive to these substances. Further, increased urinary excretion of leukotrienes was observed after antigen challenge.[29] Finally, studies have shown that cysteinyl leukotrienes may be recovered from antigen-exposed, sensitized, human airway tissue[30] and that they are released from stimulated mast cells and eosinophils,[31] which are known to accumulate in the airways of patients with asthma.

In summary, the present report shows that a new Cys LT_1-antagonist, montelukast (MK-0476), is a potent prolonged inhibitor of Cys LT_1-induced bronchoconstriction in patients with asthma. The observation that montelukast, with a dose as low as 40 mg, provides nearly complete blockade 20 to 24 hours after dosing strongly suggests that ≤40 mg of this compound once daily should demonstrate activity in clinical end point studies.

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References

1. Griffin M, Weiss JW, Leitch AG, McFadden ER, Corey EJ, Ansten KF, et al. Effects of leukotriene on the airways in asthma. N Engl J Med 1983;308:436-9.
2. Smith LJ, Greenberger PA, Patterson R, Krell RD, Bernstein PR. The effect of inhaled leukotriene D_4 in humans. Am Rev Respir Dis 1985;131:368-72.
3. Adelroth E, Morris M, Hargreave FE, O'Byrne PM. Airway responsiveness to leukotrienes C_4 and D_4 and to methacholine in patients with asthma and normal controls. New Engl J Med 1986;315:480-4.
4. Bisgaard H, Groth S, Madsen F. Bronchial hyperreactivity to leukotriene D_4 and histamine in exogenous asthma. Br Med J 1985;290:1468-71.
5. Bel EH, van der Veen H, Kramps JA, Dijkman JH, Sterk PJ. Maximal airway narrowing to inhaled leukotriene D_4 in normal subjects: comparison and interaction with methacholine. Am Rev Respir 1987; 136:979-84.
6. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow reacting substances, leukotrienes C_4 and D_4 increase the release of mucus from human airways in vitro. Am Rev Respir Dis 1982;126:449-51.
7. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes (LTC_4, LTD_4, LTE_4 and LTB_4) in human skin. J Invest Dermatol 1983;80: 115-9.
8. Hui KP, Barnes NC. Lung function improvement in asthma with a cysteinyl leukotriene receptor antagonist. Lancet 1991;337:1062-3.
9. Manning PJ, Watson RM, Margolskee DJ, Williams V, Schwartz J, O'Byrne PM. Inhibition of exercise induced bronchoconstriction by MK-571, a potent leukotriene D_4 receptor antagonist. N Engl J Med 1990;323:1736-9.
10. Margolskee DJ, Bodman S, Dockhorn R, Israel E, Kemp J, Mansmann H, et al. The therapeutic effects of MK-571 a potent and selective leukotriene D_4 receptor antagonist in patients with chronic asthma. J Allergy Clin Immunol 1991;87:309.
11. Impens N, Reiss TF, Teahan JA, DeSmet M, Rossing TH, Shingo S, et al. Acute bronchodilation with an intravenously administered leukotriene D_4 antagonist, MK-679. Am Rev Respir Dis 1993;147:1442-6.
12. Spector SL, Smith LJ, Glass M. Effect of 6 weeks of therapy with oral doses of ICI 204-219 a leukotriene D_4 receptor antagonist in subjects with bronchial asthma. Am J Respir Crit Care Med 1994;150:618-23.
13. Jones TR, Labelle M, Belley M, Champion E, Charette L, Evans J, et al. Pharmacology of montelukast sodium (SINGULAIR), a potent and selective leukotriene D_4 receptor antagonist. Can J Physiol Pharmacol 1995;73:191-201.
14. Schoors DF, DeSmet M, Reiss T, Margolskee D, Cheng H, Larson P, et al. Single dose pharmacokinetics, safety and tolerability of MK-0476, a new leukotriene D_4-receptor antagonist, in healthy volunteers. Br J Clin Pharmacol 1995;40:277-80.
15. Dubois AB, Botelho S, Comroe JH Sr. A new method for measuring airway resistance in man using a body plethysmograph. J Clin Invest 1956;35:372-35.
16. Bel EH, van der Plas KH, Sterk PJ. Inhalation challenge testing: validation of a highly efficient method of aerosol generation. Bull Eur Physiopathol Respir 1986;22(suppl):166.
17. Amin RD, Cheng H, Rogers JD. Determination of montelukast in human plasma by liquid chromatography. J Pharm Biomed Anal 1995;13:155-8.
18. Smith LJ, Glass M, Minkowitz MC. Inhibition of leukotriene D_4-induced bronchoconstriction in subjects with asthma: a concentration-effect study of ICI 204-219. Clin Pharmacol Ther 1993;54:430-6.
19. Smith LJ, Geller S, Ebright L, Glass M, Thyrum PT. Inhibition of leukotriene D_4-induced bronchoconstriction in normal subjects in the oral LTD_4 receptor antagonist ICI 204-219. Am Rev Respir Dis 1990;141: 988-92.
20. Ries AL, Clausen JL. Airway resistance. In: Wilson AF, editor. Pulmonary function testing: indication and interpretation. Orlando: Harcourt Brace, 1985; 109-23.
21. Orehek J, Nicholi MM, De Lepeleire I. Influence of a previous, deep inspiration on the spirometric measurements of provoked bronchoconstriction in asthma. Am Rev Respir Dis 1981;123:269-72.
22. Cubeddu LX, Carr ME, Fuenmayor J. Beta-blockade by oral propanolol and labetalol. Clin Pharmacol Ther 1985;37:277-83.
23. Brik A, Tashkin DP, Gong H, Dauphinee B, Lee EE.

The effect of cetirizine a new histamine H_1-antagonist on airway dynamics and responsiveness to inhaled histamine in mild asthma. J Allergy Clin Immunol 1987;80:51-6.

24. Lam S, Chan H, LeRiche JC, Chang-Yeung M, Salari H. Release of leukotrienes in patients with bronchial asthma. J Allergy Clin Immunol 1988;81:711-7.
25. Pliss LB, Ingenito EP, Ingram RH. Responsiveness, inflammation, and effects of deep breaths on obstruction in mild asthma. J Appl Physiol 1989;66: 2298-304.
26. Wardlaw AJ, Hay H, Cromwell O, Collins JV, Kay AB. Leukotrienes LTC_4 and LTB_4 in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. J Allergy Clin Immunol 1989;84:19-26.
27. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary leukotriene E_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis 1991;143:1025-9.
28. Chan-Yeung M, Chan MH, Tse KS, Salari H, Lam S. Histamine and leukotrienes release in bronchoalveolar fluid during plicatic acid-induced bronchoconstriction. J Allergy Clin Immunol 1989;84:762-8.
29. Taylor GW, Taylor I, Black P, Maltby NG, Turner N, Fuller W, et al. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. Lancet 1989;1:584-7.
30. Dahlén SE, Hansson G, Hedqvist PP, Björk T, Granström E, Dahlén B. Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C_4, D_4 and E_4. Proc Natl Acad Sci USA 1983;80:1712-6.
31. Jorg A, Henderson WR, Murphy RC, Klebanoff SJ. Leukotriene generation by eosinophils. J Exp Med 1982;155:390-402.