Respiratory pathophysiologic responses

The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo

Zuzana Diamant, MD,[a] Mieke C. Timmers,[a] Hilly van der Veen,[a] Beth S. Friedman, MD,[b] Marina De Smet, PhD,[c] Marleen Depré, PharmD,[d] Deborah Hilliard, BSc,[b] Elisabeth H. Bel, PhD,[a] and Peter J. Sterk, PhD[a]

Leiden, The Netherlands, Rahway, N.J., Brussels, Belgium, and Leuven, Belgium

Background: *The 5-lipoxygenase metabolites of arachidonic acid are likely to be involved in the pathophysiology of atopic asthma. We investigated the effect of pretreatment with MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on allergen-induced early asthmatic reactions (EARs) and late asthmatic reactions (LARs), and subsequent airway hyperresponsiveness to histamine.*

Methods: *Eight atopic men with mild to moderate asthma aged 19 to 31 years, (forced expiratory volume in 1 second [FEV_1] ≥67% of predicted value, histamine provocative concentration causing a 20% fall in FEV_1 [PC_{20}] <4 mg/ml) and documented EAR and LAR to house dust mite extract participated in a two-period, double-blind, placebo-controlled, crossover study. During each study period histamine PC_{20} was measured 2 days before and 1 day after a standardized allergen inhalation challenge test. MK-0591 was administered in 3 oral doses of 250 mg each at 24, 12, and 1.5 hours before inhalation of allergen. Biochemical activity of MK-0591 was determined by calcium ionophore A-23187–stimulated leukotriene $(LT)B_4$ biosynthesis in whole blood ex vivo and by urinary LTE_4 excretion. Airway response to allergen was measured by FEV_1 (percent fall from baseline). The EAR (0 to 3 hours) and the LAR (3 to 8 hours) were expressed as corresponding areas under the time-response curves.*

Results: *MK-0591 and placebo did not differ in their effects on prechallenge FEV_1 ($p = 0.10$). As compared with the value before pretreatment, MK-0591 blocked LTB_4 biosynthesis and LTE_4 excretion by a mean of 98% (range, 96% to 99%; $p < 0.002$) and 87% (range, 84% to 96%; $p < 0.046$), respectively, from 0 to 24 hours after allergen challenge. Both the EAR and the LAR were significantly reduced after administration of MK-0591 as compared with placebo, with a mean inhibition of 79% ($p = 0.011$) and 39% ($p = 0.040$), respectively. Allergen-induced airway hyperresponsiveness was not significantly different between the two pretreatment periods ($p = 0.37$).*

Conclusions: *In this study oral MK-0591 prevented leukotriene biosynthesis after allergen challenge in patients with mild to moderate asthma. The results of our study indicate that 5-lipoxygenase products play an important role during the EAR, whereas their contribution to the pathophysiology of the LAR seems to be of less importance. (J ALLERGY CLIN IMMUNOL 1995;95: 42-51.)*

Key words: *Asthma, leukotrienes, allergen bronchial provocation tests, bronchial hyperreactivity*

Asthma is a chronic inflammatory disease of the airways, associated with atopy and airway hyperresponsiveness to various bronchoconstrictor stimuli and characterized by episodes of wheezing, dyspnea, chest tightness, and coughing.[1] The underly-

From [a]the Department of Pulmonology, University Hospital, Leiden; [b]Merck Research Laboratories, Rahway; [c]Merck Sharp & Dohme Research Laboratories, Brussels; and, [d]the Department of Pharmacology, School of Medicine and School of Pharmacy, University of Leuven.

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Reprint requests: Zuzana Diamant, MD, Lung Function Laboratory, University Hospital, Building 1, C2P, P.O. Box 9600, NL-2300 RC Leiden, The Netherlands.

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Abbreviations used

BU: Biologic units
EAR: Early asthmatic reaction
FEV_1: Forced expiratory volume in 1 second
FLAP: 5-lipoxygenase activating protein
LAR: Late asthmatic reaction
LT: Leukotriene
PC_{20}: Provocative concentration causing a 20% fall in FEV_1

ing mechanisms of asthma are still uncertain, although the presence and activity of inflammatory cells and their mediators in the airways are reported to be involved in its development and persistence.[2, 3]

Allergen challenge is a useful model for studying human asthma in vivo.[4] Inhalation of specific allergen by sensitized patients with asthma induces acute airway narrowing within 10 to 15 minutes, accompanied by symptoms of asthma.[5, 6] This so-called early asthmatic reaction (EAR) mostly resolves spontaneously within 1 to 3 hours after allergen challenge. In about 50% of the patients a late asthmatic reaction (LAR) occurs, during which airway narrowing persists or recurs within 3 to 7 hours after inhalation of allergen.[4, 5] The LAR may last for more than 24 hours and is usually followed by transiently enhanced airway responsiveness to histamine or methacholine.[7] There is increasing evidence that inflammatory mechanisms play an important role in allergen-induced airway responses, particularly during the LAR.[4, 8] These characteristics make allergen challenge a useful tool in preclinical evaluation of antiasthma drugs with antiinflammatory properties, such as corticosteroids and receptor antagonists or biosynthesis inhibitors of inflammatory mediators.

Leukotrienes (LTs) are 5-lipoxygenase metabolites of arachidonic acid, representing a heterogeneous group of biologically active mediators that are likely to be involved in allergen-induced asthmatic responses.[9-11] The cysteinyl LTs (i.e., LTC_4, LTD_4, and LTE_4) contract airway smooth muscle, increase microvascular permeability, and stimulate secretion of mucus,[10, 11] whereas LTB_4 induces extravasation and chemotaxis of leukocytes from blood to pulmonary tissue, promoting their aggregation and degranulation.[10-12] When inhaled by human beings, cysteinyl LTs can induce features of asthma, such as airway narrowing and airway hyperresponsiveness.[13, 14] Moreover, recent biochemical observations show an upregulation of LT biosynthesis in circulating leukocytes of atopic nonasthmatic and atopic asthmatic subjects[15] and the release of LTs during spontaneous and allergen-induced asthmatic responses.[16, 17] In addition, pretreatment with specific cysteinyl LT antagonists attenuates the EAR, the LAR,[18, 19] and allergen-induced airway hyperresponsiveness at 6 hours after allergen challenge.[19] Consequently, cysteinyl LTs are likely to play an important role in the pathogenesis of asthma. However, until recently, it was not possible to determine the definite role of the 5-lipoxygenase metabolites in asthma because of the limited potency of the currently available biosynthesis inhibitors[20-23] or effective LTB_4-receptor antagonists in human beings.

MK-0591, (3-[1-(4-chlorobenzyl)-3-(*t*-butylthio)-5-(quinolin-2-yl-methoxy)-indol-2-yl]-2,2-dimethyl propanoic acid; previously L-686,708) is a novel indirect LT biosynthesis inhibitor, which acts through binding specifically to an 18 kd leukocyte membrane protein, termed *5-lipoxygenase–activating protein* (FLAP), which appears to be essential for the translocation and activation of 5-lipoxygenase.[24-26] Preliminary data in nonasthmatic volunteers showed almost complete (90% to 100%) inhibition of both LTB_4 biosynthesis in whole blood ex vivo and urinary LTE_4 excretion for up to 24 hours after a single oral dose (250 mg) of MK-0591.[27]

In this study we tested the hypothesis that MK-0591 would inhibit the EAR and the LAR to inhaled house dust mite extract in eight asthmatic subjects to whom 3 oral doses of MK-0591 (250 mg/dose) were administered as pretreatment to a standardized allergen challenge. Biochemical activity of MK-0591 was measured by calcium ionophore–stimulated LTB_4 biosynthesis in whole blood ex vivo and by urinary LTE_4 excretion. In addition, the effect of MK-0591 on allergen-induced airway hyperresponsiveness to histamine was examined.

METHODS

Subjects

Eight nonsmoking men with mild to moderate, stable asthma (aged 19 to 31 years; mean age, 24.6 years) volunteered to participate in the study (Table I). All subjects had a history of episodic chest tightness and wheezing, especially after exposure to house dust. They all denied any other relevant disease and took no regular medication, except for infrequent as needed use of inhaled short-acting β_2-agonists. Prestudy physical examination, routine laboratory data, chest x-ray film, and electrocardiogram showed no abnormalities. Their base-

TABLE I. Subject characteristics

Subject No.	Age (yr)	Height (cm)	Atopic status*	FEV_1 (% pred)†	PC_{20} histamine (mg/ml)‡	PC_{20} allergen (BU)
1	24	193	5	113.6	2.49	500
2	30	180	8	79.6	0.55	250
3	31	185	5	90.0	0.79	500
4	21	191	5	67.0	0.54	125
5	31	181	5	84.0	2.87	500
6	21	178	1	79.0	0.72	250
7	20	184	1	96.1	1.18	250
8	19	183	5	100.5	0.57	125

*Atopic status was determined by the number of wheal responses to 16 common allergen extracts (Vivodiagnost, ALK).
†Baselines in percent predicted values in the screening period.
‡PC_{20} during the screening period.

line forced expiratory volume in 1 second (FEV_1) was 67% or more of predicted value,[28] and they were all hyperresponsive to histamine (provocative concentration causing a 20% fall in FEV_1 [PC_{20}] range: 0.54 to 2.87 mg/ml).[29] All subjects showed positive wheal responses (≥3 mm) to house dust mite extract from a standardized skin prick test kit consisting of 16 common allergen extracts (Vivodiagnost, ALK, Benelux). They all had documented dual asthmatic responses to inhaled house dust mite extract in the screening period. The study was carried out in the spring. Four weeks before the start of the study, all subjects were free of symptoms of respiratory tract infection. They were asked not to use any medication during the study, except for short-acting β_2-agonists, which were allowed until 8 hours before the inhalation challenge tests. Caffeine- and alcohol-containing beverages were withheld during administration of the drug. The study was approved by the ethics committee of the University Hospital of Leiden, and all participants gave informed consent.

Study design

The study had a double-blind, crossover, and placebo-controlled design with three periods: a screening period and study periods I and II. Each period was separated by an interval of at least 7 days. The screening period consisted of 3 separate days, on which the inclusion criteria were examined. On the first screening day, PC_{20} histamine was determined. On the second screening day, the subjects underwent a duplicate skin prick test with serial dilutions of house dust mite extract. Subsequently, a diluent inhalation challenge was performed, with the criterion that FEV_1 was to remain within 10% of baseline value for 7 hours after the challenge. The allergen PC_{20} was calculated from the histamine PC_{20} and the response to a multidose skin prick test according to the method of Cockcroft et al.[6] On the third screening day, a standardized allergen inhalation challenge test with house dust mite extract was performed to document an EAR and an LAR in each subject.

Study periods I and II consisted of 5 days each. Each subject was randomly allocated to receive either MK-0591 or a matching placebo in a crossover fashion. Subjects attended the laboratory at the same time of the day (± 2 hours). The baseline FEV_1 on days 1 to 3 of each study period had to remain stable (±10%). On day 1 of each study period, baseline histamine PC_{20} was measured 48 hours before allergen challenge and had to remain within one doubling concentration between the two periods. On days 2 and 3, MK-0591 or placebo pretreatment was administered in 3 oral doses of 250 mg each at 24, 12, and 1.5 hours before the allergen challenge. After the first dose, the effect of the drug on baseline FEV_1 was recorded hourly for 2 hours. During study day 3, a standardized allergen challenge test was performed, and on study day 4, 24 hours after the allergen challenge, a histamine PC_{20} was recorded in order to determine the effect of MK-0591 on allergen-induced hyperresponsiveness. On study day 5, safety parameters were examined. Blood and urine were collected at regular intervals for determination of LTB_4, LTE_4, and routine laboratory safety values throughout both study periods. Three days after the last dose was administered in study period II, all subjects returned for follow-up safety evaluations.

Inhalation challenges

Histamine challenges were performed with histamine-acid-phosphate (Sigma Chemical Co., St. Louis, Mo.). Solutions of histamine were stored at 4° C and administered at room temperature. The tests were performed according to a standardized method.[29] In this fashion, doubling concentrations of histamine (0.03 to 8 mg/ml in phosphate-buffered saline) were aerosolized by a DeVilbiss 646 nebulizer (DeVilbiss Health Care, Inc., Somerset, Pa.) (output-0.13 ml/min) and inhaled by tidal breathing, through the mouth with the nose clipped, for 2 minutes at 5-minute intervals. After each concentration was administered, the airway response was measured by FEV_1 until a fall of at least 20% from baseline was reached, allowing calculation of the histamine PC_{20}.[29]

Allergen inhalation challenge tests were also performed according to a standardized protocol.[29] Purified

aqueous allergen extract of *Dermatophagoides pteronyssinus,* standardized by quantitative immunoelectrophoresis, (Vivodiagnost) with phenol as a preservative was prepared from the same production batch for both diluted skin prick tests and inhalation challenge tests and was stored at 4° C. Before the study, the extract was diluted in Vivodiagnost diluent (phosphate-buffered saline with 0.03% human serum albumin and 0.5% phenol). Each subject had his own stock solution of 5 ml, containing 10,000 biologic units (BU)/ml, which was diluted to 25 ml and divided into five equal portions of 5 ml, containing 2000 BU/ml, which were stored at 4° C. Immediately before inhalation, doubling dilutions from this stock, ranging from 2000 to 31.25 BU/ml, were prepared. The doses of inhaled allergen extract were calculated according to the method of Cockcroft et al.[6] Each allergen challenge was preceded by inhalation of its diluent. Consecutive doubling concentrations of allergen were aerosolized by a DeVilbiss 646 nebulizer (output-0.13 ml/min), which was connected to an inspiratory and expiratory valve box with an expiratory aerosol filter (Pall Ultipor BB50T; Pall Biomedical, Ltd., Portsmouth, England), and each concentration was inhaled by tidal breathing for 2 minutes, through the mouth with the nose clipped, at 10-minute intervals. Each subject inhaled the same 3 doses of house dust mite extract during the challenges in periods I and II, which were the three highest concentrations of allergen that had been administered during the screening period.

Response measurements

The airway responses to the inhaled aerosols were recorded by FEV_1 according to standardized lung function techniques[28] and recorded by a dry-rolling-seal spirometer (Kipp, BD 90; Delft, The Netherlands). Before inhalation of allergen, baseline FEV_1 was measured in triplicate, and the two highest values were used to calculate the mean baseline values. Airway responses were recorded at: 105, 90, 15, and 0 minutes before allergen challenge, every 10 minutes during the first hour after allergen challenge, and then hourly for 12 hours. All responses were measured in duplicate, and the highest, technically satisfactory FEV_1 value was used in the analysis.[29] If any of the subjects had severe airway narrowing (a drop in FEV_1 $\geq$50% or an FEV_1 $<$1.4 L) at any time point between 0 and 12 hours after challenge, the measurements were discontinued and treatment was instituted immediately according to recent guidelines.[29]

Drug treatment

MK-0591 was supplied by Merck, Sharp & Dohme Research Laboratories (Rahway, N.J.) as 125 mg capsules or matching placebo. The blinded medication was administered in 3 oral doses of 250 mg at 24, 12, and 1.5 hours before the allergen challenge tests.

LTB_4 biosynthesis in whole blood ex vivo

Blood was collected from each subject on screening day 2, and during both study periods on day 1 (before histamine challenge), on day 2 (immediately before pretreatment and days 3 to 5. Multiple samples were drawn on both study days 3 before pretreatment and 1, 4, 8, 12, 24, and 48 hours after allergen challenge. Stimulation of LTB_4 biosynthesis ex vivo and quantitative measurement of LTB_4 were performed as described in a previous study with some modifications.[30] LTB_4 biosynthesis was stimulated ex vivo by incubating whole blood with 50 μmol/L (final concentration) calcium ionophore, A-23187, (Sigma Chemical Co.) at 37° C for 40 minutes. In addition, at each time point the same amount of whole blood was incubated with 10% dimethyl sulfoxide in water as a control. After incubation, the samples were centrifuged at 12,000 *g* for 5 minutes. Plasma was collected and stored at −20° C until it was analyzed. LTB_4 was quantitatively measured in a competitive binding assay[30] with synthetic LTB_4 and sheep antisera against LTB_4 (kindly provided by Merck Frosst, Canada) and tritiated LTB_4 (New England Nuclear, Wilmington, Del.). The detection limit of LTB_4 for this assay was 0.9 ng LTB_4 per milliliter. The data were expressed as percentage before pretreatment, setting the (value of the) LTB_4 level to 100% (e.g., 40% of [the value of] LTB_4 before the pretreatment represents a 60% inhibition of the LTB_4 biosynthesis).

Urinary LTE_4 and creatinine excretion

Baseline urine samples were collected on screening day 2 and before pretreatment on study day 1. On study day 3, urine was collected at 3-hour intervals: 0 to 3, 3 to 6, 6 to 9, and 9 to 12 hours, after allergen challenge, respectively. Samples of spot void urine were collected on study days 4 and 5, 24 and 48 hours after allergen, respectively. Urine samples were stabilized and prepared for analysis according to a standardized method.[31] The urine was mixed by swirling, and a 40 ml aliquot was transferred to a 50 ml conical polypropylene tube and stabilized by the addition of 0.5 ml 80 mmol/L 4-hydroxy-TEMPO (Sigma Chemical Co.), and 0.5 ml 8 mol/L NaOH.[31] LTE_4 was analyzed as previously described,[31] with the following modifications: samples were spiked with tritiated LTE_4 as an internal standard and partially purified by solid-phase extraction with a disposable 500 mg C-2 column (Analytichem, Harbor City, Calif.). LTs were eluted with 2 ml of methanol, diluted to 60% methanol with aqueous mobile phase (1.6 mmol/L oxalic acid and 0.14 v/v% acetic acid, pH 5.6), and chromatography was performed.[32] Fractions of column eluant containing 0.4 ml were collected and assayed for LTE_4 directly in a competitive binding radioimmunoassay with commercially available peptidyl LT antibody (Sigma Chemical Co.). The detection limit of this assay was 6.3 pg of LTE_4 per milligram of creatinine. Urinary LTE_4 excretion values (pg LTE_4/mg creatinine) were expressed as means ± SD, during specified time

intervals from 0 to 12 hours after allergen challenge and in spot void urine samples at 24 and 48 hours after allergen challenge.

Plasma MK-0591

Plasma samples were obtained before pretreatment on days 1 and 2, and 12 hours after the second 250 mg dose, and 2.5, 5.5, 9.5, 25.5, and 49.5 hours after the third 250 mg dose (i.e., 1, 4, 8, 24 and 48 hours after allergen challenge, respectively). Samples were protected from light and stored at −20° C until they were analyzed. They were prepared for analysis by protein precipitation followed by C18 solid-phase extraction and analyzed by reverse-phase high-performance liquid chromatography with UV detection at 290 nm.[33] Intraday coefficients of variation (n = 6) between 25 and 1000 ng/ml ranged between 0.9% and 3.3%. Interday coefficients of variation (n = 13) at the same concentration ranged from 1.1% to 3.0%. The limit of quantitation was 25 ng/ml.

Analysis

Responses to inhaled allergen by FEV_1 were expressed as percentage fall from baseline values and plotted against the time (in hours). The activity of MK-0591 on the allergen-induced airway responses was determined by comparing the area under the time-response curve (AUC) and the maximum decrease from baseline for both the EAR (0 to 3 hours after allergen challenge) and the LAR (3 to 8 hours after allergen challenge) between the two pretreatment periods, MK-0591 versus placebo. In addition, changes in allergen-induced airway hyperresponsiveness between the two study periods were assessed by comparing histamine PC_{20} 48 hours before and 24 hours after allergen challenge. Natural log transformations were applied to the PC_{20} values before statistical analysis. Student's paired t test was performed to determine the difference in histamine PC_{20} between 48 hours before and 24 hours after allergen challenge.

The effect of MK-0591 on the inhibition of the LTB_4 biosynthesis in whole blood ex vivo was assessed by comparing the percent changes from values before the pretreatment between the two pretreatment periods at each of the specified time points. For the assessment of the inhibition of LTE_4, LTE_4 excretion in urine (pg LTE_4/mg creatinine) was compared between MK-0591 and placebo pretreatment periods.

All data were analyzed with analysis of variance. The model contained factors for sequence, patient within sequence, period, and pretreatment. Normality assumptions were tested with the Shapiro-Wilk statistic. Levene's statistic was used to examine the homogeneity of variance. Probability (p) values less than 0.05, two-tailed, were considered statistically significant. Unless otherwise stated, all values were expressed as means ± SD.

According to recent data,[34] the sample size of eight subjects was sufficient to detect a mean inhibition of 40% in the EAR and 30% in the LAR during administration of MK-0591 as compared with placebo (α = 0.05, two-tailed; β = 0.80, one-tailed). Furthermore, this sample size enabled us to detect a 72% difference in urinary LTE_4 excretion between the two pretreatment periods[21] and to discern a 55% and a 67% blockade from pretreatment urinary LTE_4 excretion and LTB_4 biosynthesis ex vivo, respectively.

RESULTS

Safety and baseline airway function

MK-0591 was well tolerated in all subjects. No significant changes in results of physical examination, serum hematology, chemistry, urine parameters, or electrocardiograms were observed. After 3 doses of MK-0591, baseline FEV_1 was slightly increased as compared with the value before pretreatment (mean change: 0.27 ± 0.25 L, p = 0.017), whereas placebo pretreatment did not change FEV_1 (mean change: 0.014 ± 0.23 L, p = 0.87). These changes, however, were not significantly different between MK-0591 and placebo pretreatment periods (p = 0.10). FEV_1 was also slightly higher after pretreatment with MK-0591 as compared with placebo pretreatment (mean difference: 0.25 ± 0.29 L, p = 0.047).

Histamine and allergen challenge tests

Inhaled house dust mite extract induced an EAR in all subjects, with a maximum percent fall in FEV_1 of 33.7% ± 15.5% after placebo pretreatment, and 14.5% ± 7.4% after MK-0591 pretreatment, respectively. MK-0591 produced a significant inhibition of the EAR as compared with placebo (mean AUC: 9.7% ± 11.1% and 46.5% ± 25.0% fall·hr, respectively), reducing the AUC by 79% (p = 0.011). All subjects had an LAR between 3 and 7 hours after inhalation of allergen. The maximum percent fall in FEV_1 was 30.0% ± 14.8% and 44.1% ± 15.4%, after MK-0591 and placebo pretreatment, respectively. The LAR was significantly reduced by MK-0591 as compared with placebo (mean AUC: 85.1% ± 49.0% and 140.0% ± 51.5% fall·hr, respectively), with a mean inhibition of 39% (p = 0.040) (Fig. 1). Significant inhibition was accomplished at the time points up to 7 hours after allergen challenge (p = 0.020). Two subjects, numbers 1 and 4 (Table I), had to discontinue the measurements during placebo treatment at 9 and 6 hours after allergen challenge, respectively. The reason for intervention was a fall in FEV_1 of greater than 58%. This was also the case for subject number 4 at 10 hours after allergen challenge during the MK-0591 pretreatment period.

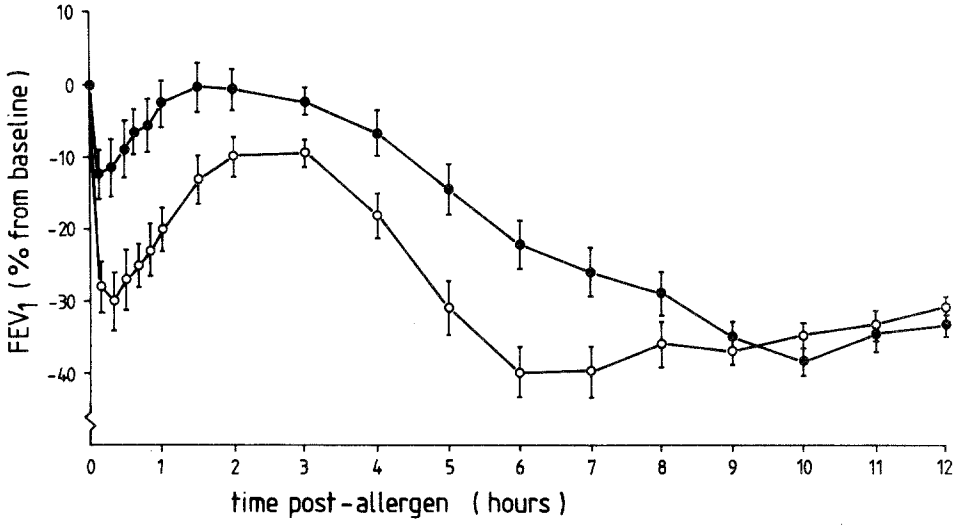

FIG. 1. Airway responses to inhaled house dust mite extract (mean ± SEM) between 0 and 12 hours after allergen challenge during placebo (*open symbols*) and MK-0591 (*closed symbols*) pretreatment periods. The EAR was significantly blocked by MK-0591, with a mean inhibition of 79% ($p = 0.011$). The LAR was also significantly reduced, with a mean inhibition of 39% ($p = 0.040$).

At 24 hours after allergen challenge, airway hyperresponsiveness to histamine was significantly enhanced after both placebo and MK-0591 pretreatment (mean change in PC_{20}: 1.20 ± 0.91 and 0.82 ± 0.78 doubling concentratons, respectively, $p < 0.02$). However, there was no significant difference in the allergen-induced hyperresponsiveness between the two pretreatment periods ($p = 0.37$) (Fig. 2). At 24 hours after challenge baseline FEV_1 was slightly but significantly reduced as compared with the prechallenge value, in both the placebo and MK-0591 pretreatment periods (mean difference ± SD: 0.79 ± 0.43 L; $p = 0.001$ and 0.69 ± 0.34 L; $p = 0.001$, respectively). These changes in baseline FEV_1 were not significantly different between the two pretreatment periods ($p = 0.43$).

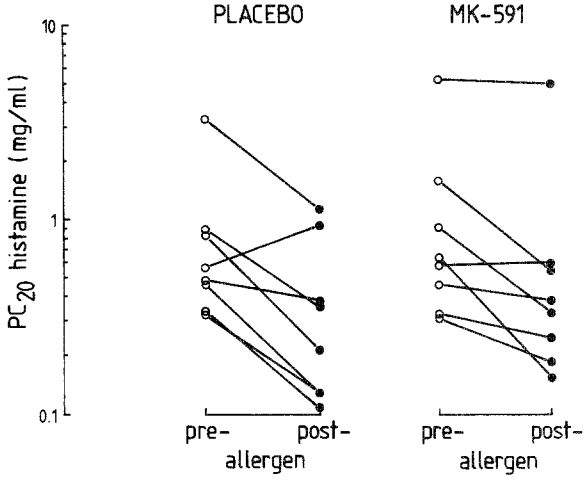

FIG. 2. Airway responsiveness to histamine 48 hours before and 24 hours after allergen challenge during placebo (*left*) and MK-0591 (*right*) period. Hyperresponsiveness to histamine was significantly enhanced after allergen challenge in both periods ($p < 0.02$). However, there was no difference in allergen-induced hyperresponsiveness to histamine between placebo and MK-0591 pretreatment periods ($p = 0.37$).

LTB_4 biosynthesis in whole blood ex vivo

The results of calcium ionophore A-23187–stimulated LTB_4 biosynthesis in whole blood ex vivo are shown in Fig. 3. Mean LTB_4 concentration before pretreatment was 87.4 ± 22.4 ng/ml. As compared with values before the pretreatment, MK-0591 almost completely inhibited LTB_4 biosynthesis in whole blood ex vivo for at least 24 hours after allergen challenge, by a mean of 98% (range, 96% to 99%; $p < 0.002$). When expressed as percent before pretreatment, maximum inhibition (98.8% ± 1.7% predose LTB_4) occurred 9.5 hours after the third 250 mg dose (i.e., 8 hours after allergen challenge) ($p = 0.001$), with significant inhibition still being present at 49.5 hours after pretreatment (i.e., 48 hours after challenge) (mean inhibition: 68.2% ± 19.0%, $p = 0.0002$).

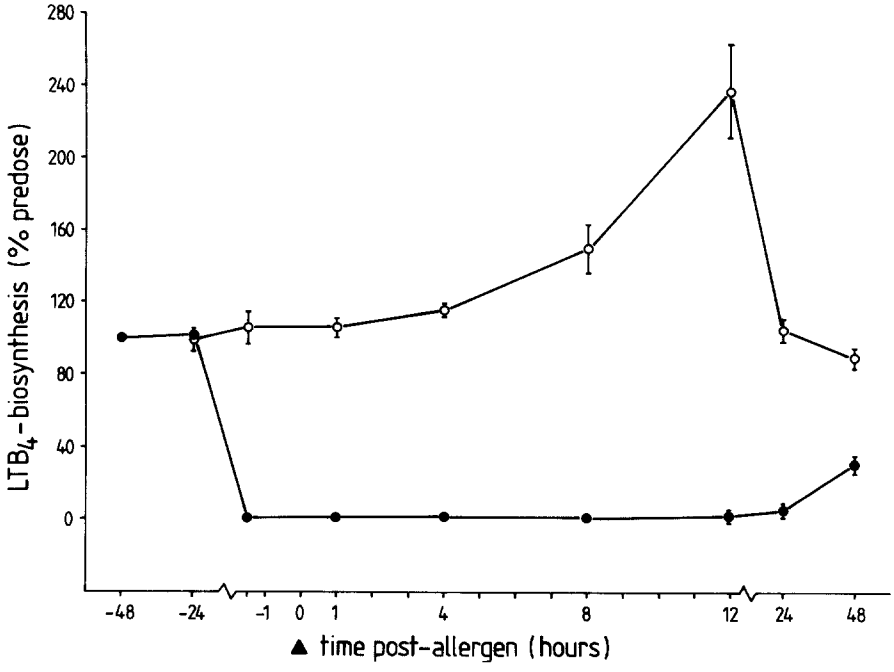

FIG. 3. LTB_4 biosynthesis ex vivo (mean ± SEM), expressed as percentage of the value before pretreatment at various time intervals after allergen challenge during placebo (*open symbols*) and MK-0591 (*closed symbols*) pretreatment periods. LTB_4 biosynthesis was inhibited by MK-0591 by at least 96% as compared with the value before pretreatment between 0 and 24 hours after allergen ($p < 0.001$).

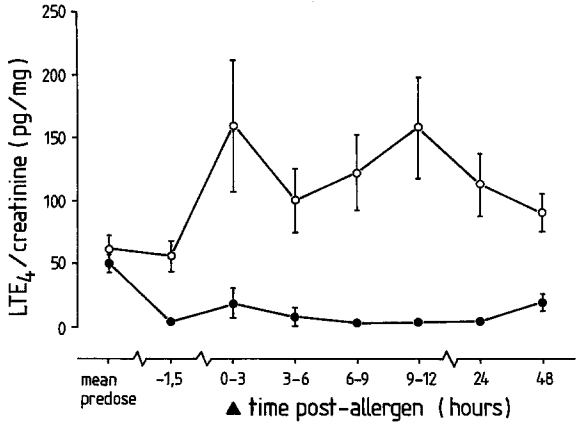

FIG. 4. Urinary LTE_4 excretion (mean ± SEM) (pg LTE_4/mg creatinine) at various time intervals after allergen challenge during placebo (*open symbols*) and MK-0591 (*closed symbols*) pretreatment periods. The LTE_4 excretion was inhibited by MK-0591 by at least 84% as compared with the value before pretreatment between 0 and 24 hours after allergen challenge ($p < 0.046$).

Urinary LTE_4 excretion

Urinary LTE_4 excretion values after allergen challenge are shown in Fig. 4. The mean baseline LTE_4 values were 49.3 ± 25.3 (screening day 2: 50.2 ± 28.3, 48 hours before allergen challenge: 48.3 ± 26.4) and 65.7 ± 27.1 (screening day 2: 65.2 ± 33.5, 48 hours before allergen challenge: 66.3 ± 27.2) expressed in picograms of LTE_4 per milligram of creatinine for MK-0591 and placebo, respectively (mean difference: 16.5 ± 14.4 pg LTE_4/mg creatinine). These values were significantly different between the two pretreatments ($p = 0.014$). MK-0591 almost completely blocked urinary LTE_4 excretion for up to 24 hours after allergen challenge, by a mean of 87% of the value before pretreatment (range, 84% to 96%; $p < 0.046$). Maximum inhibition (96.3% ± 6.0% of the value of LTE_4 level before pretreatment) occurred during the time interval of 6 to 9 hours after allergen challenge ($p = 0.006$). The inhibition slightly decreased to 61.2% ± 57.6% of the value of LTE_4 before pretreatment ($p = 0.002$) at 48 hours after allergen challenge.

MK-0591 plasma levels

On days 1 and 2 of the MK-0591 period, the plasma levels of MK-0591 were below the detection limit. On day 3, the MK-0591 concentrations were (mean ± SD) 8.2 ± 3.2, 4.9 ± 2.0, 3.1 ± 1.3, and 1.9 ± 0.9 μg/ml at 1, 4, 8, and 12 hours after allergen challenge, respectively. These levels de-

creased to 1.0 ± 0.5 and 0.3 ± 0.2 μg/ml at 24 and 48 hours, respectively. There was no significant relationship between MK-0591 plasma levels and the difference in FEV_1 between MK-0591 and placebo at any time point ($p > 0.14$).

DISCUSSION

This study shows the protective effects of a novel, specific, and long-acting 5-lipoxygenase activating protein (FLAP) inhibitor on LT release and airway responses in eight patients with mild to moderate asthma after allergen challenge. MK-0591 is the first FLAP inhibitor that produces prolonged and almost complete blockade of the allergen-induced LT biosynthesis in humans in vivo, resulting in significant inhibition of the EAR and reduction of the LAR. However, MK-0591 did not attenuate allergen-induced airway hyperresponsiveness 24 hours after allergen challenge. These results support the hypothesis that 5-lipoxygenase metabolites are important contributors to the pathophysiology of atopic asthma.

Our findings confirm and extend observations from previous studies, showing enhanced LT biosynthesis after allergen challenge, as reflected by urinary LTE_4 excretion.[17] We even showed a rise in calcium ionophore–stimulated LTB_4 biosynthesis ex vivo at 12 hours after allergen challenge, which might have been due to allergen-induced in vivo priming of leukocytes. These observations underline the rationale of studying the protective effects of LT receptor antagonists and LT biosynthesis inhibitors in the allergen challenge model. In humans in vivo, LTD_4-receptor antagonists appeared to reduce allergen-induced airway responses, but the protection was incomplete.[18, 19] LT biosynthesis inhibitors seemed to be more promising because of their capacity to inhibit the biosynthesis of both cysteinyl LTs and LTB_4. Zileuton, a selective 5-lipoxygenase inhibitor, has been shown to attenuate the asthmatic response to cold, dry air,[22] but it failed to inhibit allergen-induced airway responses.[20] MK-886, an early FLAP inhibitor, administered in 2 oral doses, attenuated the EAR and the LAR.[21] Both of these LT biosynthesis inhibitors produced incomplete inhibition of ex vivo LTB_4 (in blood) and LTE_4 (in urine).[20-22] Therefore to elucidate the precise role of LTs in the pathophysiology of asthma, more potent blockers were needed.

MK-0591 is a novel FLAP inhibitor, which is more potent and has a longer duration of action than MK-886.[24, 25] In sheep, MK-0591 produced significant inhibition of the EAR and LAR to *Ascaris suum*.[25] In our study 3 doses of MK-0591, administered orally 12 hours apart, almost completely blocked calcium ionophore–stimulated LTB_4 biosynthesis in whole blood ex vivo and urinary LTE_4 excretion after allergen challenge. It is remarkable that inhibition was still present at 50 hours after the last dose was given. This rather vigorous reduction in 5-lipoxygenase products produced 79% inhibition of the EAR (0 to 3 hours) and only 39% reduction of the LAR (3 to 8 hours), whereas airway hyperresponsiveness to histamine 24 hours after allergen challenge was not prevented. This implies that the contribution of LTs to the development of the EAR is more important than their contribution to the LAR. This point is particularly illustrated by the airway response measurements between 8 and 12 hours after allergen challenge, which were not significantly different between MK-0591 and placebo pretreatment periods. Although these data need to be interpreted cautiously because of dropout of subjects, they suggest that MK-0591 caused a delay rather than a blockade of the LAR. Consequently, it may not be surprising that allergen-induced hyperresponsiveness was not prevented by the drug.

There are at least five possible mechanisms that could account for the incomplete inhibition of the allergen-induced airway responses by MK-0591: (1) pharmacokinetics of the drug: LT biosynthesis in blood and urine might not be the same as in the lungs; (2) simultaneous blockade of other 5-lipoxygenase products, such as lipoxin A_4, which is an endogenous modulator of cysteinyl LTs[35]; (3) possible shunting of arachidonic acid metabolites to the cyclooxygenase pathway[9]; (4) a large receptor reserve for LTs[36]; and (5) other inflammatory mediators contributing to allergen-induced airway responses.[7, 37-41]

Pharmacokinetics. It is unknown to what extent MK-0591 inhibits biosynthesis of 5-lipoxygenase products in human lungs in vivo. Stevens et al.[42] recently reported that MK-0591, given intravenously to dogs before ozone inhalation, accomplished similar inhibition of the ex vivo LTB_4 biosynthesis in blood (96%) and bronchial lavage fluid (91%). This suggests an adequate activity of the compound within the lungs. Another indirect argument against insufficient dosing, as an explanation for the limited protection obtained by MK-0591, is provided by the absence of a relationship between the degree of protection and drug plasma levels. However, because in our study direct measurements in bronchoalveolar lavage were not per-

formed, further studies are needed to fully exclude this pharmacokinetic hypothesis.

Simultaneous blockade of protective 5-lipoxygenase products. Interactions between 5-lipoxygenase and 15-lipoxygenase pathways yield biologically active metabolites, termed lipoxins.[9] Christie et al.[35] have recently demonstrated that lipoxin A_4 antagonizes the bronchoconstrictor actions of LTC_4 at concentrations of lipoxin A_4 that were not bronchoconstrictive per se in patients with asthma in vivo. It cannot be excluded that concurrent blockade of this endogenous cysteinyl LT receptor antagonist could result in limited effects of 5-lipoxygenase inhibitors on airway responses after inhalation of allergen.

Possible shunting of metabolites. There are three major metabolic pathways of arachidonic acid, of which two are potentially important in asthma because of their bronchoactive derivatives.[9] These are the cyclooxygenase pathway, yielding thromboxane and prostaglandins, and the 5-lipoxygenase pathway, yielding LTs. It is possible that virtually complete blockade of the 5-lipoxygenase pathway will result in shunting of arachidonic acid metabolites[40] to, for example, the cyclooxygenase pathway. Among the different cyclooxygenase metabolites, there are some that produce bronchoconstriction, such as prostaglandin D_2 and thromboxane A_2. Because several lines of evidence indicate that cyclooxygenase products contribute to allergen-induced airway responses, shunting towards this metabolic pathway of arachidonic acid could potentially account for our findings.[38, 41, 43] However, to our knowledge, there is no experimental evidence favoring this hypothesis.

Large receptor reserve for LTs. Even after vigorous reduction in 5-lipoxygenase products, their activity in the airways could still be sufficient to induce a substantial fraction of the late response if their response is mediated by occupancy of only a small fraction of LT receptors.[36] For instance, a 99% inhibition of LT synthesis would only be equivalent to a 100-fold shift on the accompanying dose-response curve. At present, we can only speculate about this possibility, because the various subtypes of LT receptors, including their number, affinity, and specificity, are still a matter of debate.[10]

Other inflammatory mediators. Finally, apart from LTs, other inflammatory mediators including histamine,[37] platelet activating factor,[39] thromboxane,[38] and prostaglandins,[41] are reported to be involved in the pathophysiology of allergen-induced airway responses. This indicates that blockade of more than one metabolic pathway may be needed in the prevention and treatment of allergen-induced airway responses.[43]

Our results may have future clinical implications. Blocking the biosynthesis of 5-lipoxygenase products by MK-0591 appears to be partially effective in the allergen challenge model of atopic asthma. Even though this is currently the best model available, our results do not allow accurate predictions about the efficacy of MK-0591 in long-term clinical trials in patients with asthma,[7] because the effects of LT biosynthesis inhibition on the sequelae of chronic airway inflammation are still unknown. At this stage, however, our observations indicate that clinical trials with compounds such as MK-0591 are warranted.

In summary, the results of this study indicate that 5-lipoxygenase metabolites are major contributors to allergen-induced airway responses, particularly the EAR. Their contribution to the LAR appears to be of less importance. The effects of LT biosynthesis inhibitors must now be evaluated in clinical trials, including the follow-up of symptoms, lung function, airway responsiveness, sputum, bronchoalveolar lavage, and bronchial biopsies. This will elucidate the precise role of LTs in asthma and can be used to determine the perspectives of LT biosynthesis inhibitors in the treatment of asthma.

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