

# The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma

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## Summary

**Background** Cysteinyl leukotrienes are capable of inducing chemotaxis of eosinophils *in vitro* and within the airways of animals and humans *in vivo*.

**Objective** We hypothesized that montelukast (MK-0476), a potent  $\text{cysLT}_1$  receptor antagonist, would protect against allergen-induced early (EAR) and late (LAR) asthmatic responses by virtue of anti-inflammatory properties. Hence, we studied the effect of pretreatment with oral montelukast on allergen-induced airway responses. As an exploratory endpoint, changes in inflammatory cell differentials and eosinophil cationic protein (ECP) were evaluated in hypertonic saline-induced sputum.

**Methods** Twelve asthmatic men (20–34 years,  $\text{FEV}_1$  79–109% predicted, histamine  $\text{PC}_{20}\text{FEV}_1 < 4 \text{ mg/mL}$ ) with dual responses to inhaled house dust mite extract participated in a two-period, double-blind, placebo-controlled, crossover study. Three oral doses of montelukast (10 mg) or matching placebo were administered 36 and 12 h before, and 12 h post-allergen. The airway response to allergen was measured by  $\text{FEV}_1$ , and the EAR and LAR were expressed as the corresponding areas under the time-response curves ( $\text{AUC}_{0-3 \text{ h}}$  and  $\text{AUC}_{3-8 \text{ h}}$ , respectively). During each study period, sputum was induced with 4.5% NaCl 24 h before and 24 h after a standardized allergen challenge. Processed whole sputum cytospins were stained with Giemsa, and cell counts expressed as percentage nonsquamous cells. ECP was measured by FEIA in sputum supernatants.

**Results** All subjects completed the study. The changes in baseline  $\text{FEV}_1$  were not significantly different between the two pretreatments ( $P = 0.183$ ). Montelukast significantly inhibited the EAR and LAR, reducing the  $\text{AUC}_{0-3 \text{ h}}$  by 75.4% ( $P < 0.001$ ) and the  $\text{AUC}_{3-8 \text{ h}}$  by 56.9% ( $P = 0.003$ ) as compared with placebo. Sputa of nine subjects could be included in the analysis ( $< 80\%$  squamous cells). Allergen challenge significantly increased sputum eosinophils after placebo (mean change  $\pm$  SD:  $4.8 \pm 5.8\%$ ,  $P = 0.038$ ), with a similar trend after montelukast (mean change  $\pm$  SD:  $4.1 \pm 5.4\%$ ;  $P = 0.056$ ). The allergen-induced changes in sputum eosinophils and ECP, however, were not significantly different between the two pretreatments ( $P = 0.652$  and  $P = 0.506$ , respectively).

**Conclusion** We conclude that oral montelukast protects against allergen-induced early and late airway responses in asthma. However, using the present dosing and sample size, this protection was not accompanied with changes in sputum eosinophil percentage or activity, which may require more prolonged pretreatment with  $\text{cysLT}_1$  receptor antagonists.

**Keywords:** bronchial provocation tests, leukotriene receptor antagonist, sputum, eosinophils, eosinophil cationic protein

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*Clinical and Experimental Allergy*, Vol. 29, pp. 42–51. Submitted 24 November 1997; revised 27 April 1998; accepted 3 June 1998.

## Introduction

The cysteinyl leukotrienes (cysLT: LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>) are generated by mast cells, eosinophils, and basophils through oxidative degradation of arachidonic acid via the 5-lipoxygenase pathway [1]. These potent mediators possess various bronchoactive properties *in vitro*, inducing airway smooth muscle contraction [2,3], microvascular hyperpermeability and mucus hypersecretion [2]. These pro-inflammatory effects are mediated by specific membrane cysLT receptors of which two subtypes, cysLT<sub>1</sub> and cysLT<sub>2</sub> receptors, have been recognized. In human airway smooth muscle, the cysteinyl leukotrienes all activate the cysLT<sub>1</sub> receptor, which can be blocked by cysLT<sub>1</sub> receptor antagonists [4].

When inhaled by nonasthmatic or asthmatic individuals, cysLT can produce features of asthma such as airway narrowing [5,6] and airway hyperresponsiveness to histamine and methacholine [5–7]. Moreover, evidence exists that leukotriene D<sub>4</sub> is also capable of inducing chemotaxis of eosinophils isolated from human peripheral blood at a physiologically relevant concentration ( $10^{-10}$  mol/L) [8]. These chemoattractant properties have recently been confirmed by observations from *in vivo* studies in animals [9] and in humans [10,11]. In guinea-pigs, inhaled LTC<sub>4</sub> and LTD<sub>4</sub>-induced eosinophilia in the airway submucosal layer 8 h after inhalation, whereas pretreatment with a potent cysLT<sub>1</sub> receptor antagonist (MK-571) almost completely prevented the eosinophil influx [9]. In asthmatic patients, a marked increase in eosinophils and neutrophils was found in bronchial biopsy specimens 4 h after inhalation of LTE<sub>4</sub> [10]. Likewise, inhaled LTD<sub>4</sub> induced an increase in sputum eosinophils in asthmatic subjects at the same time point [11]. These observations provided ample evidence that cysteinyl leukotrienes are involved in the induction of inflammatory processes within the airway wall in humans *in vivo*.

Hence, potent leukotriene biosynthesis inhibitors and cysLT<sub>1</sub> receptor antagonists, which have been previously shown to protect against several challenges, such as allergen, aspirin and exercise [12], may have anti-inflammatory potential in the treatment of asthma. Accordingly, pretreatment of atopic asthmatics with a cysLT<sub>1</sub> receptor antagonist for 7 days has significantly decreased eosinophil and basophil numbers in the bronchoalveolar fluid 48 h after segmental allergen challenge [13]. An alternative method of examining the cellular effects of these compounds within the airways is hypertonic saline-induced sputum. Indeed, in

this way significant increases in sputum eosinophils [14–16] and ECP levels [15] have been detected 24 h [15,16] and 32 h [14] after allergen challenge.

Montelukast (MK-0476) is a novel, potent cysLT<sub>1</sub> receptor antagonist, which selectively competes with the specific binding of [<sup>3</sup>H] LTD<sub>4</sub> at the receptor site in human DMSO dU937 cells (IC<sub>50</sub> value 0.88 nmol/L) and which has been shown to be generally safe and well-tolerated in humans [17]. In a dose-finding study with oral montelukast in exercise-induced asthma, the plateau of a maximum clinical effect was reached at a dose of 10 mg once daily [18]. The primary objective of the present study was to demonstrate that pretreatment with oral montelukast (10 mg) once daily would prevent allergen-induced early (EAR) and late (LAR) asthmatic responses to inhaled house dust mite extract in asthmatic subjects. As an exploratory endpoint we added the postulate that such protective effects by montelukast would be associated with a reduction in the eosinophil number and activity in sputum induced 24 h after the challenge.

## Subjects and methods

### Subjects

Twelve male volunteers with clinically stable, intermittent or mild-persistent asthma [19] participated in the study (Table 1). All subjects had a history of episodic dyspnea, chest tightness or wheezing, especially after exposure to house dust mite, but denied any other relevant disorder. There was no history of respiratory viral infections for at least 3 weeks before and during the study, and subjects were instructed to avoid relevant allergen exposure during the study. None of the volunteers had ever smoked tobacco and none was using concomitant medication except for short-acting inhaled  $\beta_2$ -agonists as needed. They were asked to refrain from alcohol for at least 48 h before administration of the study medication, and caffeine-containing beverages were withheld 8 h before testing. Atopy to house dust mite was confirmed by a positive weal response ( $\geq 3$  mm) to a standardized skin prick test kit consisting of 10 common airborne allergen extracts (Vivodiagnost, ALK, Groningen, The Netherlands). The baseline forced expiratory volume in one second (FEV<sub>1</sub>) was  $\geq 79\%$  of predicted and all subjects showed airway reversibility after inhalation of 200  $\mu$ g salbutamol of at least 11.5% (range 11.5–23%) [20]. Moreover, all subjects were hyperresponsive to inhaled histamine (PC<sub>20</sub> histamine  $< 4$  mg/mL at screening) [21]. The study was approved by the Ethics Committee of the Leiden

**Table 1.** Subject characteristics

|    | Age (years) | Height (cm) | Atopic status* | FEV <sub>1</sub><br>% predicted‡ | PC <sub>20</sub> FEV <sub>1</sub><br>histamine<br>(mg/mL)‡ | Cumulative dose<br>inhaled HDM (BU)§ |
|----|-------------|-------------|----------------|----------------------------------|------------------------------------------------------------|--------------------------------------|
| 1  | 32          | 180         | 6              | 79                               | 0.07                                                       | 85.3                                 |
| 2  | 22          | 187.5       | 4              | 104                              | 0.31                                                       | 682.5                                |
| 3  | 27          | 192         | 5              | 109                              | 0.41                                                       | 170.6                                |
| 4  | 26          | 183.5       | 5              | 94                               | 0.60                                                       | 341.3                                |
| 5  | 22          | 184         | 6              | 88                               | 0.95                                                       | 85.3                                 |
| 6  | 20          | 190         | 2              | 92                               | 1.10                                                       | 341.3                                |
| 7  | 22          | 183         | 4              | 83                               | 1.13                                                       | 170.6                                |
| 8  | 23          | 181.5       | 5              | 88                               | 1.41                                                       | 170.6                                |
| 9  | 23          | 181         | 5              | 83                               | 1.45                                                       | 170.6                                |
| 10 | 24          | 186         | 6              | 87                               | 1.57                                                       | 341.3                                |
| 11 | 21          | 170.5       | 4              | 103                              | 1.73                                                       | 1365                                 |
| 12 | 34          | 180.5       | 6              | 79                               | 3.97                                                       | 1365                                 |

\* Atopic status as determined by the number of weal responses to 10 common allergen extracts (Vivodiagnost, ALK). †Baselines in percentage of the predicted values. ‡Provocative concentration of histamine causing a 20% fall in FEV<sub>1</sub> from baseline. §Cumulative dose of inhaled house dust mite (HDM) extract in BU (biological units) during the two allergen challenges in the study periods.

University Medical Center and all participants gave written consent.

#### Study design

Before entering the study, all subjects were seen on five separate screening visits within 7 days, during which the selection criteria were examined. On screening day 1, a questionnaire, a histamine challenge and a skin prick test were performed. On screening day 2, the subjects underwent a multidose skin prick test with house dust mite extract for determination of the skin test sensitivity [22]. Subsequently, they inhaled the allergen diluent and FEV<sub>1</sub> was measured repeatedly until 8 h after inhalation [21]. Sputum was induced 24 h before (screening day 3) and 24 h after (screening day 5) an allergen challenge [22], which was performed on screening day 4. The subjects were enrolled in the study provided that they had a documented EAR (fall in FEV<sub>1</sub>  $\geq$  20% from baseline, between 0 and 3 h post-allergen) and LAR (fall in FEV<sub>1</sub> ( $\geq$  15% from baseline between 3 and 8 h post-allergen) to inhaled house dust mite extract, and that FEV<sub>1</sub> remained stable ( $\pm$  10% from baseline) after inhalation of the diluent of allergen [21]. Furthermore, prestudy physical examination, ECG, chest X-ray, laboratory safety tests, including blood and urine, had to be within normal limits.

The study had a double-blind, placebo-controlled and crossover design and consisted of two periods of 4 consecutive days each. The subjects visited the lab at the same time of the day on all occasions ( $\pm$  2 h). All periods

(screening, period I and II) were separated by a washout interval of at least 11, and maximally 42 days [23] (Fig. 1). During study periods I and II, PC<sub>20</sub> histamine was determined on day 1. Provided that their asthma was clinically stable, and PC<sub>20</sub> histamine was within one doubling concentration of screening and/or period I, the subjects were instructed to take the study medication in the evening on study days 1 through 3 in a blinded manner. On days 2 and 4, sputum induction was performed 24 h before and 24 h after a standardized allergen challenge, respectively, the latter being performed on day 3 [21]. PC<sub>20</sub> was not repeated on day 4, since sputum was performed on that occasion [24]. At the end of each study period laboratory safety tests were repeated.

#### Inhalation challenges

##### Histamine inhalation test

Histamine challenges were performed using histamine-acid-phosphate (Sigma Chemicals, St Louis, MO, USA). The tests were performed by tidal breathing method: doubling concentrations of histamine (0.03–8 mg/mL in phosphate-buffered saline) were aerosolized by a DeVilbiss 646 nebulizer (output 0.13 mL/min; DeVilbiss, Somerset, PA, USA) and inhaled by tidal breathing, for 2 min at 5-min intervals, until FEV<sub>1</sub> dropped by  $\geq$  20% from baseline [21].

##### Allergen inhalation test

Allergen challenges were also performed according to a standardized protocol [21,22,25]. Purified aqueous allergen

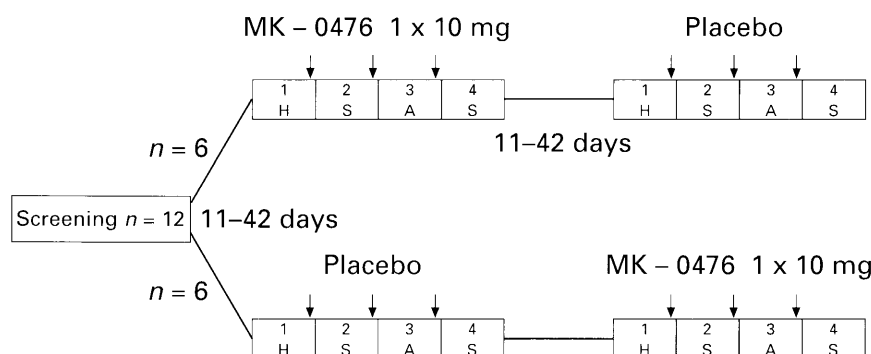


**Fig. 1.** Study design. A two-period, randomized, placebo-controlled, crossover study. Arrows indicating the dosing times of MK-0476 or placebo. H = histamine, S = sputum induction, A = allergen challenge.

extract of *Dermatophagoides pteronyssinus* (SQ 503; Vivodiagnost, ALK), with 0.5% phenol as a preservative, was prepared from the same production batch for both the multidose skin prick tests and bronchoprovocation tests. Each subject had his own stock solution of 5 mL, containing 10 000 BU/mL, which was diluted to 25 mL with Vivodiagnost diluent (phosphate-buffered saline with 0.03% human serum albumin and 0.5% phenol) and divided in five equal portions of 5 mL containing 2000 BU/mL, stored at 4°C. Immediately before inhalation, doubling dilutions from this stock were prepared ranging from 2000 to 15.63 BU/mL. PC<sub>20</sub> allergen was predicted from PC<sub>20</sub> histamine and skin-test sensitivity, derived from a multidose skin prick test, according to Cockcroft's method [22]. Starting two concentrations below the predicted PC<sub>20</sub> allergen, 3 mL of 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, Medica BV, Den Bosch, The Netherlands), and inhaled by tidal breathing for 2 min with the nose clipped at 12 min intervals, until a fall in FEV<sub>1</sub> of  $\geq 20\%$  from baseline was reached. Each subject inhaled three doses of allergen containing the same cumulative concentration during both study periods (Table 1).

### Response measurements

The airway responses to the inhaled aerosols were measured by FEV<sub>1</sub> according to standardized lung function techniques [20] and recorded by a dry-rolling-seal spirometer (Morgan Spiroflow, Rainham, UK) in series with a plotting system (Kipp, BD 90, Delft, The Netherlands). Before each test, FEV<sub>1</sub> was measured in triplicate, and the highest, technically satisfactory value was considered as baseline FEV<sub>1</sub>.

The airway response to histamine was obtained by FEV<sub>1</sub> measurements at 30 and 90 s after each dose. After each dose of histamine, the lowest, technically satisfactory FEV<sub>1</sub> value was applied in the analysis to calculate the percentage

fall from baseline [21]. The tests were discontinued once a  $\geq 20\%$  fall in FEV<sub>1</sub> from baseline was reached.

The airway response to allergen was measured in duplicate, 10 min after each inhalation of allergen, and repeated after 10, 20, 30, 40, 50, 60, 90 and 120 min and then hourly until 8 h after the last inhalation. At each time point, the highest, technically satisfactory FEV<sub>1</sub> value was applied in the analysis and expressed as percentage change in FEV<sub>1</sub> from pre-allergen baseline [21,25]. Measurements were discontinued as soon as any of the subjects developed clinically significant airway narrowing (either a drop in FEV<sub>1</sub>  $\geq$  55% from baseline or an FEV<sub>1</sub> < 1.4 L) or experienced serious discomfort. Rescue treatment could be instituted immediately according to recent guidelines [21].

### Treatment

Montelukast was supplied by Merck Sharp & Dohme Research Laboratories (Rahway, NJ, USA) as 10 mg tablets or matching placebo. The blinded medication was administered in three oral doses of 10 mg each at 36 and 12 h before, and at 12 h after allergen challenge [17,18].

### Sputum induction

Sputum was induced by inhalations of hypertonic saline (NaCl 4.5%), according to recently described protocols [15,26,27]. Hypertonic saline aerosols were generated at room temperature by a DeVilbiss Ultraneb 2000 ultrasonic nebulizer with a calibrated particle size (mass median aerodynamic diameter  $4.5\text{ }\mu\text{m}$ ) at maximal output ( $2.5\text{ mL/min}$ ). The aerosols were delivered to the subjects via a 100-cm-long tube with an internal diameter of 22 mm, and inhaled via the mouth through a two-way valve (No. 2700; Hans-Rudolph, Kansas City, MO, USA). Immediately before inhalation of the aerosols, FEV<sub>1</sub> was recorded, and for safety reasons  $200\text{ }\mu\text{g}$  salbutamol was administered by a metered dose-inhaler (Volumatic®, Allen & Hanbury Ltd, Greenford, UK). Subsequently, the subjects inhaled hypertonic saline aerosols during  $3\times 5\text{ min}$  and  $2\times 10\text{ min}$  inter-

vals, up to 35 min. After each inhalation, or as soon as the subjects experienced cough, they were asked to blow their nose, to rinse their mouth and throat with water, and to expectorate sputum into a clean plastic container by coughing. Sputum induction was discontinued after 35 min inhalation time, or as soon as an adequate,  $\pm 1$  mL sputum sample was obtained. After testing, FEV<sub>1</sub> was recorded and additional salbutamol was administered, in case of a  $\geq 10\%$  fall in FEV<sub>1</sub> from baseline.

#### *Sputum processing*

Whole sputum samples were processed according to a recently validated protocol by Fahy *et al.* [15], with modifications [26,27]. Shortly, the volume of the induced sputa samples was determined and mixed with an equal volume of 0.1% sputolysin (Dithiothreitol, Calbiochem, La Jolla, CA, USA). To ensure adequate homogenization, the samples were placed in a shaking water bath at 37°C for 15 min, once interrupted by gently mixing the sample with a plastic pipette. The homogenized sputum was centrifuged at 1400 r.p.m. at 4°C for 10 min without break. The supernatants were aspirated and frozen at  $-20^{\circ}\text{C}$  pending analysis. The cell pellets were resuspended in phosphate-buffered saline (PBS) to a final volume of 2–5 mL, followed by filtration through a gauze (pore size  $\approx 1$  mm) to remove clumps. Cell counts were performed in a haemocytometer (Tamson, Zoetermeer, The Netherlands), to obtain an estimate of the total number of cells per sample. Subsequently, the sample was diluted with PBS to a final concentration of  $\pm 0.4 \times 10^6$  cells/mL which was used for preparation of cytocentrifuge slides (50  $\mu\text{L}$ /cytospin; Shandon 3, Life Sciences International, Veldhoven, The Netherlands).

Differential cell counts of eosinophils, neutrophils, lymphocytes, macrophages, epithelial and squamous cells were performed on May-Grünwald-Giemsa-stained cytospins. In addition, mast cells were counted on Toluidine blue-stained cytospins. In each sputum sample, at least 500 nucleated cells, excluding squamous cells, were counted twice by the same blinded investigator (cytopathologist: M. V. C.) and the average percentage of each cell type was calculated and expressed as percentage nonsquamous cells. If  $> 80\%$  of the cells consisted of squamous cells, the quality of the sputum sample was judged unsatisfactory and was excluded from analysis.

Concentrations of eosinophil cationic protein (ECP) were measured in all defrosted supernatants at the same time using a FEIA (fluorescence enzyme immunoassay; Pharmacia CAP System ECP FEIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden; detection limit: 2  $\mu\text{g/L}$ ).

#### *Analyses*

The airway responses to inhaled allergen were expressed as

percentage fall in FEV<sub>1</sub> from pre-allergen baseline and plotted as time-response curves. The activity of montelukast on the allergen-induced airway responses was determined by comparing the maximum percentage fall in FEV<sub>1</sub> from baseline with the corresponding area under the time-response curve (AUC) for both the EAR (0–3 h post-allergen) and the LAR (3–8 h post-allergen) between placebo and montelukast pretreatment periods [21,25]. The AUCs were determined by the trapezoidal rule [21]. In addition, the differences in allergen-induced changes in sputum differential cell counts of mast cells, eosinophils, neutrophils, lymphocytes, macrophages, epithelial and squamous cells, and ECP levels between the two pretreatments were assessed by comparing the values of these parameters 24 h before with 24 h after allergen challenge. Since the ECP data showed a skewed distribution, log transformations were applied before analysis [26].

For the assessment of treatment differences (montelukast vs. placebo) in the parameters, repeated-measures analysis of variance (MANOVA) was applied. The model contained factors for sequence, patient within sequence, period and pretreatment. *P* values  $< 0.05$  were considered statistically significant.

The present sample size of 12 subjects would enable us to detect a  $\pm 44\%$  mean inhibition of the maximal percentage fall in FEV<sub>1</sub> during the EAR, and a  $\pm 22\%$  mean reduction of the maximal percentage fall in FEV<sub>1</sub> during the LAR between montelukast and placebo ( $\alpha = 0.05$  [two-tailed],  $\beta = 0.10$  [one-tailed]); power 0.90 [28].

Furthermore, based on a previous repeatability study in our lab, applying similar methodology of sputum induction in 10 patients with clinically stable asthma and similar subject characteristics on two separate occasions, a sample size of 12 subjects would enable us to detect a difference of 4.9% in the changes in sputum eosinophils between the two pretreatments ( $\alpha = 0.05$  [two-tailed],  $\beta = 0.10$  [one-tailed]; power = 0.90) [26].

All statistical tests were performed using the SAS version 6.10 for Windows and Statistical Package of the Social Sciences (SPSS-PC+).

## **Results**

#### *Safety*

All subjects completed the study. Montelukast was generally well tolerated in all subjects. No changes in physical examination, blood haematology and chemistry, urine, or electrocardiograms were noted.

#### *Airway calibre*

Although pre-allergen baseline FEV<sub>1</sub> was slightly increased

after montelukast, as compared with placebo ( $P=0.037$ ), the changes in baseline FEV<sub>1</sub> were not significantly different between the two pretreatments (mean change  $\pm$  SD:  $0.03 \pm 0.14$  L, and  $-0.06 \pm 0.21$  L, respectively;  $P=0.183$ ). At 24 h post-allergen, baseline FEV<sub>1</sub> was significantly reduced after both pretreatments, as compared with pre-allergen baseline (mean difference  $\pm$  SD:  $-0.21 \pm 0.32$  L,  $P=0.044$  (MK-0476), and  $-0.60 \pm 0.63$  L,  $P=0.008$  (P)). These changes in baseline FEV<sub>1</sub> were significantly smaller after montelukast as compared with placebo ( $P=0.014$ ) (Fig. 2).

#### Allergen-induced airway responses

Inhaled house dust mite extract produced dual responses in all subjects. As compared with placebo, montelukast markedly inhibited the EAR, reducing the maximal percentage fall in FEV<sub>1</sub> from baseline (mean  $\pm$  SD:  $31.3 \pm 14.7\%$  fall [placebo] and  $13.8 \pm 6.1\%$  fall [MK-0476];  $P=0.002$ ) by an average of 53.6%, and reducing the AUC<sub>0-3 h</sub> (mean  $\pm$  SD:  $49.0 \pm 34.4\%$  fall.h [placebo], and  $11.5 \pm 7.9\%$  fall.h [MK-0476];  $P<0.001$ ) by an average of 75.4% (Fig. 3). Furthermore, montelukast significantly reduced the LAR both in terms of the maximal percentage fall in FEV<sub>1</sub> from baseline (mean  $\pm$  SD:  $39.0 \pm 15.1\%$  fall [placebo], and  $26.7 \pm 12.3\%$  fall [MK-0476];  $P=0.008$ ) by an average of 36.4%, and as the AUC<sub>3-8 h</sub> (mean  $\pm$  SD:  $128.1 \pm 66.0\%$

fall.h [placebo], and  $64.6 \pm 40.1\%$  fall.h [MK-0476],  $P=0.003$ ) by an average of 56.9% (Fig. 3).

#### Allergen-induced changes in sputum cell differentials

Adequate sputum samples at all study days were obtained from nine subjects only. Subjects one, four, and ten were excluded from analysis because their sputum samples contained  $>80\%$  squamous cells on most study days. The average ( $\pm$  SD) percentage of squamous cells in sputum was  $25.2 \pm 26.4\%$ . The cell viability of all sputum samples was good (range 60.2–77.9%). Since there were hardly any mast cells in the samples, they are not reported in the analysis.

Pre-allergen, there was no significant difference in the percentage sputum eosinophils between placebo and montelukast pretreatments ( $P=0.367$ ). During the placebo pretreatment period, there was a significant rise in the percentage sputum eosinophils after allergen challenge (mean change  $\pm$  SD:  $4.8 \pm 5.8\%$ ;  $P=0.038$ ). A similar trend did not reach significance during montelukast treatment ( $4.1 \pm 5.4\%$ ;  $P=0.056$ ). However, these allergen-induced changes in the percentage eosinophils were not significantly different between placebo and montelukast ( $P=0.652$ ; Table 2, Fig. 4). During montelukast there was a significant reduction in sputum bronchial epithelial cells (mean difference  $-11.0 \pm 8.9\%$ ,  $P<0.006$ ). There were no significant changes in any of the other inflammatory cell differentials between placebo and montelukast ( $P \geq 0.15$ ).

#### Allergen-induced changes in ECP levels

Sputum ECP levels were significantly increased after allergen challenge during both periods (placebo and montelukast) as compared with pre-allergen ( $P<0.050$ ) (Table 2). There was no significant difference in the increases in sputum ECP levels between placebo and montelukast pretreatment periods ( $P=0.506$ ).

#### Discussion

The results of the present study demonstrate that pretreatment with montelukast (MK-0476), a potent and selective cysLT<sub>1</sub> receptor antagonist, provides considerable protection against allergen-induced early and late airway responses in asthmatic subjects, after two once daily doses. It is unlikely that these protective effects were predominantly caused by bronchodilator effects, since two doses of montelukast did not significantly change pre-allergen baseline FEV<sub>1</sub> as compared with placebo. The present findings can be explained by effective antagonism

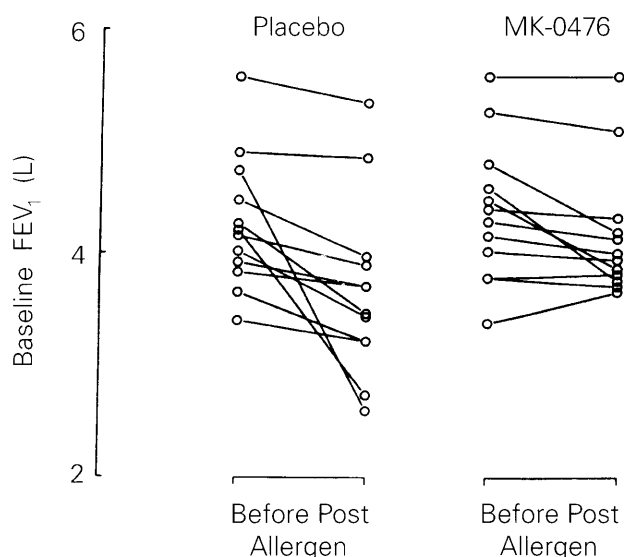


**Fig. 2.** Baseline FEV<sub>1</sub> (L) before and 24 h after allergen challenge during placebo and montelukast (MK-0476) pretreatment periods. At 24 h post-allergen, baseline FEV<sub>1</sub> was reduced after both pretreatments, as compared with pre-allergen baseline (placebo:  $P=0.008$ ; MK-0476:  $P=0.044$ ). These changes in baseline FEV<sub>1</sub> were significantly different between the two pretreatments ( $P=0.014$ ).

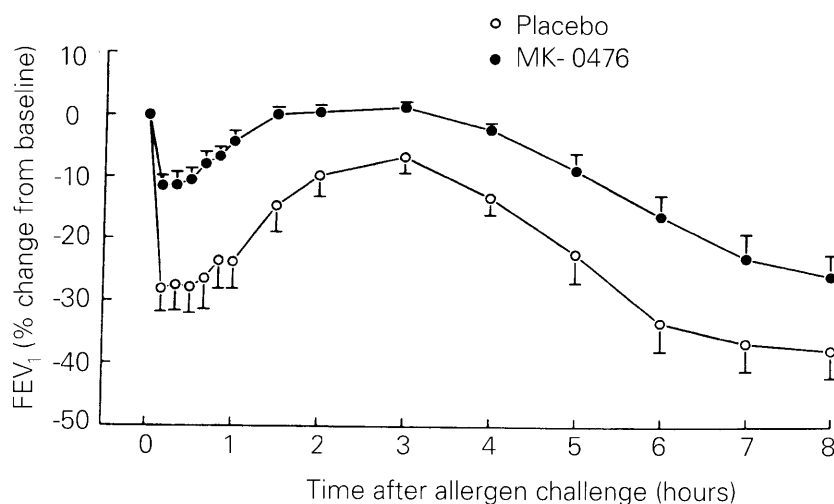


**Fig. 3.** Time-response curves in percentage change from pre-allergen baseline  $FEV_1$  (mean  $\pm$  SEM) 0–8 h after allergen challenge during placebo and montelukast (MK-0476) pretreatment periods. Montelukast markedly inhibited the EAR reducing the maximal percentage fall in  $FEV_1$  from baseline by an average of 53.6% and reducing the  $AUC_{0-3h}$  by an average of 75.4%. In addition, montelukast also decreased the LAR, reducing the maximal percentage fall in  $FEV_1$  from baseline by an average of 36.4%, and the  $AUC_{3-8h}$  by on average 56.9%.

of the cysteinyl leukotrienes released following allergen challenge. Our results do not favour anti-inflammatory effects of montelukast when given during such short period of time, since we were unable to demonstrate protection against allergen-induced increases in number and activity of sputum eosinophils, using the present dosing and sample size.

Our findings confirm and extend observations obtained from previous allergen challenge studies with potent  $cysLT_1$  receptor antagonists in asthmatic subjects. Given in one single oral dose (40 mg) 2 h before challenge, zafirlukast has been shown to protect against the EAR and the LAR reducing the maximal percentage fall in  $FEV_1$  from baseline by 81% and 54%, respectively, and even against the allergen-induced airway hyperresponsiveness to histamine at 6 h after inhalation [29]. Another potent  $cysLT_1$  receptor antagonist, MK-571, administered intravenously (450 mg) before and up to 8 h after the challenge, also provided adequate protection against allergen-induced airway responses, blocking the  $AUC_{0-3h}$  by 88% and the  $AUC_{3-10h}$  by 63% [30]. However, the oral route of administration appears to be more suitable for the management of day-to-day asthma. Therefore, when comparing our data with findings from these allergen studies, montelukast has been shown to possess a favourable pharmacodynamic profile and similar degree of inhibition of the EAR and the LAR.

Allergen inhalation induces an inflammatory response within the airways in which several pro-inflammatory cells and mediators are implicated [1,31–34]. The early response predominantly involves IgE-triggered mast cell mediator release, whereas in the late response activated eosinophils appear to be the most important effector cells, releasing pro-inflammatory mediators including ECP and leukotriene  $D_4$  [1,32,33]. So far, only a few allergen challenge studies have reported on the effects of antileuko-

triene therapy on cellular or soluble inflammatory parameters in humans after single or short-term multiple dose administration [13,35,36]. In asthmatic individuals, 8 days of  $4 \times 600$  mg daily pretreatment with an oral 5-lipoxygenase inhibitor, zileuton, significantly reduced eosinophil numbers in BAL fluid as well as the  $LTE_4$  production in BAL and urine, 24 h after segmental allergen instillation [35]. Likewise, 7 days of pretreatment with oral zafirlukast 160 mg b.i.d. provided a significant decrease in eosinophils and basophils in BAL fluid from asthmatic subjects, 48 h after a segmental allergen challenge [13]. Taken together, preliminary evidence exists from BAL studies in asthmatics that antileukotriene drugs may possess anti-inflammatory properties in allergen challenge, when administered for at least 1 week.

When applying a validated methodology of sputum induction [15,26,27], we were unable to detect an effect of three doses of montelukast on the allergen-induced rise in the percentage eosinophils and ECP levels in sputum. We expected at least a difference of 4.9% in the changes in sputum eosinophils between the two pretreatments, but we found a difference of only 0.8%. Several factors could account for this: (1) the limited rise in allergen-induced sputum eosinophils during placebo period; (2) too short pretreatment with montelukast; or (3) insufficient power for detecting such a small difference in the nine remaining subjects.

First, in our study the allergen-induced rise in the percentage sputum eosinophils appeared to be rather low as compared with other allergen studies [14–16]. Several factors could account for these different findings, such as a greater number of subjects [14], a different methodology or timing of sputum induction [14,16], or a different method of allergen provocation testing [15]. Taken together, these differences in methodology and performance may account

**Table 2.** Sputum cell differentials and ECP pre- and post-allergen during placebo and montelukast (MK-0476) pretreatment periods

|        | Eosinophils (%) | Neutrophils (%) | Lymphocytes (%) | Macrophages (%)   | ECP ( $\mu\text{g/L}$ ; CV) |
|--------|-----------------|-----------------|-----------------|-------------------|-----------------------------|
| P pre  | $2.7 \pm 2.6$   | $28.2 \pm 21.7$ | $4.3 \pm 3.5$   | $57.1 \pm 19.8$   | 155.6 (42%)                 |
| P post | $7.5 \pm 6.1^*$ | $37.1 \pm 15.1$ | $4.3 \pm 5.0$   | $42.8 \pm 13.5^*$ | 641.8 (32%)*                |
| M pre  | $5.5 \pm 10.4$  | $24.7 \pm 17.9$ | $2.5 \pm 2.2$   | $52.7 \pm 18.9$   | 72.9 (50%)                  |
| M post | $9.5 \pm 11.5$  | $38.9 \pm 28.5$ | $3.9 \pm 3.2$   | $44.1 \pm 24.9$   | 502.6 (42%)*                |

\* Significantly different from prechallenge values ( $P < 0.05$ ). Cell differentials in percentage nonsquamous cells (mean  $\pm$  SD). ECP in geometric mean (coefficient of variation = CV in percentage). P pre: placebo pretreatment period before allergen challenge. P post: placebo pretreatment period after allergen challenge. M pre: MK-0476 pretreatment period before allergen challenge. M post: MK-0476 pretreatment period after allergen challenge.

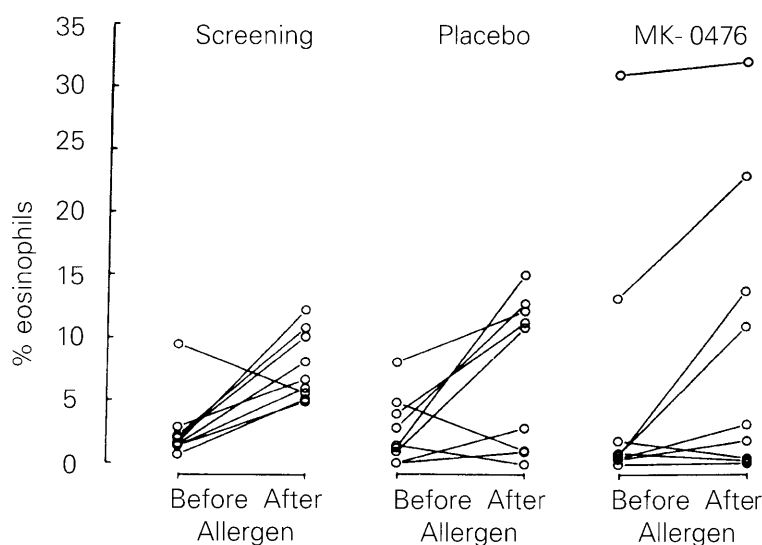
for at least some of the different findings between our study and the others. However, the observation that a significant and similar rise in the percentage sputum eosinophils was found during both the screening and placebo period, is in favour of a reproducible methodology in the present study.

Second, inhibition of inflammatory markers after allergen challenge has only been achieved after a pretreatment period of at least 7 days [13,35]. Hence, for optimal blockade of eosinophil recruitment into the airways, montelukast may also require a more prolonged pretreatment instead of the 36-h period in our study. This resembles recent observations with inhaled steroids. A single dose of inhaled beclomethasone ( $500 \mu\text{g}$ ) 30 min before allergen did produce a (complete) inhibition of the late response, but failed to protect against sputum eosinophilia 7–48 h post-challenge [37]. However, pretreating patients with inhaled beclomethasone ( $400 \mu\text{g/d}$ ) for 7 days not only reduced the allergen-induced airway responses, but attenuated the allergen-induced airway hyperresponsiveness and sputum

eosinophilia as well [16]. Correspondingly, 4 weeks of treatment with montelukast has been shown to reduce eosinophils in induced sputum by 48% from patients with mild asthma [38].

And third, we based our sample size of 12 subjects on a repeatability study of the inflammatory markers in whole samples of induced sputum in patients with similar subject characteristics [26] and on previous sputum data in allergen challenge [15]. At the end of the study, however, sputum samples of only nine subjects were appropriate for analysis due to an excess ( $>80\%$ ) of squamous cells in three subjects. When comparing the present allergen-induced changes in the percentage eosinophils between the screening and placebo periods, a sample size of nine subjects would have been sufficient to detect a difference of at least 6.0% in the changes in sputum eosinophils between the two pretreatments at 90% power. However, we found a difference of 0.8% in these changes. This indicates that the power of the study was insufficient to provide a definite answer on

**Fig. 4.** Percentage sputum eosinophils 24 h before and 24 h after allergen challenge during screening, placebo and montelukast (MK-0476) pretreatment periods. Pre-allergen percentage sputum eosinophils were not significantly different between these three periods ( $P = 0.504$ ). After allergen challenge, there was a significant rise in the percentage sputum eosinophils during the placebo period ( $P = 0.038$ ), with a similar trend during the MK-0476 period ( $P = 0.056$ ). The changes in the percentage sputum eosinophils were not significantly different between the pretreatment periods ( $P = 0.652$ ).



the mode of action of montelukast, due to a limited allergen-induced rise in the percentage of sputum eosinophils.

In summary, we have shown that the potent  $\text{cysLT}_1$  receptor antagonist montelukast provides protection against the allergen-induced airway responses in humans *in vivo*. This finding underlines the importance of the role of cysteinyl leukotrienes in both the early and the late asthmatic responses, and demonstrates that montelukast may be effective in the treatment of allergic asthma. However, pretreatment with three oral once daily doses of montelukast before and during allergen challenge provided insufficient information on its effects on sputum cellular markers and/or activity in the present sample size of nine subjects. Hence, more prolonged pretreatment in larger groups with montelukast should clarify its potential anti-inflammatory effect in allergen challenge.

### Acknowledgements

The authors thank Mrs H. van der Veen and Mr A. C. van der Linden for their technical assistance in the study.

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