Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial

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ABSTRACT: Leukotrienes are pro-inflammatory mediators which may contribute to tissue, sputum, and blood eosinophilia seen in allergic and inflammatory diseases, including asthma. Montelukast is a cysteinyl leukotriene$_1$ (CysLT$_1$) receptor antagonist which improves asthma control; the aim of this study was to investigate its effect on induced sputum eosinophils.

Montelukast 10 mg (n=19) or placebo (n=21) were administered orally once in the evening for 4 weeks to 40 chronic adult asthmatic patients, aged 19–64 yrs, in a double-blind, randomized, parallel group study. Patients were included if, at prestudy, they had >5% sputum eosinophils, symptomatic asthma with a forced expiratory volume in one second ≥65% of the predicted value and were being treated only with "as needed" inhaled β$_2$-agonists. In addition to sputum eosinophils, blood eosinophils and clinical endpoints were also assessed.

Four weeks of montelukast treatment decreased sputum eosinophils from 7.5% to 3.9% (3.6% decrease, 95% confidence interval (CI) -16.6–0.4). In contrast, placebo treatment was associated with an increase in sputum eosinophils from 14.5% to 17.9% (3.4% increase, 95% CI -3.5–9.8). The least squares mean difference between groups (-11.3%, 95% CI -21.1– -1.4) was significant (p=0.026). Compared with placebo, montelukast significantly reduced blood eosinophils (p=0.009), asthma symptoms (p=0.001) and β$_2$-agonist use (p<0.001) while significantly increasing morning peak expiratory flow (p=0.001). Montelukast was generally well tolerated in this study, with a safety profile similar to the placebo.

These results demonstrate that montelukast decreases airway eosinophilic inflammation in addition to improving clinical parameters. Its efficacy in the treatment of chronic asthma may be due, in part, to the effect on airway inflammation.
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Asthma is associated with increased eosinophils in the airways, lung tissue and peripheral blood, which can correlate with asthma severity [1]. Airway eosinophilia has been observed in chronic stable asthma, after allergen inhalation and during exacerbations [2–5]. When activated by various stimuli, eosinophils cause inflammation by releasing toxic products including oxygen radicals, basic proteins, cytokines and cysteinyl leukotrienes (CysLTs) [6]. Bronchial inflammation is considered to be a cause of symptoms and airflow limitation in asthma.

CysLTs are chemoattractants for eosinophils *in vitro* [7]. In guinea-pigs, leukotriene (LT)D$_4$ causes eosinophil influx into the bronchoalveolar lavage (BAL), lung tissue, and conjunctiva, all of which are inhibited by (LT) receptor antagonists [8, 9]. In humans, inhalation of LTE$_4$ and D$_4$ increase bronchial tissue and sputum eosinophils [10, 11]. Inhibition of LT synthesis decreases airway eosinophils in asthmatic patients [12] and blunts the increase in BAL eosinophils after segmental allergen challenge in allergic patients [13].

Montelukast is a potent, specific, oral cysteinyl leukotriene$_1$ (CysLT$_1$) receptor antagonist which improves the signs and symptoms of asthma [14–18]. Montelukast treatment also significantly decreases blood eosinophils [15].

Recently, induced sputum has been introduced as a reliable, valid, and responsive method to safely obtain airway secretions [19]. In contrast to BAL and bronchial biopsy, induced sputum has the additional advantage of being noninvasive, thus allowing repeated measurements during treatment [3, 4, 19–23].

In this study, induced sputum eosinophils were measured to investigate the effect of 4 weeks of daily treatment with montelukast on airways inflammation. In this randomized, placebo-controlled, double-blind trial, the effect of treatment on blood eosinophils and clinical outcomes were also evaluated.

Patients and methods

Patients

Symptomatic adult asthma patients capable of producing induced sputum at a prestudy visit, with a proportion of eosinophils >5%, were consecutively enrolled in the study

(table 1). The diagnosis of asthma was established by symptoms of asthma and by an improvement in the prebronchodilator forced expiratory volume in one second ($FEV1$) $\geq 15\%$ after salbutamol (200 µg). The asthma was mild as indicated by an $FEV1$ between 60 and 85% of predicted. All patients had a minimum level of daytime symptoms (weekly score of at least 32 out of a possible 168), and required inhaled β_2-agonists on an as-needed basis (weekly average, 1 puff·day^{-1}) as recorded on a daily diary card completed during the week before allocation. The asthma was stable; there had been no exacerbations or need for any other treatment for one month, and no hospitalizations for asthma occurred within 3 months of the prestudy visit. Specifically, no patient had previously used an antileukotriene drug. Patients were otherwise healthy, were nonsmokers for at least 1 yr (with a smoking history of no more than 10 pack-yrs), consumed their usual diet and maintained their usual exercise regimen. None had an unresolved sinus or upper respiratory tract infection within 3 weeks before the prestudy visit. Females of childbearing potential had a negative test result for serum β-human chorionic gonadotropin (β-hCG) at the prestudy visit and agreed to use contraception during the study. The Ethical Review Committees and Institutional Review Boards of all participating centres approved the study and all subjects gave written informed consent.

Study design

This six-centre, randomized, double-blind, placebo-controlled, parallel-group trial was comprised of 6 weekly visits: the first (prestudy visit) for patient selection, the second (baseline visit) for allocation to treatment (after a 1-week baseline period), and the remaining four to collect efficacy and safety data during the treatment period. At the prestudy visit, informed consent was obtained and sputum was induced. Patients were given placebo study medication in a single-blind manner and were instructed to continue their prestudy treatment, to record symptoms, the use of as-needed β_2-agonists, and morning and evening peak expiratory flow (PEF) on a daily diary card. Additionally, patients refrained from inhaled salbutamol and caffeinated beverages for at least 6 h, and any antihistamine medication for at least 48 h, before a study visit, and contacted a study physician if a deterioration in symptoms or PEF occurred. Qualifying patients underwent allergy skin testing and sputum induction, and were allocated randomly according to a

computer-generated schedule to receive either montelukast 10 mg or matching image placebo tablets once daily at bedtime. Sputum was induced again 1 and 4 weeks after treatment allocation. Clinical efficacy measurements ($FEV1$, PEF, daytime asthma symptoms) were performed over the 4-week treatment period. Blood safety tests (including eosinophil counts) were performed at prestudy and 2 and 4 weeks after allocation.

Clinical measurements

Patients recorded the number of as-needed β_2-agonist puffs used, morning and evening PEF (the best of three measures using a Mini-Wright peak flow meter (Clement Clark Inc., Columbus, OH, USA)), and daytime asthma symptom scores on a daily diary card validated for its measurement and linguistic properties [24]. Spirometry was performed according to American Thoracic Society criteria [25] between 06:00 h and 09:00 h, ~8–10 h after the previous (bedtime) dose of study drug. For each time point, the best $FEV1$ value from at least three measurements was used for analysis. Reversibility was determined by administering salbutamol 200 µg through a spacer device (Aerochamber^{TM}; Monaghan Medical Corporation, Plattsburgh, NY, USA) and measuring $FEV1$ 10–30 min later. Allergy skin tests were carried out by the skin prick technique with a minimum of five aeroallergen extracts appropriate to the geographical location. A weal diameter at least 3 mm larger than the diluent control was considered positive.

Sputum and blood examination

Study site personnel were trained to perform sputum induction and processing in a standardized manner. Sputum was induced as described by PIN et al. [22] with an aerosol of hypertonic saline (3, 4 and 5%) generated by a Fisoneb^{TM} ultrasonic nebulizer (Canadian Medical Products Ltd., Markham, Ontario, Canada). Sputum separated from saliva was processed as described by PIZZICHINI et al. [19]. Briefly, sputum was treated by adding four volumes of 0.1% dithiothreitol (DTT), sputalysin 10% (Calbiochem Corp., San Diego, CA, USA) followed by four volumes of Dulbecco's phosphate-buffered saline (D-PBS). The suspension was filtered through a 48-µm nylon gauze (BBSH Thompson, Scarborough, Ontario, Canada) and total cell counts of leukocytes and cell viability (trypan blue exclusion method) were determined. The cell suspension was adjusted to 1.0×10^6 cells·mL^{-1} and cytocentrifuge preparations were made using 60 µL of the cell suspension (Shandon III cytocentrifuge, Shandon Southern Instruments, Sewickly, PA, USA). Four cytospins were made, air dried, coded and sent to a central laboratory for reading (St. Joseph's Hospital, Hamilton, Ontario, Canada). Two were stained by Wright's stain for a differential cell count on at least 400 nonsquamous cells, and two were fixed in Carnoy's fixative and stained with toluidine blue for a differential cell count on at least 1,500 metachromatic cells (mast cells and basophils). Squamous cells were measured as a percentage of the total cell population; results of the other cells were expressed as a percentage of the total nonsquamous cell count.

Blood was collected in venoject tubes and sent to a central laboratory (Covance Central Laboratory Services

Table 1. – Mean baseline patient characteristics

Parameter	Montelukast	Placebo
Subjects M/F	12/7	12/9
Age (range) yrs	31 (19–64)	28 (19–62)
Atopic n	18	20
Asthma symptom score	2.5±0.83	2.17±0.70
β_2-agonist use puffs·day^{-1}	4.70±2.9	4.10±2.8
PEFa.m. L·min^{-1}	424±72.2	429±81.8
$FEV1$ % pred	69.2±10.9	68.9±14.7
Sputum eosinophils %	6.9±9.1	14.1±14.2
Blood eosinophils 10^6 cells·mL^{-1}	0.36±0.19	0.51±0.29

Data are presented as mean±SD. M: male; F: female; PEFa.m.: morning peak expiratory flow; $FEV1$: forced expiratory volume in one second.

Table 2. – Sputum cell counts

Cell type	Treatment	Cells		Difference in least-squares mean* %	95% CI of difference %
		Baseline	Last visit		
Eosinophil	Placebo	14.54±14.40	17.90±19.79		
	Montelukast	7.53±9.52	3.88±4.67	-11.27	(-21.11– -1.44)[+]
Neutrophil	Placebo	35.83±19.45	33.12±22.88		
	Montelukast	41.47±28.27	45.85±29.32	7.23	(-7.50–21.96)
Lymphocyte	Placebo	1.80±1.38	1.68±1.19		
	Montelukast	1.41±1.54	1.49±1.08	0.19	(-0.61–0.99)
Macrophage	Placebo	47.19±17.52	46.96±21.50		
	Montelukast	49.32±26.93	48.33±28.64	-1.23	(-17.99–10.53)
Bronchial epithelial cell	Placebo	0.43±0.86	0.35±0.52		
	Montelukast	0.28±0.43	0.46±0.88	0.29	(-0.40–0.98)
Metachromatic cell	Placebo	0.11±0.12	0.11±0.19		
	Montelukast	0.08±0.07	0.08±0.06	0.00	(-0.09–0.08)

Data are presented as mean±SD, single value or range. *: analysis of covariance. CI: confidence interval. [+]: p=0.026.

Inc., Indianapolis, IN, USA), which analysed blood chemistry, haematology and differential (including eosinophil) cell counts.

Slide reading

All slides were independently evaluated by two readers blinded to treatment, study site and visit. The average value for each cell type was calculated. A previous validation study using the same readers employed in this study demonstrated that the one week concordance correlation coefficient [26] within reader was >0.98, while between readers was >0.99 [19].

Statistical analysis

The percentage change in sputum eosinophils from baseline (the allocation visit) to the Week-4 value (primary hypothesis) was analysed using an analysis of covariance (ANCOVA) model including factors for centre, treatment, and baseline. The ANCOVA model was used to adjust differential regression to the mean effects due to the imbalance in baseline values. While sputum eosinophils had to be >5% at the prestudy visit, some baseline (allocation visit) values were <5%. Therefore, a confirmatory analysis was performed on patients with baseline sputum eosinophils >3% (considered significantly elevated [27]) to exclude baseline values too low to show meaningful decreases during treatment. Treatment by baseline interaction was also evaluated as was the change from baseline to the Week-1 (first visit after allocation) value using the same model. The average treatment period change from baseline for peripheral blood eosinophils was also compared between treatment groups using this model. Postallocation sputum eosinophil data were excluded from analysis if prespecified criteria confounding efficacy measurements, such as an upper respiratory tract infection, occurred. For clinical endpoints, the average treatment period changes (daytime symptom score, PEF) or average per cent changes (FEV1, β2-agonist use) were summarized (mean±SD) and compared between treatment groups using an analysis of variance (ANOVA) model including factors for treatment and centre. The between treatment differences were summarized as least squares means and 95% confidence intervals (CI) using the ANOVA model. All available data were used for the analysis of

clinical endpoints. A p-value ≤0.05 was considered statistically significant.

The study was designed with a treatment group sample size of n=18 to have 80% power (SD=65% (log scale) at an α=0.05, two-tailed test) to detect a reduction of 50% in the change from baseline in the percentage of eosinophils between treatment groups.

Results

Patient allocation and exclusion from sputum analysis

Forty eligible patients were randomized to active treatment (19 to montelukast, 21 to placebo). Three patients in the montelukast group did not have Week-4 sputum samples available. Two of these patients were discontinued, one owing to an adverse event (abdominal pain) and one owing to a protocol deviation. Data from one placebo patient were excluded from sputum analysis because an upper respiratory tract infection occurred within 5 days after randomization (prespecified criteria). All allocated patients were included in clinical endpoint and safety analyses.

Effect of montelukast on sputum and blood cell counts

The characteristics of sputum cells in each group were examined before treatment. Eleven patients in the montelukast group and 17 in the placebo group had sputum eosinophils >3% at baseline visit. Compared with montelukast, the proportion of sputum eosinophils at baseline was higher in the placebo group whether including all subjects (table 2) or analysing only patients with sputum eosinophils >3% (mean eosinophils of 10.6 and 17.1% for montelukast and placebo, respectively).

While there was some variability in response, the effects of treatment on sputum eosinophils in most patients within each group were similar. Four weeks of treatment with montelukast significantly reduced the percentage of sputum eosinophils. In contrast, there was an increase in sputum eosinophils after treatment with placebo (table 2, fig. 1). These effects were evident after the first week (decrease of 2.6% and increase of 2.0% from baseline for montelukast and placebo, respectively, p=0.177) and progressed over the remaining weeks of therapy. The mean (95% CI)
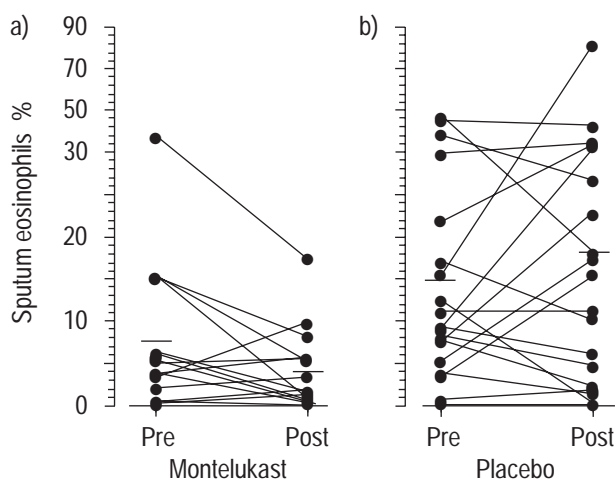
Fig. 1. – The effect of: a) montelukast (n=16); and b) placebo (n=20) on sputum eosinophils at baseline (Pre) and after 4 weeks (Post) of therapy. Each point is the mean from two blinded readers. Horizontal bars represent mean values. The percentage of eosinophils (mean±SD) decreased from 7.53±9.52 to 3.88±4.67 in the montelukast group and increased from 14.54±14.40 to 17.90±19.79 in the placebo group (p=0.026).
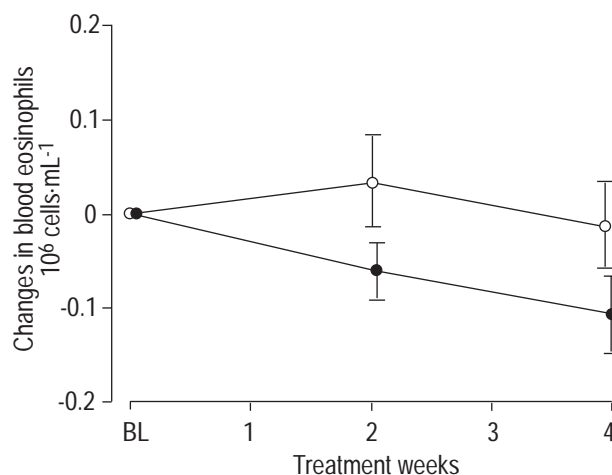

Fig. 2. – The effect of montelukast (●) and placebo (○) on peripheral blood eosinophils at baseline (BL) and 2 and 4 weeks after allocation. Vertical bars represent mean±SEM. Over the 4-week period, montelukast caused a significant (p=0.009) reduction compared with placebo in peripheral blood eosinophils.

changes in sputum eosinophils were -3.6% (-16.6–0.4) and 3.4% (-3.5–9.8) in the montelukast and the placebo group, respectively. Similar changes were observed in the patients with sputum eosinophils >3%.

Over the four week treatment, montelukast significantly reduced the number of peripheral blood eosinophils compared with placebo (fig. 2). The mean difference from baseline was -0.09 (decrease of 25%) and -0.01 (decrease of 2%) in the montelukast and placebo groups, respectively (p=0.009).

Effect of montelukast on clinical endpoints

Compared with placebo, montelukast treatment produced a significant reduction (mean (95% CI)) in the daytime asthma symptoms -0.7 (-1.1– -0.3) (p=0.001) and β_2-agonist use -8.1% (-100.4– -35.7), (p<0.001), and an increase in morning PEF 39.0 (16.8–61.2) L·min^{-1}, (p=0.001). There was no statistically significant difference between treatments in FEV1 (p=0.395) (fig. 3). The only significant correlation between changes in sputum eosinophils and changes in clinical endpoints was seen with per cent change in FEV1 (r=-0.41, p=0.012).

Adverse experiences

Montelukast was generally well tolerated in this study. There were few clinical adverse experiences and their frequency in the montelukast and placebo groups were similar. Three patients were discontinued due to clinical adverse experiences. One placebo patient was withdrawn due to worsening asthma. One montelukast patient was withdrawn due to sarcoidosis believed by the investigator to have been present at the prestudy visit; another montelukast patient was withdrawn because of abdominal pain. Laboratory adverse experiences were also infrequent and similar between montelukast and placebo. No patient was discontinued due to a laboratory adverse experience.

Discussion

In this randomized trial, daily treatment with montelukast for 4 weeks reduced sputum and blood eosinophils and improved clinical asthma control compared with placebo. A small effect on sputum eosinophils was seen after 1 week and this was significant after 4 weeks of therapy. These results raise the possibility that the decreases in sputum eosinophils might have contributed to the clinical improvement.

This is the first report of the effect of montelukast on airway eosinophilic inflammation in humans. The decrease in eosinophils was identified by the repeated examination of induced sputum, a method which is noninvasive and has been shown to be successful, reliable and responsive to change with treatment [28]. The effect of montelukast on blood eosinophils has also been demonstrated in previous studies [16, 17].

The results, not confounded by other asthma treatments, were obtained in patients with chronic asthma and with a relatively small sample size. Montelukast treatment, despite a lower baseline, decreased sputum eosinophils, while an increase was observed in the placebo group. The direction of the changes, despite baseline differences, provided strong evidence for a treatment effect by working against changes expected *via* regression to the mean [29]. In the absence of a treatment effect, the regression to the mean would cause changes in the opposite direction, reducing the treatment effect. Future studies might consider stratifying patients by baseline eosinophil percentages, to ensure they are similar in the treatment groups.

Standard, study-wide methodology to ensure sputum induction and processing consistency was employed; all technicians were trained centrally. The use of two independent and highly trained haematology technicians to read the cytospins minimized potential sources of bias. A validation of these same two slide readers had previously been shown to be highly repeatable and concordant [19].

An inhibition of eosinophil chemotaxis by montelukast may explain the results in this study. CysLTs have been shown to attract eosinophils, and LT antagonists could
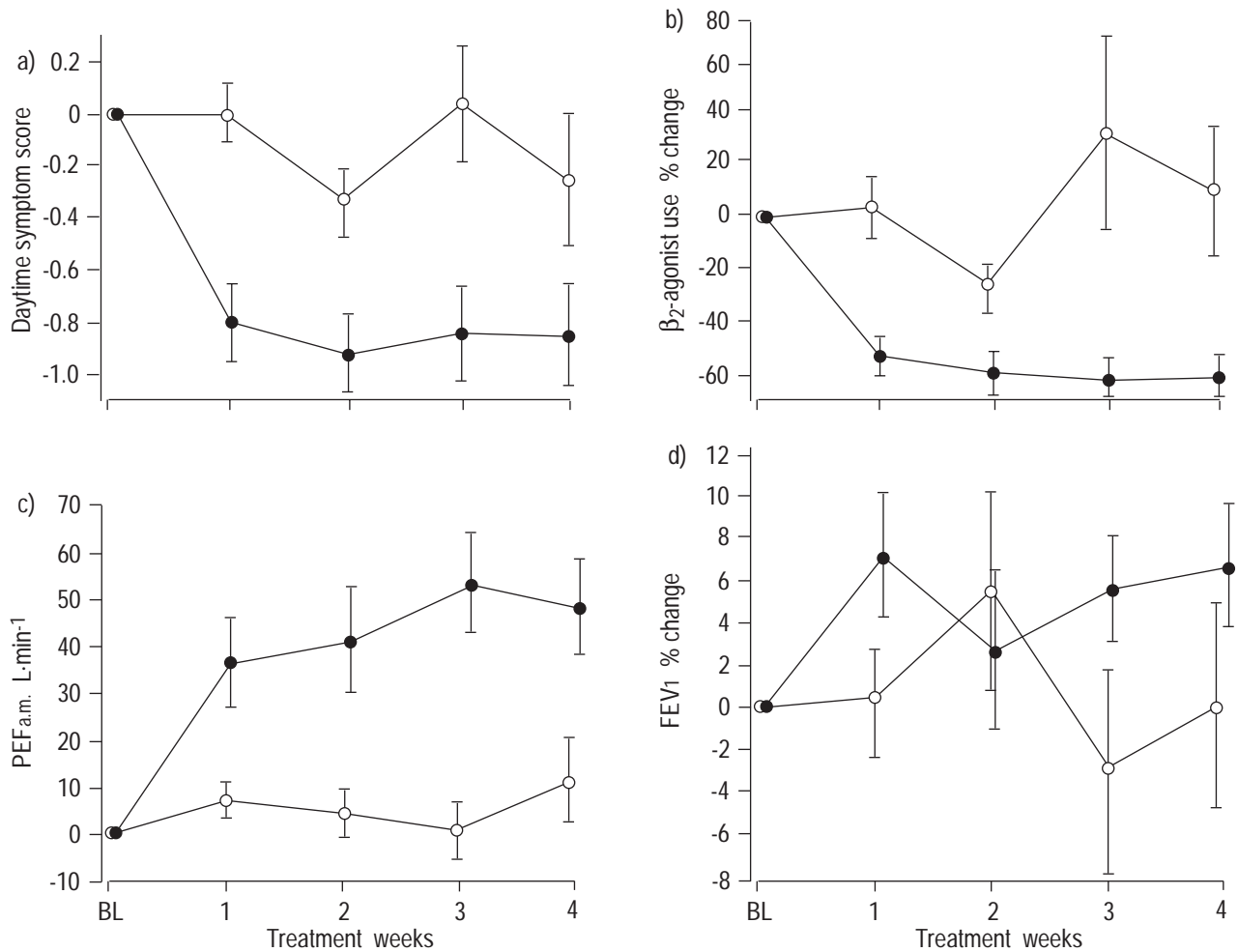
Fig. 3. – The effect of montelukast ($\bullet$) and placebo ($\circ$) on: a) daytime symptoms; b) β_2-agonist use (puffs·day^{-1}); c) morning peak expiratory flow (PEFa.m.); and d) forced expiratory volume in one second (FEV1) over the 4-week treatment period. Vertical bars represent mean±SEM change from baseline (BL). Treatment with montelukast caused significant reductions in daytime symptoms (p=0.002) and β_2-agonist use (p<0.001) and increases in PEFa.m. (p=0.001).

potentially block these effects. For example, SPADA et al. [7] showed LTD$_4$ to be a potent and selective chemoattractant for human eosinophils at physiologically relevant concentrations. Using radiolabelled eosinophils in guinea-pig conjunctiva, CHAN et al. [9] showed administration of LTD$_4$ induced a 2.5-fold increase in conjunctival radioactivity (a measure of eosinophil chemotaxis) in vivo. Utilizing aerosolized LTD$_4$, UNDERWOOD et al. [8] demonstrated inhaled LTs elevated BAL eosinophils in guinea-pig airways; increased eosinophil numbers were confirmed histologically in the bronchial epithelium and subepithelium. Finally, LAITINEN et al. [10] showed inhaled LTE$_4$ increased eosinophils (but not mast cells, lymphocytes, plasma cells or macrophages) in the lamina propria of the airways of patients with asthma.

Corticosteroids and antileukotriene agents have been shown to affect sputum eosinophils in patients with asthma. In a 3-week placebo-controlled study, inhaled beclomethasone (1,000 µg daily) decreased sputum eosinophils by 73%, (45% decrease with placebo treatment, net difference 28%) [30]. Similarly, inhaled beclomethasone (1,000 µg·day^{-1}) reduced sputum eosinophils in a 4-week study comparing beclomethasone with salmeterol [31], and treatment with budesonide (400 µg·day^{-1} for 7 days) attenuated the

increase in eosinophils after antigen challenge [3]. In the present study, the 48% decrease in sputum eosinophils after montelukast treatment (compared with the 23% increase after placebo) is similar in magnitude (net difference of 71%) to the effects seen with corticosteroids in other studies; however, direct within-study comparisons are necessary to confirm this interpretation. An additional, interesting observation in the present trial is the large improvement observed in most clinical endpoints (PEF, β_2-agonist use, symptoms) which, on average, are greater than the effect seen in other montelukast trials [16, 17]. The explanation for this observation is unknown (and may be a chance variation), but may be due to the selection of patients with more substantial airway eosinophilic inflammation. However, the correlation between improvements in sputum eosinophils and clinical endpoints was generally modest; the relationship between changes in clinical endpoints and airways eosinophils is unknown and awaits further clinical trials.

This study was neither designed nor powered to prospectively determine the correlation between sputum markers and clinical outcomes. Therefore, the weak correlations between improvement in sputum eosinophils and clinical outcomes is likely to be an effect of the small range of

airflow limitation, symptoms and β_2-agonist use observed in these mildly symptomatic asthmatic patients. However, it has been reported in prednisone-dependent asthmatics [32] that the improvement in clinical parameters after treatment of asthma with prednisone preceded the complete resolution of sputum inflammatory markers. Additionally, during a programmed reduction in prednisone dose, the inflammatory markers in sputum exacerbated before the clinical outcomes. The improvement of clinical parameters before the improvement of airway inflammation was also observed after treatment in nonprednisone-dependent asthmatics with a severe exacerbation of asthma [33]. These observations indicate that changes in clinical parameters may not be completely explained by changes in airway inflammation.

In summary, these results have shown that montelukast, a leukotriene receptor antagonist, reduced sputum and blood eosinophils, and improved clinical endpoints of asthma. The reduction in eosinophilic inflammation may contribute to the beneficial effects of montelukast in chronic asthma.

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