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Failure of montelukast to reduce sputum eosinophilia in high-dose corticosteroid-dependent asthma

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ABSTRACT: Sputum eosinophilia is a sensitive predictor of benefit from corticosteroid treatment. Montelukast is a cysteinyl leukotriene antagonist, which also reduces sputum and blood eosinophils. The present study examined the possibility that montelukast has an added eosinophil-lowering effect in subjects with asthma who are corticosteroid responsive but relatively corticosteroid resistant.

A total of 14 clinically stable adults with asthma requiring minimum treatment with a high-dose inhaled steroid or prednisone, with baseline sputum eosinophilia (⩾5%), were randomised to receive 4 weeks of 10 mg montelukast or placebo daily in a double-blind crossover trial. The primary outcome was the effect of treatment on the percentage of sputum eosinophils. Secondary outcomes were changes in the blood eosinophil count, symptoms, forced expiratory volume in one second, peak expiratory flow and the need for salbutamol.

The median (interquartile range, *i.e.* 75th–25th centile) for sputum eosinophils at baseline was 15.7% (22). The effect of adding montelukast was not significantly different from that of placebo, sputum eosinophils being 9.3% (18.9) after montelukast and 11.3% (22.8) after placebo. No difference was detected on secondary outcomes. No crossover interactions were observed.

In conclusion, the addition of montelukast to existing high-dose corticosteroid therapy in subjects with asthma with elevated sputum eosinophils does not provide additional attenuation of airway eosinophilia.

KEYWORDS: Montelukast, prednisone-dependent asthma, sputum cell counts

Asthma is clinically defined by the presence of variable airflow limitation, but is also associated with airway inflammation [1]. The treatment of asthma is directed towards both of these components. Whilst airway inflammation is heterogeneous, sputum eosinophilia predicts improvement with corticosteroid treatment [2]. The improvement is associated with the reversal of sputum eosinophilia and improvement in airway responsiveness and airflow limitation. Sputum eosinophilia is also a sensitive marker of eosinophilic exacerbations of asthma and control of it reduces these [3–5]. Hence, it is reasonable to consider that control of airway eosinophilia should be an objective of the treatment of asthma.

In a minority of patients with asthma, high-dose corticosteroid is required to maintain optimum treatment. In these patients, a drug with an added anti-inflammatory effect might allow a reduction in the corticosteroid dose needed. Another drug which lowers airway eosinophilia is montelukast, a cysteinyl leukotriene-receptor antagonist (LTRA). Cysteinyl leukotrienes promote bone marrow eosinophilopoiesis and eosinophil chemotaxis into the airways, increase the surface expression of adhesion molecules on eosinophils and blood vessels facilitating their transmigration through endothelial barriers, and prolong eosinophil survival by upregulating gene expression of mediating cytokines and chemokines, such as interleukin (IL)-5 and eotaxin [5–9]. Montelukast lowers sputum and blood eosinophils in steroid-naive asthma, and in asthma requiring lower doses of inhaled steroid [10–12]. Its effect in asthma requiring maintenance treatment with higher doses of inhaled steroid or prednisone has not been investigated. Prednisone treatment has been observed as having no effect on bronchoalveolar lavage (BAL) fluid eicosanoid levels [13]. The current authors hypothesised that montelukast would have an added eosinophil-lowering effect in these patients.

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Therefore, the ability of montelukast to reduce sputum eosinophilia in patients with asthma, who required chronic treatment with prednisone or higher doses of inhaled steroid and in whom additional corticosteroid had been shown to abolish the eosinophilia, was examined. The study was placebo controlled, double-blind, randomised and crossover. The primary outcome was the change in the sputum eosinophil count after 4 weeks of treatment. Secondary outcomes were changes in the blood eosinophil count, symptoms, forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF) and the need for salbutamol.

MATERIAL AND METHODS

Subjects

A total of 14 adults with asthma were recruited from 30 adults who were screened between February and December 1999 in the clinics of the Firestone Institute for Respiratory Health (Hamilton, ON, Canada; table 1). Asthma was defined by symptoms of episodic wheeze, chest tightness and dyspnoea, plus an improvement of $\geq$12% or 200 mL in FEV_1 after 200 μg salbutamol or a course of additional prednisone within the last year. All subjects had been monitored by sputum cell counts (in addition to symptoms and spirometry) to decide the minimum treatment to prevent frequent exacerbations. All subjects required treatment with daily high-dose inhaled steroid (budesonide $\geq$800 μg; n=14) or additional prednisone (n=11) to optimise symptoms and spirometry. In this state, all but two had chronic airflow limitation (FEV_1/vital capacity: <70% predicted). Four subjects were ex-smokers of $\geq$10 pack-yrs. None had any other evidence of chest disease, including emphysema (based on computerised tomography of the thorax and carbon dioxide gas transfer), reversible, or confounding causes for steroid insensitivity, or intolerance to nonsteroid anti-inflammatory drugs [14]. Those with an upper respiratory tract infection and those requiring antibiotics within 4 weeks of commencing the study were excluded. At entrance into the study, the subjects were clinically stable, but not necessarily ideally treated. Most had some daily symptoms (table 2) and an FEV_1 below predicted and below their best value in the last year (table 1). The patients' corticosteroid dose had been unchanged for a minimum of 2 months. All subjects had sputum eosinophilia of >5% (normal <2%), which had been reversed by previous increases in corticosteroid treatment. The study was approved by the hospital research ethics board and all subjects gave written informed consent.

Study design

The present study consisted of a randomised, double-blind, crossover study of two periods with four visits over 10 weeks and no intervening washout period. At the first visit, subjects' characteristics were documented and allergy skin tests carried out. The subjects began to record daily symptoms, medication use, and morning and evening PEF in a diary card, which was continued throughout the study. After a 2-week run-in period, baseline data (visit two) were collected, including symptom severity, spirometry, induced sputum cell counts, blood eosinophil count and liver function tests. Sputum and blood measurements were made blind to clinical details. The subjects were randomised to receive either montelukast 10 mg before bed or placebo for 4 weeks. They then crossed over to receive the alternative medication for a further 4 weeks. The procedures performed at the second visit were repeated at the end of each treatment. A washout period was not included

TABLE 1 Subject characteristics

Patient No.	Subject characteristics								Best value in last 12 months					
	Age yrs	Sex	Smoker pack-yrs	Atopy	P mg·d^{-1}	ICS μg·d^{-1}	LABA	FEV_1 % pred V1	FEV_1 % pred	FEV_1/VC %	FEV_1 ΔS mL	FEV_1 ΔS %	FEV_1 ΔP mL	FEV_1 ΔP %
1	58	M	20	No	9	1600	No	48	76	46	410	23	1230	55
2	60	M	76	Yes	20	3200	Yes	47	100	60	270	12	230	10
3	68	F	0	Yes	7.5	1600	Yes	64	71	59	150	12	250	21
4	67	M	4	Yes	10	2400	Yes	50	68	65	220	25	320	30
5	73	M	0	No	4	800	No	38	64	59	330	24	370	27
6	42	M	0	Yes	10	3200	No	66	71	55	150	6	880	53
7	58	M	1.6	No	10	1600	No	29	40	36	310	33	100	9
8	45	F	0	Yes	0	2400	No	75	92	78	0	0	640	48
9	64	F	10	No	12.5	1200	Yes	51	55	43	180	18	180	15
10	74	M	5	Yes	0	1600	No	54	79	66	480	23	530	21
11	69	F	10	No	12.5	1600	Yes	68	72	63	360	23	ND	ND
12	72	F	1	Yes	0	1600	Yes	72	86	68	280	11	490	16
13	54	F	0	Yes	5	1600	Yes	55	66	58	320	37	220	17
14	52	M	0	Yes	5	1600	No	89	111	77	380	18	1030	42
Mean±SD	61.1±10.2				7.5±5.7	1857.1±694.7		61.5±15.4	75±18	59.5±11.9	274.3±125.2	18.9±10	487.9±342	27.8±15.6

Atopy: $\geq$1 positive ($\geq$3 mm) weal with allergy skin-prick tests; P: prednisone; ICS: inhaled corticosteroid (budesonide equivalent dose); LABA: long-acting β_2-agonist; FEV_1: forced expiratory volume in one second; V1: visit one; VC: vital capacity; ΔS: change with salbutamol 200 μg; ΔP: change with added prednisone; M: male; F: female; ND: not done.

TABLE 2 Inflammatory and clinical outcomes at baseline, with montelukast and placebo

Inflammatory	Baseline	Montelukast	Placebo	p-value
Sputum total cell count 10^6 cells·g^{-1}#	6.3 (5.2)	5.7 (6.7)	7.1 (13.8)	0.53
Eosinophils %#	15.7 (21)	9.3 (18.9)	11.3 (22.8)	0.14
Neutrophils %	54.5±16.7	42.8±25.8	45.7±22.4	0.21
Blood eosinophils 10^9 cells·mL^{-1}	0.38±0.24	0.35±0.25	0.41±0.28	0.12
Clinical				
Symptom score¶	29.7±5.5	29.4±4.1	30.3±3.0	0.58
Salbutamol use μg·day^{-1}	73.1 (175.5)	57.1(171.9)	63.7 (170.1)	0.26
FEV1				
Postbronchodilator L	1.96±0.80	2.05±0.80	1.98±0.76	0.19
%	63.2±17.3	66.8±16.1	64.7±17.3	0.41
Morning PEF L·min^{-1}	340±95	339±101	339±99	0.90
Evening PEF L·min^{-1}	347±97	348±96	334±86	0.70
Diurnal PEF variation				
Amplitude % mean	6.92±6.32	13.15±17.44	7.5±5.96	0.79
Lowest morning %	75.8±11.3	79.5±12.3	75.7±13.8	0.22

Data are presented as mean±SD. The baseline is equivalent to the second visit. PEF: peak expiratory flow, calculated mean over the last 7 days. Diurnal PEF variation calculated as 1) amplitude % mean=(maximum–minimum)/mean of last 7 days, and 2) lowest morning PEF %=subject's personal best of last 7 days. The p-value was calculated by ANCOVA comparing montelukast to placebo with baseline as covariate. #: median (interquartile range); ¶: five worst, 35 best.

to ensure that the study was completed in a reasonable time to minimise any risk of a secular trend with time, which might result in a period–treatment interaction. In addition, the study design took into account that the comparison of montelukast and placebo would be made at the end of 4 weeks, when any carry-over effect should be negligible [15].

Procedures

Subject characteristics were documented with a structured questionnaire. Symptoms (shortness of breath, chest tightness, wheeze, cough and sputum) were recorded using a validated seven-point Likert scale, with a score of five being the worst and 35 the best [16]. Allergy skin tests were performed by the modified prick technique [17] with 14 common allergen extracts. Spirometry was performed with a Koko spirometer (PDS Instrumentation, Louisville, CO, USA), according to the American Thoracic Society specifications [18], before and 10 min after 200 μg salbutamol was inhaled through an Aerochamber (Trudell Medical International, London, ON, Canada). The FEV1 was recorded as the best of three reproducible values (within a maximum change of 5%). Reference values were taken from CRAPO *et al.* [19]. PEF was recorded as the best of three blows using a mini-Wright peak flow meter (Clement Clarke Inc., Mason, OH, USA). Diurnal variation of PEF was calculated using two methods. The first used the amplitude % mean (maximum value minus the minimum value, divided by the mean) of the 7 days before each visit. The second method used the lowest morning PEF expressed as a percentage of personal best over the same period of time [20]. Assessment of compliance was by pill count and weighing of inhaled medication canisters at each visit. Compliance with regular treatment for each subject was also checked against prescription records at the patients' pharmacy.

Sputum was induced and processed as described by PIZZICHINI *et al.* [3]. Peripheral blood was examined for cell counts and serum bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase.

Statistical analysis

Descriptive statistics were used to summarise the clinical characteristics of the subjects. Dependent variables with a non-Gaussian distribution, including the primary outcome, were log transformed before analysis. Measurements are reported as mean±SD unless otherwise specified. Repeated measures of ANOVA were used to compare the main effect of montelukast with placebo in a model with factors for treatment, treatment sequence, and baseline measures as covariates [15]. This model was adjusted for the differential regression to the mean effects due to imbalance in baseline values. Significance was accepted at $p<0.05$ (two-tailed).

A sample size of 14 subjects was calculated to provide an 80% power of detecting ($\alpha=0.05$, two-tailed) with a $\geqslant$50% difference in the sputum eosinophil count between treatment groups [21]. This difference was considered to be clinically significant *a priori*.

RESULTS

All 14 subjects completed the study. Compliance was questionable in one subject (patient No. 13) based on the pharmacy prescription profile and pill count; data from this subject were excluded from subsequent analysis.

Montelukast had no significant effect on the sputum eosinophil count ($p=0.14$) or other total and differential cell counts compared with placebo (table 2). The sputum eosinophil counts after 4 weeks with either treatment were similar regardless of the treatment sequence allocated, indicating

no treatment or carry-over effect ($p=0.38$). Similarly, no significant differences were seen with montelukast compared with placebo on blood eosinophil counts, symptom scores, FEV_1 diurnal variation of PEF or need for salbutamol.

One subject (patient 5) developed drug-induced hepatitis after 4 weeks' treatment with montelukast. The cause was attributed to montelukast because there was no history of other causes; the patient's pre-treatment liver function tests and follow-up liver ultrasonography were normal, and the serum bilirubin, ALT, AST and alkaline phosphatase, which rose to 10 $\mu mol \cdot L^{-1}$, 326 $U \cdot L^{-1}$, 262 $U \cdot L^{-1}$ and 318 $U \cdot L^{-1}$, respectively, returned to normal within 3 days of stopping the montelukast treatment.

DISCUSSION

In the current study, the addition of montelukast 10 mg daily for 4 weeks did not reduce sputum eosinophils, improve symptoms or airway function compared with placebo in subjects with asthma. The patients selected for the study had current sputum eosinophilia; they were corticosteroid responsive and the eosinophilia had been previously abolished by adequate corticosteroid treatment. However, the patients were relatively steroid resistant as indicated by the need for daily treatment with high-dose maintenance corticosteroid. The results may argue against montelukast having a steroid sparing effect in patients with these characteristics.

The lack of benefit is supported in a different study by the failure of montelukast to reduce elevated blood eosinophil counts in subjects needing treatment with moderate-to-high doses of inhaled steroid [21]. The result is contrary to the effect of montelukast in lowering sputum and blood eosinophils in subjects with asthma who were not being treated with inhaled steroids or being treated with low doses [10–12]. Hence, montelukast seems incapable of lowering eosinophils in subjects who are relatively corticosteroid resistant and need daily treatment with prednisone or higher doses of inhaled steroid.

The strength of the present study is that it has a randomised, controlled double-blind design. Possible weaknesses that need discussion include the dose and duration of montelukast treatment, the lack of a washout period between treatments in the crossover design, possible regression to the mean in sputum eosinophilia, and the power of the sample size to detect a difference between the two treatments. With respect to dose, it is possible that a higher dose of montelukast might have been more effective in the patients in the present study, just as previous treatment with higher doses of corticosteroid had been. This is a consideration for future study. However, against this possibility are the observations of ALTMAN *et al.* [22] who found, in a dose-ranging study in moderate-to-severe asthma (FEV_1 59–62% predicted), that montelukast 10 mg daily, over 6 weeks, produced similar improvement to doses of 100 or 200 mg in blood eosinophil counts, symptoms, FEV_1 and PEF. With respect to duration of treatment, there has been no study in subjects needing higher doses of corticosteroid treatment. However, in those needing lower doses, montelukast reduces sputum eosinophils after 1 and 4 weeks of treatment [10, 23].

A washout period was omitted from the present study for reasons stated previously in the Methods section. The current authors do not believe that this is a weakness of the study, since montelukast binds reversibly to the leukotriene receptor, reaches steady state by day 2 and has a plasma terminal half-life of 5.3 h (up to 6.6 h in the elderly); this suggests that its clinical effects last, at most, for 33 h (five half-lives) [24]. Furthermore, several previous crossover trials examining the efficacy of adding montelukast to placebo in subjects on corticosteroids have omitted a washout period without evidence of carry-over interactions [25–27]. For example, a two-period, 16-week crossover trial, with a 2-week run-in phase, comparing the effect of adding montelukast or placebo to subjects with mild asthma already on fluticasone proprionate, found similar insignificant reductions in inflammatory cells on bronchial biopsy [27].

The subjects in the present study were selected to have sputum eosinophilia of $\geqslant 5\%$ to provide a signal of response to treatment [28]. This could have resulted in a regression to the mean and contributed to the nonsignificant result. However, because of the crossover design, regression to the mean should occur equally with both treatments, allowing any genuine additional anti-inflammatory effect of montelukast to be detected. In addition, the statistical model used for the analysis adjusted for the differential regression to the mean effects due to imbalance in baseline values [29]. Finally, the lack of an eosinophil lowering effect of montelukast does not seem to be due to an underpowered study. A retrospective power analysis, using the data obtained in the current study, demonstrated that the trial had a 93% power to detect a 50% difference, and an 80% power to detect a 30% difference in sputum eosinophils between montelukast and placebo.

The results of the present study, therefore, do not support an added effect of montelukast on eosinophilic bronchitis in subjects with the characteristics investigated. Here, it is relevant to ask if sputum eosinophilia is important in the reversal of effects. On the one hand, there is evidence that sputum eosinophilia is a sensitive predictor of eosinophilic exacerbations of asthma [3–5]. It is abolished, in association with clinical improvement, by adequate steroid treatment [2, 30] and the control of it reduces exacerbations [5, 31]. On the other hand, it has been observed that *i.v.* humanised monoclonal anti-IL-5 antibody failed to prevent allergen-induced late asthmatic responses despite attenuation of blood and sputum eosinophilia [32]. Hence, while sputum eosinophils are an important marker of eosinophilic airway inflammation, the cell itself may be a bystander in the inflammatory response rather than an effector participant in it. It is possible that montelukast may exert a greater effect on the mediators of eosinophil maturation, differentiation and activation in the airway. It may also reduce other functionally relevant cells, such as mast cells, basophils and lymphocytes and noncellular components of airway inflammation, such as airway oedema, which are not primary outcomes of the present study.

As a secondary objective, the current authors also examined the possibility of a clinical benefit of montelukast. Here, the sample size or population studied could have contributed to the lack of effect. The subjects had mild symptoms and, in general, only a mild reduction in FEV_1 from their best values.

Half of the subjects were also being treated with a long-acting β_2-agonist, which may have produced maximal benefit with respect to bronchodilator reversibility. However, a higher dose of corticosteroid had produced clinical improvement (table 1). The current authors have also reported that in similar patients treated with a higher dose of prednisone for a week, symptoms improved and the FEV1 returned to previous best values [3]. Furthermore, a larger study of 100 subjects with moderate-to-severe asthma, of similar design, powered to detect a difference in symptoms score and mean change in PEF of 10–15 $L \cdot min^{-1}$, also failed to find a difference after treatment for 2 weeks [25].

The current authors conclude that the addition of 10 mg montelukast daily for 4 weeks to existing high-dose corticosteroid therapy does not provide additional attenuation of airway eosinophilia. This argues against the possibility that this dose and duration of treatment will have a steroid-sparing effect in patients with this type of asthma.

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