

Effects of mediator antagonism on mannitol and adenosine monophosphate challenges

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Summary

Background Airway hyper-responsiveness (AHR) to indirect stimuli is a useful non-invasive surrogate inflammatory marker in the evaluation of asthma, while histamine and cysteinyl leukotrienes are important inflammatory mediators.

Objective To evaluate AHR to indirect bronchoconstrictor stimuli and time taken to recover following single doses of montelukast 10 mg and desloratadine 5 mg in combination, montelukast 10 mg alone and placebo.

Methods Fifteen mild-to-moderate persistent asthmatics completed a randomized, double-blind, cross-over study. Patients received encapsulated montelukast 10 mg/desloratadine 5 mg combination, montelukast 10 mg alone and placebo, 10–14 h prior to challenge on two separate occasions. The mannitol threshold dose, AMP threshold concentration and recovery times after challenge were measured along with lung function.

Results Compared to placebo, montelukast/desloratadine conferred improvements ($P < 0.05$) in adenosine monophosphate (AMP) threshold concentration and mannitol threshold dose: a 3.2-fold (95% CI 2.2–4.6) and 2.4-fold (95% CI 1.7–3.3) difference, respectively, while compared to montelukast this amounted to a 2.0-fold (95% CI 1.2–3.4) and 1.5-fold (95% CI 1.1–2.4) improvement, respectively. Montelukast was not significantly different from placebo. Both montelukast/desloratadine and montelukast compared to placebo, shortened recovery following both challenges ($P < 0.05$): a 27-min (95% CI 17–37) and 29-min (95% CI 20–36) reduction, respectively, for AMP, and a 27-min (95% CI 17–37) and 26-min (95% CI 17–35) reduction, respectively for mannitol.

Conclusion The dissociated effects of single doses of montelukast alone but not montelukast/desloratadine combination on AHR and recovery time, highlights the relative roles of histamine in initiating the bronchoconstrictor response and cysteinyl leukotrienes in sustaining it. Similar improvements in AHR and recovery time were observed following both indirect bronchoconstrictor stimuli.

Keywords bronchoprovocation, histamine, leukotrienes

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Introduction

Suppression of the inflammatory cascade is an integral component in the management of asthma. Cysteinyl leukotrienes are important pro-inflammatory mediators in the pathogenesis of asthma causing bronchial smooth muscle contraction and proliferation in addition to recruitment of inflammatory cells [1]. Histamine, a potent bronchoconstrictor released from airway mast cells, is also implicated in the asthmatic inflammatory process. In light of these observations, combined therapy which antagonizes the effects of both mediators, has received

recent interest [2]. For example, in patients with both asthma and allergic rhinitis, the combination of cetirizine 10 mg plus montelukast 10 mg was as effective as orally inhaled budesonide 400 µg plus intranasal budesonide 200 µg on lung function, symptoms and reliever use [3]. Reicin et al. also showed that the combination of montelukast 10 mg plus loratadine 20 mg was significantly better than montelukast 10 mg alone, on lung function parameters in persistent asthmatics [4].

Bronchial provocation tests are widely used to assess the presence of airway hyper-responsiveness (AHR) and response to treatment in the laboratory. They can be performed using direct and indirect bronchoconstrictor stimuli, while the latter may have more clinical relevance [5, 6]. Bronchoprovocation using an indirect stimulus such as adenosine monophosphate (AMP) causes degranulation of primed mast cells and subsequent release of inflammatory mediators. Mannitol is a novel bronchoconstrictor stimulus which can also be used to assess

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AHR [7]. When given as a dry powder, inhaled mannitol increases the surface osmolarity of the bronchial mucosa resulting in the release of inflammatory and bronchoconstrictor mediators from a variety of cells. It has been shown that a higher than conventional daily dose of fexofenadine (as two 180 mg doses over 14 h) decreased airway sensitivity to mannitol but lengthened recovery time, while a higher than conventional daily dose of montelukast (as three 10 mg doses over 36 h) shortened recovery time after mannitol challenge [8].

The main objective of our study was to evaluate effects of single clinically recommended doses of an antihistamine plus leukotriene receptor antagonist (LTRA) in combination and LTRA alone compared to placebo. The primary end-points were AHR to two separate indirect bronchoconstrictor stimuli (AMP and mannitol) and time taken to spontaneously recover. This also allowed us to observe whether similar effects of treatment occurred using both mannitol and AMP challenges.

Materials and methods

Study design

Mild-to-moderate persistent atopic asthmatics were enrolled from our database of asthma volunteers into a double-blind, randomized, placebo-controlled, cross-over trial. Subjects attended the department on eight occasions at least 5 days apart. The first two occasions served as screening days to assess eligibility and determine AHR to AMP and mannitol.

Visits to the department occurred at the same time of day, 10–14 h after taking a 10-pm dose of randomized treatment (i.e. between 8 am to 12 noon). Subjects were given identical encapsulated montelukast 10 mg and desloratadine 5 mg in combination, montelukast 10 mg alone or placebo in a double-blind, randomized fashion. On study days, inhaled corticosteroid and short-acting β_2 -agonist were withheld for 8 h. The inhaled corticosteroid dose remained unchanged, and any other second line anti-asthma medication including long-acting β_2 -agonists, antihistamines, leukotriene receptor antagonists and theophyllines were not allowed during the study.

Patients

Inclusion criteria were: forced expiratory volume in 1 second (FEV_1) > 60% predicted, provocative concentration AMP causing a 20% fall in FEV_1 (PC_{20}) $\leq$ 200 mg/mL, provocative dose of mannitol causing a 20% fall in FEV_1 (PD_{20}) $\leq$ 315 mg, recovery time after each challenge of at least 15 min and a positive skin prick test to at least one aeroallergen. For 3 months before the screening, patients were required to use short acting β_2 -agonists only, or maintained on a constant daily dose of inhaled corticosteroid ($\leq$ 800 μ g), have no history of respiratory tract infection and no oral corticosteroid use. All subjects gave written consent and the Tayside committee on medical research ethics gave approval. The Medicines Control Agency approved the use of mannitol and desloratadine.

Study visits

On each visit to the laboratory patients had their FEV_1 measured in triplicate and repeated 10 min later to confirm repeatability. Following spirometry, subjects proceeded to have either an AMP or mannitol challenge on three occasions each in random sequence.

Lung function

Spirometry was carried out according to American Thoracic Society criteria [9]. This was performed using a vitalograph compact spirometer (Vitalograph Ltd, Buckinghamshire, UK) with a pneumotachograph head which was calibrated daily.

AMP challenge

AMP challenge testing was performed as previously described [10]. In brief, AMP was administered in doubling concentrations (from 0.09 mg/mL to 800 mg/mL) at 90-s intervals until a fall in $FEV_1 \geq 20\%$ was recorded. A log dose–response curve was constructed followed by linear interpolation to obtain the AMP PC_{20} .

Mannitol challenge

The preparation of dry powder mannitol and full description of mannitol bronchial challenge has previously been described [7]. In brief, subjects were asked to inhale doses of 0 (empty capsule acting as placebo), 5, 10, 20, 40, 80, 160, 160 and 160 mg of mannitol. At 60-s intervals after completion of inhalation, spirometry was measured in triplicate and the highest FEV_1 was recorded. Once a 20% fall in FEV_1 from the empty capsule was reached or a cumulative dose of 635 mg had been given, the bronchial challenge ceased. The mannitol PD_{20} was calculated by linear interpolation of the log dose–response curve.

Recovery

Once a 20% fall in FEV_1 from baseline for either challenge had been achieved, recovery was monitored by performing two repeatable FEV_1 manoeuvres at 2, 4, 7, 10, 15, 20, 25, 30 and then 10-min intervals thereafter. This was repeated until subjects had a FEV_1 within 5% of their baseline value or once 60 min had elapsed when they were given 400 μ g of salbutamol.

Statistical analysis

The study was powered at 80% to show a difference of one doubling dilution/dose shift in 12 patients. Data were analysed using a 'Statgraphics' software package (STSC Software Publishing Group, Rockville, MD, USA). Previous data using mannitol have used PD_{15} [7, 8], however, we elected to use mannitol PD_{20} since AMP bronchial challenge data are commonly expressed as PC_{20} . The AMP PC_{20} and mannitol PD_{20} data were logarithmically transformed to normalize their distributions. Recovery times were found to exhibit a normal distribution. An overall analysis of variance was performed followed by Bonferroni corrected multiple-range testing, set at 95% confidence limits (two-tailed, $P < 0.05$). All comparisons are denoted as significant at $P < 0.05$ to not confound the overall alpha error.

Results

Seventeen patients responsive to AMP and mannitol were initially enrolled of whom 15 completed the study. One subject was withdrawn because of subsequent unresponsiveness to mannitol following initial screening; the other was withdrawn because the recovery time (< 15 min) was too rapid after both AMP and mannitol challenges. The baseline demographic data of subjects completing the study are shown in Table 1. The intrasubject

Table 1. Demographic data at screening

Gender	Age (years)	FEV ₁ (% predicted)	FEF _{25–75} (% predicted)	ICS	ICS dose (µg/day)	SPT	AMP PC ₂₀ (mg/mL)	AMP recovery (min)	Mannitol PD ₂₀	Mannitol recovery (min)
F	44	80	39	BDP	400	3	170	60+	170	60+
F	21	98	73	BDP	500	4	8.9	50	32	50
F	36	79	61	BUD	400	4	0.19	15	2	50
F	20	123	102	FP	400	2	13	25	48	40
F	46	75	40	None		7	25	40	75	60+
M	32	65	38	BDP	400	5	32	20	11	60+
F	25	98	72	None		1	137	40	205	60+
F	21	75	44	BDP	500	1	7	60+	64	15
M	55	66	48	BDP	400	2	20	50	18	60+
F	44	104	66	BDP	400	3	69	60+	36	60+
M	25	85	52	BDP	800	1	200	40	189	50
M	66	70	54	None		2	46	60+	255	60+
F	49	92	51	BDP	200	4	30	60+	255	50
M	41	81	50	BDP	200	6	109	60+	176	30
F	26	82	37	BDP	800	4	20	60+	106	60+
10 F	37	85	55		450	3	27	46	60	50
5 M	(4)	(4)	(5)		(49)	(0.5)	(14)	(5)	(28)	(5)

Summary statistics are shown in bold: values are shown as means (SE) except AMP PC₂₀ and mannitol PD₂₀ which are shown as geometric means (geometric SE). BDP, beclomethasone; BUD, budesonide; FP, fluticasone; ICS, inhaled corticosteroid; SPT, number of positive skin prick tests. Values of 60+ represent patients who had an FEV₁ > 5% from baseline after 60 min.

Table 2. Values following treatment with placebo, montelukast alone and montelukast/desloratadine combination

Treatment	FEV ₁ (% predicted)	FEF _{25–75} (% predicted)	AMP PC ₂₀ (mg/mL)	AMP recovery (min)	Mannitol PD ₂₀ (mg)	Mannitol recovery (min)
Placebo	79 (4)	54 (5)	25 (10)	49 (4)	54 (15)	47 (4)
Montelukast	85 (4)*	58 (5)	39 (11)	20 (4)*	82 (20)	21 (4)*
Montelukast/desloratadine	88 (4)*	63 (5)*	79 (24)*†	22 (4)*	127 (33)*†	20 (3)*

Values are shown as means (SE) except AMP PC₂₀ and mannitol PD₂₀ which are shown as geometric means (geometric SE). *Denotes significant ($P < 0.05$) difference from placebo; †denotes significant ($P < 0.05$) difference from montelukast.

variability between screening and placebo challenges (as coefficient of variation) was 1.8% for mannitol PD₂₀ and 3.9% for AMP PC₂₀.

Airway hyper-responsiveness

Compared to placebo, montelukast/desloratadine combination conferred significant ($P < 0.05$) improvements in AMP PC₂₀ and mannitol PD₂₀ amounting to 3.2-fold (95% CI 2.2–4.6) and 2.4-fold (95% CI 1.7–3.3) differences, respectively. Montelukast/desloratadine combination was significantly better compared to montelukast alone in AMP PC₂₀ and mannitol PD₂₀: 2.0-fold (95% CI 1.2–3.4) and 1.5-fold (95% CI 1.1–2.4) differences, respectively. Montelukast alone was not significantly different from placebo for either challenge (Table 2 and Fig. 1).

Figure 2 shows the interchangeability between challenges in terms of doubling dose/dilution shifts after single doses of montelukast/desloratadine in combination and montelukast alone.

Recovery time

Compared to placebo, both montelukast/desloratadine combination and montelukast alone significantly shortened the recovery time following both challenges to a similar degree. This amounted to a 27-min (95% CI 17–37) and 29-min (95% CI

20–36) reduction, respectively, for AMP, and a 27-min (95% CI 17–37) and 26-min (95% CI 17–35) reduction, respectively, for mannitol (Table 2 and Fig. 3). There was no significant difference between montelukast/desloratadine and montelukast alone.

Lung function

For spirometry (Table 2), treatment with montelukast/desloratadine and montelukast conferred significant improvements in FEV₁ compared to placebo amounting to a 9% predicted (95% CI 4–14) and 6% predicted (95% CI 1–11) difference, respectively. For FEF_{25–75}, the improvement between montelukast/desloratadine vs. placebo was significant: a 10% predicted (95% CI 1–19) difference. There was no significant difference between montelukast/desloratadine vs. montelukast alone.

Discussion

This study has demonstrated that following mannitol and AMP bronchial challenges, montelukast/desloratadine combination and montelukast alone shortened the recovery, while montelukast/desloratadine but not montelukast reduced airway

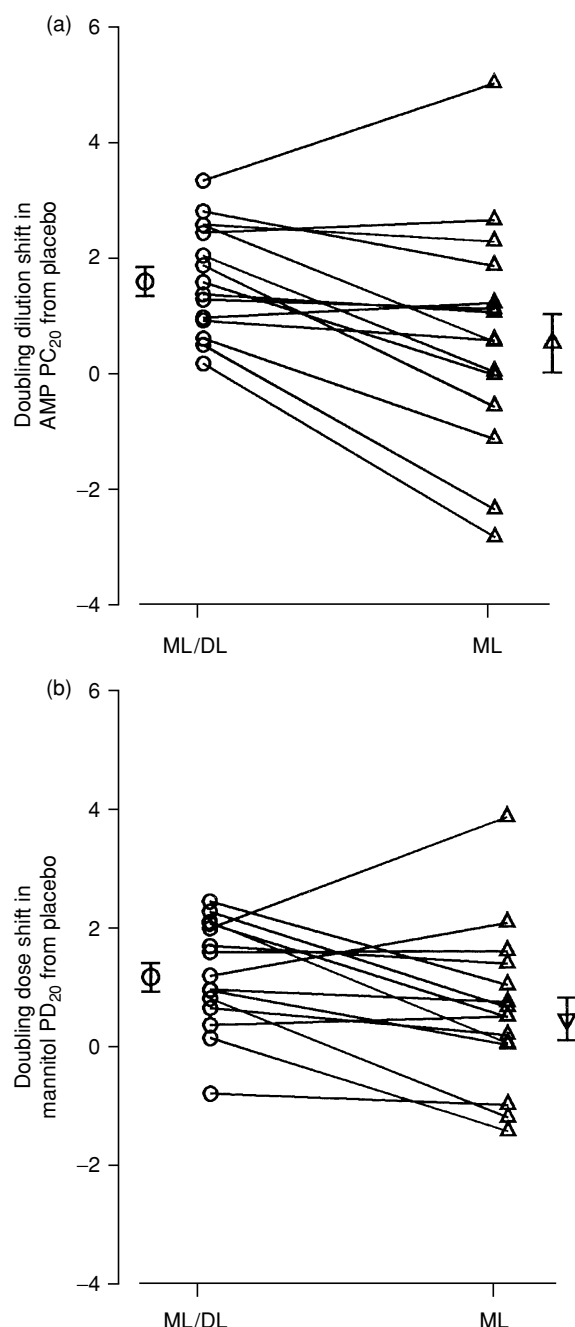


Fig. 1. Individual values with means (SE) as doubling dilution/dose shift from placebo following (a) AMP challenge and (b) mannitol challenge.

sensitivity. The dissociated effects of montelukast/desloratadine combination and montelukast alone highlight the relative roles of histamine in initiating the bronchoconstrictor response and cysteinyl leukotrienes in maintaining the response following bronchial challenge.

The effects of antagonizing both the histamine and leukotriene pathways in allergic asthma are of particular relevance. Roquet et al. highlighted the importance of both inflammatory pathways in asthmatics, when the combination of zafirlukast 80 mg twice daily plus loratadine twice daily 10 mg for 1 week was more effective than either agent alone in attenuating the

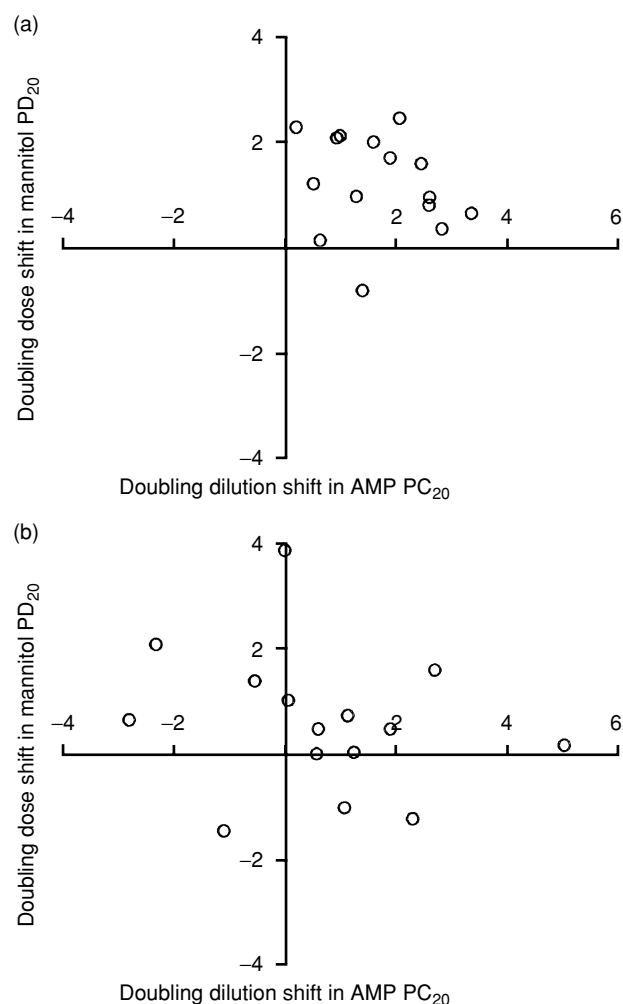


Fig. 2. Doubling dilution/dose shifts from placebo in AMP PC_{20} and mannitol PD_{20} after treatment with (a) montelukast/desloratadine in combination and (b) montelukast alone.

early and late asthmatic response following allergen challenge [11]. In a study by Wilson et al. in patients with both asthma and seasonal allergic rhinitis, the AMP PC_{20} was improved by 2.8-fold with montelukast 10 mg plus cetirizine [3], and by 2.9-fold with montelukast 10 mg alone in another study [12]. Rorke et al. also demonstrated in mild to moderate asthmatics that after two doses of montelukast 10 mg, AMP-induced bronchoconstriction was improved by 2.4-fold compared to placebo [13].

Similar to Brannan et al. [8], we did not demonstrate improvements in airway sensitivity to mannitol or AMP following administration of montelukast alone. This is perhaps surprising given that LTRAs as monotherapy are known to attenuate the effects of various bronchoconstrictor stimuli [14–17]. Indeed, a meta-analysis in which chronic dosing with LTRA was compared to placebo demonstrated an overall estimated shift in AHR of approximately one doubling dose/dilution [18]. However, it is important to point out that in the present study we examined the effects of single doses, which may partly explain the lack of effect of montelukast alone on airway sensitivity. In this respect, we have previously shown that chronic dosing with montelukast conferred significant additive effects to inhaled corticosteroids in terms of in attenuating AHR to AMP and

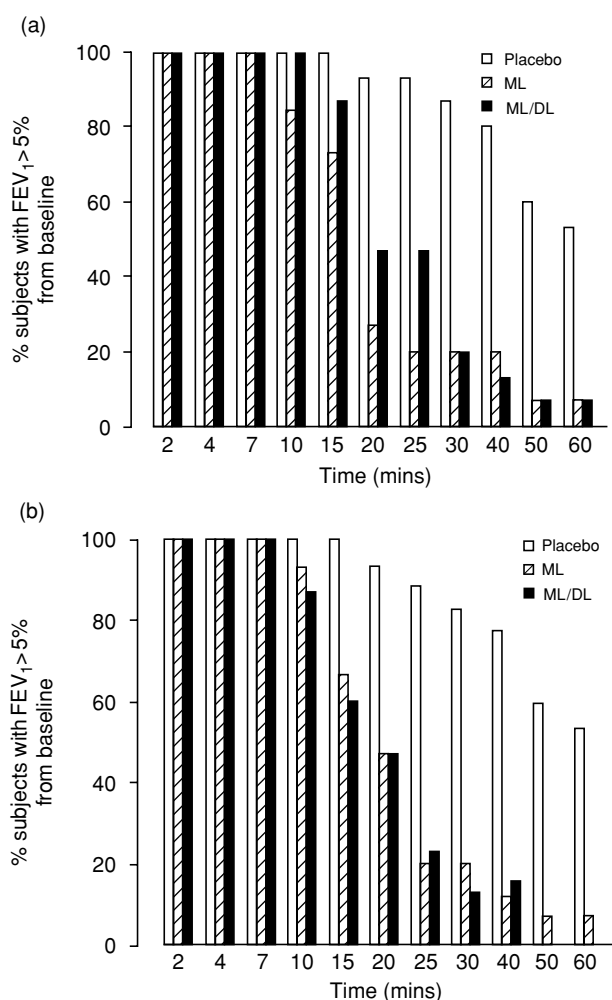


Fig. 3. Percentage of patients during recovery with a fall in $FEV_1 > 5\%$ from baseline following (a) AMP challenge and (b) mannitol challenge.

reducing time taken to recover [19]. Indeed, these effects were mirrored by commensurate improvement in other surrogate inflammatory makers including peripheral blood eosinophils and exhaled nitric oxide.

We did not include a further randomized limb with antihistamine alone, as patients were already attending for eight bronchial challenges in the present protocol. Other studies have shown antihistamines to attenuate AHR to AMP [20, 21] or mannitol [8] challenge. For example, Lee et al. observed that single doses of desloratadine 5 mg, levocetirizine 5 mg and fexofenadine 180 mg all improved the AMP PC_{20} compared to placebo by approximately one doubling dilution, but prolonged the time taken to recovery [21]. Brannan et al. also reported delayed recovery after mannitol challenge in patients receiving fexofenadine at a dose of 360 mg over 14 h [8]. This was attributed to administering a higher dose of mannitol after fexofenadine treatment (and resultantly a higher PD_{15}), which in turn would have released more leukotrienes and thus prolonged the recovery. Our observed effects of montelukast on recovery to mannitol and AMP are similar to those described with montelukast on recovery after exercise [22]. The most likely explanation of a shortened recovery time with montelukast/

desloratadine combination is that any increased leukotriene release associated with a higher dose of mannitol or AMP, would have been counteracted by the presence of leukotriene receptor antagonism.

We elected to use two different indirect bronchoconstrictor stimuli in our study, namely mannitol and AMP, as these agents have not previously been evaluated in the same study. Furthermore, we wished to discover whether following mediator antagonism, similar effects would be observed with both mannitol and AMP. It should be pointed out that while this has not previously been shown, our results are perhaps not surprising as the modes of action of AMP and mannitol are similar. Indeed, Fig. 2(a) shows a degree of interchangeability of the relative shifts in AHR of AMP and mannitol. We also found after single doses of montelukast/desloratadine, a similar magnitude of effect with both AMP and mannitol in terms of airway sensitivity and recovery. This in turn suggests a possible more widespread role for mannitol in assessing AHR, especially as it is well tolerated and easily carried out [7]. Indeed, monitoring AHR to mannitol has been shown to be useful in guiding the reduction of inhaled corticosteroid doses [23].

Bronchial provocation with indirect stimuli is considered to be particularly relevant to real life situations as cold air, exercise and allergen also act in a similar fashion. In particular, shifts in AMP PC_{20} are more closely related to underlying airway eosinophilic inflammation and associated with symptoms of atopic asthma than direct bronchoconstrictor stimuli such as methacholine [24, 25]. Wilson et al. showed that in asthmatics treated with budesonide in a dose escalation study, AMP was more sensitive than methacholine in detecting differences in AHR by approximately one doubling dilution [26].

In summary, our findings show beneficial effects on AHR and recovery time after single doses of desloratadine/montelukast in combination and a reduction in recovery time after single doses of montelukast alone. This highlights the relative roles of histamine in initiating, and leukotrienes in sustaining the bronchoconstrictor response, respectively. Further chronic dosing studies using mediator blockade are required in more severe patients to fully establish their role in asthma management. We have also demonstrated that in terms of shifts in AHR and improvements in recovery time, bronchoprovocation with mannitol produces similar results as AMP.

Acknowledgements

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Conflicts of interest

GPC, KH, SJF, AMW and BJL have all received funding from Merck (who make montelukast) for attending postgraduate educational meetings.

References

- 1 Lipworth BJ. Leukotriene-receptor antagonists. *Lancet* 1999; 353:57–62.
- 2 Sampson AP, Rorke S. Combination therapy with anti-mediator drugs in allergic disease. *Clin Exp Allergy* 2001; 31:11–7.
- 3 Wilson AM, Orr LC, Sims EJ, Dempsey OJ, Lipworth BJ. Antiasthmatic effects of mediator blockade versus topical corticosteroids in allergic rhinitis and asthma. *Am J Respir Crit Care Med* 2000; 162:1297–301.
- 4 Reicin A, White R, Weinstein SF et al. Montelukast, a leukotriene receptor antagonist, in combination with loratadine, a histamine receptor antagonist, in the treatment of chronic asthma. *Arch Intern Med* 2000; 160:2481–8.
- 5 van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000; 16:514–33.
- 6 Cockcroft DW. How best to measure airway responsiveness. *Am J Respir Crit Care Med* 2001; 163:1514–5.
- 7 Anderson SD, Brannan J, Spring J et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997; 156:758–65.
- 8 Brannan JD, Anderson SD, Gomes K et al. Fexofenadine decreases sensitivity to and montelukast improves recovery from inhaled mannitol. *Am J Respir Crit Care Med* 2001; 163:1420–5.
- 9 American Thoracic Society. Standardization of Spirometry. 1994 Update. *Am J Respir Crit Care Med* 1995; 152:1107–36.
- 10 Tan KS, McFarlane LC, Lipworth BJ. Loss of normal cyclical beta 2 adrenoceptor regulation and increased premenstrual responsiveness to adenosine monophosphate in stable female asthmatic patients. *Thorax* 1997; 52:608–11.
- 11 Roquet A, Dahlen B, Kumlin M et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997; 155:1856–63.
- 12 Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ. A comparison of topical budesonide and oral montelukast in seasonal allergic rhinitis and asthma. *Clin Exp Allergy* 2001; 31:616–24.
- 13 Rorke S, Jennison S, Jeffs JA et al. Role of cysteinyl leukotrienes in adenosine 5'-monophosphate induced bronchoconstriction in asthma. *Thorax* 2002; 57:323–7.
- 14 Dempsey OJ, Kennedy G, Lipworth BJ. Comparative efficacy and anti-inflammatory profile of once-daily therapy with leukotriene antagonist or low-dose inhaled corticosteroid in patients with mild persistent asthma. *J Allergy Clin Immunol* 2002; 109:68–74.
- 15 Diamant Z, Grootendorst DC, Veselic-Charvat M et al. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. *Clin Exp Allergy* 1999; 29:42–51.
- 16 Leigh R, Vethanayagam D, Yoshida M, et al. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. *Am J Respir Crit Care Med* 2002; 166:1212–7.
- 17 Yoshida S, Ishizaki Y, Shoji T et al. Effect of pranlukast on bronchial inflammation in patients with asthma. *Clin Exp Allergy* 2000; 30:1008–14.
- 18 Currie GP, Lipworth BJ. Bronchoprotective effects of leukotriene receptor antagonists in asthma: a meta-analysis. *Chest* 2002; 122:146–50.
- 19 Currie GP, Lee DK, Haggart K, Bates CE, Lipworth BJ. Effects of montelukast on surrogate inflammatory markers in corticosteroid treated asthmatics. *Am J Respir Crit Care Med* 2002; DOI: 10.1164/rccm.200209-1116OC.
- 20 Rafferty P, Beasley R, Southgate P, Holgate S. The role of histamine in allergen and adenosine-induced bronchoconstriction. *Int Arch Allergy Appl Immunol* 1987; 82:292–4.
- 21 Lee DKC, Bates CE, Currie GP, Lipworth BJ. Comparative bioactivity of modern H1-anti-histamines in asthma. *J Allergy Clin Immunol* 2003; 111: 337–41.
- 22 Leff JA, Busse WW, Pearlman D et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 1998; 339:147–52.
- 23 Leuppi JD, Salome CM, Jenkins CR et al. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. *Am J Respir Crit Care Med* 2001; 163:406–12.
- 24 van den Berge M, Meijer RJ, Kerstjens HA et al. PC (20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC (20) methacholine. *Am J Respir Crit Care Med* 2001; 163:1546–50.
- 25 de Meer G, Heederik D, Postma DS. Bronchial Responsiveness to Adenosine 5'-Monophosphate (AMP) and Methacholine Differ in Their Relationship with Airway Allergy and Baseline FEV (1). *Am J Respir Crit Care Med* 2002; 165:327–31.
- 26 Wilson AM, Lipworth BJ. Dose-response evaluation of the therapeutic index for inhaled budesonide in patients with mild-to-moderate asthma. *Am J Med* 2000; 108:269–75.