

Effects of montelukast on patients with asthma after experimental inoculation with human rhinovirus 16

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Background: Leukotrienes are induced by viral infections.

Objectives: To determine whether treatment with montelukast would improve asthma disease control in patients with mild allergic asthma during an experimentally induced rhinovirus infection.

Methods: Patients with mild allergic asthma were randomized to receive treatment with either montelukast or placebo, and 7 days later both groups were inoculated with human rhinovirus 16. Patients were evaluated at baseline, during the acute infection phase, and during the recovery phase for asthma and cold symptoms by questionnaire. Sputum, nasal lavage fluid, and blood were analyzed for viral shedding and cellular inflammation, and peak expiratory flow was measured daily.

Results: A total of 19 patients (11 in the placebo group and 8 in the active group) completed the study. No significant differences were found in asthma control and cold symptom scores between the control and treatment groups. The change in peak expiratory flow from the randomization to acute illness phase was greater in the placebo group than the treatment group (mean, -22 vs 0 L/min; $P = .05$). During the recovery phase, the percentage of sputum eosinophils increased in the placebo group and remained at baseline levels in the montelukast group (median, 2.7% vs 0.2% ; $P = .05$ between groups).

Conclusions: In this pilot study, montelukast did not improve asthma control or cold symptom scores caused by experimental rhinovirus infection. Analysis of secondary outcomes suggests that montelukast may prevent reductions in lung function and increases in sputum eosinophils caused by common cold infections. Further studies are needed to determine whether these effects are associated with clinically significant improvements in health outcomes during natural colds.

Trial Registration: clinicaltrials.gov Identifier: NCT00359073.

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INTRODUCTION

Viral respiratory tract infections are a major cause of asthma exacerbations in adults and children.^{1,2} Human rhinoviruses (HRVs) are frequently identified, especially among patients with severe exacerbations requiring hospitalization.^{3,4} The precise mechanism by which HRV elicits asthma exacerbations has not been fully explained, but many theories exist.⁵ The role of leukotrienes in the process has been postulated because elevations have been noted during viral infections, including those caused by HRVs.⁶ Furthermore, leukotriene receptor antagonists

have been shown in a few studies to reduce asthma symptoms or exacerbations with colds in children.^{7–9}

These studies suggest the possibility that montelukast, a leukotriene receptor antagonist, could reduce the severity of HRV illnesses in patients with asthma and have explored the mechanism by which this occurs. To test this hypothesis, we conducted a pilot study in which a group of patients with allergic asthma were randomized to receive either montelukast or placebo and then experimentally inoculated with HRV-16. The primary outcome was the infection-related change in asthma control diary scores. Secondary outcomes included cold symptoms and analysis of secretions for viral shedding, leukotrienes, and cellular inflammation. Experimental infection with HRV-16 was used to minimize confounding from response variation to the many strains of HRV and other viruses found in the community.

METHODS

Study Participants

Between November 2006 and December 2008, 171 individuals were recruited by advertisement and underwent serologic testing for HRV-16; 63 had no detectable antibody to HRV-16, and 42 seronegative individuals consented to the study procedures. At visit two, 19 individuals were removed from the study for the following reasons: 13 had no evidence of

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methacholine reactivity (provocative concentration of methacholine producing a 20% decrease in forced expiratory volume in 1 second [FEV₁] >8 mg/mL) or reversible airway obstruction, 3 had FEV₁ below the study entry criteria, 2 had clinical symptoms of a cold that day, and 1 had been taking omalizumab since the first visit. At visit three, 3 additional individuals dropped out; 2 could not make the visits, and 1 had a low FEV₁. Twenty individuals (8 who received montelukast treatment and 12 who received placebo treatment) completed the study. One patient was not included in the statistical analysis because of a positive test result for HRV-16 antibody at the randomization visit. Therefore, 19 individuals were included in the statistical analysis.

The study included healthy adults between 18 and 65 years of age who had a diagnosis of mild persistent asthma for greater than 6 months, were allergic based on at least one positive skin prick test result to a standard panel of common allergens (wheal size >3 mm greater than negative control), were able to produce induced sputum, and had a prebronchodilator FEV₁ of 80% or higher than predicted at baseline. Study participants used only inhaled short-acting β -agonist inhalers as needed. Individuals qualified based on a postbronchodilator reversibility of 12% or higher or bronchial hyperresponsiveness to provocative concentration of methacholine producing a 20% decrease in FEV₁ of 8 mg/mL or less.¹⁰ Individuals were excluded if they met any of the following criteria: history of severe asthma exacerbations with respiratory infections, serum HRV-16 antibody titer of 1 or higher at screening, use of asthma controller medication such as montelukast or inhaled corticosteroid, current smoker or more than 5-pack-year history, currently receiving immunotherapy, pregnant, breastfeeding, or currently participating in another clinical trial. All participants provided written informed consent, and the study was approved by University of Wisconsin Health Sciences Institutional Review Board. The trial was registered on clinicaltrials.gov.

Study Design

The study was a randomized, double-blind, placebo-controlled trial designed to test whether montelukast would lessen asthma symptoms associated with experimental inoculation of seronegative volunteers with mild allergic asthma with HRV-16. At screening, individuals underwent a physical examination, allergy skin prick testing, blood draw for HRV-16–neutralizing antibody, urine pregnancy test, and pulmonary function tests before and after inhalation of albuterol, and diary cards were distributed. Specimens were collected at baseline, during the acute cold phase, and on recovery (Table 1). Peak flow meters were issued at the beginning of the study. Participants filled out peak flow, asthma, and cold symptom diaries daily for 7 days before randomization, 7 days before inoculation, and then 14 days after inoculation. Nasal lavage, sputum, and blood were collected at each visit after screening. Cell counts with differential were performed on nasal lavage, sputum, and blood samples. Viral titers and RNA were quantitated in nasal lavage samples. An attempt

Table 1. Study Design

	Screen	Randomize	Acute cold		Convalescence		
Visit	1	2	3	4	5	6	7
Day	–14	–7	0	3	8	14	21
Randomize		X					
HRV-16			X				
inoculation							
Nasal lavage		X	X	X	X	X	X
Sputum		X	X	X	X	X	X
induction							
Blood draw		X	X	X	X	X	X
Spirometry	X	X	X	X	X	X	X

Abbreviation: HRV-6, human rhinovirus 16.

was made to measure cysteinyl leukotriene in the sputum, but based on quality control measures, leukotriene values were not stable after freezing.

HRV-16 Inoculation

None of the study participants had neutralizing antibody to HRV-16 at the time of screening, although one individual had a low titer of HRV-16–neutralizing antibody detected in the serum obtained on the day of inoculation (1.4). This patient was excluded from our analysis. Samples on the day of inoculation were tested for other respiratory viruses by multiplex polymerase chain reaction (PCR) (Respiratory Multi-Code Assay; EraGen BioSciences, Madison, Wisconsin).¹¹ If an HRV was detected, partial sequencing was performed to determine strain.¹² HRV-16 inoculation was performed with 1,000 units of a tissue culture infectious dose that produces pathological change in 50% of cell cultures (TCID₅₀) administered by spraying 0.25 mL by atomizer into each nostril as previously described.¹³

Cold and Asthma Symptom Scores

The Jackson scale was used to assess the 13 cold symptoms (cough, nasal discharge, sneezing, stuffy nose, sore throat, headache, malaise, chilliness, shaking chills, fever, laryngitis, aching joints or muscles, and watery or burning eyes) on a 4-point scale (0, not present; 1, mild; 2, moderate; and 3, severe).¹⁴ The symptoms were scored twice daily, and the highest value for each symptom was used to calculate the total daily highest symptom score (TDHSS; maximum possible daily score of 39). Cold symptoms were also recorded on the validated Wisconsin Upper Respiratory Symptom Survey 21 (www.fammed.wisc.edu/wurss).¹⁵

Asthma symptoms were assessed twice a day with patients completing a validated daytime diary card before bed and a nocturnal diary card on awakening.¹⁶ For the daytime diary, study participants answered 4 questions: how often they experienced asthma symptoms (0, none of the time; 6, all of the time), how much their asthma symptoms bothered them (0, not at all; 6, severely bothered), how much activity they could do (0, more than usual; 6, less than usual), and how often their asthma affected their activities (0, none of the

time; 6, all of the time). Daily scores were calculated as the average of the 4 questions, and an overall score for the week was assessed as the average of the daily scores. For the nocturnal diary, study participants recorded how often they woke up with asthma symptoms (0, none; 1, once; 2, more than once; 3, awake all night). Weekly average scores were computed from the nocturnal diary. In addition, study participants measured peak flows and recorded albuterol use on awakening and just before bed.

Nasal Lavage, Sputum Induction, and Virology

Nasal lavage,¹³ sputum induction,¹⁴ and processing of samples¹⁴ were performed as reported previously. Viral titers from nasal lavage were calculated after 4 tissue culture tubes containing WI38 cells (human lung diploid cells; ViroMed, Minnetonka, Minnesota) were inoculated for each serial 10-fold dilution of sample (10^0 – 10^{-7}) and incubated while rolling at 33°C for 10 days. Tubes were read at 24 hours and then every other day up to day 10. TCID₅₀ was calculated as the concentration that was capable of infecting 50% of the tubes. Viral titers were expressed as TCID₅₀ per milliliter. Cell counts and differentials were made from nasal lavage and sputum samples after treatment with 0.1% dithiothreitol.

Total RNA was extracted from nasal lavage samples and reverse transcribed, and the quantity of HRV RNA was measured as reported previously.¹⁷ The standard curve was developed by extracting HRV-16 dilutions of known concentration ranging from 1 PFU to 1×10^6 PFU; 1 PCR unit on the standard curve was defined as 1 PFU (approximately 130 copies of RNA).

Statistical Analysis

Group characteristics and outcomes, including height, weight, age, peak flow, sputum and nasal cell counts and percentages, cold and asthma symptom scores, viral shedding and RNA, and albuterol puffs, were compared using the Wilcoxon rank sum test. Categorical outcomes, including race, sex, and detection of nasal eosinophils, were compared using the Fisher exact test. For daily-recorded data (symptom scores, peak flow, and albuterol use), both the mean and maximum were calculated for each of 4 study weeks (baseline, randomization, acute phase, convalescent phase) and compared between groups. Continuous outcomes are summarized as median (interquartile range [IQR]). A 2-sided $P < .05$ was regarded as statistically significant.

RESULTS

Characteristics of the Study Participants

Twenty individuals completed the study; 9 received montelukast and 11 received placebo (Table 2). One individual from the montelukast group had no detectable virus in nasal secretions, but that individual had a virus detected in nasal lavage by reverse-transcription PCR at inoculation and was therefore excluded from the analysis.

When comparing the placebo and montelukast groups, patients were predominately in their 20s (21 [20–23] vs 20

Table 2. Demographics of the Study Participants

	Placebo (n = 11)	Montelukast (n = 8)	P value
Height, median (IQR), cm	173 (169–177)	176 (174–183)	.20
Weight, median (IQR), kg	70 (62–90)	73 (65–80)	.84
Age, median (IQR), y	21 (20–23)	20 (19–21)	.50
Sex, F/M, No.	4/7	3/5	>.99
Race, white/African American, No.	10/1	8/0	>.99
PEF, median (IQR), mL/min	445 (402–566)	469 (439–519)	.49

Abbreviations: IQR, interquartile range; PEF, peak expiratory flow.

[19–21] years of age; $P = .50$) and were mainly white (10 white and 1 African American vs 8 white, $P > .99$). There was a similar male-female ratio, with 4 females and 7 males in the placebo group and 3 females and 5 males in the montelukast group ($P > .99$). Height (173 [169–177] vs 176 [174–183] cm; $P = .20$) and weight (70 [62–90] vs 73 [65–80] kg; $P = .84$) were not significantly different.

Cold Symptoms

Individuals in the placebo and montelukast groups had similar peak TDHSSs for cold during HRV infection (5.9 [4.2–10.1] vs 7.3 [5.6–7.9]; $P = .74$). When comparing the placebo group with the montelukast group, both had an increase in cold symptom scoring from randomization to acute illness, and there was no significance in the change observed from randomization to the acute phase between these 2 groups (4.9 [4.0–8.2] vs 5.3 [4.3–6.2]; $P = .70$). Similar results were seen when assessing the Wisconsin Upper Respiratory Symptom Survey diary completed by both groups. Mean symptom scores during acute illness in the placebo group (15.8 [9.0–32.1]) were not significantly different from the montelukast group (12.9 [11.0–24.5]; $P > .99$). Again, there was an increase in symptom scores from randomization to acute illness and no significant difference from randomization to the acute phase between the placebo and montelukast groups (11.5 [7.3–31.4] vs 12.7 [9.2–23.7]; $P = .90$).

Viral Shedding

Individuals in the placebo and montelukast groups had similar peak viral shedding during the infection (3.5 [2.5–4.5] vs 2.5 [2.3–4.5] log₁₀ TCID₅₀/mL; $P = .50$). The placebo and montelukast groups had similar levels of nasal HRV-16 RNA at the peak of the acute cold on day 3 (7.9 [7.2–8.6] vs 8.2 [6.8–8.8] log₁₀ PCR units; $P = .84$) and on recovery on day 10 (6.1 [5.2–6.8] vs 5.9 [5.7–6.7] log₁₀ PCR units; $P = .97$). There was a significant decrease in nasal HRV RNA between the acute cold and recovery phases in both groups (placebo: $P = .002$; montelukast: $P = .04$).

Asthma Symptoms and Peak Flows

No individual developed a severe asthma exacerbation during this study that required oral corticosteroids, urgent or emergency department care, or hospitalization. The placebo and

Table 3. Effect of Montelukast on Virus-Induced Changes in Sputum Eosinophils^a

Visit	Placebo (n = 11)	Montelukast (n = 8)	P value
2 (day -7)	0.3 (0.0–1.2)	0.7 (0.4–6.0)	.30
3 (day 0)	0.3 (0.3–1.7)	0.7 (0.7–1.3)	.68
4 (day 3)	0.5 (0.2–3.4)	0.3 (0.0–1.0)	.62
5 (day 8)	0.4 (0.3–0.7)	0.3 (0.0–0.6)	.31
6 (day 14)	2.0 (0.3–2.7)	0.3 (0.1–0.3)	.05
7 (day 21)	0.9 (0.3–2.4)	0.0 (0.0–1.5)	.25

^a Data are presented as median (interquartile range) percentage of total cell count.

montelukast groups had no significant differences in mean daily asthma symptoms at baseline (1.0 [0.2–1.4] vs 0.3 [0.0–3.8]; $P = .83$), at randomization (0.3 [0.0–1.6] vs 0.5 [0.0–2.0]; $P = .90$), during the acute illness phase (2.3 [0.0–4.9] vs 2.1 [0.1–4.1]; $P = .83$), or during convalescence (0.1 [0.0–1.3] vs 0.7 [0.0–2.6]; $P = .77$) (Fig 1). Albuterol use was similarly low between the placebo and montelukast groups at baseline (0.0 [0.0–0.1] vs 0.1 [0.0–0.2] puffs; $P = .42$) and during the acute cold (0.0 [0.0–0.5] and 0.3 [0.1–0.6] puffs; $P = .32$).

Peak expiratory flows (PEFs) obtained did not differ between the two groups at any one point in time (Fig 2). However, when comparing change in mean PEF from randomization to acute illness, the infection-induced decrease in mean PEF was greater in the placebo group (-31 [-37 to -6] vs 5 [-13 to 19] L/min; $P = .05$). The infection-induced decrease in maximum PEF was similarly affected in the placebo and montelukast groups (-16 [-27 to -15] vs -3 [-7 to 1] L/min; $P = .01$).

Cellular Inflammation

Montelukast treatment had no effects on eosinophil counts during the acute cold (Table 3). During the recovery phase (14 days after inoculation) eosinophils were increased in the placebo group but not in the treatment group (2.0% [0.3%–2.7%] vs 0.3% [0.1%–0.3%]; $P = .05$; Table 3). Eosinophils were also detectable in nasal lavage in some individuals, but no significant difference was observed between the two groups during any point in the study.

In sputum, neutrophils were detectable and no significance difference was observed between the two groups at baseline during the acute infection or during convalescence. In nasal lavage samples, a significant difference between the placebo and montelukast groups was observed in neutrophil counts both 3 days 80% [72%–88%] vs 91% [87%–93%]; $P = .05$) and 8 days (91% [84%–91%] vs 92% [91%–94%]; $P = .05$) after inoculation with HRV-16.

DISCUSSION

Previous studies have shown that starting treatment with montelukast before a known viral illness season can lessen asthma symptoms.⁸ The primary goals of this study were to use a model of experimental infection to determine whether

montelukast would reduce virus-induced asthma and cold symptoms in patients with mild allergic asthma and to explore possible mechanisms for beneficial effects. In fact, montelukast had no significant effects on virus-induced asthma or cold symptoms; however, there were some changes in preselected secondary outcomes. When patients were prescribed montelukast before inoculation with HRV-16, a decrease in PEF was observed in the placebo group between randomization and the acute cold period. The group of patients taking montelukast did not have a decrease in their PEF, which suggests montelukast had a protective effect on lung function. There was no appreciable effect on viral shedding. These findings suggest that leukotrienes may be involved in virus-induced reductions in lung function, and this effect is not mediated by a reduction in viral replication.

Montelukast also affected the course of eosinophilic inflammation. Eosinophils tend to increase during the convalescence phase after an acute upper respiratory tract infection in patients with allergic airway disease,¹⁸ and in the montelukast group, there was a decrease in the number of sputum eosinophils during the convalescence phase. This finding is in agreement with other studies showing that montelukast can affect eosinophilic inflammation in the airways. For example, a recent study conducted in Korea showed a significant decrease in eosinophil-derived neurotoxin levels in patients prescribed montelukast after a respiratory syncytial virus infection. Furthermore, montelukast-induced reductions in eosinophil-derived neurotoxin levels were associated with a significant decrease in wheezing episodes in follow-up.¹⁹ Furthermore, the presence of eosinophils in the airways has been identified as a risk factor for more severe colds in previous studies.^{10,20} These findings suggest that montelukast effects to reduce airway eosinophils could contribute to clinical benefits in the context of viral infection.

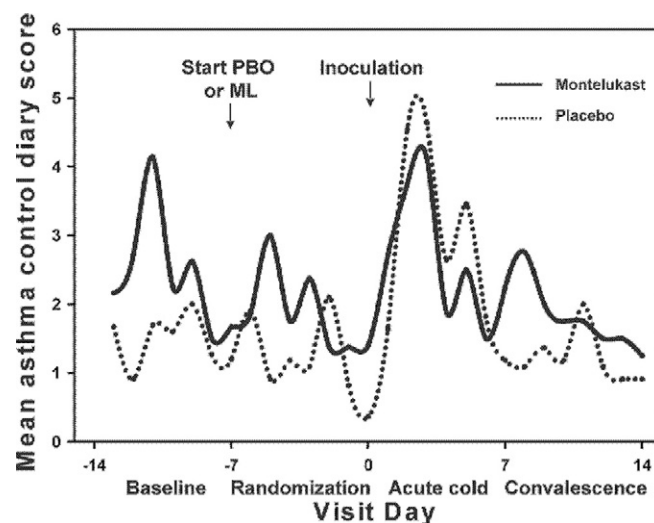


Figure 1. Mean asthma symptom scores at baseline, after randomization, during the acute cold, and during recovery in study participants taking montelukast (solid line) and those taking placebo (dotted line).

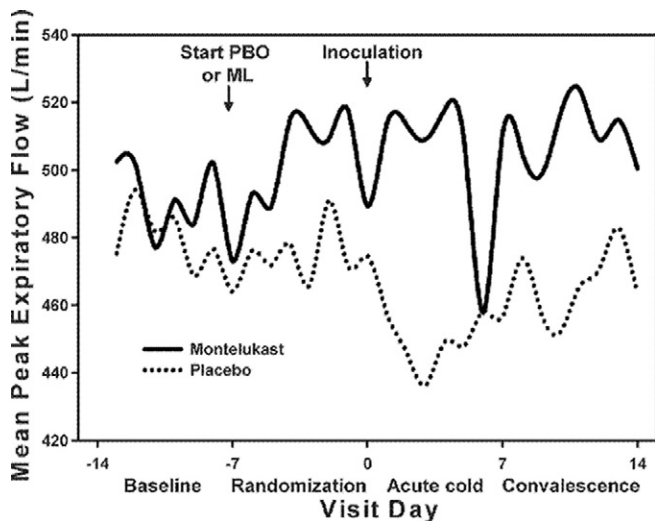


Figure 2. Median morning peak flows at baseline, after randomization, during the acute cold, and during recovery in study participants taking montelukast (solid line) and those taking placebo (dotted line).

Previous studies of montelukast effects during acute colds have produced conflicting results. In patients with acute respiratory syncytial virus bronchiolitis, montelukast had no effects on respiratory symptoms, including wheezing and cough.^{21,22} In contrast, school-age children with mild allergic asthma were treated with either montelukast or placebo before starting school in the fall.²³ This was done in anticipation of exposure to HRV on returning to school. Children were monitored for symptoms after a natural community-acquired cold, and montelukast therapy was associated with a significant decrease in days with worsening asthma symptoms and unscheduled physician visits. Furthermore, in preschool children with recurrent wheeze, taking montelukast at the first sign of upper respiratory tract illness produced a modest reduction in wheezing, trouble breathing, and activity limitation.²⁴ These findings suggest that cysteinyl leukotrienes contribute to cold-induced asthma exacerbations. In the present study, we serially monitored cold symptom scores and viral shedding after inoculation and found no evidence that montelukast affected the course of upper respiratory tract illness. This leads us to believe montelukast does not potentiate antiviral pathways that are important for HRV infections. In our study, there were no differences in lower airway symptoms either, although lower airway symptoms are generally mild after experimental inoculation of volunteers with mild asthma, and this low signal may have impaired our ability to detect montelukast effects on virus-induced chest symptoms.

Advantages of this study design are that standardized inoculation procedures limit the variability related to infection dose, strain, and timing. Inoculating patients with HRV allows for precise monitoring of viral shedding, inflammatory changes, and symptoms at specified intervals relative to the time of inoculation. We also excluded patients taking other

controller medications that could mask effects of virus on the upper or lower airways. One limitation to this study is that the study participants had mild asthma and were relatively stable at the time of inoculation. Responses to infection would be more pronounced in patients with more severe or poorly controlled asthma. Patients with moderate to severe asthma may be most likely to benefit from prevention of asthma exacerbation from viral illnesses, and this limits our ability to generalize our findings to all patients with asthma. In addition, this was a pilot study, and the small sample size limits the statistical power of the study and does not allow for subgroup analysis.

Overall, we found that montelukast did not improve asthma control or cold symptom scores caused by experimental rhinovirus infection. Secondary findings provide further evidence that montelukast can attenuate eosinophilic inflammation in the airway and also indicate that leukotriene inhibitors may protect lung function during viral respiratory infections. On the basis of these findings and other studies linking blood and sputum eosinophils to more severe viral illnesses,²⁰ additional studies may be warranted to determine whether montelukast might be useful in preventing asthma exacerbations with viral illness in patients who have eosinophilic airway inflammation.

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