

## The Effect of Montelukast on Bronchial Provocation Tests and Exhaled Nitric Oxide Levels in Asthmatic Patients

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### Abstract

**Background:** Leukotriene antagonist therapy in asthmatic patients alleviates symptoms and improves exercise tolerance, however the effect of these drugs on bronchial provocation tests and exhaled nitric oxide levels are less clearly established.

**Objective:** To determine the effect of montelukast treatment on airway hyperresponsiveness to exercise, methacholine and adenosine-5'-monophosphate and on exhaled nitric oxide levels in steroid-naive asthmatics.

**Methods:** Following a 2 week run-in period, 20 mild to moderate asthmatics were enrolled in an open label 6 week trial of oral montelukast-sodium therapy. Bronchial hyperreactivity (exercise, methacholine and adenosine-5'-monophosphate challenges) and exhaled nitric oxide levels were measured before and after the 6 week period.

**Results:** Montelukast treatment resulted in a significant improvement in exercise tolerance: median  $\Delta$ FEV<sub>1</sub> 20.0% (range 0–50) prior to treatment vs. 15.0% (range 0–50) post-treatment ( $P = 0.029$ ). A significant difference was also observed for exhaled NO following therapy: median NO 16.0 ppb (range 7–41) vs. 13.0 (range 4.8–26) ( $P = 0.016$ ). No change was seen in baseline lung function tests (FEV<sub>1</sub>, MEF<sub>50</sub>) or in the bronchial responsiveness (PC<sub>20</sub>) for methacholine and adenosine-5'-monophosphate.

**Conclusions:** This study demonstrates that the leukotriene antagonist montelukast-sodium reduces bronchial hyperreactivity in response to exercise and reduces exhaled nitric oxide levels but has little effect on bronchial responsiveness to methacholine and adenosine challenges.

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Leukotriene receptor antagonist therapy in asthmatic patients is associated with improvement in symptom scores, reduction in the need for breakthrough bronchodilator therapy, and improved exercise capacity [1,2]. This class of drugs has also been shown to exert anti-inflammatory effects, as demonstrated by a reduction in eosinophil numbers both in peripheral blood [2] and induced sputum samples [3]. Studies indicate, however, that leukotriene receptor antagonists are less effective than inhaled steroids [4] in alleviating symptoms. Several studies in adults and children have demonstrated that leukotriene antagonists attenuate the fall in forced expiratory volume in the first second after exercise [1,5].

There are, however, little data regarding the effect of this drug on other bronchial provocation tests.

Levels of the gas nitric oxide are elevated in the exhaled air of asthmatic patients as compared to non-asthmatic controls [6], and these levels may mirror airway inflammation [7,8]. Exhaled NO is increased following allergen challenge and reduced following treatment with steroids, but is not changed by treatment with bronchodilators [9–11]. Several investigators have suggested that exhaled NO can be used to monitor disease activity [7,8].

In the present study we examined the effect of a 6 week course of oral montelukast on exercise, methacholine and adenosine challenges as well as on exhaled NO levels in steroid-naive asthmatic patients.

### Patients and Methods

#### Subjects

Twenty asthmatic patients from the outpatient pulmonary clinic of Hadassah University Hospital, Jerusalem, were recruited to participate in the study. Steroid-naive patients with mild persistent asthma were considered suitable for participation. Inclusion criteria were as follows: age 10–70 years, symptoms occurring more than twice a week but less than once daily, the presence of night-time symptoms at least twice a month, and treatment with short-acting bronchodilators as needed only. Exclusion criteria were pregnancy, emergency room visits due to asthma during the preceding 3 months, the presence of an upper respiratory tract infection including sinusitis in the month prior to enrolment, active cardiovascular disease or liver function disturbances, the use of inhaled or oral steroids during the preceding 3 months, and the use of long-acting bronchodilators, theophylline or cromolyn in the 2 weeks prior to enrolment.

The participants were given diaries in which they documented daily daytime symptoms, disturbance of daily activity and daily bronchodilator use. Night-time disease activity was assessed by documenting the use of night-time bronchodilators and the number of awakenings due to asthma symptoms. After a 2 week run-in period without medications, patients were given montelukast sodium 10 mg or 5 mg (age <15) in a once daily dose for a period of 6 weeks. All participants were examined and performed spirometry at enrollment (week -2), following run-in (week 0), at week +2, and at the completion of the study (week +6). Bronchial provocation challenges using methacholine, exercise and adeno-

NO = nitric oxide

sine-5'-monophosphate, and measurement of exhaled NO were performed prior to starting the study drug (week 0) and at the end of the study (week +6). Bronchial provocation tests were performed in random order and in a blinded fashion such that results of previous challenges were unknown to the patient and to the laboratory technician performing the study. A minimum of 2 hours washout time was observed between each challenge. Exhaled NO was measured three times in each patient and the mean value was used for the study.

The participants gave written informed consent, and the institutional ethics committee approved performance of the study.

### Symptom analysis

The asthma symptom record was kept from 2 weeks prior to starting the drug and during the entire 6 week period of drug ingestion. For purposes of analysis, we obtained a composite score for each parameter in each patient using the 2 week period prior to taking the study drug (baseline) and compared this with the 2 week period at the end of the study period (weeks 4–6).

### Spirometry and bronchial challenges

Spirometry was performed using a pneumotachograph-based system (Vitalograph Compact Spirometer, Vitalograph Ltd., Buckingham, UK). Bronchial challenges were performed as previously described [12]. Methacholine (Spectrum Chemical Corp, Gardena, CA, USA) and adenosine-5'-monophosphate (Sigma-Aldridge, Rehovot, Israel) solutions were prepared in doubling concentrations ranging from 0.03 to 16 mg/ml for MCH and 0.39 to 400 mg/ml for AMP. Solutions were administered via a nebuliser chamber (Hudson RCI, Temecula, CA) with an airflow rate of 5 L/min for 2 minutes and FEV<sub>1</sub> was measured 30, 90 and 180 seconds after each dose. The dose was successively doubled until a fall in FEV<sub>1</sub> of 20% from baseline was obtained. The concentration of MCH or AMP causing a 20% fall in FEV<sub>1</sub> from the baseline value (PC<sub>20</sub>) was derived from a plot of percent fall in FEV<sub>1</sub> against logMCH or logAMP concentrations. Exercise challenge was performed with subjects running on a treadmill at a speed of 6 km/hour for 6 minutes. FEV<sub>1</sub> was measured before and 3, 5, 10 and 15 minutes after exercise. The maximal fall in FEV<sub>1</sub> ( $\Delta$ FEV<sub>1</sub>) as compared to baseline was measured.

### Measurement of exhaled NO

NO was measured using a chemiluminescence analyser (LR 2000, Logan Research, Rochester, UK), which is sensitive to NO from 2 to 4,000 parts per billion by volume. Patients performed a slow vital capacity maneuver starting from total lung capacity for 20–30 seconds. No nose clip was used according to recommended guidelines [13,14]. Exhalation was performed against resistance, and mouth pressure and flow rate were constant for the duration of the maneuver (5 cm H<sub>2</sub>O, 6 L/min) using a visual aid for patient guidance. NO and CO<sub>2</sub> were sampled continuously via a side-arm

attached to the mouthpiece with flow rate of 250 ml/minute. Three successive recordings were made and the mean value was recorded. NO levels were recorded during the plateau phase and coincided with the plateau phase for exhaled CO<sub>2</sub>. Calibration was performed using 0 and 110 ppb calibration gas cylinders. Ambient NO levels were measured daily and were always found to be <3 ppb. Reference values for exhaled NO (mean  $\pm$  SE) in known steroid-naïve asthmatics and non-atopic non-asthmatic controls in our laboratory are 21.5  $\pm$  3.0 and 5.9  $\pm$  0.5 ppb respectively.

### Data analysis

Analysis was performed using paired non-parametric statistics (Wilcoxon signed rank test) from data obtained before and following treatment. A value of  $P \leq 0.05$  was considered significant.

### Results

Of the 20 subjects who enrolled 19 completed the study. One patient was withdrawn from the study because of an acute exacerbation that necessitated the administration of systemic steroids. Mean patient age was 18 years (range 10–48 years) and 7 patients were less than 16 years old. Ten of the 19 patients were male.

Values for FEV<sub>1</sub>, maximal expiratory flow rate, provocation studies and exhaled NO at baseline and at the end of the study period are listed in Table 1. There was no significant difference in FEV<sub>1</sub> or in MEF<sub>50</sub> before and at the end of the 6 week study period. No statistically significant difference was observed in PC<sub>20</sub> for methacholine or adenosine-5'-monophosphate in response to 6 weeks of montelukast [Figure 1].

In contrast, a statistically significant difference was observed for exercise prior to and after treatment: median  $\Delta$ FEV<sub>1</sub> 20.0% (range 0–50) vs. 15.0% (range 0–50) respectively ( $P = 0.029$ ). A significant difference was also observed for exhaled NO following therapy: median NO 16.0 ppb (range 7–41) vs. 13.0 (range 4.8–26) ( $P = 0.016$ ) [Figure 1]. We found no correlation between changes in  $\Delta$ FEV<sub>1</sub> following exercise and the reduction in levels of exhaled NO.

Although treatment with montelukast resulted in an improvement in clinical parameters of disease activity, these data are of limited value because no placebo arm was used in this study.

ppb = parts per billion

MEF<sub>50</sub> = maximal expiratory flow rate at 50% of vital capacity.

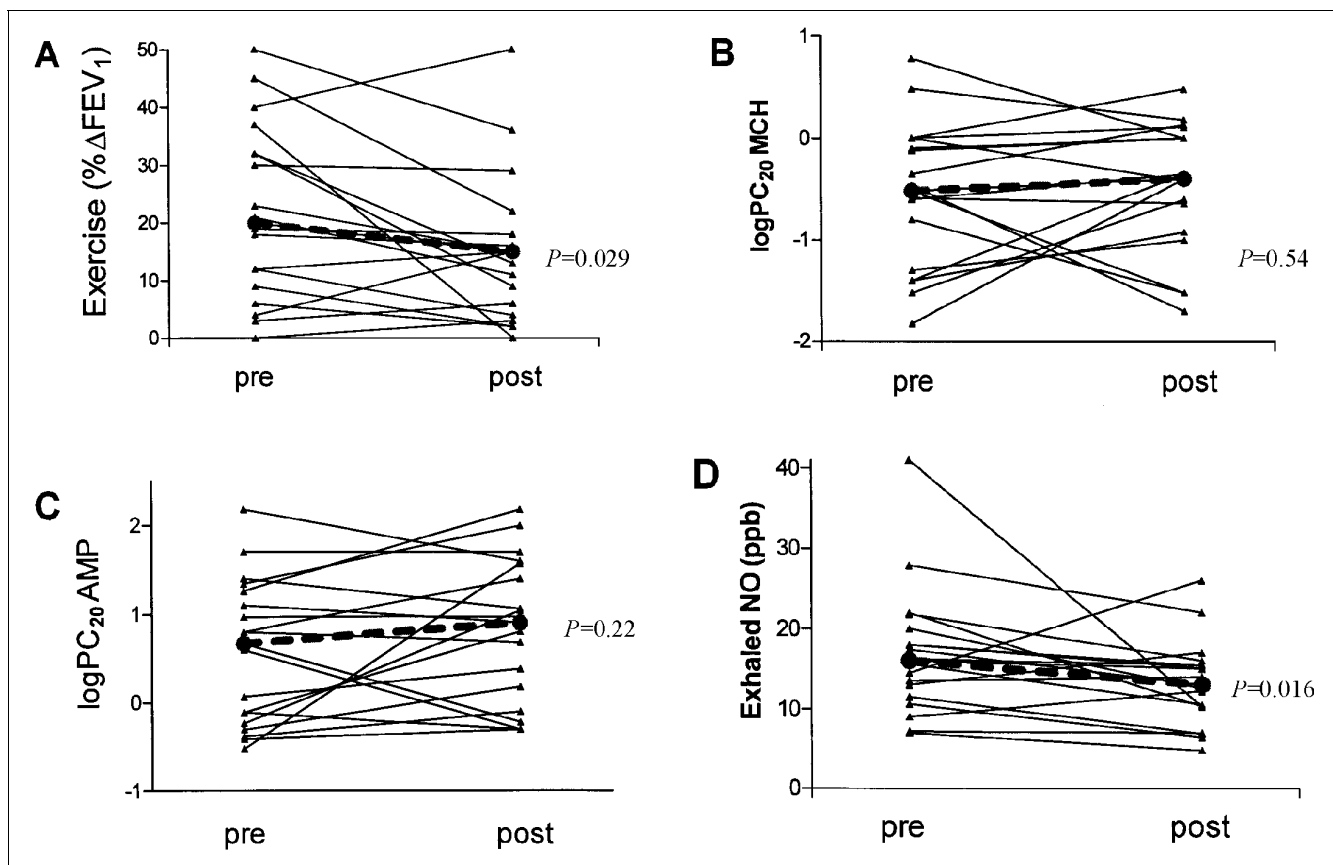
**Table 1.** Spirometry, bronchial provocation tests and exhaled NO before and after montelukast

	Baseline	Final	P value
FEV <sub>1</sub> (% predicted)	81.0 (70–113)	87 (70–112)	0.46
MEF <sub>50</sub> (% predicted)	62.0 (35–108)	65.0 (33–107)	0.60
Exercise (% $\Delta$ FEV <sub>1</sub> )	20.0 (0–50)	15.0 (0–50)	0.029
PC <sub>20</sub> MCH (mg/ml)	0.30 (0.015–6.0)	0.40 (0.02–3.0)	0.54
PC <sub>20</sub> AMP (mg/ml)	4.7 (0.30–150)	7.9 (0.5–150)	0.22
Exhaled NO (ppb)	16.9 (7–41)	13.2 (4.8–26)	0.016

MCH = methacholine

AMP = adenosine-5'-monophosphate

FEV<sub>1</sub> = forced expiratory volume in the first second



**Figure 1.** Values obtained before and after a 6 week course of montelukast treatment: **[A]** % $\Delta$ FEV<sub>1</sub> following exercise, **[B]** logPC<sub>20</sub> for methacholine (MCH), **[C]** logPC<sub>20</sub> for adenosine-5'-monophosphate (AMP), **[D]** exhaled nitric oxide (NO). Median values are shown in bold.

## Discussion

In a 6 week open-label, non-placebo-controlled trial of oral montelukast in non-steroid-treated asthmatic patients, there was a significant reduction in  $\Delta$ FEV<sub>1</sub> following exercise as well as a significant fall in exhaled nitric oxide levels. In contrast, no change was observed in baseline FEV<sub>1</sub> or in bronchial hyperreactivity in response to methacholine or adenosine tests.

Several large studies have documented an improvement in exercise capacity in response to leukotriene antagonist therapy. In a double-blind placebo-controlled study of 110 asthmatic patients receiving montelukast for 12 weeks, Leff et al. [1] observed a reduction from 36.5% to 22.2% in  $\Delta$ FEV<sub>1</sub> following exercise. Villaran and co-workers [15] noted an even more dramatic decrease in exercise-induced  $\Delta$ FEV<sub>1</sub> in 197 patients treated for 8 weeks with montelukast (33% to 17%). This improvement was greater than that seen with salmeterol (33% to 23%) and no tolerance to the beneficial response to montelukast was observed during the study period. Studies in children treated with montelukast demonstrate a similar protective effect to that seen in adults [16]. Zafirlukast, another leukotriene receptor antagonist, has also been shown to attenuate the exercise-induced fall in FEV<sub>1</sub> [17]. The mean fall in FEV<sub>1</sub> prior to treatment was lower in our patients than described in these studies. This may be because a positive exercise test was not a prerequisite to participate in our study, thus several patients had an initial small or absent fall in FEV<sub>1</sub> post-exercise resulting in a

lower mean % $\Delta$ FEV<sub>1</sub>. Although the protective effect of montelukast against exercise-induced bronchospasm is statistically significant, it is questionable whether this effect is of clinical significance.

Leukotriene antagonist drugs have been shown to attenuate both the early and late-phase bronchoconstrictor effect induced by allergen [18]. In contrast to published data regarding exercise and allergen, little has been reported on the effect of these drugs on other bronchial provocation tests. Rasmussen et al. [19] found that treatment with an inhaled leukotriene D<sub>4</sub> antagonist (L-648,051) improved the PC<sub>20</sub> for methacholine in 10 asthmatic patients when compared to placebo [19]. Treatment with pranlukast has been shown in several studies to reduce bronchial hyperreactivity in response to methacholine [18,20]. Fujimura et al. [21] did not observe any change in PC<sub>20</sub> for methacholine following 1 week of treatment with ONO-1078. A single dose of montelukast improved the PC<sub>20</sub> for adenosine from 42 mg/ml to 115 mg/ml in 12 asthmatic patients [22]. The addition of salmeterol had an additive effect to that of montelukast (PC<sub>20</sub> 247 mg/ml). In many of these studies, the patient populations were heterogeneous since some subjects received concomitant inhaled steroids.

Leukotriene antagonist drugs have an anti-inflammatory effect in that they consistently reduce both blood and sputum eosinophil numbers in asthmatic patients [2,3] and reduce the number of activated eosinophils (EG2+ cells) and lymphocytes (CD3+ and CD4+ cells) in bronchial biopsies from asthmatic patients [20].

Exhaled nitric oxide levels seem to mirror the degree of airway inflammation in asthmatic patients; i.e., levels increase in parallel with acute exacerbations and fall in response to anti-inflammatory medication. In a double-blind crossover 2 week study performed in 26 children aged 6–15 treated with montelukast, exhaled NO fell by 15% within 2 days and by 20% after 2 weeks (from 35.5 to 29.1 ppb) [23]. Several of the subjects in this study were receiving inhaled steroids in addition to other medications, which could confound results obtained for exhaled NO. In a smaller study (12 children), 4 weeks of montelukast reduced exhaled NO from 83 to 58 ppb [24]. Lipworth et al. found that zafirlukast reduced levels of exhaled NO when added to inhaled corticosteroid therapy [25]. In our study, all subjects received the study drug and inhaled beta-agonists only, and therefore NO levels were not influenced by additional confounding factors.

It is not clear why we failed to observe an improvement in bronchial hyperreactivity for methacholine and adenosine in our study, although  $\Delta FEV_1$  for exercise did improve. Methacholine is not specific for asthma and exerts its effect by acting directly on airway smooth muscle. In contrast, adenosine is thought to induce bronchoconstriction indirectly, as does exercise (in part via activation of mast cells). It is possible that the fall in  $FEV_1$  in response to exercise is leukotriene-mediated, while the mechanism of adenosine-induced bronchoconstriction is dependent on alternative pathophysiologic pathways.

In conclusion, a 6 week course of oral montelukast therapy resulted in a reduction in  $\% \Delta FEV_1$  following exercise and a significant fall in exhaled nitric oxide levels, but had no effect on  $FEV_1$  or on bronchial hyperreactivity in response to methacholine and adenosine tests.

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