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Exhaled Nitric Oxide Before and After Montelukast Sodium Therapy in School-Age Children With Chronic Asthma: A Preliminary Study

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Summary. Exhaled nitric oxide (ENO) is a surrogate marker of airway inflammation in asthma. In 12 children aged 6–11 years with mild to moderate persistent asthma, ENO concentrations were measured before and after 4 weeks of treatment with montelukast sodium, a leukotriene receptor antagonist, and 2 weeks after withdrawal of therapy.

Baseline ENO levels (mean and 95% confidence interval) were significantly elevated in patients with asthma compared to age-matched nonasthmatic control subjects, with levels of 83 (42–123) vs. 13 (11–15) ppb ($P < 0.001$). After treatment with montelukast sodium, there was a significant ($P < 0.01$) reduction in ENO to 58 (27–89) ppb which again rose to 69 (38–99) ppb 2 weeks after treatment was withdrawn. During treatment, the fall in ENO was accompanied by nonsignificant improvements in prebronchodilator forced expiratory volume in 1 s (FEV_1) from 81–85% predicted or reductions in use of albuterol from a mean of 2.5 to 1.6 puffs/day. Individual ENO measurements and change in ENO concentrations with treatment did not correlate with either pulmonary function changes or use of bronchodilator.

These data show that ENO is elevated in children with relatively mild asthma treated with bronchodilator alone, and that treatment with montelukast sodium for 4 weeks results in a significant reduction in ENO concentrations, even in the absence of significant changes in pulmonary function. These findings suggest an anti-inflammatory role for leukotriene D_4 receptor antagonism in the treatment of children with mild to moderate asthma. **Pediatr Pulmonol. 1999; 28:402–407.**

Key words: exhaled nitric oxide; leukotrienes; montelukast; asthma; controlled randomized clinical trial; children.

INTRODUCTION

Exhaled nitric oxide (ENO) is increasingly recognized as a noninvasive marker of airway inflammation, and has been shown to be elevated in untreated asthma in adults[1,2] and children.[3–8] Although precise correlations with objective indices of inflammation remain to be established, ENO levels readily fall after therapy with corticosteroids.[9–13] Activated inflammatory cells produce NO in increased amounts due to the expression of inducible NO synthase (iNOS), and importantly, these activated cells produce cytokines that induce this enzyme in respiratory epithelium.[14,15] Accordingly, increased expression of inducible iNOS has been demonstrated in the bronchial epithelium of asthmatics.[2,16–18]

The cysteinyl leukotrienes are important mediators of asthma. They are produced and released by inflammatory cells present in the airways of asthmatic patients and are potent mediators of bronchoconstriction, mucus secretion, and vascular permeability.[19,20] Leukotriene pathway modifiers, both LTD_4 receptor antagonists[21–23] and 5-lipoxygenase inhibitors,[24] have been shown to improve asthma control. Of interest, while first-dose effects of these medications are often seen on pulmonary function,[21,23,24] significant additional improvement in symptoms and pulmonary function is often observed over the course of weeks to months, suggesting that these agents have slower-onset anti-inflammatory effects on airway disease.[22,24] Furthermore, in a recent study in children using montelukast sodium, a newly released LTD_4 receptor antagonist, a significant decrease was observed in the numbers of circulating eosinophils, a pivotal cell in the asthmatic inflammatory process of asthma.[21]

The goal of this study was to determine whether exhaled nitric oxide (ENO) concentration, a sensitive, non-

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invasive marker of inflammation, is useful as an outcome measure in children with relatively mild persistent chronic asthma following therapy with montelukast sodium, given in addition to beta-agonist bronchodilator. We also sought to determine whether ENO is a more sensitive measure of airway inflammation than FEV_1 in children with mild chronic asthma in whom FEV_1 often remains unchanged with introduction of therapy.[8,13,25] We found that relative to healthy control children, ENO levels were significantly elevated in children with mild to moderate persistent asthma managed with bronchodilator alone. Furthermore, treatment with montelukast sodium resulted in a significant reduction in ENO concentrations in the absence of significant changes in pulmonary function or use of rescue bronchodilator.

MATERIALS AND METHODS

Study Populations

All asthmatic subjects were enrolled in the multicenter protocol 065-01/SNG386, "A Randomized, Open Label, Cross-Over Study Comparing Parent/Guardian Preference for Montelukast Sodium Tablets or Cromolyn Sodium Aerosol (MDI) Treatment in Their Children aged 6 to 11 With Chronic Asthma." The present study was conducted as a single-site protocol addendum for the measurement of ENO before and after montelukast treatment. The asthmatic subjects, 6 to 11 years old, had a clinical history of mild to moderate stable asthma, but were otherwise judged to be in good health on the basis of history and physical examination. Inclusion criteria were the presence of symptoms requiring beta-agonist therapy on at least 7 of the 14 days during the run-in period, evidence of airway reversibility (≥12% improvement in FEV_1) determined either twice during the run-in period, or once during the run-in period and once within 1 year prior to study start, and an FEV_1 between 60–85% of predicted values[26] following withholding beta-agonist for 6 h or more on two occasions during the run-in period. Patients were excluded if they had used inhaled, oral, intramuscular, or intravenous corticosteroids, theophylline, leukotriene synthesis inhibitors or receptor antagonists, nedocromil, cromolyn, or nonsedating antihistamine within the past 2 weeks (3 months for astemizole) prior to the study; if they had required ≥3 short courses of systematic corticosteroids within the previous 6 months; and if there was clinically active sinusitis, upper respiratory tract infection, or gastroesophageal reflux. Nine of the 12 asthmatic subjects had histories of allergic rhinitis. Nasal corticosteroids were permitted at a constant dose during the course of the study.

Twelve healthy, age and gender matched control subjects with no history of asthma, rhinitis, or viral infection during the previous 4 weeks, on no medications, and without evidence of airflow obstruction on examination and spirometry, were recruited for comparison of ENO levels. All subjects demonstrated the ability to reproducibly perform the slow vital capacity maneuver required for ENO analysis (see below). The protocol was reviewed and approved by the National Jewish Medical and Research Center Institutional Review Board, and all subjects and their parents provided informed consent.

Abbreviations

CI	Confidence interval
ENO	Exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
iNOS	Inducible nitric oxide synthase
LTD_4	Leukotriene D_4
NO	Nitric oxide
ppb	Parts per billion
SEM	Standard error of the mean

Study Design

Asthmatic subjects were followed for a 2-week run-in period during which information regarding symptoms and beta-agonist usage was collected by diary and in which entry criteria were met. ENO concentration was measured on three occasions: immediately before starting montelukast sodium, following 4 weeks of treatment with montelukast sodium (5 mg chewable tablet) administered once daily at bedtime, and after a 2-week post-treatment washout period. Given the previously observed stability in ENO values for normal subjects over time,[27] ENO for normal control subjects was measured during a single clinic visit.

Measurement of NO

Each asthmatic subject performed spirometry and then received two puffs of albuterol by metered dose inhaler (180 mcg). ENO levels were measured 20 min post-bronchodilator to remove artifactual lowering of ENO due to bronchoconstriction and/or spirometry.[28] The measurement of ENO was done using a chemiluminescent analyzer (Model 280 NOA, Sievers Instruments, Inc., Boulder CO). The analyzer was calibrated daily using ambient air entrained via a NO scrubber for the zero NO gas and a standard calibration gas of 19.8 ppm. Without a nose clamp, subjects inhaled to total lung capacity through a paper mouthpiece fitted to a one-way valve (Hans Rudolph, Kansas City, MO) from a reservoir bag filled with medical air containing <5 ppb NO to eliminate contamination by environmental NO. The subjects then exhaled against a fixed resistor at a constant pressure of 20 mm Hg, a maneuver that closes the velum of the posterior nasopharynx and excludes nasal NO con-

tamination.[27] The fixed mouth pressure and expiratory resistance created a constant expiratory flow rate of 40 mL/s, thus reducing variability as the level of ENO is highly flow-dependent.[27] ENO was derived from the plateau phase of the single-breath NO profile vs. time. Measurements were performed until three values with ≤10% variability were obtained. These values were then averaged for each visit. In asthmatic subjects, post-bronchodilator spirometry was performed after ENO was measured.

Statistical Analysis

ENO values deviated significantly from a normal distribution, therefore, log transformed data were used in the analyses. A repeated measures analysis of variance (ANOVA) was used to compare data, and $P < 0.05$ was regarded as statistically significant. The normal range for ENO was defined as the log transformed mean ±2 standard deviations of the mean. Both the mean ENO concentration and range for controls subjects were found to be quite similar to control groups described in other series.[27,29] The Pearson correlation coefficient was used to test the correlation between baseline ENO and pre- and post-bronchodilator FEV_1 and albuterol use. Similarly, post-treatment ENO and changes in ENO levels over the course of montelukast sodium treatment were tested for correlation with pulmonary function and albuterol use.

RESULTS

Twelve asthmatic subjects with mild to moderate persistent asthma and 12 normal control subjects were recruited for this study. Baseline characteristics are shown in Table 1. Baseline mean ENO concentration for the asthmatic group was 83 ppb (42–123, 95% CI) which was significantly higher ($P < 0.001$) than the mean ENO level of 13 ppb (11–15, 95% CI) for the age and gender matched control subjects (Table 2). In Figure 1, ENO values are shown at baseline, following montelukast sodium treatment and after the washout period for individual asthmatic subjects. Following montelukast sodium treatment, mean ENO fell significantly to 58 ppb (27–89, 95% CI), representing a mean ENO decrease of 24 ppb ($P < 0.01$). As shown, ENO values at baseline for 2 patients were within the range of normal subjects (upper limit of 20 ppb, see Materials and Methods), while all others were elevated. For 7 of the 10 patients with elevated baseline ENO values, a fall of greater than 20% was observed during montelukast treatment. ENO concentrations rose during the washout phase following montelukast treatment to 69 ppb (38–99, 95% CI), a level which was not significantly different from either end of treatment or baseline levels.

TABLE 1—Baseline Characteristics[1]

	Asthmatic subjects	Normal subjects
Age (years), mean ± SD	9.3 ± 1.6	9.2 ± 1.7
Male:female	8:4	8:4
Caucasian:black	9:3	11:1
FEV_1 % predicted before β_2 agonist, mean ± SD[2]	81 ± 12	104 ± 8
FEV_1 % predicted post-β_2 agonist, mean ± SD[2]	97 ± 12	
Puffs/day, mean ± SD[3]	2.5 ± 2.1	

[1]N = 12 subjects in each group.
[2]Determined as percent predicted.[26]
[3]Albuterol use averaged for the 2-week period prior to montelukast sodium treatment.

TABLE 2—Comparison of ENO in Asthmatic and Control Subjects[1]

	Mean (95% CI)	Median (2.5–97.5%ile)
Asthmatics	83 (42–123)	70 (10–236)
Controls	13 (11–15)	13 (8–18)

[1]Difference between patients and controls is significant ($P > 0.001$) by ANOVA.

During montelukast sodium therapy, a small but nonsignificant increase in prebronchodilator FEV_1 and a reduction in need for rescue bronchodilator were demonstrated (Table 3). Correlations of individual patients' baseline ENO and pre- and post-bronchodilator FEV_1 and $beta_2$-agonist use were not significant (data not shown). Similarly, end of treatment ENO levels and changes in ENO concentrations over the course of montelukast sodium treatment were not significantly correlated with pulmonary function or beta-agonist use.

DISCUSSION

This is the first study to examine ENO levels during treatment of children with asthma with montelukast sodium. With each subject serving as his or her own control, a significant fall in ENO was demonstrated on montelukast treatment, and a nonsignificant rebound of ENO towards pretreatment values was seen in the short washout period following withdrawal of the LTD_4 receptor antagonist. In contrast, pulmonary function and rescue medication use were not significantly changed, although these indicators showed favorable trends during treatment with montelukast sodium. These findings suggest either that ENO is more sensitive than pulmonary function in detecting changes in airway inflammation, or that ENO concentrations are assessing different aspects of the pathological features of asthma. Alternatively, the lack of correlation between fall in ENO and clinical improvement may relate to the severity of disease being too mild (see below), the length of the trial too short, or the small number of patients studied.

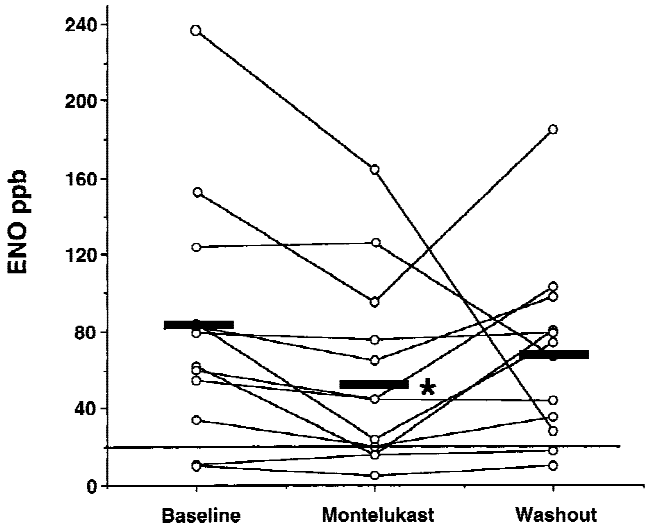

Fig. 1. ENO (ppb) is measured at baseline, at the end of treatment with montelukast sodium, and following the washout period. Data for individual patients are shown by connected circles (○). Mean ENO concentration (bar) was 83 ppb (42–123, 95% CI) before treatment, 58 ppb (27–89, 95% CI) after 4 weeks of treatment with montelukast sodium, and 69 ppb (38–99, 95% CI) after a 2-week washout period. ENO following montelukast treatment (*) was significantly different from baseline ENO ($P < 0.01$).

The fall in ENO after montelukast is in keeping with many publications showing that ENO levels are high in untreated asthma of adults[1,2] and children[3–8] and that they fall following therapy with corticosteroids, but not after placebo treatment (unpublished data). The reduction in ENO levels following introduction of inhaled corticosteroids is rapid, occurring within a few days,[10,13,25] and even faster after oral steroids.[11,12] It is the decline following corticosteroid therapy that supports the contention that ENO is a surrogate marker of airway inflammation.

To date, little information is available regarding ENO concentrations in the treatment of asthma with other medications, including the new leukotriene pathway modifying agents. In a study by Tamaoki et al.,[30] the addition of pranlukast, an LTD_4 receptor antagonist, to the regimen of patients requiring high doses of inhaled corticosteroids allowed tapering by half of inhaled corticosteroids, whereas the addition of placebo did not. They showed that ENO levels rose significantly as inhaled corticosteroids were withdrawn in patients receiving placebo, whereas no increase in ENO levels were seen during the withdrawal phase in patients receiving pranlukast. As montelukast has also been shown to decrease peripheral blood eosinophils,[21] we speculate that cysteinyl leukotrienes play a role in the inflammatory cell recruitment that ultimately results in cytokine production and induction of nitric oxide synthase.[17,31]

TABLE 3.—Treatment Outcomes[1]

	Baseline	Montelukast	*P* value
FEV_1 % predicted before β_2 agonist[2]	81 ± 4	85 ± 4	0.34
FEV_1 % predicted post-β_2 agonist[2]	97 ± 4	95 ± 4	0.28
Puffs/day[3]	2.5 ± 0.6	1.6 ± 0.5	0.13

[1]Data shown as means ± SEM.
[2]Determined as percent predicted.[26]
[3]Albuterol use averaged for the 2 weeks before, or for the 4-week period during montelukast sodium treatment.

Our data from this preliminary study of a limited number of subjects demonstrate that ENO fell on average by approximately 33% following treatment with montelukast sodium in 7 of 10 children with elevated ENO. The magnitude of this fall is not unlike that seen following the introduction of inhaled corticosteroid in adult patients in one study where mean ENO concentrations measured under similar conditions fell from 85 to 40 ppb.[10] In a study by Kharitonov et al.,[13] mild asthmatics started on inhaled corticosteroids (vs. placebo) were found to have a 25% decrease of ENO levels within 7 days that continued to decrease to approximately 40% from baseline by the third week of the trial. As we sampled ENO concentrations only before and at the end of 4 weeks of treatment with montelukast sodium, we have no data to suggest how quickly the reduction in ENO occurred, or whether new steady-state levels had been achieved.

The children recruited for this study fall into the category of mild to moderate persistent asthma as defined by the National Asthma Education and Prevention Program (NAEPP) Expert Panel Report II, Guidelines for the Diagnosis and Management of Asthma.[32] While most published data on ENO levels have looked at pediatric patients with acute exacerbations of asthma, little information is available for ENO concentrations in children with relatively stable persistent asthma.[4,7,8,11] Furthermore, recent data would suggest that ENO appears related to atopic status,[29,33] which may explain some of the variability in baseline ENO demonstrated in our study population, though this was not formally addressed. The data here demonstrate that in 10 out of 12 children with relatively mild disease, ENO was elevated above the normal range, and on average by a factor of 5 over the mean seen in normal subjects. As suggested above, the relatively mild presentation of these patients (mean FEV_1 prebronchodilator of 81% and albuterol usage of 2.5 puffs/day) may also explain the finding that while ENO concentrations were significantly reduced by montelukast sodium treatment, pulmonary function and use of bronchodilator were not. These findings agree with Kharitonov et al.[13] who, in spite of showing a significant change in ENO levels, showed no significant change in FEV_1 before and after introduction of inhaled corticoste-

roids in study subjects, a finding likely attributable to the mild nature of their disease. Furthermore, in another study of inhaled corticosteroid dose titration, increasing or decreasing budesonide dosage by 200 mcg/day resulted in changes in ENO concentrations, with an ENO decrease of approximately 40% or an increase of 100%, respectively.[25] In this latter study, ENO measurements were shown to be more sensitive to changes in inhaled steroid doses than were measurements of spirometry, peak flows, need for rescue medication, or diary recordings.

Based on our findings and those of others, ENO appears to be a sensitive, rapidly changing surrogate marker of airway inflammation in asthma that offers the advantage of ease of performance and noninvasive measurements. Optimally, changes in this surrogate marker of inflammation need to be corroborated with traditional airway findings obtained invasively. However, doing so in relatively mild disease and in children in whom chronic asthma is an evolving disease is not practical.

The contribution of ENO measurements to the clinical management of asthma has not yet been fully defined, but could include measurements at diagnosis and during screening visits when one could assess the degree of airway inflammation, and during follow-up of disease when fluctuations in ENO could help assess disease activity and possibly predict future deterioration. Unfortunately, interpretation of the current ENO literature is difficult due to the various methods used to measure ENO concentrations, which are highly flow-dependent and require the exclusion of nasal nitric oxide.[27,34] The results of a recent American Thoracic Society Workshop on the Standardization of Measurement of ENO are soon to be published and will set the standard for ENO measurement in adults and children. As the authors participated in the workshop, we note that this study was conducted in accordance with the upcoming recommendations for the measurement of ENO in subjects 6 years of age and older.

In conclusion, the findings reported here demonstrate that ENO levels are elevated in children with mild to moderate persistent asthma, managed with bronchodilator alone. ENO concentrations fell significantly following 4 weeks of treatment with montelukast sodium. Our data add to the growing body of evidence that LTD_4 receptor antagonists have anti-inflammatory properties.

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