

# Inhibitory effect of a leukotriene receptor antagonist (montelukast) on neurokinin A-induced bronchoconstriction

Nunzio Crimi, MD, Corrado Pagano, MD, Filippo Palermo, BSc,  
Claudio Mastruzzo, MD, PhD, Gaetano Prosperini, MD, Maria P. Pistorio, BSc, and  
Carlo Vancheri, MD, PhD *Catania, Italy*

**Background:** Tachykinins are potent contractors of human airways producing a dose-related bronchoconstriction when administered by means of inhalation to asthmatic subjects.

**Objective:** The aim of this study was to examine the effective role played by leukotrienes (LTs) in neurokinin A (NKA)-induced bronchoconstriction in asthmatic patients.

**Methods:** To address this question, we investigated the protective effect of a selective cysteinyl LT receptor antagonist, montelukast, against inhaled NKA and determined  $\text{LTE}_4$  excretion in the urine.

**Results:** Inhaled NKA in the absence of any drug treatment produced a concentration-related bronchospasm with a geometric mean provocative concentration required to produce a 15% decrease in  $\text{FEV}_1$  from the postsaline baseline value ( $\text{PC}_{15}$ ) value of  $290.9 \mu\text{g/mL}$  (+SE,  $407.1 \mu\text{g/mL}$ ; -SE,  $207.84 \mu\text{g/mL}$ ). Montelukast pretreatment significantly increased ( $P < .01$ ) the  $\text{PC}_{15}$  NKA value ( $708.8 \mu\text{g/mL}$ ; +SE,  $890.47 \mu\text{g/mL}$ ; -SE,  $564.15 \mu\text{g/mL}$ ) in comparison with placebo ( $394.4 \mu\text{g/mL}$ ; +SE,  $491.88 \mu\text{g/mL}$ ; -SE,  $248.16 \mu\text{g/mL}$ ) and produced a shift of the NKA concentration-response curve to the right in all the subjects studied. When compared with placebo, montelukast did not have a significant protective effect against methacholine challenge; the geometric mean  $\text{PC}_{15}$  values obtained were  $0.87$  and  $0.96 \text{ mg/mL}$  with placebo and montelukast, respectively. Although we have not observed any increase in urinary  $\text{LTE}_4$  excretion after NKA inhalation, we have shown that pretreatment of asthmatic subjects with montelukast elicits a significant protection against NKA-induced bronchoconstriction.

**Conclusion:** In asthmatic subjects NKA-induced bronchoconstriction is indirectly caused by the release of LTs, and this mechanism could explain some of the antiasthmatic and anti-inflammatory effects of LT antagonists. (*J Allergy Clin Immunol* 2003;111:833-9.)

**Key words:** Neurokinin A, *cysLT* receptor antagonist, bronchial asthma, leukotrienes

## Abbreviations used

Cys-LT: Cysteinyl leukotriene

LT: Leukotriene

NKA: Neurokinin A

$\text{PC}_{15}$ : Provocative concentration required to produce a 15% decrease in  $\text{FEV}_1$  from the postsaline baseline value

SP: Substance P

Sensory neuropeptides, such as substance P (SP) and neurokinin A (NKA), are localized in the sensory c-fibers of the airway wall.<sup>1</sup> Although the nerve fibers containing tachykinins are less dense in human airways compared with those in rodents, there is convincing evidence for the involvement of neuropeptides in the pathogenesis of asthma. Tachykinins, in fact, might cause contraction of airway smooth muscle, increased vascular permeability, mucus secretion, and activation of cholinergic neurotransmission.<sup>2,3</sup> Several studies have shown that tachykinins are potent contractors of human airways producing a dose-related bronchoconstriction when administered by means of inhalation to asthmatic subjects.<sup>3-5</sup> Furthermore, studies on necroscopic tissue, bronchoalveolar lavage fluid, and induced sputum have demonstrated increased amounts of SP in the airways of asthmatic patients compared with that seen in control subjects and have also shown that tachykinins express a wide variety of immunomodulatory effects on inflammatory cells, such as eosinophils, neutrophils, lymphocytes, monocytes, and mast cells.<sup>6-12</sup> Although most of the biologic responses elicited by sensory neuropeptides are mediated by 2 specific receptors, NK1 and NK2, the mode of action by which tachykinins elicit bronchoconstriction and neurogenic inflammation in asthma is not completely understood. It has been shown that the bronchoconstrictor effect of SP and NKA in asthmatic patients is inhibited by a pretreatment with nedocromil sodium, suggesting that the effect of tachykinins is not due to a direct stimulation of the airway smooth muscles but possibly through a cholinergic reflex activation or likely through the induction of mast cell mediators, such as histamine.<sup>13,14</sup> Indeed, a release of histamine in bronchoalveolar lavage fluid recovered after

From the Department of Internal and Specialistic Medicine, Section of Respiratory Diseases and Infectious Diseases, University of Catania, Catania. Montelukast and its matched placebo were supplied by Merck Sharpe & Dome. No funding was supplied by this company for conducting the study. Received for publication March 20, 2002; revised November 17, 2002; accepted for publication November 26, 2002.

Reprint requests: Nunzio Crimi, MD, Department of Internal and Specialistic Medicine, Section of Respiratory Diseases, University of Catania, Via Passo Gravina 187, 95125, Catania, Italy.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.161

TABLE I. Patient characteristics

| Patient no. | Sex | Age (y) | Baseline FEV <sub>1</sub> , L<br>(% predicted) | PC <sub>15</sub> methacholine baseline<br>(mg/mL) | PC <sub>15</sub> NKA baseline<br>(μg/mL) | Atopy |
|-------------|-----|---------|------------------------------------------------|---------------------------------------------------|------------------------------------------|-------|
| 1           | M   | 23      | 3.40 (79)                                      | 0.21                                              | 125                                      | W-D   |
| 2           | M   | 18      | 3.50 (110)                                     | 1.97                                              | 556                                      | W-D   |
| 3           | M   | 20      | 4.65 (113)                                     | 1.12                                              | 421                                      | W     |
| 4           | M   | 45      | 3.40 (83)                                      | 1.31                                              | 316                                      | W-D   |
| 5           | M   | 19      | 3.35 (74)                                      | 0.82                                              | 35                                       | W     |
| 6           | F   | 30      | 2.97 (82)                                      | 0.28                                              | 187                                      | W     |
| 7           | M   | 19      | 4.66 (104)                                     | 1.91                                              | 793                                      | W     |
| 8           | M   | 19      | 3.16 (71)                                      | 0.3                                               | 359                                      | W     |
| 9           | M   | 26      | 5.01 (112)                                     | 1                                                 | 857                                      | W     |
| Mean        |     | 24.3    | 3.78 (92)                                      | 0.76                                              | 290.9                                    |       |
| ± SEM       |     | 2.91    | 0.25 (5.8)                                     | 0.57-1.0                                          | 207.84-407.10                            |       |

W, Wall pellitory grass; D, *Dermatophagoides* species.

NKA infusion has been shown in rats.<sup>9</sup> Despite a well-established effect of SP in causing histamine release from cutaneous mast cells, more controversial is the effect of the peptide on pulmonary mast cells. However, antihistaminic drugs are not effective in preventing SP-induced bronchoconstriction in asthmatic patients.<sup>15-17</sup> In addition, recent reports indicate that most of the functional effects of tachykinins might be mediated through the release of mediators other than histamine. Ellis and Undem<sup>18</sup> have demonstrated that the cysteinyl leukotriene 1 (Cys-LT1) receptor antagonist pobilukast is an effective inhibitor of the contraction and plasma extravasation induced by tachykinins in the isolated tracheas of guinea pigs. More recently, Yang et al<sup>19</sup> have reported that hyperpnea-induced bronchoconstriction in an animal model of exercise-induced asthma is followed by an increase in biliary leukotriene (LT) D<sub>4</sub> levels and can be attenuated by tachykinin receptor antagonists and, most importantly, by a pretreatment with an LTD<sub>4</sub> antagonist, suggesting a release of Cys-LTs induced by tachykinins. Moreover, Turner et al<sup>20</sup> demonstrated that bradykinin, which is reported to release tachykinins, also evokes Cys-LT synthesis in the airways. The aim of this study was to examine in asthmatic subjects the mechanism of action of tachykinins and particularly the effective role played by LTs in NKA-induced bronchoconstriction. To address this question, we investigated the protective effect of a selective antagonist of Cys-LTs, montelukast, against inhaled NKA and determined LTE<sub>4</sub> excretion in the urine as a measure of Cys-LT synthesis within the lung.

## METHODS

### Subjects

Nine asthmatic subjects (8 men and 1 woman; mean ± SEM age, 24.3 ± 2.9 years) with stable asthma participated in a double-blind, placebo-controlled, randomized cross-over study (Table I). Randomization was performed by using an appropriate table of random numbers. Subjects were nonsmokers, and all were atopic, as defined by positive skin prick test responses (>3-mm wheal response) to one or more of 6 common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, wall pellitory grass, mixed grass pollen, cat fur, and dog hair). At the beginning of the study, all subjects

were asymptomatic, with a baseline FEV<sub>1</sub> value greater than 70% of predicted values. None had received oral corticosteroids, theophylline, antihistamines, or sodium cromoglycate within the preceding 4 weeks. Inhaled bronchodilators and steroids were discontinued for at least 12 hours and 12 weeks, respectively, before each visit to the laboratory. Subjects were not studied within 6 weeks of an upper respiratory tract infection or exacerbation of their asthma, and all visits to the laboratory were carried out at the same time of day to avoid change in the response caused by diurnal rhythm and outside the pollen season. The study was approved by the local ethics committee, and all subjects provided written informed consent.

### Bronchial provocation test

Airway caliber was recorded before and during the provocation as FEV<sub>1</sub> by using a Turbin Spirometer (μkit, COSMED), with the better of 2 consecutive measurements being used for analysis. Methacholine (Sigma Chemical Co) and NKA (Peninsula Laboratories Ltd) were made up in PBS and 0.9% sodium chloride (1% albumin solution) to produce a range of increasing doubling concentrations of 0.03 to 16.00 mg/mL and 3.9 to 500 μg/mL, respectively. The aqueous solutions were administered as aerosols generated from a starting volume of 3 mL in a disposable Inspiron Mini-nebulizer (C.R. Bard International) driven by compressed air at 8 L/min. Under these conditions, the nebulizer had an output of 0.48 mL/min and generated an aerosol with a mass median particle diameter of 4.7 μm. Wearing a nose clip, subjects inhaled the aerosolized solutions in 5 breaths from end-tidal volume to full inspiratory capacity through a mouthpiece. Subjects were trained to take 3 seconds to reach full inspiratory capacity.

### Study design

The study consisted of 2 distinct phases (Fig 1).

**Phase 1.** Subjects attended the laboratory on 2 separate occasions at least 72 hours apart to undertake concentration-response studies with either inhaled methacholine or NKA in the absence of any drug treatment. On the first occasion, after a 15-minute resting period, 3 baseline measurements of FEV<sub>1</sub> were made at intervals of 3 minutes, followed by inhalation of 0.9% sodium chloride and further FEV<sub>1</sub> measurements repeated at 1 and 3 minutes. Provided that FEV<sub>1</sub> had not decreased by greater than 10% of the baseline value, a methacholine concentration-response study was carried out. After administration of each methacholine concentration, FEV<sub>1</sub> was measured at 1 and 3 minutes. Increasing doubling concentrations of methacholine were inhaled at 5-minute intervals until FEV<sub>1</sub> had decreased by greater than 15% of the postsaline baseline value. The decrease in FEV<sub>1</sub> after each concentration of agonist was expressed

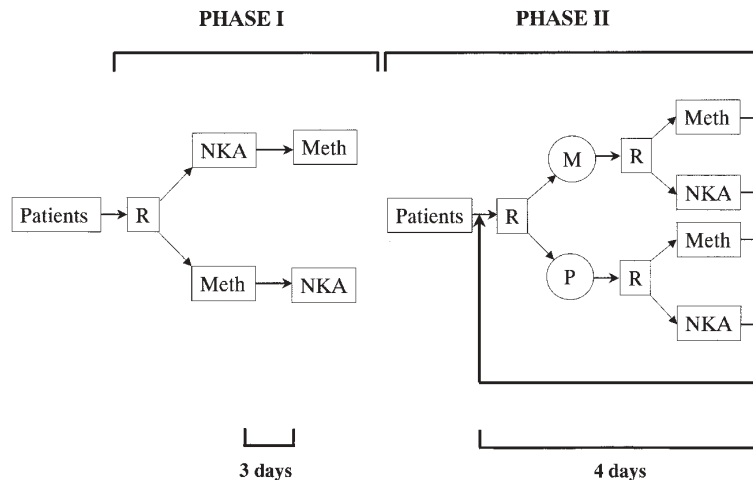


**FIG 1.** Study design: a 2-phase, randomized, placebo-controlled, crossover study. In the first phase a concentration-response study was performed either with methacholine or NKA in the absence of any drug treatment. During the second phase, each subject recruited went through all possible combinations of drug administration and challenge procedures. *R*, Random; *Meth*, methacholine; *M*, montelukast; *P*, placebo.

**TABLE II.** Urinary concentration of LTE<sub>4</sub> before and after NKA and methacholine bronchial challenge

| Methacholine |             |             |           | NKA          |              |              |              |
|--------------|-------------|-------------|-----------|--------------|--------------|--------------|--------------|
| Placebo      |             | Montelukast |           | Placebo      |              | Montelukast  |              |
| Before       | After       | Before      | After     | Before       | After        | Before       | After        |
| 82.06 ± 18.5 | 88.3 ± 13.6 | 90 ± 26.1   | 94 ± 29.4 | 76.97 ± 23.4 | 72.34 ± 17.7 | 82.95 ± 22.6 | 77.58 ± 18.4 |

Values are given as nanograms per millimole of creatinine (mean ± SEM).

as a percentage of the higher of the 2 postsaline baseline FEV<sub>1</sub> recordings. The percentage decrease in FEV<sub>1</sub> was plotted against the cumulative concentration of agonist on a logarithmic scale, and the provocative concentration required to produce a 15% decrease in FEV<sub>1</sub> from the postsaline baseline value (PC<sub>15</sub>) was determined by means of linear interpolation. The bronchial provocation test with inhaled NKA was undertaken in a similar manner to that described for methacholine. FEV<sub>1</sub> measurements were recorded 1 and 3 minutes after inhalation of each concentration of NKA, and the corresponding PC<sub>15</sub> FEV<sub>1</sub> values were derived.

**Phase 2.** Subjects attended the laboratory on 4 separate occasions at least 4 days apart to undertake concentration-response studies with methacholine and NKA after receiving either oral montelukast or matched placebo (Merck Sharp & Dohme). To ensure an adequate drug level and achieve a better saturation of Cys-LT<sub>1</sub> receptors, we studied the effect of the drug, administering 2 tablets of 10 mg of montelukast in a double-blind manner and in random order 3 hours before the start of each challenge. It has been shown that montelukast achieves the plasmatic peak approximately 3 hours after administration.<sup>21</sup> Urine for analysis of LTE<sub>4</sub> was collected before and then 2 hours after challenge on each study day at the same time. After collection, 20-mL aliquots of urine were frozen at -80°C until the assay was performed. Urinary LTE<sub>4</sub> was measured by using a commercially available enzyme immunoassay (Cayman Chemical Co), according to the manufacturer's instructions. The assay is sensitive to 25 pg/mL LTE<sub>4</sub>. All samples were determined in duplicate. Creatinine was measured in all urine samples by using a colorimetric alkaline picrate method with a standard procedure and commercially available kit (Beckman Coulter). The results were expressed as nanograms of immunoreactive LTE<sub>4</sub> per millimole of creatinine in each urine sample (Table II).

## Data analyses

Results are expressed as means ± SEM unless otherwise specified, and a *P* value of less than .05 was accepted as the minimum level of statistical significance. Pretreatment and posttreatment baseline values of FEV<sub>1</sub> before bronchial challenge were compared between and within study days by means of 2-way ANOVA, followed by the Neumann-Keuls test for specific means comparisons where appropriate. Values of PC<sub>15</sub> methacholine and NKA after treatment with placebo and montelukast were logarithmically transformed to normalize their distribution and were compared by means of the Student *t* test for paired data. The PC<sub>15</sub> values, after a logarithmic conversion, were expressed as the geometric mean ± SEM. The protective effect of montelukast against NKA challenge was expressed as the dose ratio, which was derived by dividing the PC<sub>15</sub> value after administration of montelukast by that found after placebo administration. We also compared the percentage decrease of FEV<sub>1</sub> after montelukast to the value obtained at the same provocation concentration found after placebo with a Wilcoxon signed-rank test. Airway response was also evaluated by measurement of the area under the concentration-response curve, which was calculated by using the trapezoidal rule. The area values were compared by using the appropriate 2-way ANOVA.

## RESULTS

The inhalation of methacholine and NKA caused a temporary and mild feeling of chest tightness to 2 patients and 1 patient, respectively. The decrease in FEV<sub>1</sub> in the remaining patients was devoid of clinically evident discomfort. There was no significant difference in mean

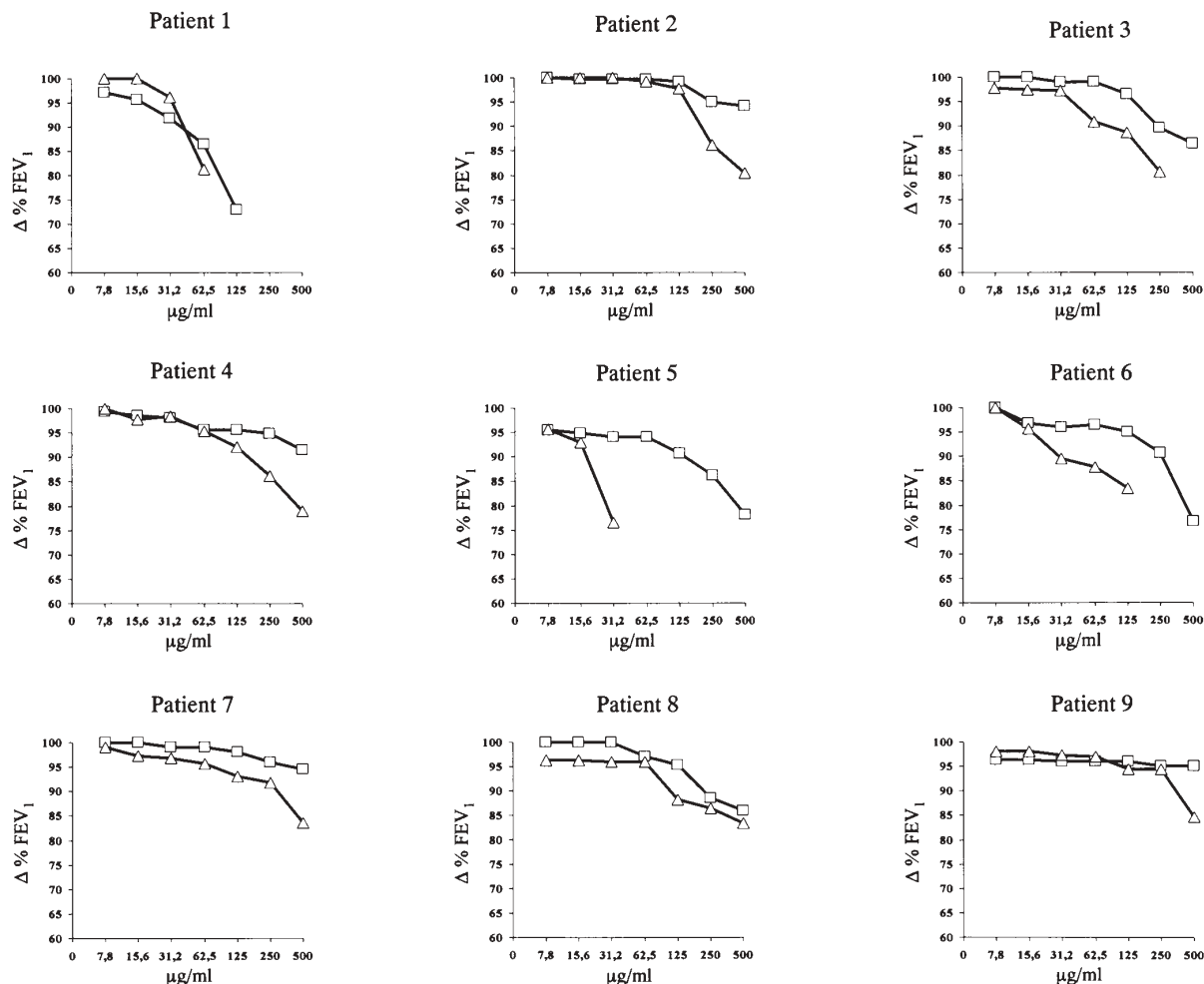


FIG 2. Effect of montelukast (filled squares) compared with placebo (open triangles) on the concentration-response curves to inhaled NKA in 9 asthmatic subjects.

± SEM baseline values of FEV<sub>1</sub> between any of the study days. Administration of montelukast did not cause any significant change in FEV<sub>1</sub> from baseline either on methacholine or NKA study days. Baseline mean values of FEV<sub>1</sub> were  $3.71 \pm 0.31$  and  $3.79 \pm 0.32$  L on methacholine and NKA study days. After montelukast treatment, FEV<sub>1</sub> mean values were  $3.57 \pm 0.32$  and  $3.79 \pm 0.3$  L, respectively. Inhaled methacholine and NKA in the absence of any drug treatment produced a concentration-related bronchospasm with a geometric mean PC<sub>15</sub> value of 0.76 mg/mL (+SE, 1.00 mg/mL; -SE, 0.57 mg/mL) and 290.9 μg/mL (+SE, 407.1 μg/mL; -SE, 207.84 μg/mL), respectively (Table III). Placebo administration did not produce any significant increase in methacholine and NKA responsiveness in comparison with baseline values, and the PC<sub>15</sub> geometric mean value was 0.87 mg/mL (+SE, 1.22 mg/mL; -SE, 0.63 mg/mL) and 394.4 μg/mL (+SE, 491.88 μg/mL; -SE, 248.16 μg/mL), respectively (Table III).

Montelukast pretreatment significantly ( $P < .01$ ) increased the PC<sub>15</sub> NKA value (geometric mean, 708.8 μg/mL [+SE, 890.47 μg/mL; -SE, 564.15 μg/mL]) in

comparison with placebo (geometric mean, 394.4 μg/mL [+SE, 491.88 μg/mL; -SE, 248.16 μg/mL]) and produced a shift of the NKA concentration-response curve to the right in all subjects studied (Table III and Fig 2).

When compared with placebo, montelukast did not have a significant protective effect against methacholine challenge: the geometric mean PC<sub>15</sub> values obtained were 0.87 mg/mL (+SE, 1.22 mg/mL; -SE, 0.63 mg/mL) and 0.96 mg/mL (+SE, 1.24 mg/mL; -SE, 0.74 mg/mL) with placebo and montelukast, respectively (Table III).

When reported as the concentration ratio, montelukast afforded 2.02-fold protection against NKA-induced bronchoconstriction.

In 6 subjects after montelukast pretreatment, inhaled NKA reached the maximum concentration of 500 μg/mL without any evident bronchoconstrictor activity. To avoid evident side effects after higher concentrations of NKA, we calculated the PC<sub>15</sub> values in these subjects, assuming an arbitrary dose of 1000 μg/mL as provocative. In addition, we expressed the results as the percentage decrease in FEV<sub>1</sub>. The provocative concentration of inhaled NKA produced a mean decrease in FEV<sub>1</sub> of  $-18.34\% \pm 0.89\%$

**TABLE III.** Effect of pretreatment with montelukast and placebo on NKA and methacholine responsiveness

| Patient no.    | PC <sub>15</sub> NKA (μg/mL) |         |             | PC <sub>15</sub> methacholine (mg/mL) |         |             |
|----------------|------------------------------|---------|-------------|---------------------------------------|---------|-------------|
|                | Baseline                     | Placebo | Montelukast | Baseline                              | Placebo | Montelukast |
| 1              | 125                          | 106     | 127         | 0.21                                  | 0.16    | 0.35        |
| 2              | 556                          | 659     | 1000        | 1.97                                  | 2.71    | 2.43        |
| 3              | 421                          | 333     | 1000        | 1.12                                  | 1.63    | 1.28        |
| 4              | 316                          | 554     | 1000        | 1.31                                  | 1.97    | 1.83        |
| 5              | 35                           | 50      | 543         | 0.82                                  | 0.86    | 0.94        |
| 6              | 187                          | 205     | 655         | 0.28                                  | 0.34    | 0.46        |
| 7              | 793                          | 886     | 1000        | 1.91                                  | 1.43    | 1.63        |
| 8              | 359                          | 684     | 1000        | 0.3                                   | 0.3     | 0.3         |
| 9              | 857                          | 964     | 1000        | 1                                     | 1.69    | 1.61        |
| Geometric mean | 290.9                        | 394.4   | 708.8*      | 0.76                                  | 0.87    | 0.96        |
| Mean +SE       | 407.10                       | 491.88  | 890.47      | 1.00                                  | 1.22    | 1.24        |
| Mean -SE       | 207.84                       | 248.16  | 564.15      | 0.57                                  | 0.63    | 0.74        |

\**P* < .01 versus baseline and placebo.

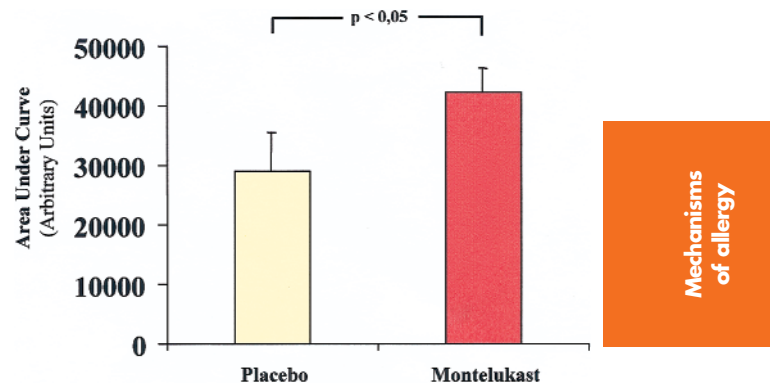
after placebo; considering the same provocative concentration for each patient, the mean decrease in FEV<sub>1</sub> after montelukast was  $-7.87\% \pm 1.45\%$  (*P* < .005).

Moreover, the area of the concentration-response curve to NKA obtained after montelukast administration was significantly greater than either area obtained after placebo (*P* < .05) or the baseline value (Fig 3).

There was no statistically significant increase in urinary LTE<sub>4</sub> excretion after NKA and methacholine inhalation in comparison with baseline values and on the placebo and montelukast study days (Table II).

## DISCUSSION

In this study we hypothesized that NKA-induced bronchoconstriction in asthmatic subjects is indirectly caused by the release of LTs and that this mechanism could explain some of the antiasthmatic and anti-inflammatory effects of LT antagonists. Tachykinins are considered mediators released by local and central reflexes during allergic and nonallergic reactions, but it is still debated whether they act alone or rather through the production of other bronchoactive and proinflammatory mediators. The protective effect exerted by sodium cromoglycate and nedocromil sodium on the bronchoconstriction induced by SP and NKA indicates that the functional effect played by tachykinins could be due to the activation of mast cells and the subsequent release of bronchoactive mediators.<sup>13,14</sup> Indeed, some studies have shown that SP enhances mast cell release of mediators, such as TNF- $\alpha$  and histamine.<sup>10,11</sup> On the other hand, a number of other studies have not been able to demonstrate histamine release from human mast cells after SP administration. Moreover, it has been shown that antihistaminic drugs, such as astemizole and terfenadine, do not prevent tachykinin-induced bronchoconstriction in human patients, suggesting that histamine does not play a determinant role in tachykinin-induced bronchoconstriction.<sup>16,17</sup> Recently, several reports have suggested that tachykinins could also interact with LTs, inflammatory mediators thought to play an important role in the patho-

**FIG 3.** Mean values of the area under the concentration-response curve to inhaled NKA after placebo and montelukast treatment.

genesis of asthma. In the guinea pig ileum LTD<sub>4</sub> causes release of SP and the contractile response, so induced, is at least in part inhibited by SP antagonists, showing that the effect of LTD<sub>4</sub> is mediated by SP.<sup>22</sup> The infusion of LTD<sub>4</sub> results in an increase of SP and NKA immunoreactivity in the lungs of guinea pigs, and the effect of the stimulation of capsaicin-sensitive nerves on smooth muscle contraction is partially inhibited by LTD<sub>4</sub> antagonists.<sup>20,22,23</sup> Moreover, Ishikawa et al<sup>24</sup> have shown that both capsaicin chronic treatment, which causes tachykinin depletion, and NK-receptor antagonist pretreatment reduce airway microvascular leakage and bronchoconstriction induced by the inhalation of LTD<sub>4</sub>. In another study performed in normal and asthmatic subjects, Diamant et al<sup>25</sup> demonstrated an increase of the bronchoconstriction induced by LTD<sub>4</sub> when these subjects were pretreated with thiorphan, an inhibitor of neutral endopeptidase, the enzyme that degrades tachykinins. All these data confirm the hypothesis that LTs might substantially affect tachykinin release. New emerging data have shown that tachykinins might also be, in turn, responsible for LT release. Turner et al<sup>20</sup> have shown that tachykinins mediate a substantial portion of bradykinin-induced airway narrowing in the guinea pig, as evidenced

by the reduction of bradykinin-induced bronchoconstriction in animals pretreated with NK1 and NK2 receptor antagonists. Interestingly, in the same study pranlukast, a potent LTD<sub>4</sub> antagonist, partially attenuated bradykinin-induced bronchoconstriction, suggesting that bradykinin evokes LT synthesis through the release of tachykinins. In addition, Yang et al<sup>19</sup> have demonstrated that tachykinins indirectly mediate hyperpnea-induced bronchoconstriction by triggering Cys-LT synthesis in the guinea pig airways. The blockade of hyperpnea-induced bronchoconstriction by selective antagonists of LTD<sub>4</sub> and NK receptors indicated that tachykinins might evoke airway narrowing indirectly through the release of Cys-LT. The abolition of an increase in biliary Cys-LT after NK antagonists pretreatment provides more support for the role exerted by LTs in the bronchoconstriction induced by tachykinins, although it is not clear yet what cellular source and through which mechanism tachykinins might induce the release of LTs. However, it is interesting to note that eosinophils are found in the airway nerves of patients with asthma, they have tachykinins receptors, and there is evidence showing that tachykinins might activate eosinophils.<sup>26-28</sup>

In the present study we have shown that pretreatment of asthmatic subjects with montelukast, a potent Cys-LT receptor antagonist, elicits significant protection against NKA-induced bronchoconstriction. We did not find any protective activity of montelukast on methacholine airway response. It is conceivable to hypothesize that an acute single dose of montelukast does not affect the most critical determinants of airway response to methacholine, such as interaction between receptor and agonist, increased airway smooth muscle contractile properties, and airway smooth muscle amounts. The protection afforded by montelukast against NKA challenge occurred in all subjects studied and amounted to an approximately 2-fold displacement of the concentration-response curves to the right. In spite of these data, we have not been able to demonstrate an increase of urinary LTE<sub>4</sub> 2 hours after NKA-induced bronchoconstriction. However, it is not possible to exclude that NKA could act in upregulating the signal transduction pathways of LT receptors rather than increasing LT release, and this would explain the lack of increase of urinary LTE<sub>4</sub> after NKA challenge. There is evidence *in vivo* showing an increase in the excretion of urinary LTE<sub>4</sub> after allergen challenge.<sup>29-32</sup> However, in a number of different situations, the bronchoconstriction induced by Cys-LTs is not necessarily followed by an increase in urinary LTE<sub>4</sub> excretion. During the late-phase response, the recruitment of eosinophils and the subsequent release of peroxidase might convert Cys-LTs to LTB<sub>4</sub> isomers, which are not detected by means of the currently available immunoassay for LTE<sub>4</sub>.<sup>33</sup> Adenosine monophosphate and cold air cause bronchoconstriction in asthmatic subjects, likely through the release of Cys-LTs, as shown by the protective effect exerted by LTD<sub>4</sub> receptor antagonists. Nevertheless, these situations are not accompanied by increased urinary LTE<sub>4</sub> excretion.<sup>34-36</sup> In subjects with severe allergic rhinitis,

despite Cys-LTs being recovered from nasal washings after allergen challenge, no LTE<sub>4</sub> urinary excretion was noted.<sup>37</sup> In these conditions and possibly after NKA inhalation, the local level of Cys-LTs generated might be sufficient to determine a functionally relevant effect, being too low or transient to change significantly the whole-body production reflected by urinary LTE<sub>4</sub> excretion. It must also be considered that by collecting urine 2 hours after NKA challenge, we might have missed the urinary increase in LTE<sub>4</sub>, although in the literature it is reported that after allergen challenge, this time point should ensure a good recovery of urinary LTs.<sup>28,29</sup>

To our knowledge, this is the first study showing in human patients that montelukast, a selective Cys-LT antagonist, abrogates the airway response to NKA, lending further support to the hypothesis that tachykinins might elicit bronchoconstriction indirectly through the release of Cys-LTs. Several stimuli, including allergens, viruses, pollutants, smoking, and chemical irritants, act to stimulate sensory nerves in the airways and induce local release of tachykinins from airway sensory fibers. On the basis of our findings, tachykinins stimulate the release of Cys-LTs and, according to the literature, Cys-LTs might in turn induce the release of tachykinins, establishing a vicious circle that might mediate several features of asthma, including airway smooth muscle contraction, mucus secretion, and increased vascular permeability.

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