

Protection against exercise-induced bronchoconstriction by montelukast in aspirin-sensitive and aspirin-tolerant patients with asthma

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Summary

Background Montelukast, a cysteinyl-leukotriene receptor antagonist, was reported to have a protective effect against exercise-induced bronchoconstriction (EIB). Aspirin-induced asthma (AIA) is characterized by overproduction of cysteinyl-leukotrienes.

Objective The aim of the study was to compare the response to exercise and the effect of montelukast on EIB in AIA as compared to aspirin-tolerant asthma (ATA).

Methods A placebo-controlled, double blind, cross-over randomized study was performed in 19 AIA and 21 ATA patients with stable asthma. A single dose of montelukast (10 mg) or placebo (PL), was given orally one hour prior to exercise challenge. FEV₁ was measured before and 5, 10, 15 min after exercise and then at 15-minute intervals for 4 h. Urinary LTE₄ excretion and blood eosinophil count were measured at baseline, 2 h and 4 h following exercise challenge.

Results Positive bronchial response to exercise was observed in 47.5% of all patients studied. Exercise led to almost identical maximal fall in FEV₁ in AIA and ATA patients ($23.5\% \pm 6.8\%$ vs. $21.8\% \pm 12.0\%$, respectively; $P=0.7$). Montelukast, as compared to PL, significantly attenuated EIB in 63.2% of 19 patients with positive exercise test preceded by PL. The mean of maximum fall in FEV₁ from the pre-exercise value was $10.2\% \pm 13.8$ after montelukast as compared to $22.5\% \pm 10.2$ after placebo ($P<0.001$). No significant differences between protective effect of montelukast was observed in AIA as compared to ATA patients ($P=0.63$, ANOVA). Urinary LTE₄ excretion showed no change following exercise, irrespective of the result of the test in all subjects.

Conclusion Patients with AIA and ATA react similarly to exercise challenge and obtain similar protection against EIB by montelukast.

Keywords exercise-induced bronchoconstriction, leukotrienes, aspirin-induced asthma, montelukast

Submitted 14 January 2002; revised 12 March 2002; accepted 28 May 2002

Introduction

Exercise-induced bronchoconstriction (EIB) is one of the most common clinical manifestations of reactive airways disease [1]. The cause of EIB is not completely understood. The hypothetical initiating events include dehydration of the mucosal lining in the airways and rapid airway cooling followed by re-warming. This process triggers activation of mast cells from which histamine and cysteinyl-leukotrienes (cys-LTs) are released [1, 2]. Cysteinyl-leukotrienes (LTC₄, LTD₄ and LTE₄), synthesized from arachidonic acid through the 5-lipoxygenase pathway, are potent inflammatory mediators that induce bronchial smooth muscle constriction, airway oedema, and mucus hypersecretion [3]. They activate inflammatory cells through interaction with at least two high-affinity G-protein coupled receptors, termed Cys-LT₁ and Cys-LT₂, which were

recently cloned and characterized [4, 5]. Following EIB some researchers reported on an increase in urinary LTE₄ excretion [6, 7], however, others did not observe change in blood [8] or urinary [9] cys-LTs levels.

Inhibitors of leukotriene synthesis [10, 11] and leukotriene-receptor antagonists [6, 12–15] were shown to exert a protective effect against exercise-induced bronchoconstriction. It can be hypothesized that a subpopulation of patients with asthma, characterized by aspirin intolerance [16], might profit most from the protective effect of montelukast against EIB. These patients are characterized by cys-LTs overproduction. At baseline they excrete high amounts of cys-LTs in the urine [17]. Following ingestion of aspirin, they release large quantities of cys-LTs into the lungs and further increase the urinary LTE₄ excretion [18]. Bronchial biopsy studies revealed a profound overexpression of LTC₄ synthase in the AIA as compared to aspirin-tolerant asthmatics and normal subjects [19]. A variant of allele C of LTC₄ synthase [20] was found to be more common in severe aspirin-sensitive than in aspirin-tolerant patients or normal subjects [21].

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We therefore compared the protective effect of montelukast, a potent leukotriene receptor antagonist, on EIB in two groups of patients with bronchial asthma: aspirin-intolerant and aspirin-tolerant. We also assessed whether the protective effect of montelukast is related to leukotriene production.

Materials and methods

Patients

The study was performed in 19 asthmatic patients sensitive to aspirin (AIA; average age, 40.7 years) and 21 asthmatics who tolerated aspirin well (ATA; average age, 35.4 years). The patients' characteristics (taking into account their response to EIB, see below) are presented in Table 1. The diagnosis of aspirin intolerance was confirmed by oral aspirin provocation tests [22], performed during the 12 months preceding the study. All ATA patients occasionally used aspirin without any adverse reactions. The patients had stable asthma and their baseline FEV₁ was >60% of the predicted value on each study day. The subjects were instructed to withhold medications that decrease bronchial responsiveness prior to exercise challenge according to ATS [23]. They were allowed to use inhaled (at a dose ≤ 2000 µg budesonide per day) and systemic corticosteroids (at a dose < 10 mg prednisone per day). None of them was treated with leukotriene receptor antagonists.

Study design

We performed a double-blind, placebo-controlled, cross-over randomized trial. Exercise challenge tests preceded by placebo and/or montelukast were carried out in 3-day intervals. Single dose placebo or montelukast (one tablet, 10 mg) were given orally one hour prior to exercise challenge test. FEV₁ was measured at baseline (before placebo or montelukast administration) and again at 5, 10, 15, 20, 30 min following exercise challenge, and then at 15-minute intervals for 4 h. Blood

samples for eosinophil count and urine samples for LTE₄ excretion were collected at baseline (before placebo or montelukast administration), and 2 and 4 h after exercise challenge. The exercise challenge was considered positive when FEV₁ dropped by 10% or more as compared to baseline. The bronchial response to exercise was calculated as percentage fall in FEV₁.

$$\text{Index} = \frac{\text{baseline FEV}_1 - \text{lowest FEV}_1 \text{ after exercise}}{\text{baseline FEV}_1} \times 100\%$$

The study was approved by the University Ethics Committee, and informed consent was obtained from each patient.

Pulmonary function tests

Spirometry was performed on a flow-integrating computerized pneumotachograph (Pneumoscreen, E. Jaeger, Friedberg, Germany).

Eosinophil count

Eosinophils were counted using a light microscope, after dissolving the whole blood sample in Pilot's solution [24].

Measurement of urinary LTE₄ excretion

Baseline urine sample was collected at about 8 AM before placebo or montelukast administration. The previous passing urine was at 6 AM. During each following 2-h interval the patient drank 200 mL of water. The total volume of urine samples were measured and 50 mL aliquots were immediately frozen. Before being frozen, the urine was centrifuged and antioxygenating agent (4-OHTEMPO) was added in the ratio of 100 µL of the solution per 10 mL of urine. These urine samples were frozen to -80 °C until the analysis. Creatinine was measured in all urine samples by a colorimetric method.

LTE₄ was measured in unpurified urine samples by direct enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) as previously reported [17].

Table 1. Clinical characteristics of the patients studied

Patients' characteristics	AIA, EIB positive <i>n</i> = 7	AIA, EIB negative <i>n</i> = 12	ATA, EIB positive <i>n</i> = 12	ATA, EIB negative <i>n</i> = 9
Age, years	40.9 (35–47)	40.7 (23–60)	33.4 (19–54)	38.0 (21–60)
Male/Female	3/4	11/1	7/5	4/5
Duration of asthma, years	11.1 (3–20)	9.5 (0.5–28)	9.2 (3–22)	10.6 (3–40)
Positive prick tests ±	3/4	2/10	8/4	7/2
Total IgE, IU/mL	147 (57–191)*	62.9 (29.7–222.5)*	441.5 (159–569)*	119 (58.7–598)
Inhaled steroids, <i>n</i>	7	11	10	5
Dose µg/d	900 (200–1500)	1072.7 (100–1600)	1275 (400–2250)	1100 (500–1500)
Oral steroids, <i>n</i>	2	2	3	1
Dose mg/d	5 (4–6)	4 (2–6)	6 (2–8)	8 (8–8)
Baseline FEV ₁ (% predicted)	PL 86.1 (65.7–113.6) M 84.6 (70.8–103.8)	PL 84.8 (64.4–104.0) M 86.5 (60.7–106.3)	PL 83.3 (61.0–110.2) M 80.3 (60.3–104.7)	PL 90.2 (73.1–111.6) M 91.1 (70.2–111.7)
Baseline FEV ₁ (L)	PL 2.77 (1.82–4.26) M 2.72 (1.96–4.11)	PL 3.16 (2.12–4.29) M 3.22 (1.93–4.55)	PL 3.03 (1.82–4.63) M 2.93 (1.71–4.40)	PL 3.04 (1.89–3.85) M 3.07 (1.88–4.06)
Baseline urinary LTE ₄ excretion (pg/mg creatinine)	PL 635 (326–714)* M 722 (410–1057)*	PL 1032 (451–1645)* M 904 (432–1832)*	PL 497 (158–794)* M 492.5 (174–838)*	PL 170 (131–211)* M 267 (153–322)*
Baseline eosinophil count/mm ³	PL 509 (284–605)* M 608 (571–789)*	PL 635 (257–837) M 623 (320–895)*	PL 389 (186–727)* M 459 (233–634)*	PL 172 (75–243)* M 165 (115–402)

PL: placebo; M: montelukast. The values in the table represent mean and range, except for serum IgE values, urinary LTE₄, eosinophil count. *Median (25%–75% percentiles, lower and upper quartile).

Exercise challenge test

Exercise was performed on a treadmill, according to American Thoracic Society guidelines [23]. Heart rate was monitored from a three-lead electrocardiographic configuration. The patients inspired dry air of temperature 20–25 °C, and humidity of 50% or less. During the exercise challenge, the patient had a clip on the nose to ensure breathing through the mouth only. Total duration of exercise was 6–8 min. The initial low speed and grade were progressively increased during the first 2–3 min of exercise until the heart rate achieved 80–90% of the predicted maximum (calculated as $220 - \text{age in years}$). The obtained heart rate was maintained for at least 4–6 min.

Indications for withdrawing from these tests were in accordance with regulations by American Heart Association.

Statistical analysis

Summary statistics were expressed as mean (M) and standard deviation (SD) for symmetric distributed data and median or geometric mean (GM) and 25% and 75% percentiles for non-symmetric (skewed) distributed data. Multi-way ANOVA model was used for multiple-group comparisons. Logarithmic transformation was used when needed as variance stabilizing transformation. Fisher's exact test was used for dichotomous response data for two independent random samples. A P -value ≤ 0.05 was considered statistically significant.

Results

At baseline the AIA ($n=19$) and ATA ($n=21$) groups were similar in most clinical parameters, except for urinary LTE_4 values ($P=0.001$) and eosinophil count ($P=0.01$) which were significantly higher in the former than in the latter group.

Out of 40 patients studied, in 19 (47.5%) the exercise challenge test preceded by placebo was positive. The baseline clinical characteristic of the patients with AIA and ATA, divided into subgroups with positive and negative response to EIB is presented in Table 1. In these patients, who showed positive response to exercise the maximum decrease in FEV_1 was observed 10 min after the exercise ($M \pm \text{SD}$, $22.5\% \pm 10.2$). When the patients studied were split into AIA and ATA, positive exercise challenge test following placebo led to at least 10% decrease in FEV_1 in seven patients with AIA and 12 patients with ATA ($n=12$). The maximum fall in FEV_1 following exercise was almost identical in those two groups ($23.5\% \pm 6.8\%$ vs. $21.8\% \pm 12.0\%$, respectively; $P=0.7$).

The percent decrease in FEV_1 in the first 60 min after positive exercise challenge preceded by placebo, the maximum decrease in FEV_1 and the time from the maximum decrease in FEV_1 to the return to within 5% of baseline FEV_1 , separately for AIA and ATA patients, with positive response to EIB are presented in Fig. 1.

	Positive challenge test preceded by placebo	
	AIA $n=7$	ATA $n=12$
Pre-exercise FEV_1 (% of predicted)	86.1 ± 16.1	83.3 ± 12.4
Area under the curve	9.8 ± 11.2	8.0 ± 6.3
Maximal decrease in FEV_1 after exercise (Index-%)	23.5 ± 6.8	21.8 ± 12.0
Time from maximal decrease in FEV_1 to return to within 5% of baseline FEV_1 (min)	33.4 ± 17.8	26.9 ± 22.06

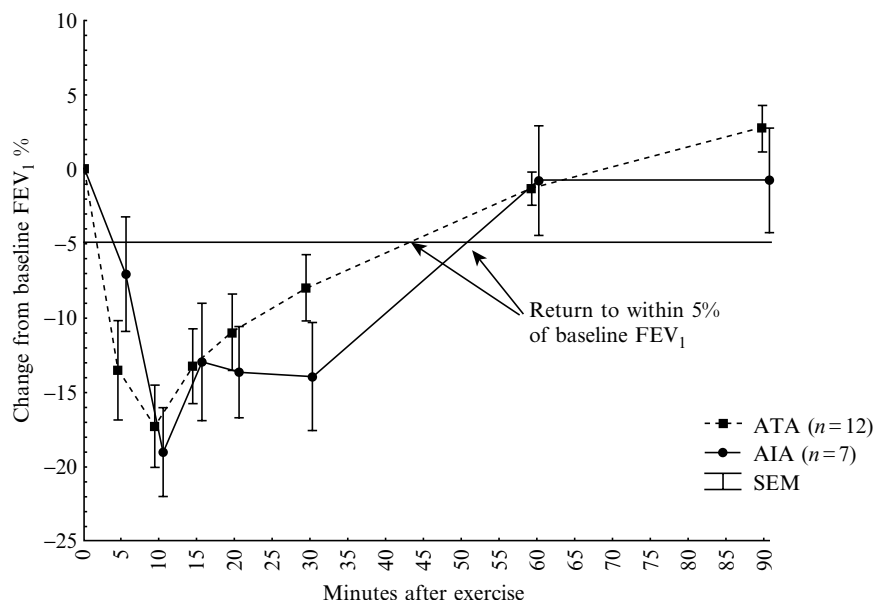


Fig. 1. The area under the curve for the percent decrease in FEV_1 in the first 60 min after exercise, the maximal decrease in FEV_1 after exercise, and the time from the maximal decrease in FEV_1 to the return to within 5% of the FEV_1 value before exercise in AIA and ATA groups with positive exercise challenge test preceded by placebo.

Montelukast attenuated or completely prevented EIB in 12 of 19 (63.2%) patients who had positive challenge after placebo ($P < 0.001$, ANOVA). Similar protective effect was found in aspirin-sensitive and aspirin-tolerant asthmatics ($P = 0.63$, ANOVA). The effect of montelukast on post-exercise FEV₁ in both study groups ($n = 19$) with positive response to the challenge preceded by placebo is presented in Fig. 2. The mean of maximum fall in FEV₁ from the pre-exercise value was $10.2\% \pm 13.8$ after montelukast as compared to $22.5\% \pm 10.2$ after placebo ($P < 0.001$).

The mean changes in urinary LTE₄ excretion and eosinophil count following exercise challenge in AIA and ATA patients, taking into account their response to this challenge are presented in Figs 3 and 4.

In all patients studied ($n = 40$) the urinary LTE₄ excretion both baseline and following exercise preceded by placebo, was not related to the positive or negative results of the test

($P = 0.72$, ANOVA). In 19 subjects with positive result of exercise test, neither montelukast nor placebo had any influence on the leukotriene excretion into urine ($P = 0.64$, ANOVA), no matter whether the patients belonged to the AIA or ATA group ($P = 0.17$, ANOVA).

However, AIA patients showed a higher level of the urinary LTE₄ excretion at each examination interval ($P < 0.001$), which was caused by higher baseline levels as compared to the ATA group. Moreover, only in AIA patients who were negative in exercise challenge preceded by montelukast and placebo, the decrease in the urinary LTE₄ excretion 2 h after exercise was noted ($P < 0.001$) with the tendency to return to baseline levels 4 h following the challenge ($P = 0.013$). There were no significant changes in urinary LTE₄ excretion after the exercise in the ATA group, independent of the results of the challenge test.

Eosinophil count showed a significant decrease two ($P = 0.04$) and four ($P = 0.01$) hours after challenge as compared to

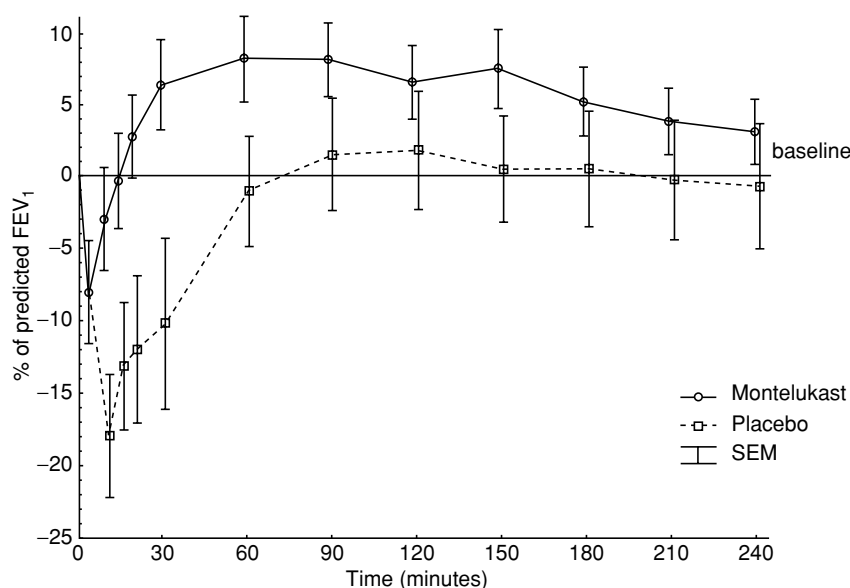


Fig. 2. Mean FEV₁ values in asthmatics following exercise challenge test after pre-treatment with montelukast or placebo (in all patients presented, the exercise challenge following placebo was positive).

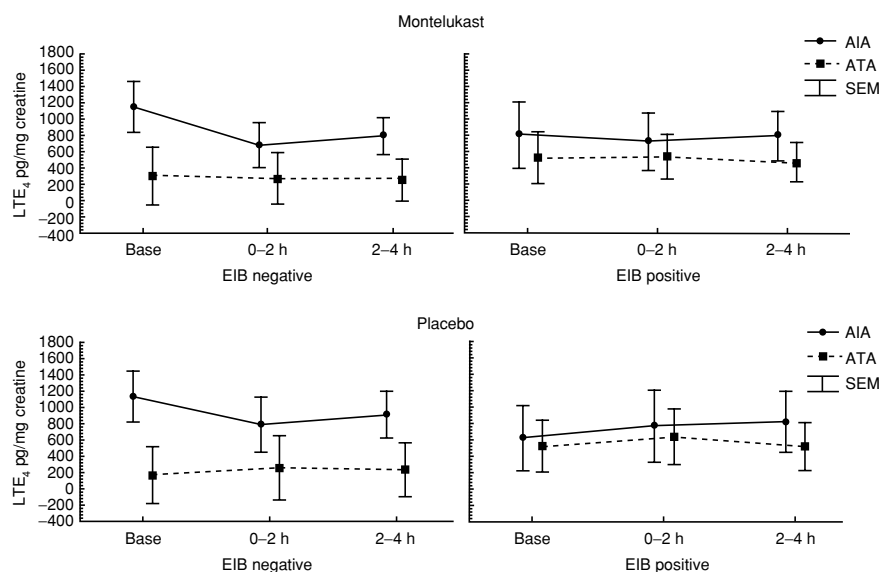


Fig. 3. Urinary LTE₄ levels before and following exercise challenge test preceded by placebo and montelukast in AIA and ATA groups separately with positive or negative result of exercise challenge test.

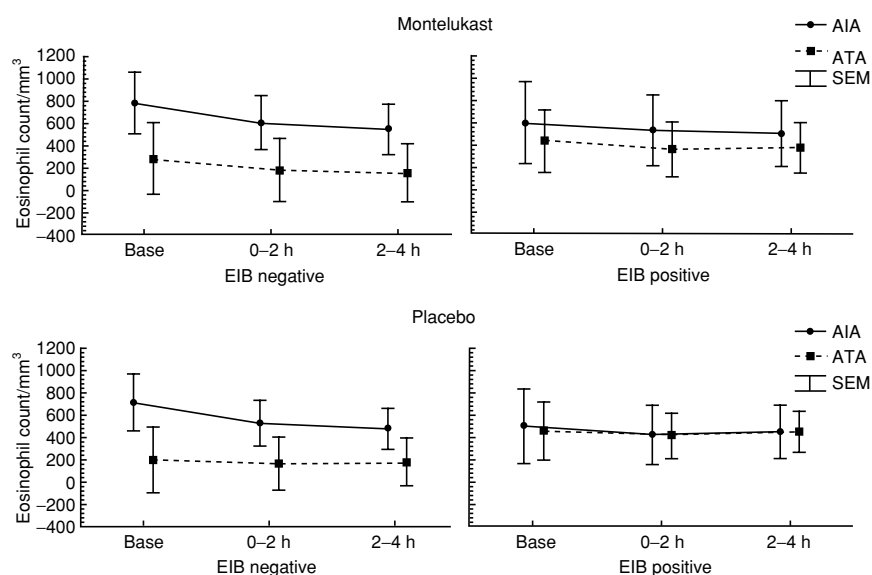


Fig. 4. Eosinophil count before and following exercise challenge test preceded by placebo and montelukast in AIA and ATA groups separately with positive or negative result of exercise challenge test.

baseline values, in those 21 subjects with negative results of the test, preceded by placebo.

In 19 patients with positive exercise challenge test eosinophil count showed similar tendency following the challenge ($P=0.05$, ANOVA). This fall was not related to the pre-treatment (montelukast or placebo, $P=0.99$, ANOVA), or the type of asthma (AIA or ATA, $P=0.52$, ANOVA).

In ATA patients exclusively, the higher levels of both baseline urinary LTE_4 excretion ($P=0.037$, ANOVA) and baseline blood eosinophil count ($P=0.047$, ANOVA) were associated with positive response to exercise challenge. However, in the AIA group, baseline urinary LTE_4 excretion ($P=0.53$, ANOVA) and baseline blood eosinophil count ($P=0.87$, ANOVA) had no influence on positive response to exercise.

Discussion

Our study is the first which compares the results of the exercise challenge in AIA and ATA patients. We observed that AIA and ATA patients reacted similarly to exercise challenge.

The study revealed that pretreatment with a single dose of montelukast effectively attenuates or completely prevents exercise-induced bronchoconstriction. The magnitude of the protection in our study is consistent with that reported by other authors [6, 12–15]. Leukotriene antagonists provide substantial protection against EIB for many, but not all, patients with asthma. Defining the subpopulations of asthmatics who may benefit most from this therapy is a challenge [25, 26]. Contrary to expectations, our study revealed a lack of difference in the protection offered by montelukast on EIB between AIA and ATA patients. Perhaps, in aspirin-intolerant asthmatics, tachyphylaxis and/or down-regulation of cys-LT_1 receptors results from increased baseline leukotriene overproduction. The results here presented corroborate recent reports on similar clinical response of aspirin-tolerant and aspirin-intolerant patients to montelukast therapy [27–29].

The AIA patients are characterized by higher baseline levels of urinary LTE_4 excretion as compared to ATA patients [16, 17]. This, however, did not influence the results of exercise

challenge tests. Similarly, in all the subjects the baseline urinary LTE_4 excretion was not related to the positive or negative results of the exercise challenge. This might suggest that cysteinyl leukotrienes are not major mediators of EIB. There are a number of contradictory reports concerning the relation between leukotrienes and EIB [6, 7, 9]. The reasons for these discrepancies are not clear, but probably are associated with the sampling time of leukotrienes, patients' age group (children or adults), and the fact that only about 10%–20% of cys-LTs produced in the body are released into urine [30].

The higher baseline levels of urinary LTE_4 excretion were associated with positive response to exercise challenge in ATA patients only. The inability to predict the result of an exercise challenge test in AIA patients could possibly be explained by different sources of leukotrienes in AIA as compared to ATA. In the AIA group, the leukotrienes are supposed to be released mainly by eosinophils [31], and in ATA other cells, such as mast cells are likely to play a predominant role in EIB.

Similar findings were observed in case of eosinophil count. At baseline, the blood eosinophil levels had no influence on results of the exercise challenge in the AIA group, however, in ATA patients the higher baseline levels of eosinophil count predisposed to positive exercise challenge. Perhaps, this phenomenon could be explained by differences in the biochemical activity of the enzymes participating in cys-LTs synthesis. For example, the expression of LTC_4S mRNA in eosinophils of AIA patients is up-regulated at base, as compared to ATA [21].

In all subjects studied, blood eosinophil count, following positive or negative exercise challenge showed the tendency to decrease, as already reported by Venge et al. [32]. However, only in the patients with negative results of the tests, this decrease was statistically significant. The reason for this eludes us; probably assessment of eosinophils in the bronchial mucosa and the lung tissue would give a better insight into this phenomenon.

In summary, patients with AIA and ATA react similarly to exercise challenge and obtain similar protection against EIB by montelukast. Urinary LTE_4 excretion shows no change following positive exercise challenge test in both study groups. In ATA, but not in AIA patients, higher baseline levels of both

urinary LTE₄ and blood eosinophil count are associated with positive response to exercise challenge.

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