Effect of Montelukast on Basophil Releasability in Patients with Asthma

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Abstract

Background: The anti-inflammatory effect of montelukast, a leukotriene receptor antagonist, in patients with bronchial asthma is not entirely clear. Basophils can release a variety of mediators, including histamine and leukotriens, which most likely play an active role in the late allergic response.

Objectives: To study the effect of montelukast (10 mg/day) on histamine and cysteinyl leukotriene release from basophils taken from 12 mild atopic asthmatic patients who took the drug for 4 weeks.

Methods: Basophils were withdrawn at baseline, and after 48 hours, 1 week, and 4 weeks of therapy. Histamine was measured by a radioenzymatic method and leukotrienes by immunologic assay. Histamine and cysLT release was measured spontaneously and following stimulation with interleukin-3 and anti-immunoglobulin E. Spirometry and symptom score were measured before and during treatment.

Results: During the treatment with montelukast there were no significant changes in spontaneous, IL-3 and anti-IgE-induced histamine release. cysLT release decreased significantly only after 4 weeks of treatment (from 2899 ± 550 pg/ml at baseline to 2225 ± 430 pg/ml at 4 weeks, $P = 0.02$).

Conclusions: Montelukast does not seem to affect the release of histamine from basophils but mildly inhibits the cysLT release seen after 4 weeks of treatment.

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The cysteinyl leukotrienes (LTC4, LTD4 and LTE4) are generated by mast cells, eosinophils and basophils through oxidative degradation of arachidonic acid via the 5-lipoxygenase pathway [1,2]. These potent mediators possess various broncho-active properties that induce airway muscle contraction, microvascular hyperpermeability and mucous hypersecretion [1,2]. When inhaled by asthmatic patients, cysLT can produce features of asthma, such as airway bronchoconstriction, bronchial hyperactivity to histamine and methacholine, and induction of inflammatory processes within the airway wall in humans [3,4].

The clinical efficacy of cysLT receptor antagonists in the treatment of asthma and allergic rhinitis is thought to result primarily from their competitive antagonism of cysLT receptor sites located on airway smooth muscle and vasculature [4]. In addition, cysLTRA exert anti-inflammatory properties by altering the response of immune cells, which may also account for their therapeutic effectiveness [5]

Basophils are among the cells playing a major role in the pathogenesis of asthma and allergic diseases. Upon activation, basophils release preformed histamine and proteases from secretory granules, generate the arachidonic acid-derived cysLT and subsequently transcribe, translate and secrete several pro-inflammatory cytokines and chemokines [6]. The basophil release of histamine and cysLT is used as an important research tool for investigating the effects of various drugs on the basophil function in asthma and allergic conditions [7–9].

Although some *in vitro* studies suggested that cysLT or their agonists may affect the function of the various immune cells participating in allergic reactions, including basophils, no study examined directly the *in vivo* effect of cyLTRA therapy on human basophil releasability [10–12]. In the present work we examined the effect of treatment with the cysLTRA montelukast on the histamine and cysLT release from basophils obtained from asthmatic patients.

Patients and Methods

Patients

The study group comprised 12 patients with mild intermittent asthma (according to American Thoracic Society criteria). The eight males and four females had a mean age of 24 ± 4 years. Their baseline forced expiratory volume in 1 second was 87% ± 7% and their forced vital capacity 104% ± 6%.

They were all atopic, as determined by clinical history and routine skin test to common environmental allergens. All subjects gave informed consent, and experimental protocols were approved by the Tel Aviv Sourasky Medical Center. None of the study participants had received oral, nasal, inhaled or systemic corticosteroids during the preceding month, nor had they received any other treatment for asthma apart from inhaled short-term β2-agonists. None of the patients had had asthma exacerbations or upper respiratory tract infections in the previous month. Current smokers or ex-smokers of more than 10 pack-years and patients with FEV1 < 70% predicted were excluded.

Experimental design

After the screening visit eligible patients were allocated to 4 week treatment periods with montelukast (10 mg daily) given

CysLT = cysteinyl leukotriene
IL = interleukin
Ig = immunoglobulin
cysLTRA = cysLT receptor antagonists
FEV1 = forced expiratory volume in 1 second

at bedtime. Symptom scores were recorded daily by the patients and included salbutamol use (1–5 points), night asthma (1–5 points), day asthma (1–5 points), and exercise-induced asthma (1–5 points). For basophil releasability tests, blood was withdrawn around 8 a.m. on the day before the treatment began and 48 hours, 1 week and 4 weeks following the start of the treatment. The withdrawn blood was divided into two equal parts – one for histamine releasability and the second for cysLT release. Spirometry was performed before the treatment period and 4 weeks later, at the same time of day. No other treatment was given, except for salbutamol on demand with the last dose given more than 12 hours prior to the blood test. The patients' medical condition was stable throughout this period and they did not require any additional therapy.

Materials

The following materials were used: EDTA and Dextran (Sigma, St. Louis, MO, USA), Hank's balanced salt solution ph 7.3 without calcium and magnesium, and RPMI medium (Biological Industries, Beit HaEmek, Israel); HEPES 24 mM, CaCL2 2 mM and MgCL2 2 mM (Sigma) were added to RPMI; anti-IgE (Bio-Makor, Rehovot, Israel); IL-3 (National Biological Standards Board, UK); histamine dihydrochloride (Fluka Biochemica AG, Buchs, Switzerland); adenosyl-methionine, S-[methyl-14C] 50–60 mCi/mmol, and 20 mCi/ml in H2SO4 (Nen Dupont de Nemours, Dreiech, Germany).

Preparation of basophils

For histamine release the blood was anti-coagulated with 10 mM EDTA and mixed with a 0.25 volume of 6% dextran in saline. Erythrocytes were allowed to sediment at room temperature for 60–90 minutes after which leukocyte-rich plasma was removed. The leukocytes were pelleted by centrifugation at 400x*g* for 10 min at room temperature, washed twice in HBSS pH 7.3 and resuspended in 1 ml of RPMI medium containing 2 mM C2Cl2 and 0.5 mM MgCl2. Total leukocytes were counted with a hemocytometer, and a differential count for basophil percentage determination was done on a Giemsa-stained cytocentrifuge preparation.

The cell suspension, each one containing 10^4 basophils, was placed in disposable 35 mm polystyrene tissue culture dishes. One of each of the stimulants (i.e., anti-IgE or IL-3 20 ng/ml) was added. The mixtures were incubated at 30°C for 24 hours, after which the cells were centrifuged and the cell-free supernatants were collected and stored at -20°C until the histamine was assayed.

Histamine assay

Aliquots of the test supernatants were assayed for histamine content by using an enzymatic-isotope assay as described elsewhere [13]. The net percentage of released histamine was calculated using the total histamine release from cells boiled for 10 min and corrected for histamine release and spontaneously from unstimulated cells.

CAST enzyme-linked immunosorbent assay test

The second blood sample was collected into heparin-containing tubes. The measurements of cysLT release were performed using the CAST-ELISA kit (Bulhmann Laboratories AG, Allschwil, Switzerland), according to the manufacturer's instructions. Briefly, leukocytes were isolated by dextran sedimentation for 90 min, transferred into fresh tubes and harvested by centrifugation (130x*g*, 15 min). The cells were then resuspended in the stimulation buffer (Bulhmann Laboratories AG) containing 20 ng/ml IL-3. CysLT production was induced by monoclonal antibody against the high affinity IgE receptor. Cells were stimulated for 40 min at 37°C and release of cysLT was measured in cell supernatant fluid using the ELISA kit (Bulhmann Laboratories AG).

Statistics

Paired *t*-test was used to compare results. Multivariance analysis was used to evaluate the effect of therapy on various Data. *P* less than 0.05 was considered significant.

Results

All the patients completed the study. There was a significant improvement in symptom scores ($P < 0.03$) (from a score of 13 ± 3 to 18 ± 3), but no significant change was seen in the mean FEV1 (mean baseline of 75.5 ± 5.7% to 78 ± 6.6% at 4 weeks) and FVC (mean baseline of 95.5 ± 7.6 to 96.3 ± 7.2% at 4 weeks) of the group. The mean histamine release following each stimulus did not change significantly as a result of taking montelukast. The spontaneous histamine release was (mean ± SD) 12% ± 4% at baseline and 12% ± 4% at 48 hours, 14% ± 7% at 1 week, and 10% ± 4% at 4 weeks. IL-3 increased histamine release but, again, there was no significant change in release: 23% ± 10% at baseline, 19% ± 7% at 48 hours, 20% ± 11% at 1 week and 15% ± 6% at 4 weeks [Table I].

Anti-IgE, similar to IL-3, increased histamine release without significant change during treatment: 21% ± 7% at baseline, 19% ± 7% at 48 hours, 21% ± 9% at 1 week and 16% ± 8% at 4 weeks. Leukotriene release from basophils showed the following trend [Figure 1]: 2899 ± 550 pg/ml at baseline, 3301 ± 1385 pg/ml at 48 hours, 3109 ± 851 pg/ml at 1 week, and 2225 ± 430

Table 1. Histamine release from basophils (ng/ml) during the study

		Total		Anti-IgE		IL-3		Spontaneous
	SD	BHR	SD	BHR	SD	BHR	SD	BHR
Baseline	42.2	125.8	11.7	25.2	11.4	26.6	4.2	14.8
After 48 hr	38.7	135.8	13.2	25.8	10.5	25.3	8.0	17.0
After 1 week	38.7	131.7	10.8	27.0	10.4	24.6	7.9	17.9
After 4 weeks	44.6	131.3	13.5	21.0	14.2	20.0	6.4	13.5
ANOVA		NS		NS		NS		NS

BHR = basophil histamine release (percent from total histamine released), SD = standard deviation, NS = not significant.

HBSS = Hank's balanced salt solution
ELISA = enzyme-linked immunosorbent asssay
FVC = forced vital capacity

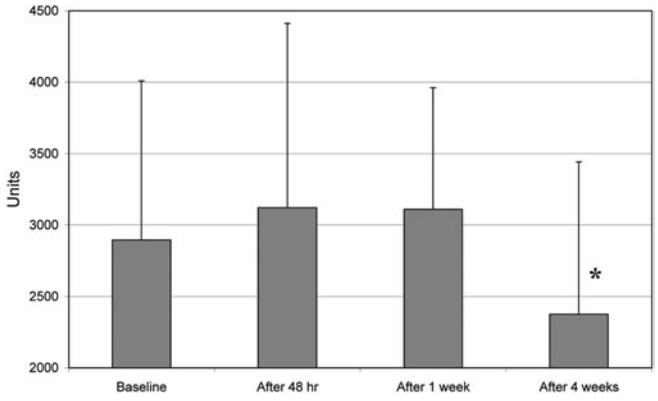

Figure 1. Leukotriene release before and following treatment with montelukast (pg/ml)

pg/ml at 4 weeks. The change at 4 weeks was significantly different from baseline ($P = 0.02$) and from the levels measured at the first week ($P = 0.007$). Multivariance analysis for the whole group showed a P value of 0.04.

Discussion

For the last two decades, inflammation has been perceived as the major pathogenic force for the development and chronicity of asthma. Such inflammation has been characterized by a TH2-type pattern, with an increase in CD4 lymphocytes, eosinophils and their respective cytokines and mediators. This concept led to the recommendation to use anti-inflammatory drugs to treat patients with asthma. Corticosteroids, which are well-proven drugs with anti-inflammatory effects, ameliorate symptoms and physiologic parameters but have several undesirable side effects. Leukotriene receptor antagonists were initially developed because of the known ability of the leukotriene to induce bronchoconstriction, but studies have suggested that they also induce an anti-inflammatory response [3–5]. Because they were free of the side effects associated with corticosteroids, cysLTRA were introduced as a substitute or a supplement to corticosteroids in the treatment of asthma. Despite their wide use in asthma and their growing use in other allergic diseases such as allergic rhinitis and urticaria, their effect on various aspects of inflammation and allergic response has not been adequately studied.

Atopic diseases, such as asthma, are associated with the production of IgE antibodies that bind to the high affinity IgE receptor on basophils and mast cells. Upon interaction with an antigen, these cells degranulate and release stored and newly formed mediators into the microenvironment, contributing to the early as well as the late symptoms of allergic reactions [1,6]. While mast cells are considered responsible for the early events after allergen challenge, basophils are considered to be the key participants in the late-phase allergic reaction and in the inflammation that develops after the immediate allergic reaction [6,14].

While histamine is the main preformed mediator of basophils and can be released immediately upon cellular activation, basophils also produce and release a large amount of leukotrienes, whose role in bronchial asthma has been clearly established [4,6]. That the human basophil is one of the major immune cells participating in allergic diseases such as asthma and that its mediators are the target of many therapeutic agents prompt the important question whether cysLTRA can modulate basophil function.

Several studies have been carried out on the effects of drugs on the release of histamine and leukotrienes in asthma and allergic disease [7–9]. This approach has helped clarify the complex biochemical mechanisms underlying the release of these mediators in response to immunologic and non-immunologic stimuli. Significant differences have been documented with regard to the pharmacological agents that modulate the release of preformed (histamine) and *de novo* synthesized (CysLT) mediators [15].

Our study is the first to examine the release of histamine and cysLT from basophils of asthmatic patients who were treated with the cysLTRA, montelukast. The patients had mild, stable, atopic asthma that was improved by montelukast treatment, but we found no effect of montelukast on histamine release during the study period except for a mild decrease in leukotriene release after 4 weeks of treatment.

The results of this study are in agreement with our previous works in which we showed that histamine release from basophils does not correlate with asthma phenotype or severity and that histamine releasability from basophils is more pronounced in the airways than in the blood of asthmatics [13,16,17]. Antihistamine, a widely used drug in various allergic diseases, antagonizes the effects of histamine on H1 receptor. It was shown in *in vitro* studies that high concentrations of antihistamines inhibit the release of histamine and cysLT from human basophils, but these studies found no correlation between the potency of H1 receptor-blocking activity and the inhibition of mediator release [18–20]. Similar findings were reported previously by our group regarding the effects of cromoline and nedocromil, which inhibit histamine release from triggered metachromatic cells but not from peripheral basophils [16,17].

The fact that we found no correlation between cysLT release and histamine release should not come as a surprise. Despite the similar cellular source of histamine and cysLT, several studies have demonstrated that the release of cysLT is not always coupled with the release of histamine [21] and that the histamine secretion and leukotriene C4 production from basophils seem to be independent events [12,22,23].

The expression of the cysLT receptor on various cell types capable of synthesizing cysLT, including basophils, suggests that the cysLT may act in both an autocrine and a paracrine fashion [12]. Several studies have shown that cysLT from one cell type can influence the production of cysLT mediators in another type of cell, and that cysLT may also play a role in modulating the release process itself [24,25]. cysLTRA inhibit the production or expression of a variety of other mediators that may contribute to asthmatic inflammation. These include other cytokines, such as tumor necrosis factor and IL-6, endothelin, adhesion molecules, nitric oxide, and reactive oxygen intermediates [2–5]. We

hypothesize that the treatment of our patients with montelukast caused a gradual decrease in inflammation and in cytokine production, including cysLT, after 4 weeks of treatment.

Our study shows that histamine release from basophiles is not decreased by cysLTRA and that cysLT release is reduced only mildly after 4 weeks of treatment. Studies that will increase our understanding of the mechanisms governing the synthesis and release of basophils' cysLT and their antagonist on the basophils themselves or other effector cells are essential.

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The things we admire in men – kindness and generosity, openness, honesty, understanding and feeling – are the concomitants of failure in our system. And those traits we detest – sharpness, greed, acquisitiveness, meanness, egotism and self-interest – are the traits of success. And while men admire the quality of the first they love the product of the second.

John Steinbeck (1902-68), American author. His novels deal with the social and economic conditions of his native California, most notably *East of Eden* and *Grapes of Wrath*. He won the Nobel Prize for Literature in 1962