ENHANCED PROSTAGLANDIN E₂ SECRETION IN SPUTUM FROM ASTHMATIC PATIENTS AFTER ZAFIRLUKAST THERAPY

Jaw-Ji Tsai, Yung-Chang Su, Shu-Chen Chan, Chia-Kuan Ho, and Ta-Cheng Feng

Cysteinyl-leukotrienes (C-LTs) are the lipooxygenase products of an arachidonic acid metabolite and have been postulated to play a significant role in the etiology of asthma [1, 2]. C-LTs are synthesized by mast cells, eosinophils, basophils, macrophages, and monocytes. They cause constriction of human airway smooth muscle, increase the secretion of bronchial mucus, and increase vascular permeability resulting in mucosal edema. They are present in the bronchoalveolar lavage fluid of patients with asthma, which suggests their participation in the events underlying the disease. Therefore, considerable interest has focused on developing C-LT receptor antagonists [3, 4]. Results from in vitro and in vivo trials have shown that zafirlukast is a potent and selective C-LT receptor antagonist [5, 6]. Although zafirlukast has been reported to have a therapeutic effect in asthma, the clinical relevance of zafirlukast on the modulation of inflammatory mediators remains unclear.

It has been reported that prostaglandin E₂ (PGE₂) is generated by human epithelial cells in response to a variety of stimuli [7]. Increased concentrations of PGE₂ have been detected in the bronchoalveolar lavage fluid of asthmatic subjects [8]. The eosinophil-derived...
modulate PGE2, LTE4, and ECP production through antagonism of the C-LT receptor in patients with bronchial asthma. The clinical efficacy of zafirlukast was evaluated by measuring pulmonary function before and after therapy. Induced sputum was collected and analyzed to determine concentrations of PGE2, LTE4, and ECP, and eosinophil count.

**Materials and Methods**

**Patients**

The selection criteria for patients with bronchial asthma were based on the American Thoracic Society standards for diagnosis of asthma [11]. Selected patients had mild persistent asthma with frequent asthma symptoms at night and after exercise that were reversible by β2-agonist therapy; they received therapy with zafirlukast for a period of 6 weeks. All patients were allowed to continue using their current treatment throughout the study, including inhaled β2-agonists, inhaled glucocorticoids, and aminophylline. Trial medication was supplied by Astra Zeneca Pharmaceuticals at a dose of 20 mg/tablet. Patients were asked to take one tablet every 12 hours, at least 1 hour before or 2 hours after a meal (based on a recent review [12]).

**Pulmonary function test and sputum collection**

Pulmonary function testing was performed using a MicroLab spirometer (Micro Medical Ltd, Rochester, Kent, UK). The peak expiratory flow rate (PEFR) as a percentage of predicted value was recorded at the clinic before and 3 and 6 weeks after therapy. Blood samples and induced sputum were collected after PEFR measurements. Sputum was induced with an aerosol of 3% hypertonic saline generated by an ultrasonic nebulizer. A short-acting β2-agonist, Berotec inhaler 200 µg/metered dose (Boehringer Ingelheim Taiwan Limited), was used to inhibit possible bronchoconstriction caused by the saline aerosol. Each patient was asked to blow their nose, rinse their mouth, and swallow saliva, and cough sputum into a container. When the sputum was expectorated, 0.1% dithiothreitol (Sigma Chemical Co, St. Louis, MO, USA) was added to break disulphide bonds in the mucus. A total cell count was obtained by hemocytometer. Smears of cytospins were made and stained with Wright stain. The supernatant was collected after centrifugation at 1,500 rpm and 14°C for 10 minutes, and stored at –70°C for mediator determination. Total protein concentration in sputum was measured using the Lowry method as previously described [13]. All data are represented as pg/mL or µg/L in 1 g protein.

**PGE2, LTE4, and ECP assays**

LTE4 was assayed in duplicate with an enzyme immunoassay using an LTE4 assay kit (Cayman Chemical, Ann Arbor, MI, USA). All samples were diluted appropriately in assay buffer. Either standard or sample (50 µL) was mixed with 50 µL of acetylcholinesterase-linked LTE4 (tracer) and 50 µL of anti-LTE4 antibodies. These mixtures were incubated overnight at room temperature, and 200 µL of developer was added to each well for 60 to 90 minutes' incubation. After washing in phosphate-buffered saline, the plates were read at 412 nm. A normal standard curve was constructed with known concentrations of LTE4. The detectable range was from 7.8 to 1000 pg/mL.

The PGE2 immunoassay was also performed in duplicate with a double antibody immunoassay using PGE2 assay kits (R&D Systems, Minneapolis, MN, USA). All procedures followed the manufacture’s instructions and were similar to the LTE4 assay described above. The detectable range of PGE2 was from 39 to 5000 pg/mL.

ECP was assayed using a fluoroenzyme immunoassay (the Pharmacia CAP system, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

**Statistical analysis**

Paired Student’s t-test was used to compare data before and after zafirlukast therapy. A p value of less than 0.05 was considered statistically significant.

**Results**

A total of 30 asthmatic patients who attended the Allergy Clinics at Cathay General Hospital, Taipei, were recruited for participation in this study; however, two patients dropped out because of respiratory tract infection.

PEFR measurements were compared before and after treatment with zafirlukast. PEFR improved from 74.3 ± 4.6% predicted value at baseline to 79.6 ± 5.3% predicted value at the end of the third week, and significantly improved to 82.0 ± 4.8% predicted value (p = 0.017) at the end of the sixth week (Fig. 1).

The serum concentrations of ECP before and after treatment were significantly different (13.7 ± 2.4 vs 10.3 ± 2.1 µg/L, p = 0.025) (Table 1).
Table 1. The baseline pulmonary function and the effect of zafirlukast on eosinophil cationic protein (ECP) concentration in the sera

<table>
<thead>
<tr>
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<th>Mean</th>
<th>SEM</th>
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<tbody>
<tr>
<td>Age</td>
<td>29–69</td>
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<tr>
<td>Pulmonary function (percent predicted)</td>
<td></td>
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<tr>
<td>FVC</td>
<td>86.5</td>
<td>3.7</td>
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<td>PEFR</td>
<td>75.7</td>
<td>4.3</td>
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<tr>
<td>Serum ECP (µg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>13.7</td>
<td>2.4</td>
</tr>
<tr>
<td>After</td>
<td>10.3*</td>
<td>2.1</td>
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M:F ratio = 10:18; mean ± standard error of the mean (SEM). FVC = forced vital capacity; PEFR = peak expiratory flow rate. *p = 0.02.

The eosinophil count and ECP concentration in sputum decreased after treatment (p = 0.14 and p = 0.16, respectively), but were not statistically different from baseline (Table 2). The sputum PGE\textsubscript{2} concentration increased significantly after 6 weeks of treatment (p = 0.01; Table 2). The concentration of LTE\textsubscript{4} increased after 6 weeks of treatment, but this was not significant. The correlation between PEFR and sputum PGE\textsubscript{2} before and after treatment did not reach statistical significance (R\textsuperscript{2} = 0.01 and R\textsuperscript{2} = 0.002, respectively).

Discussion

This study investigated the relationship between arachidonic acid metabolites and C-LT receptor antagonists in asthma by comparing LTE\textsubscript{4} and PGE\textsubscript{2} concentrations and PEFR before and after zafirlukast therapy.

Our results showed that zafirlukast therapy improved PEFR and enhanced PGE\textsubscript{2} secretion in the sputum within 6 weeks.

It has been reported that inhalation of PGE\textsubscript{2} can block the early and late bronchoconstriction response to inhaled allergen, abolish allergen-induced increases in bronchial reactivity, and attenuate exercise-induced bronchoconstriction [14, 15]. Increased secretion of PGE\textsubscript{2} in the sputum may play a role in the improvement in PEFR in asthmatic patients. Although there was no correlation between PEFR and PGE\textsubscript{2} concentration before and after therapy in this study, this may have been due to the pre-existence of a high concentration of PGE\textsubscript{2} in sputum before therapy.

LTE\textsubscript{4} has been reported to be a spasmogenic mediator in asthma. Increased amounts of LTE\textsubscript{4} have been reported in bronchoalveolar lavage fluid 24 hours after instillation of ragweed antigen into a lobar bronchus in allergic subjects [16]. In this study, we did not find any

Fig. 1. Peak expiratory flow rate (PEFR) as percentage of predicted value before and 3 and 6 weeks after zafirlukast therapy. *p = 0.017 compared to baseline.

Fig. 2. Concentrations of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and leukotriene E\textsubscript{4} (LTE\textsubscript{4}) before and after zafirlukast therapy. *p < 0.01 compared to baseline.

Table 2. Effect of zafirlukast on eosinophil count and concentrations of eosinophil cationic protein (ECP), prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), and leukotriene E\textsubscript{4} (LTE\textsubscript{4}) in sputum

<table>
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<th>Baseline</th>
<th>After treatment</th>
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<tr>
<td>Sputum eosinophil count (%) leukocytes</td>
<td>18.9 ± 2.3</td>
<td>15.9 ± 2.1</td>
</tr>
<tr>
<td>Sputum ECP (µg/L)</td>
<td>169.7 ± 34.4</td>
<td>159.7 ± 29.1</td>
</tr>
<tr>
<td>Sputum PGE\textsubscript{2} (pg/mL)</td>
<td>112.7 ± 14.0</td>
<td>176.8 ± 32.8*</td>
</tr>
<tr>
<td>Sputum LTE\textsubscript{4} (pg/mL)</td>
<td>241.9 ± 38.7</td>
<td>249.5 ± 39.5</td>
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Mean ± standard error of the mean in 28 individuals; *p = 0.01.
change in LTE₄ secretion in sputum after zafirlukast therapy. Although the total amount of LTE₄ was similar after therapy, the receptor antagonist could have contributed to the improvement in asthma symptoms. Whether the modulation of PGE₂ secretion is a secondary effect of C-LT receptor blockage remains to be determined. Further investigation is necessary to clarify the relationship between C-LT receptor agonist and PGE₂ production.

The serum ECP concentration has been reported to increase in patients with atopic disorder and clinical improvement is associated with its decrease [17, 18]. The effect of zafirlukast in the modification of cellular influx and activation response to antigen challenge has been reported [4]. In the study, we found a significant decrease in ECP concentration in serum but not in sputum, which indicated that zafirlukast has a systemic anti-inflammatory effect. The decreases in sputum ECP concentration and eosinophil count were not significant after zafirlukast therapy, which might have been due to the persistence of C-LT in the sputum. It has been reported that C-LT is an important inflammatory mediator for eosinophil chemotaxis and activation [19, 20]. Whether or not this modulation of ECP concentration is due to antagonism of leukotrienes on eosinophils remains to be determined.

In summary, our results suggest that zafirlukast exhibits a combination of effects in asthma. The antagonism of C-LT’s proinflammatory activities was found to prevent eosinophilic inflammation and inhibit the release of ECP. The antagonism of C-LT–induced bronchial smooth muscle constriction improved pulmonary function. The increase in PGE₂ secretion also had an additive effect on bronchodilation. The anti-inflammatory and bronchodilatory effects of zafirlukast found in this study suggest its efficacy in the therapy of asthma.

ACKNOWLEDGMENTS: This study was supported by Astra Zeneca, Taiwan.

References


11. Standards for diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987;136:225–44.


