

Efficacy of leukotriene receptor antagonist in bronchial hyperresponsiveness and hypersensitivity to analgesic in aspirin-intolerant asthma

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Summary

Background Albeit its exact pathogenesis is still ambiguous; aspirin-intolerant asthma is one of several types of asthma for which antileukotriene therapy is useful, because it is widely accepted that bronchial over-production of leukotrienes may be involved in its pathogenesis. Pranlukast (8-[p-(4-phenylbutyloxy) benzol] amino-2-(tetrazol-5-yl)-4-oxo-4H-1-benzopyran hemihydrate), a selective cysteinyl leukotriene receptor antagonist, is now widely used in the treatment of asthma.

Objective This study was designed to investigate the protective effect of pranlukast on airway sensitivity to sulpyrine provocation testing, bronchial responsiveness to methacholine provocation testing, and to investigate whether this protective activity is associated with a reduction in aspirin-induced excretion of urinary LTE₄ (uLTE₄), a marker of the cysteinyl leukotriene (LT) overproduction that participates in the pathogenesis of aspirin-induced asthma.

Methods We assessed the effects of pretreatment with pranlukast on bronchoconstriction precipitated by inhalation of methacholine and sulpyrine in 16 adult patients with mild or moderate aspirin-intolerant asthma; those who were in stable clinical condition and were hypersensitive to sulpyrine provocation testing were allocated to this study. A double-blind, randomized, crossover design was used. uLTE₄ was measured using combined reverse-phase high-performance liquid chromatography (rp-HPLC)/enzyme immunoassay.

Results Pranlukast protected against analgesic-induced bronchoconstriction through mechanisms that were not related to the bronchodilator property, but were related to the improvement both of bronchial hyperresponsiveness and hypersensitivity to analgesic ($P < 0.005$ and $P < 0.0001$). Pranlukast showed little effect on excretion of uLTE₄.

Conclusion These results support the hypothesis that cysteinyl leukotriene is one of the most important components in the pathogenesis of aspirin-intolerant asthma. Pranlukast improves not only hypersensitivity to analgesic, but also bronchial hyperresponsiveness in aspirin-intolerant asthma. It is also possible that pranlukast has another anti-asthmatic effect besides that of a leukotriene receptor antagonist.

Keywords: aspirin-intolerant asthma, bronchial hyperresponsiveness, leukotriene, pranlukast, sulpyrine provocation test

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Introduction

Acetylsalicylic acid and other non-steroidal anti-inflammatory agents with anticyclooxygenase activity induce severe, sometimes life-threatening asthmatic attacks in about 10% of adults with asthma [1,2]. Although aspirin-intolerant asthma has been widely recognized as a clinical entity since the beginning of this century, the exact pathological mechanism for the production of this syndrome still remains obscure. Some possible mechanisms have been proposed for aspirin-intolerant asthma, such as bronchial over-production of leukotrienes and inhibition of prostaglandin synthesis [3–5]; overproduction of bronchoconstrictive prostaglandins such as prostaglandin D₂ metabolite 9 *a*, 11 *b*-prostaglandin F₂ [6,7]; and chronic viral infection [8], and some other hypothesis may be involved in the pathogenesis of aspirin-intolerant asthma. Current evidence indicates that cyclooxygenase inhibition and increased production of cysteinyl-leukotrienes play important roles in these obstructive reactions.

Pranlukast (8-[*p*-(4-phenylbutoxy) benzol] amino-2-(tetrazol-5-yl)-4-oxo-4*H*-1-benzopyran hemihydrate), a selective receptor antagonist of cysteinyl leukotrienes (–C₄, –D₄, and –E₄), is intrinsically potent but has poor oral bioavailability [9]. Short-term clinical studies have shown that pranlukast is well tolerated and is effective in reducing bronchial hyperresponsiveness [10,11] and allergen-induced bronchoconstriction [12]. Some previous reports indicated

that pranlukast and other leukotriene antagonists inhibited the bronchoconstriction (fall in FEV₁) in aspirin-intolerant asthmatics [13,14]. The aim of the present study is to confirm previous observations on the protective activity of pranlukast against aspirin-intolerant asthma and to investigate the mechanism of protective effect of pranlukast.

Materials and methods

Subjects

We studied 16 adult patients with clinical histories of aspirin-intolerant asthma. Sulpyrine and lysine aspirin and/or tolmetin sodium provocation test was positive in all of them. All patients were non-smokers and diagnosed with mild or moderate asthma according to American Thoracic Society criteria [15]. The patients were in stable condition and had been free of symptoms of respiratory infection for at least 6 weeks (Table 1). Patients using oral or inhaled steroids, inhaled cromolyn, pranlukast or any other drugs that interfere with leukotriene production or responsiveness were excluded. Other treatments were all withheld for at least 12 h before the challenge testing, except for slow-release theophylline, which was withheld at least 24 h in advance [16]. The study was conducted according to the ethical standards of our medical centre, which requires informed consent from each patient.

Table 1 Characteristics of patients with aspirin-induced asthma at time of entry into the study. Patient numbers do not indicate order of entry into the study

Patient number	Sex	Age (years)	Atopy	FEV ₁ (% pred.)	FVC (% pred.)	Eosinophils (%)	IgE (RAST) (U/mL)	Medication
1	M	37	HD, Gr	76	97	9	466	T, B
2	M	21	HD, C, Gr	87	103	7	475	B
3	M	41	Cd	76	99	8	260	B
4	M	33	Cd	70	96	4	132	T, B
5	F	39	Gr, HD	70	97	5	133	T, B
6	F	33	C	76	89	4	37	B
7	F	54	HD, Cd	74	118	8	340	T, B
8	F	27	Cd	69	101	4	245	T, B
9	M	46	None	84	94	5	151	T, B
10	M	52	None	76	95	2	86	T, B
11	M	44	None	72	96	3	54	T, B
12	F	48	None	73	95	5	102	T, B
13	F	41	None	88	88	4	89	T, B
14	F	43	None	70	97	6	63	T, B
15	F	36	None	70	91	6	116	T, B
16	F	56	None	68	82	10	77	T, B

HD, house dust; C, cat; Gr, grass; Cd, candida; T, theophylline; B, inhaled β_2 -agonist on demand

Study design

To investigate the bronchodilator response to a single oral dose of pranlukast as a preliminary test, FEV₁ and FVC were measured for all subjects 1 h before oral administration of pranlukast or placebo, 1, 2, 3 and 4 h after oral administration of pranlukast or placebo on the preliminary test days.

One week later, we performed two kinds of bronchial challenge tests, methacholine provocation test and sulpyrine provocation test, to evaluate the involvement of cysteinyl-leukotrienes on bronchial hyperresponsiveness and hypersensitivity to analgesics in aspirin-intolerant asthma. Each interval among these two provocation tests and control studies were at least 2 weeks. Each bronchial challenge developed by Yoshida *et al.* was administrated as previously described [17], and according to the standard method of the Japanese Allergology Society [18]. On the first study day, all patients were given provocation testing in the form of pretreatment with either pranlukast (Ono pharmaceutical Co. Ltd, Osaka, Japan) or matching placebo in a double-blind, randomized manner before bronchial challenge testing. On the second study day, subjects were crossed over to receive alternative treatment and followed the identical procedure at the same time of day. On each study day before a light breakfast, FEV₁ and FVC were measured for baseline, and then 225 mg of pranlukast capsule or placebo were given orally as pretreatment 60 min before methacholine or sulpyrine provocation testing.

Methacholine provocation testing

Bronchial responsiveness was evaluated by methacholine provocation. This test was employed methacholine chloride (Sigma, St Louis, MO, USA), dissolved in saline to make solutions of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/mL administrated by a jet nebulizer driven by compressed air (Nihon Shoji Co. Ltd, Osaka, Japan).

Each study day began with inhalation of nebulized saline to rule out non-specific obstructive reaction; FEV₁ was measured 5 min before, and 5, 10 and 15 min after completion of inhalation. Saline was inhaled first for 2 min and FEV₁ was measured on a flow-integrating computerized pneumotachograph (Discom-21FX II, Chest M.I. Co. Ltd, Tokyo, Japan). After confirmation that the change in FEV₁ from the baseline after inhalation of saline was 10% or less in all subjects, inhalation of methacholine was started. If FEV₁ did not fall more than 10%, the subject entered the study properly. All patients underwent bronchial provocation on two occasions, separated by at least 2 weeks. One hour after administration of a capsule of pranlukast or placebo, the inhalation spirometry was performed. Methacholine was inhaled for 2 min by tidal breathing with a nose

clip, immediately followed by spirometry. Methacholine of increasing concentrations was successively inhaled until FEV₁ occurred fell by 20% or more. The measured values were plotted on semilogarithmic graph paper, and the methacholine provocative concentration producing a 20% fall in the FEV₁ was calculated as PC₂₀methacholine. The provocation test was terminated by the administration of an inhaled bronchodilator if FEV₁ fell by more than 35% from baseline. The higher of the first two satisfactory recordings was taken for analysis.

Sulpyrine provocation testing

Sulpyrine provocation testing is widely used for diagnosis of aspirin-intolerant asthma, because its sensitivity is much better than that of lysin-aspirin provocation testing, even though its specificity is worse. This testing was performed using almost the same conditions as methacholine provocation testing mentioned above. Freshly pulverized sulpyrine (Astra Japan Inc., Osaka, Japan) was dissolved each day in saline to produce concentration of 10, 20, 100, 250, 500 and 1250 mg/mL, which are in accordance with our daily protocol of sulpyrine in inhalation testing to diagnose aspirin-intolerant asthma. As one subject was too sensitive to challenge at these doses of sulpyrine, the sulpyrine provocation testing was performed at 4 and 10 mg/mL. Subjects were asked to inhale the sulpyrine solution from 10 (or 4) mg/mL for 3 min. If FEV₁, measured at the time points mentioned above after the inhalation did not drop by more than 10% of the initial value, a higher concentration of sulpyrine was given. The provocation was stopped when FEV₁ fell by 20% or more from the baseline or when the maximum dose of sulpyrine was reached. Dose-response relations for sulpyrine were constructed and used for calculation of PC₂₀sulpyrine. For statistical evaluation, PC₂₀sulpyrine values were set as equal to the highest concentration of sulpyrine given.

Analysis of uLTE₄

uLTE₄ was measured on the schedule of sulpyrine provocation testing days by the compound rp-HPLC (Class LC-10 system, Shimadzu, Kyoto, Japan)/enzyme immunoassay as previously described [17]. ³H-LTE₄ (168.4 Ci/mmol; NEN, DuPont, Dreierich, Germany) was used as an internal standard; antibody to LTE₄ (Cayman Chem, Ann Arbor, MI, USA) showed a cross-reactivity of 10–25% vs LTC₄-LTD₄ and <0.01% vs LTB₄. Creatinine concentration was measured with a standard assay kit (Sigma) and the results, corrected according to the observed recovery, were expressed as picograms of LTE₄ per milligram of creatinine. Urine was collected before challenge, at 2 h and 4 h after challenge. Each single sample was stored at –20 °C until assay.

Table 2 Bronchial responses in baseline FEV₁ and FVC affected by administration of placebo and pranlukast. Asterisks indicate statistically significant difference: * $P < 0.05$. N.S. not significant

Interval from treatment	Placebo FVC	FEV ₁	Pranlukast (+) FVC	FEV ₁
60 min before†	3.18 ± 0.42	2.26 ± 0.22	3.22 ± 0.42	2.24 ± 0.26
60 min after	3.24 ± 0.40	2.27 ± 0.23	3.17 ± 0.40	2.30 ± 0.30
120 min after	3.16 ± 0.43	2.26 ± 0.21	3.19 ± 0.41	2.38 ± 0.28
180 min after	3.17 ± 0.42	2.24 ± 0.20	3.20 ± 0.43	2.44 ± 0.23*
240 min after	3.17 ± 0.41	2.25 ± 0.23	3.18 ± 0.40	2.46 ± 0.32*

The representative data are shown as mean ± SD values. *Significantly greater than baseline (60 min before), $P < 0.05$. †Baseline

Statistical analysis

Statistical evaluation was performed with StatView-J 4.02 for Macintosh (Abacus Concepts, Inc., Berkeley, CA, USA).

All PC₂₀ values were logarithmically transformed before analysis, and summary statistics were expressed as mean ± standard deviation (SD). Student's paired *t*-test was used to compare mean differences. A *P*-value less than or equal to 0.05 (two-sided) was considered significant.

Results

None of the patients had a severe obstructive reaction to either methacholine and sulpyrine. All subjects could be followed for longer than 120 min. Bronchial responses to pranlukast are presented in Table 2. Although baseline FVC

was not significantly affected by administration of pranlukast or placebo before each provocation test, FEV₁ significantly increased at 1, 2, 3 and 4 h after administration of pranlukast. Thus we confirmed a bronchodilator effect with pranlukast between 180 (2.44 ± 0.23 after pranlukast vs 2.24 ± 0.26 after placebo, $P < 0.05$) and 240 min (2.46 ± 0.32 after pranlukast, $P < 0.05$) in this study. After pretreatment with pranlukast or placebo, challenge with both methacholine and sulpyrine caused an obstructive reaction in all patients (Table 3).

In the provocation challenge with methacholine, values of PC₂₀methacholine transformed logarithmically were elevated significantly (Table 3 and Fig. 1).

Mean log PC₂₀methacholine was 2.93 ± 0.57 after pranlukast vs 2.61 ± 0.53 after placebo ($P < 0.005$).

On the other hand, values of PC₂₀sulpyrine were obviously improved after pranlukast, except for two subjects. Furthermore, all values of logarithmically transformed

Table 3 Individual data for PC₂₀ methacholine and PC₂₀ sulpyrine

Patient no.	PC ₂₀ methacholine (mg/mL)		PC ₂₀ sulpyrine (mg/mL)	
	Placebo	Pranlukast	Placebo	Pranlukast
1	0.08	0.08	10	100
2	1.25	1.25	20	100
3	0.16	0.31	10	100
4	2.50	5.00	20	100
5	0.31	0.31	10	100
6	1.25	1.25	20	20
7	0.08	0.16	10	20
8	0.63	1.25	10	10
9	0.63	1.25	20	100
10	0.31	1.25	20	100
11	0.16	1.25	10	250
12	0.63	5.00	20	100
13	2.50	10.0	250	< 1250
14	0.63	0.31	10	100
15	0.63	0.63	10	100
16	0.04	0.31	4	20

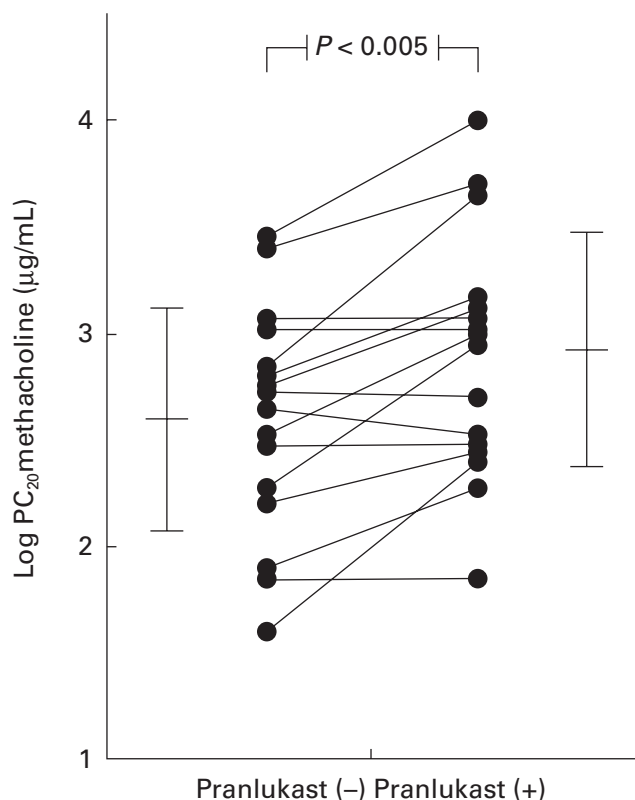


Fig. 1 Individual results of PC_{20} methacholine at baseline and after pranlukast treatment. Both results are shown as the mean, with the bar indicating SD. All PC_{20} values were logarithmically transformed before analysis, and summary statistics were expressed as mean $\pm$ SD

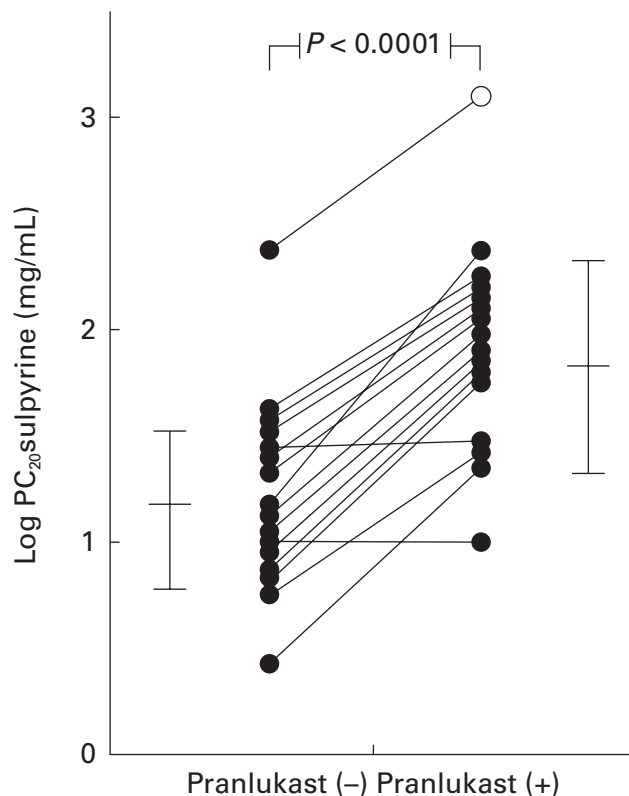


Fig. 2 Individual results of PC_{20} sulpyrine at baseline and after pranlukast. Both results are shown as the mean, with the bar indicating SD. All PC_{20} values were logarithmically transformed before analysis, and summary statistics were expressed as mean $\pm$ SD. Open circle: one subject failed to produce a 20% decrease in FEV_1 after inhalation of the highest sulpyrine dose

PC_{20} sulpyrine were elevated significantly (Table 3, Fig. 2). Mean log PC_{20} was 1.90 ± 0.49 after pranlukast vs 1.18 ± 0.38 after placebo ($P < 0.0001$). One subject failed to produce a 20% decrease in FEV_1 after inhalation of the highest sulpyrine dose. Oral administration of pranlukast improves bronchial hyperresponsiveness and protects against sulpyrine-induced bronchoconstriction in one out of 16 patients.

Mean baseline $uLTE_4$ was 717 ± 274 pg/mg creatinine before pranlukast vs 770 ± 115 pg/mg creatinine on the placebo day (not significant). After placebo, challenge with sulpyrine caused a significant increase in $uLTE_4$, reaching a maximum value of 2304 ± 528 pg/mg creatinine (Fig. 3, $P < 0.05$ compared with baseline). After pranlukast, the maximum level of $uLTE_4$ was 2204 ± 612 pg/mg creatinine, which was slightly lower than placebo but not significant. Furthermore, no significant correlation was observed between the maximum fall in FEV_1 or the concentration of sulpyrine inhaled and the corresponding maximum increase in $uLTE_4$ after placebo, or between the protection afforded by pranlukast against FEV_1 decrease and $uLTE_4$ excretion.

None of the subjects reported any adverse effect after pranlukast administration, nor could they discriminate between pranlukast and placebo.

Discussion

Although the pathogenesis of aspirin-intolerant asthma remains unproved, it is a widely accepted hypothesis that bronchial overproduction of leukotrienes and inhibition of prostaglandin (PG) synthesis, most likely due to removal of inhibitory PGE_2 are involved in the pathogenesis of this disease [3–5]. The inhibitory effect of acetylsalicylic acid on the cyclooxygenase increases formation of bronchoconstrictor leukotrienes via shunting of unmetabolized arachidonic acid into metabolism by the 5-lipoxygenase. The severity and rapidity of bronchospasm, which are induced by cyclooxygenase-inhibiting drugs in aspirin-intolerant asthma, are directly related to the dose and potency of the drug to inhibit cyclooxygenase. Knapp *et al.* showed that excretion of LTE_4 was increased during aspirin-intolerant

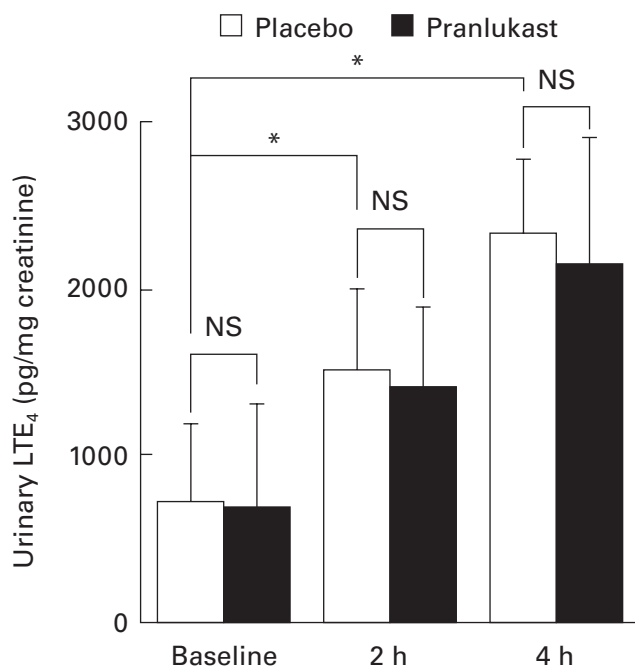


Fig. 3. Mean urinary LTE_4 before and during the observation period after placebo or pranlukast. * $P < 0.05$; NS, not significant

asthma episodes, but the degree of increase for individual patients did not correlate with the degree of bronchospasm or inhibition of platelet thromboxane B_2 formation [19]. Thus, although the endogenous synthesis of potent bronchoconstrictor leukotrienes increases during aspirin-induced bronchospasm, it is unlikely that a direct shunting of unmetabolized arachidonate into leukotriene synthesis represents the mechanism of aspirin-intolerant asthma. Szczeklik and colleagues reported a statistically significant rise in peptido-leukotrienes, IL-5 and eosinophils in bronchoalveolar lavage of patients with aspirin-intolerant asthma after bronchial challenge with aspirin [6]. It has also been reported that, after bronchial instillation of aspirin, biopsies showed a decrease in the number of mast cells and activation of eosinophils [20]. These findings suggested that activation of eosinophils and degranulation of mast cells are early events associated with aspirin-intolerant reactions.

Our results revealed for the first time in aspirin-intolerant asthma that pranlukast not only suppresses hypersensitivity to analgesic ($P < 0.0001$) (Fig. 2), but also improves bronchial hyperresponsiveness ($P < 0.005$) (Fig. 1) and thus prevents bronchoconstriction. Some previous reports indicated that pranlukast prevented the activation of human peripheral blood eosinophils by inhibiting LTC_4 and eosinophil cationic protein release [21]. Pranlukast might have an effect to prevent eosinophilic inflammation.

Bronchial asthma is widely recognized as a chronic inflammatory disease [16] and it is likely that there is

continuous release of some inflammatory mediators influencing bronchial hyperresponsiveness. It has been reported that bronchial hyperresponsiveness is reduced by long-term inhalation of cromolyn sodium, which is a well-established inhibitor of inflammatory mediators [22]. Indeed, Fujimura *et al.* found that 1 week's administration of pranlukast (225 mg twice a day) attenuated bronchial hyperresponsiveness in 11 patients with asthma (not with aspirin-intolerant asthma) [10]. Bronchial inflammation should be involved in bronchial hyperresponsiveness. Though pranlukast does not have a direct bronchodilator effect, our data confirm that 8.9–9.8% improvement in FEV_1 was obtained between 3 and 4 h after a single oral dose of pranlukast ($P < 0.05$). Since pranlukast improved bronchial hyperresponsiveness before the baseline pulmonary function would improve in most subjects, it has been our hypothesis that a sub-threshold concentration of cysteinyl leukotrienes in the bronchial tissues may contribute to the development of bronchial hyperresponsiveness in asthma. Therefore, data procured in our study can be interpreted in two ways: (1) the cysteinyl-leukotrienes are involved in bronchial hyperresponsiveness in aspirin-intolerant asthma directly and/or indirectly, and (2) some other mechanisms, for example, activation of eosinophils and degranulation of mast cells, may be involved in the pathogenesis of aspirin-intolerant asthma.

After pretreatment of pranlukast, the maximum level of uLTE_4 was slightly lower than placebo but not significantly (Fig. 3). In addition no significant correlation was observed between the maximum fall in FEV_1 or the concentration of sulpyrine inhaled and the corresponding maximum increase in uLTE_4 after placebo, or between the protection afforded by pranlukast from FEV_1 decrease and uLTE_4 excretion. These results are consistent with those of Obata *et al.* who reported that pranlukast showed little or no effect on the activities of cyclooxygenase, 5-lipoxygenase and thromboxane synthesis in isolated guinea pig tissues [23].

In conclusion, our findings suggest that cysteinyl leukotrienes play a pivotal role in pathogenesis of aspirin-intolerant asthma and that pranlukast is a potent drug for the treatment of this disease.

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References

- 1 Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to

- asthma attacks in aspirin-sensitive patients. *Br Med J* 1975; 1:67–9.
- 2 Lee TH. Mechanism of aspirin sensitivity. *Am Rev Respir Dis* 1992; 145:34–6.
- 3 Houjou S, Dobashi K, Nakazawa T. Aspirin-induced asthma. *Jpn J Clin Med* 1994; 3:355–8.
- 4 Power I. Aspirin-induced asthma. *Br J Anaest* 1993; 71:619–21.
- 5 Szczeklik A, Schmitz SM. Aspirin-induced asthma: from pathogenesis to therapy. *Allergol Immunopathol* 1993; 21: 35–40.
- 6 Szczeklik A, Sladak Dworski K et al. Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996; 154:1608–14.
- 7 O'Sullivan S, Dahlen B, Dahlen SE, Kumlin M. Increased urinary excretion of the prostaglandin D₂ metabolite 9 alpha, 11 beta-prostaglandin F₂ after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J Allergy Clin Immunol* 1996; 98:421–32.
- 8 Szczeklik A. Aspirin-induced asthma as a viral disease. *Clin Allergy* 1988; 18:15–20.
- 9 Obata T, Nanbu F, Kitagawa T et al. ONO-1078: an antagonist of leukotrienes. *Adv Prostaglandin Thromboxane Leukot Res* 1987; 17A:540–3.
- 10 Fujimura M, Sakamoto S, Kanno Y, Matsuda T. Effect of a leukotriene antagonist, ONO-1078, on bronchial hyperresponsiveness in patients with asthma. *Respir Med* 1993; 87:133–8.
- 11 Taki P, Suzuki R, Tori K, Matsumoto S, Takiguchi H, Takagi K. Reduction of the severity of bronchial hyperresponsiveness by the novel leukotriene antagonist 4-oxo-8-[4-(4-phenylbutoxy) benzoyl amino]-2-(tetrazol-5-yl) -4H-l-benzopyran hemihydrate. *Drug Res* 1994; 44:330–3.
- 12 Taniguchi Y, Tamura G, Honma M et al. The effect of an oral leukotriene antagonist, ONO-1078, on allergen-induced immediate bronchoconstriction in asthmatic subjects. *J Allergy Clin Immunol* 1993; 92:507–12.
- 13 Yamamoto H, Nagata M, Kuramitsu K et al. Inhibition of analgesic-induced asthma by leukotriene receptor antagonist ONO-1078. *Am J Respir Crit Care Med* 1994; 150:254–7.
- 14 Cristie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK & F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991; 144:957–8.
- 15 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary diseases (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
- 16 Chai H, Farr RS, Froehlich LA et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:323–7.
- 17 Yoshida S, Amayasu H, Sakamoto H et al. Cromolyn sodium prevents bronchoconstriction and urinary LTE₄ excretion in aspirin-induced asthma. *Ann Allergy Asthma Immunol* 1998; 2:171–6.
- 18 Makino S, Kobayashi S, Miyamoto T, Shida T, Takahashi S. Standard for allergen bronchial provocation tests in asthma and hypersensitivity pneumonitis. *Jpn J Allerg* 1982; 31:1074–6.
- 19 Knapp HR, Sladek K, Fitzgerald GA. Increased excretion of leukotriene E₄ during aspirin-induced asthma. *J Lab Clin Med* 1992; 119:48–51.
- 20 Nasser SMS, Christie PE, Pfister R et al. Effect of endobronchial challenge on inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Thorax* 1996; 51:64–70.
- 21 Kobayashi S, Yoshida K, Mori A et al. Inhibitory effect of ONO-1078, a novel leukotriene receptor antagonist, on peripheral blood eosinophils. *Jpn J Allergology* 1996; 45:1166–71.
- 22 Altounyan REC. Review of the clinical activity and modes of action of sodium cromoglycate. In: Pepys MB, Edwards JH, eds. *The Mast Cell – Its Role in Health and Disease*. London: Pitman Medical, 1979:199–216.
- 23 Obata T, Okada Y, Motoishi M et al. In vitro antagonism of ONO-1078, a newly developed anti-asthma agent, against peptide leukotrienes in isolated guinea pig tissues. *Jpn J Pharmacol* 1992; 60:27–237.