

## Effect of pranlukast, an oral leukotriene receptor antagonist, on leukotriene D<sub>4</sub> (LTD<sub>4</sub>) challenge in normal volunteers

T C O'Shaughnessy, P Georgiou, K Howland, M Dennis, C H Compton, N C Barnes

### Abstract

**Background** – There is increasing evidence to show that leukotrienes are important mediators in asthma. Leukotriene receptor antagonists protect against antigen and exercise challenges in patients with chronic asthma. A study was undertaken to investigate the activity of the leukotriene receptor antagonist pranlukast (SB 205312, ONO-1078) in blocking bronchoconstriction induced by leukotriene D<sub>4</sub> (LTD<sub>4</sub>) inhalation. The selectivity of pranlukast was evaluated using histamine challenge.

**Methods** – Pranlukast, 450 mg twice daily, was given to eight healthy non-smoking men for five days in a randomised, double blind, placebo controlled, crossover study. The specific airways conductance (sGaw) was measured before and after bronchial provocation with inhaled LTD<sub>4</sub> at 3.5 hours after the first dose and at 3.5 and 9.5 hours after the last dose of pranlukast on the morning of day 5. The concentration of LTD<sub>4</sub> required to produce a fall in sGaw of 35% (PC<sub>35</sub>) was calculated. Subjects also underwent a histamine challenge 3.5 hours after a single dose of pranlukast, 450 mg, or placebo.

**Results** – A single dose of pranlukast produced a 10.6 fold increase in PC<sub>35</sub>sGaw (95% confidence interval (CI) 4.4 to 25.5; p<0.001) for LTD<sub>4</sub> at 3.5 hours after dosing compared with placebo. Three and a half hours after the morning dose of pranlukast on day 5 the PC<sub>35</sub>sGaw for LTD<sub>4</sub> was increased 25.9 fold (95% CI 10.8 to 62.2; p<0.001) and was still increased sevenfold (95% CI 2.9 to 16.7; p<0.001) relative to placebo 9.5 hours after administration of the morning dose. No significant differences were noted for the PC<sub>35</sub>sGaw to histamine for pranlukast compared with placebo.

**Conclusions** – This study shows that pranlukast is a potent and selective LTD<sub>4</sub> receptor antagonist in humans which blocks LTD<sub>4</sub> challenge after initial and repeated administration when given twice daily for five days.

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Keywords: leukotriene receptor antagonist, asthma, pranlukast, SB 205312, ONO-1078.

Asthma is characterised by variable and reversible airflow obstruction, bronchial hyper-

responsiveness, and airway inflammation. During the past decade a growing body of evidence has shown that leukotrienes play an important part in the pathogenesis of asthma. Moreover, the cysteinyl leukotrienes LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> have been shown potently to constrict bronchial smooth muscle, stimulate mucous secretions, mediate inflammation, and possibly induce bronchial hyperresponsiveness.<sup>1-3</sup>

The cysteinyl leukotrienes are released in response to immunological and non-immunological stimuli from mast cells, eosinophils, macrophages, and other inflammatory cells that are implicated in asthma. Bronchoconstriction is stimulated directly through leukotriene receptors found on bronchial smooth muscle and other cells.<sup>4-7</sup>

Urinary levels of the leukotriene metabolite LTE<sub>4</sub> are increased in several subpopulations of patients who have asthma – namely, aspirin-sensitive patients in the resting state and after aspirin challenge, wheezing patients, and patients who have atopic asthma after allergen challenge.<sup>4-12</sup> Leukotrienes are also found in the bronchoalveolar lavage fluid of patients with asthma.<sup>13</sup>

A number of studies have demonstrated the effectiveness of specific cysteinyl leukotriene receptor antagonists during early and late responses to asthma induced by allergens, exercise, and cold air, as well as to chronic asthma.<sup>14-17</sup> Pranlukast (SB 205312, ONO-1078) has been shown in animal studies to be a potent and selective cysteinyl leukotriene receptor antagonist.<sup>18,19</sup> Results of allergen challenges in asthma patients investigated in Japan also have confirmed the activity and selectivity of pranlukast.<sup>20</sup>

In the present study we have examined the protective effect of pranlukast on bronchoconstriction induced by LTD<sub>4</sub> inhalation after a single 450 mg dose and after 4.5 days of 450 mg daily oral dosing in normal subjects. This dose was chosen as it was a candidate dose regimen based on Japanese evidence on efficacy and safety. To investigate the selectivity of pranlukast we assessed the response to histamine challenge after a single dose.

### Methods

#### STUDY POPULATION

The study population consisted of eight healthy male volunteers of mean age 30.9 years (range, 26-39). The subjects were non-smokers with

Department of Respiratory Medicine, The London Chest Hospital, Bonner Road, London E2 9JX, UK

T C O'Shaughnessy  
N C Barnes

Department of Clinical Pharmacology, SmithKline Beecham, New Frontiers Science Park, Harlow, Essex CM19 5AW, UK

P Georgiou  
K Howland  
C H Compton

Department of Drug Metabolism and Pharmacokinetics, SmithKline Beecham, PO Box 1539, King of Prussia, Pennsylvania 19406, USA  
M Dennis

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Correspondence to:  
Dr N C Barnes.

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normal lung function. None of the study subjects was taking any regular medication and non-steroidal anti-inflammatory drugs were not allowed during the study period. Subjects who developed upper respiratory tract infections within six weeks of the start of the study were excluded. All of the men were familiar with pulmonary function testing and all gave written informed consent for participation in the study, which was approved by the ethics committee of the Royal Brompton National Heart and Lung Hospitals.

#### MATERIALS

Leukotriene D<sub>4</sub> (Cascade Biochem Ltd, University of Reading, UK) was stored at  $-70^{\circ}\text{C}$  until immediately before use, at which time it was dissolved in buffered normal saline solution. The starting LTD<sub>4</sub> concentration was  $2 \times 10^{-6}$  mol/l. During the challenges this concentration was increased by 3.2 fold ( $0.5 \log_{10}$ ) increments up to a maximum of  $2 \times 10^{-4}$  mol/l.

Histamine acid phosphate (Northwick Park Hospital, London, UK) was also dissolved in buffered normal saline solution, and a starting concentration of 0.25 mg/ml ( $0.8 \mu\text{mol/l}$ ) was used. During the challenges the histamine concentrations were increased by doubling, up to a maximum concentration of 32 mg/ml.

#### AEROSOL DELIVERY

The challenge aerosols were inhaled using a Wright nebuliser (Clement Clarke, Harlow, Essex, UK) that contained 2 ml of test solution propelled by compressed air at a flow rate of 7 l/min. Subjects inhaled the aerosol by tidal breathing by mouth for two minutes, in a manner previously described.<sup>21,22</sup> The nebuliser delivered its contents at a rate of 0.16 ml/min. The same flow rate and type of nebuliser were used throughout the study.

#### SAFETY SCREEN

The initial screening of volunteers included a full physical examination, routine haematology and biochemistry tests, and an electrocardiogram. Haematology and biochemistry tests were performed again on each study day and at the end of the study.

#### STUDY PROTOCOL

The study was divided into two parts and was conducted using a randomised, double blind, crossover design in each part which included at least a one week washout period between the two dosing periods in part 1 of the study and between the end of part 1 and start of part 2. In part 1 each subject was given pranlukast, 450 mg, or placebo in two separate repeat dosing periods consisting of four days of twice daily dosing and a single dose on the morning of day 5. LTD<sub>4</sub> challenges were performed at 3.5 hours after dosing on day 1, and at 3.5 and 9.5 hours after dosing on day 5. In part 2 each subject received a single 450 mg dose of

pranlukast or placebo on two occasions separated by at least one week. A histamine challenge was performed 3.5 hours after each of these two doses.

On day 1 of the study subjects presented at the laboratory, having fasted and abstained from caffeinated beverages since midnight during the preceding night. Baseline measurements of forced expiratory volume in one second (FEV<sub>1</sub>) were made three times with a rolling seal spirometer (P K Morgan, UK). Specific airways conductance (sGaw) was measured five times using a whole body plethysmograph (Morgan Data Analysis System Version 3.02; P K Morgan) and a mean sGaw value was calculated. The subjects then ate a standard breakfast. Thirty minutes later they were given the study medication: pranlukast, 450 mg, or matching placebo. Three and a half hours later sGaw was measured again and the subjects then underwent LTD<sub>4</sub> inhalation challenge.

For the LTD<sub>4</sub> challenges subjects first inhaled buffered normal saline for two minutes. After an additional two minutes sGaw was measured five times and the mean value was calculated and used in subsequent analyses as a baseline value. Subjects then inhaled increasing concentrations of LTD<sub>4</sub>. The sGaw was measured five times at two and five minutes after each inhalation until either a 35% fall in sGaw from the values after saline was achieved or the aerosol containing the highest LTD<sub>4</sub> concentration was administered. If the decrease in sGaw was greater than 10% but less than 35% the lung function tests were repeated every 3–5 minutes until sGaw values returned to 90% of the post-saline values. The next concentration was not administered until this occurred.

On study day 5 an LTD<sub>4</sub> challenge was performed according to the same procedure at 3.5 hours and 9.5 hours after the morning dose of pranlukast or placebo.

Histamine challenge tests were performed using the same procedure as was used for the LTD<sub>4</sub> challenges, except that a single set of measurements of sGaw was taken after two minutes, before proceeding to the next concentration.

#### PHARMACOKINETICS

In part 1 of the study blood was drawn from the antecubital vein into a heparinised syringe before breakfast on each study day, immediately before and after each LTD<sub>4</sub> challenge at 3.5 hours after dosing on days 1 and 5, and before the challenge at 9.5 hours after dosing on day 5. Plasma was stored at  $-70^{\circ}\text{C}$  until plasma concentrations of pranlukast could be assayed by high pressure liquid chromatography.<sup>20</sup> The assay is linear up to 1000 ng/ml, with a lower limit of quantification of 10 ng/ml, and is accurate and reproducible to within  $\pm 10\%$ .

#### STATISTICAL ANALYSIS

The PC<sub>55</sub> was calculated from the log concentration-response curve between the two points on either side of a 35% fall in sGaw by

Table 1 Baseline lung function tests

Treatment		FEV <sub>1</sub> (l)	sGaw (l/s/kPa)
Screen		4.36 (0.49)	1.5 (0.4)
Part 1			
Day 1			
	Pre-dose pranlukast	4.34 (0.71)	1.8 (0.7)
	Pre-dose placebo	4.27 (0.49)	1.6 (0.5)
+ 3.5 h	Pre-challenge pranlukast	–	1.9 (0.8)
+ 3.5 h	Pre-challenge placebo	–	1.8 (0.5)
Day 5			
	Pre-dose pranlukast	4.18 (0.51)	1.8 (0.9)
	Pre-dose placebo	4.34 (0.60)	1.5 (0.4)
+ 3.5 h	Pre-challenge pranlukast	–	1.9 (1.0)
+ 3.5 h	Pre-challenge placebo	–	1.5 (0.4)
+ 9.5 h	Pre-challenge pranlukast	–	1.9 (1.0)
+ 9.5 h	Pre-challenge placebo	–	1.7 (0.5)
Part 2			
	Pre-dose pranlukast	4.28 (0.56)	1.9 (0.8)
	Pre-dose placebo	4.12 (0.68)	1.8 (0.9)
Follow up		4.28 (0.65)	1.9 (0.9)

Values are mean (SD).

FEV<sub>1</sub> = forced expiratory volume in one second; sGaw = specific airways conductance.

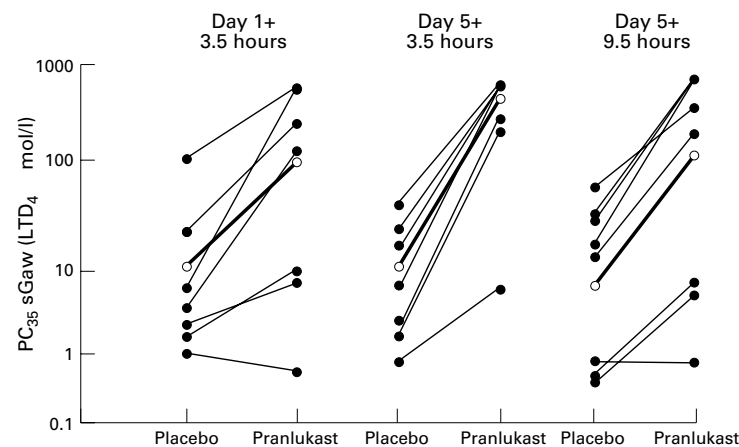


Figure 1 Changes in PC<sub>35</sub> for the LTD<sub>4</sub> challenges; ○ = geometric means.

linear interpolation.<sup>22</sup> For those curves where the maximum dose was reached without achieving a 35% reduction in sGaw, a conservative estimate was made through extrapolation of the slope of the dose-response curve during active treatment, using the slope of the corresponding placebo response. The upper limit of extrapolation was 640 mmol/l for LTD<sub>4</sub> and 64 mg/ml for histamine.

The data were analysed in each part of the study by analysis of variance for the two period crossover design, fitting a linear model with terms for subject, period, treatment, and, for part 1, time and treatment by time interaction. Logarithmic transformation was applied to PC<sub>35</sub> values prior to statistical analysis.

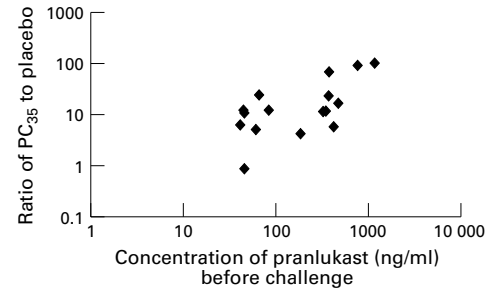


Figure 2 Relationship between shift in PC<sub>35</sub> for LTD<sub>4</sub> challenges and plasma level of pranlukast before challenge on day 5 ( $r=0.62$ ; Spearman correlation = 0.59).

## Results

There was no significant variation in the baseline FEV<sub>1</sub> and sGaw values on the different study days and no change in pulmonary function either after 3.5 hours or 4.5 days of treatment (table 1). The percentage of baseline sGaw was plotted against the log dose of LTD<sub>4</sub> for each challenge.

Subjects treated with pranlukast tolerated significantly higher concentrations of LTD<sub>4</sub> at 3.5 hours after dosing on day 1 and at 3.5 and 9.5 hours after dosing on day 5 than subjects treated with placebo before a 35% decrease in sGaw was observed (fig 1).

On day 1 the geometric mean PC<sub>35</sub>sGaw for LTD<sub>4</sub> at 3.5 hours after a single 450 mg dose of pranlukast increased 10.6 fold compared with placebo (95% CI 4.4 to 25.5;  $p<0.001$ ). On day 5, after repeat dosing, the geometric mean PC<sub>35</sub>sGaw for LTD<sub>4</sub> at 3.5 hours after pranlukast increased 25.9 fold (95% CI 10.8 to 62.2;  $p<0.001$ ) compared with placebo. Moreover, compared with placebo, a sevenfold increase (95% CI 2.9 to 16.7;  $p<0.001$ ) in PC<sub>35</sub>sGaw for LTD<sub>4</sub> was still present even at 9.5 hours after dosing on day 5 (table 2).

There was no significant difference in the histamine concentrations for PC<sub>35</sub>sGaw after a single dose of pranlukast compared with placebo (shift 1.3 fold; 95% CI 0.6 to 2.7;  $p=0.38$ ) (table 2).

A positive correlation was observed between log plasma concentrations of pranlukast in blood samples taken immediately before LTD<sub>4</sub> challenge (3.5 and 9.5 hours after dosing on day 5) and the log shift in the PC<sub>35</sub> ratio of pranlukast to placebo ( $r=0.62$ ; 95% CI 0.19 to 0.86;  $p<0.01$ ; fig 2).

No changes in haematological or biochemical parameters were noted during the study. The

Table 2 Geometric mean concentrations of LTD<sub>4</sub> and histamine required to produce a fall in specific airways conductance of 35% (PC<sub>35</sub>sGaw)

Treatment regimen	LTD <sub>4</sub> (μmol/l)			Histamine (mg/ml)
	Day 1 + 3.5 hours	Day 5 + 3.5 hours	Day 5 + 9.5 hours	Dosing + 3.5 hours
Placebo	8.1	11.6	13.0	5.34
Pranlukast 450 mg twice daily	85.4	301.2	90.7	7.05
Estimated ratio	10.6	25.9	7.0	1.3
95% confidence interval	(4.4 to 25.5)	(10.8 to 62.2)	(2.9 to 16.7)	(0.6 to 2.7)
p value (estimated ratio)	<0.001	<0.001	<0.001	NS

only side effects reported were mild abdominal discomfort and loose stools in one subject taking pranlukast and mild abdominal discomfort in one subject taking placebo.

### Discussion

As in previous studies, the present study demonstrates that LTD<sub>4</sub> is approximately 1000 times more potent than histamine in causing bronchoconstriction in normal subjects.<sup>21</sup> The study also confirms the reproducibility of LTD<sub>4</sub> challenge in normal individuals.<sup>23</sup> The data on day 5 at 3.5 and 9.5 hours on the placebo day showed no evidence of tachyphylaxis to leukotriene challenge.

Administration of pranlukast produced a significant 10.6 fold shift in the LTD<sub>4</sub> dose-response curve at 3.5 hours after dosing on day 1. At 3.5 hours on day 5, when plasma concentrations of pranlukast are expected to be at steady state, the shift in the dose-response curve was greater at 25.9 fold. This represents an underestimate of the true increase in the shift of the dose-response curve, given that a conservative method of extrapolation was used. These results indicate that the broncho-protective effect of pranlukast is increased after repeat dosing for five days and that this effect is maintained for 9.5 hours. This shift in the LTD<sub>4</sub> dose-response curve is greater than the first generation of LTD<sub>4</sub> dose antagonists<sup>21</sup> and comparable to shifts produced by second generation leukotriene receptor antagonists such as zafirlukast.

The effectiveness of pranlukast during LTD<sub>4</sub> challenge correlates positively with pranlukast plasma concentrations. The relationship between plasma concentrations of pranlukast and the ability of the drug to block the bronchoconstriction induced by LTD<sub>4</sub> inhalation suggests that higher plasma concentrations of pranlukast are associated with larger shifts in the LTD<sub>4</sub> dose-response curve, a relationship not previously demonstrated with other LTD<sub>4</sub> receptor antagonists.<sup>21</sup>

The repeated LTD<sub>4</sub> challenges in this study showed that pranlukast has a prolonged protective effect against bronchoconstriction induced by LTD<sub>4</sub> inhalation consistent with a twice daily dosing regimen. In addition to demonstrating a long duration of inhibition, this study showed no evidence of short term tachyphylaxis due to pranlukast. We believe this study to be the first reported to use repeated LTD<sub>4</sub> challenge and longer term dosing to help to define the duration of action and to demonstrate the absence of short term tachyphylaxis.

As expected, pranlukast had no effect on bronchoconstriction induced by histamine inhalation compared with placebo, demonstrating its specificity of action as an LTD<sub>4</sub> receptor antagonist.

In conclusion, this study shows that pranlukast is a selective and potent orally active LTD<sub>4</sub> receptor antagonist. The effectiveness of

pranlukast during LTD<sub>4</sub> challenge is positively correlated with plasma concentrations, and the pharmacodynamics of the drug appear to be suited to twice daily dosing, with no evidence of tachyphylaxis after short term treatment for 4.5 days.

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