Effect of inhaled Piriprost (U-60, 257) a novel leukotriene inhibitor, on allergen and exercise induced bronchoconstriction in asthma

J S MANN, C ROBINSON, A Q SHERIDAN, P CLEMENT, M K BACH, S T HOLGATE

From Medicine I, Southampton General Hospital, Southampton, and Upjohn Company, Kalamazoo, Michigan, USA

ABSTRACT The leukotrienes, a group of oxidative metabolites of arachidonic acid, have potent pharmacological actions on human airways. We have investigated the effects of a leukotriene synthesis inhibitor, piriprost (U-60, 257) administered by inhalation on allergen and exercise induced bronchoconstriction in 12 subjects with allergic asthma. Subjects underwent diagnostic challenges with allergen and treadmill exercise to define the strengths of the stimuli required to reduce the FEV₁ to about 25% of baseline (PS₂₅). On separate study days subjects inhaled either piriprost 1 mg or vehicle placebo, followed 15 minutes later by the PS₂₅ allergen or exercise. The FEV₁ was measured at regular intervals before and after challenge up to 60 minutes. After allergen challenge in six subjects peak expiratory flow (PEF) was measured for the following 20 hours. When compared with placebo, inhalation of piriprost had no significant protective effect on the fall in FEV₁ at any time point within 60 minutes of allergen or exercise challenge. In the four subjects with a documented late asthmatic reaction 2–12 hours after allergen challenge piriprost had no protective effect when compared with placebo. In the subjects who recorded PEF over 20 hours after allergen challenge there was no significant difference between piriprost and placebo. Piriprost was appreciably more irritant to the respiratory tract than was placebo. On the assumption that inhaled piriprost was bioavailable in the airways, this study casts doubt on any theory of a pivotal role for leukotrienes in the pathogenesis of acute exercise and allergen induced airway bronchoconstriction in asthma.

The sulphidopeptide leukotrienes represent some of the most potent proinflammatory mediators that have so far been described in man. Leukotrienes C₄ (LTC₄) and D₄ (LTD₄) are up to 1000 times more potent than histamine as contractile agonists for human bronchi in vitro.¹ ² When given to man by inhalation leukotrienes are powerful bronchoconstrictors in normal and asthmatic subjects, although asthmatic subjects appear to be relatively less reactive to leukotrienes than to histamines.³ ⁴ In addition to their dynamic effects on airway smooth muscle, leukotrienes cause constriction and increased permeability of the microvasculature and stimulate mucus secretion from bronchial mucus glands.⁵

Although there is convincing evidence that the IgE dependent stimulation of human lung results in the synthesis and release of LTC₄ and LTD₄,⁶ ⁷ there is still debate about whether these mediators originate from mast cells.⁹ A recent study¹⁰ has shown that IgE dependent stimulation of bronchoalveolar lavage cells containing 0·1–1·0% mast cells initiates the release of large amounts of histamine and prostaglandin (PG) D₃ in parallel but only a small quantity of LTC₄, which does not correlate with histamine release.

In addition to the sulphidopeptide leukotrienes, the 5S, 12R-dihydroxyeicosatetranoeic acid LTB₄ is believed to be important in inflammatory reactions.¹¹ This compound is a potent chemotactic factor for neutrophils¹² and eosinophils,¹³ and its release may contribute to the cellular infiltrate that characterises the late asthmatic reaction, increased non-specific
bronchial responsiveness, and more chronic forms of asthma.

There is therefore considerable interest in the pharmacological potential of drugs that may inhibit the synthesis or block the actions of leukotrienes. Piriprost (6,9-deoxy-6,9-(phenylimino)-Δ6,8-prostaglandin I2 (U-60,257), an analogue of prostacyclin, is a potent inhibitor of 5-lipoxygenase product formation in rat peritoneal mononuclear cells,15 human asthmatic lung fragments16 and dispersed cells,17 neutrophil leucocytes,18 and eosinophils.19 At higher concentrations it is a receptor antagonist of sulphidopeptide leukotrienes.20 In vivo, piriprost inhibits the IgE dependent bronchoconstriction induced by inhaled Ascaris suum in sensitised rhesus monkeys21 and it is a potent inhibitor of canine tracheal mucus secretion.22 In this paper we report a double blind evaluation of a single inhaled dose of piriprost to protect against allergen and exercise induced bronchoconstriction in asthmatic subjects.

Methods

Subjects

Nine men and three women with asthma (mean (SEM) age 24 (1·3) y) took part in the study. On entry all patients were within 15% of their ideal weight and had normal haemoglobin concentration, peripheral leucocyte count, liver function, urea and electrolyte concentrations, and urine analysis, and had a normal chest radiograph and electrocardiogram. All patients were using intermittent inhaled β2 adrenoceptor agonists, but none required chronic treatment with corticosteroids, long acting antihistamines, or theophylline preparations. All subjects had positive skin reactions to extracts of at least two common allergens. Written informed consent was obtained from each patient and the study was approved by the Southampton University Hospital's ethical subcommittee.

Bronchial Provocation and Airway Measurements

Exercise was carried out at room temperature on a treadmill adjusted to a speed of 6 km/h. The incline of the treadmill was set at 5–15% and exercise performed for five minutes.23 The bronchial provocation with allergen was carried out by a method modified from that of Chai et al.24 Extracts of grass pollen or house dust mite (Bencard, Brentford, Middlesex) were administered as a single nebulised dose from an Inspiron Mini-neb nebuliser (Inspiron, CR Bard, Sunderland) driven by air at a flow rate of 81 min⁻¹. This delivers 0·33 ml of aerosolised solution a minute. The subjects were asked to take five deep breaths from residual volume to total lung capacity, during which aerosolised allergen (mass median particle diameter 4·7 μm) was inhaled.

The same exercise and allergen challenges were used on each of the study days. Before and at regular time points after each challenge FEV1 was measured with a Vitalograph dry wedge spirometer, the better of two recordings being used for analysis.

Piriprost 2 mg (Upjohn Co, Kalamazoo, Michigan, USA) was dissolved in 200 μl of 10 mg/ml tris (hydroxymethylamino)methane buffered saline, to which 3·8 ml of nitrogen purged 0·9% saline solution was added. The nebulisers were sealed under a blanket of nitrogen, stored at 4°C, and protected from light until used. Vehicle solutions were prepared similarly but without piriprost. Neither the investigator nor the subjects were aware of the contents of the nebulisers.

Blood Measurements

At the start and immediately after completion of each of the protocols routine urine analysis was undertaken and blood withdrawn for full blood count; platelet count; liver function tests; and determination of the concentrations of urea, electrolyte, and blood glucose.

Study Protocol

Subjects attended the laboratory on six separate days at least one week apart. Before each visit all treatment was omitted for 12 hours. On three consecutive visits subjects underwent a five minute exercise challenge on the treadmill. On the remaining study days allergen bronchial provocation was performed. An initial test was performed to determine the strength of exercise and allergen stimuli required to produce a 25% fall in FEV1. After three baseline measurements of FEV1, subjects underwent exercise challenge to define the scope of the treadmill necessary to induce bronchoconstriction to a maximum 25% fall in FEV1. On the first allergen day increasing concentrations of the allergen extract to which the subject was most sensitive on skin prick testing were administered by inhalation at five minute intervals until a 25% fall in FEV1 occurred.

On the drug study days subjects attended the laboratory to inhale either piriprost (0·5 mg/ml in a total volume of 4 ml) or matched vehicle placebo. Subjects inhaled the drug or placebo for six minutes, breathing tidally through a mouthpiece from a preweighed Mini-neb disposable nebuliser. The nebuliser was reweighed after use to ensure that 1 mg drug had been delivered. Fifteen minutes after inhalation of the aerosols baseline recordings of FEV1 were made. Subjects then underwent either exercise challenge or allergen provocation followed by measurements of FEV1 at 5, 10, 15, 30, 45, and 60 minutes. After aller-
FEV<sub>1</sub> (% fall from baseline)

Fig 1  Effect of inhaled piriprost (1 mg) (●—●) or placebo vehicle (○——○) on the immediate change in FEV<sub>1</sub> after treadmill exercise. Each point represents the mean and standard error for observations made on 12 subjects. There were no significant differences between piriprost and placebo at any time point.

Fig 2  Effect of inhaled piriprost (1 mg) (●—●) or placebo vehicle (○——○) on the immediate changes in FEV<sub>1</sub> after allergen provocation (time zero). Each point represents the mean and standard error for observations made on 12 subjects. There were no significant differences between piriprost and placebo at any time point.

GEN CHALLENGE

Gen challenge nine subjects recorded peak expiratory flow rate measurements (PEF) with a Mini-Wright peak flow meter for the next 20 hours to monitor any late asthmatic reaction or change in morning PEF. Blood pressure and heart rate were measured before and after the study.

ANALYSIS OF DATA

The responses of FEV<sub>1</sub> to allergen and exercise provocation were plotted as absolute values and as percentage changes from baseline after drug or placebo, and mean values were calculated. The area under individual time course-response curves was calculated by trapezoid integration. A two way analysis of variance was used to compare the effect of drug and placebo on FEV<sub>1</sub> at each time point after challenge and on maximum fall in FEV<sub>1</sub> for each challenge irrespective of time. A decrease of more than 15% in PEF from the prechallenge baseline occurring 3–10 hours after allergen challenge was considered to indicate a late reaction.

RESULTS

Baseline measurements of FEV<sub>1</sub> after placebo or piriprost were not significantly different when patients attended for exercise (mean (SEM) 3.11 (0.11) and 3.06 (0.08)) and allergen (3.12 (0.12) and 3.23 (0.12)) provocation. After exercise the FEV<sub>1</sub> fell in all subjects to a minimum, irrespective of time after provocation, of 2.41 (0.18) (19.8% fall from baseline) after inhaled piriprost, and 2.51 (0.19) (18.7% fall) after placebo. There was no significant difference between piriprost and placebo in the maximum fall in

Table 1  Effect of piriprost and placebo on minimum peak expiratory flow (1 min<sup>-1</sup>) recorded during an allergen induced rate reaction in four subjects

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Minimum PEF 3–12 h</th>
<th>Piriprost</th>
<th>Minimum PEF 3–12 h</th>
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<td></td>
<td>2 h after challenge</td>
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<td>2 h after challenge</td>
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<tr>
<td>450</td>
<td>180 (10 h)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>410</td>
<td>240 (6 h)</td>
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<tr>
<td>500</td>
<td>440 (12 h)</td>
<td>520</td>
<td>470 (11 h)</td>
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<td>370</td>
<td>290 (5 h)</td>
<td>400</td>
<td>295 (3 h)</td>
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<tr>
<td>500</td>
<td>420 (13 h)</td>
<td>500</td>
<td>460 (5 h)</td>
</tr>
<tr>
<td>Mean (SEM) 455 (31)</td>
<td>333 (61)</td>
<td>458 (32)</td>
<td>366 (58)</td>
</tr>
</tbody>
</table>

*Time of minimum PEF recording.
FEV₁ irrespective of time after challenge, percentage fall from baseline, time taken to achieve the maximum fall in FEV₁ at each time point (fig 1), rate of recovery of FEV₁ after challenge, or the area under the fall in FEV₁-time response curve.

Inhaled allergen also provoked an immediate fall in FEV₁ to a minimum, irrespective of time after challenge, of 2-29 (0-23)1 (27-6% of baseline) on the piritrast study day and 2-23 (0-2)1 (26-9% fall from baseline) on the placebo day. Again, there was no significant difference between these values on the drug and placebo study days, or the percentage fall of FEV₁ from baseline, time taken to achieve the maximum fall in FEV₁ at each time point (fig 2), rate of recovery of FEV₁ after challenge, or area under the allergen fall in FEV₁-time response curve.

Four of the 12 patients experienced a late reaction 5–10 hours after allergen provocation after recovery from the immediate response. There was no difference between piritrast and placebo in the late asthmatic reaction as reflected by absolute falls in PEF or percentage change in this measurement from baseline (table 1).

In the six patients who recorded PEF at 8.30 am the day after allergen challenge there was no significant difference between post-piritrast and post-placebo values in PEF (345 (53)1 min⁻¹ and 361 (42)1 min⁻¹), or in the fall in peak flow rate overnight (98 (34) and 77 (12)1 min⁻¹) (table 2).

At no time during the study was any abnormality detected in blood or urine biochemistry or in blood pressure or heart rate after drug administration. During exercise provocation six patients complained of throat irritation or burning and three of wheezing after piritrast, whereas only one complained of wheezing, with no other symptoms, after placebo. In the allergen study one patient complained of cough, one of an altered sense of taste, eight of throat irritation or burning, and two of wheezing after piritrast, whereas only one had mild symptoms of rhinorrhoea during the placebo period (p < 0-01).

Discussion

In the present study piritrast, administered as an aerosol locally to the airways of 12 patients with asthma, did not confer any protection against the immediate bronchoconstrictor response induced either by inhaled allergen or by exercise. In a smaller group of subjects we were unable to show any protection from piritrast against the allergen induced late asthmatic reaction or in the fall in PEF on the morning after allergen challenge.

There are several possible interpretations of our findings. Firstly, at the dose used and administered by inhalation, piritrast may not have been bioavailable in the airways, because of either chemical instability, rapid absorption from the bronchi into the circulation, or enzymatic inactivation before it reached its site of action. In view of the documented instability of the drug, considerable care was taken in the preparation of the piritrast solutions; under the conditions we used the drug is stable for at least 10 hours. Nebulisation of a 1% solution prepared in the same manner, was sufficient to attenuate bronchoconstriction in sensitised rhesus monkeys challenged with Ascaris suum. Thus in non-human primates piritrast is unlikely to be inactivated when delivered to the airways. The dose administered in our subjects was about one fifth that administered to the monkeys and possibly insufficient drug was given to produce a pharmacological effect. In guinea pigs sensitised with ovalbumin, however, a calculated dose of inhaled piritrast of 5 µg per animal (1-5 µg/kg) delivered to the airways was sufficient to attenuate allergen induced bronchoconstriction. This dose was in the same range as the dose given to the asthmatic subjects in the present study. With the method of nebulisation we used 12% of the solution leaving a Mini-neb nebuliser reaches the lungs and 90% of this material is deposited in the airways. There is no information on how the pharmacokinetics of inhaled piritrast in man compare with those in the monkey, although it

<table>
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<tr>
<th>Placebo</th>
<th>2 h after challenge</th>
<th>18 h after challenge*</th>
<th>Piriprost</th>
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<td>360</td>
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<tr>
<td>Mean (SEM) 421 (31)</td>
<td></td>
<td>443 (29)</td>
<td>345 (53)</td>
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*Recorded at 8.30 am on the day after allergen challenge.
remains possible that reduced bioavailability may have occurred as a result of a short pulmonary-systemic transit time.28

Another possibility is that piriprost did not act on mediator secreting cells in the airways. This seems an unlikely explanation for our negative results, since piriprost has been shown to inhibit both the immunological and the ionophore dependent generation of LTC4 from human enzymatically dispersed lung cells17 and lung fragments from normal20 and asthmatic16 subjects, although in the case of dispersed cells reversal of inhibition was observed.17

The earliest phase (0–10 min) of the immediate asthmatic reaction can to a great extent be accounted for by the spasmogencic effect of released histamine29 30 and in vivo may be antagonised by potent H1 histamine receptor antagonists such as astemizole.31 The latter part of the response (10–30 min) may largely be attributed to the release of newly generated mast cell dependent mediators, which include PGD2, LTC4 and LTD4. In the present study the maximum degree of bronchoconstriction and the rate of recovery after allergen or exercise was not influenced by piriprost. The degree to which leukotrienes contribute to the immediate airway response to inhaled allergen in asthma has not been defined. If an H1 receptor antagonist, a cyclo-oxygenase inhibitor, and the addition of exogenous arachidonate were necessary for demonstrating optimal bioactivity of piriprost against allergen induced bronchoconstriction in guinea pigs,26 32 then similar steps may be necessary to show a similar drug effect in man. Moreover, the amount of LTC4 generated by IgE dependent challenge of human bronchoalveolar lavage cells is relatively small by comparison with the amount of mast cell derived PGD2 and histamine.10 By comparison with normal subjects, the airways of asthmatic patients are less hyperreactive to LTC4 than to inhaled histamine2 or methacholine,5 the difference from normal subjects being smaller with LTC4 than with the latter two substances.

After allergen provocation some patients develop late bronchoconstrictor responses accompanied by an increase in non-specific airways reactivity six to nine hours after challenge,53 associated with a peripheral blood eosinophilia and a large increase in the number of eosinophils recovered by bronchoalveolar lavage.34 35 It might be expected therefore that a drug capable of inhibiting the leukotriene generation pathway could also inhibit allergen induced release of LTB4 and hence recruitment of secondary effector cells for the late reaction. In the present study four of the nine patients who recorded PEF during 12 hours after allergen challenge developed late asthmatic reactions, but this was similar after piriprost and placebo, despite the in vitro capacity of piriprost to inhibit

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Effect of piriprost, a leukotriene inhibitor, on allergen and exercise induced bronchoconstriction in asthma


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