Effect of a 5-lipoxygenase inhibitor and leukotriene antagonist (PF 5901) on antigen-induced airway responses in neonatally immunized rabbits

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1 The effect of a single intratracheal dose (10 mg) of PF 5901 (2-[3(1-hydroxyhexyl) phenoxy]methyl] quinoline hydrochloride, a specific inhibitor of the 5-lipoxygenase pathway of arachidonic acid metabolism and a leukotriene D₄ antagonist) on airway changes induced in response to *Alternaria tenuis* aerosol challenge was assessed in adult rabbits neonatally immunized. Leukotriene generation was determined *in vivo* by measuring leukotriene B₄ (LTB₄) levels in bronchoalveolar lavage (BAL) fluid and *ex vivo* by measuring calcium ionophore-stimulated production of LTB₄ in whole blood.

2 While PF 5901 (10 mg) had no significant effect on the acute bronchoconstriction induced by antigen, this dose was sufficient to inhibit significantly the increase in airway responsiveness to inhaled histamine 24 h following antigen challenge ($P < 0.05$).

3 Total leucocyte infiltration into the airways induced by antigen, as assessed by bronchoalveolar lavage, was significantly inhibited by pretreatment with PF 5901 (10 mg). However, the pulmonary infiltration of neutrophils and eosinophils induced by antigen was unaltered by prior treatment with PF 5901 (10 mg).

4 PF 5901 (10 mg) had no effect on *ex vivo* LTB₄ synthesis in whole blood. However, the antigen-induced increase in LTB₄ levels in BAL 24 h following challenge was significantly inhibited ($P < 0.05$).

5 We suggest from the results of the present study that the antigen-induced airway hyperresponsiveness to inhaled histamine in immunized rabbits is mediated, at least in part, by products of the 5-lipoxygenase metabolic pathway, and is not dependent on the extent of eosinophil or neutrophil influx into the airway lumen.

Keywords: Antigen; leukotrienes; inflammation; airway hyperresponsiveness

Introduction

Asthma is considered a combination of features including airway obstruction with spontaneous and drug-induced reversibility, increased airway responsiveness to exogenous and endogenous stimuli and airway inflammation. A number of animal models have been established to mimic various aspects of the allergic asthmatic response (including airway hyperresponsiveness) and to investigate the mechanisms contributing to such changes. One such model is the neonatally immunized rabbit. It has been shown that rabbits neonatally immunized with antigen and adjuvant within 24 h of birth and repeatedly exposed to antigen during the first 3 months of life, preferentially produce IgE antibodies (Pinckard *et al.*, 1972). As adults these rabbits respond to antigen exposure by eliciting both an acute and late-onset airways obstruction (Larsen *et al.*, 1987; Shampain *et al.*, 1982), exacerbations of airway responsiveness (Bloom *et al.*, 1988) and pulmonary inflammatory cell infiltration (Marsh *et al.*, 1985). Given the similarities between this model and allergic asthma in man, the rabbit has been used as a test system to investigate the many and varied mechanisms contributing to allergic inflammation and asthma (Marsh *et al.*, 1985; Murphy *et al.*, 1986; Coyle *et al.*, 1989; 1990; Riccio *et al.*, 1993).

Metabolism of arachidonic acid via the enzymes phospholipase A₂ and 5-lipoxygenase leads to the formation of the leukotrienes, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE), all of which exert potent inflammatory effects. Leukotrienes belong to a family of structurally-related compounds of which the

most active are the sulphidopeptide, cysteinyl-containing leukotrienes (leukotriene C₄ (LTC₄), LTD₄ and LTE₄) and the dihydroxy acid LTB₄. The peptido-leukotrienes have been shown to induce many of the features of asthma both in animals and man, including bronchoconstriction, mucus hypersecretion, increased vascular permeability (reviewed in Barnes *et al.*, 1988), pulmonary inflammatory cell recruitment (Laitinen *et al.*, 1993) and airway hyperresponsiveness (Arm *et al.*, 1988; O'Hickey *et al.*, 1991). LTB₄ and 5-HETE are potent stimulators of leucocytes, including the chemotaxis, chemokinesis, adherence and aggregation of polymorphonuclear leucocytes (Ford-Hutchinson *et al.*, 1980; Ford-Hutchinson, 1991). *In vitro* 5-HPETE potentiates histamine release from human basophils (Peters *et al.*, 1982).

Peptido-leukotrienes have been detected in nasal secretions after allergen challenge *in vivo* (Creticos *et al.*, 1984; Freeland *et al.*, 1989), in bronchoalveolar lavage (BAL) fluid after local antigen challenge (Diaz *et al.*, 1989), urine after inhaled antigen provocation (Taylor *et al.*, 1989) and in pooled plasma from subjects with acute asthma (Zawrzewski *et al.*, 1985). Peptido-leukotrienes have also been detected in BAL fluid (Wardlaw *et al.*, 1989), sputum (Lam *et al.*, 1988), urine (Taylor *et al.*, 1989), plasma (Okubo *et al.*, 1987) and nasal secretions (Ferreri *et al.*, 1988) of asthmatic patients. In addition, fragments of lung (Dahlen *et al.*, 1983) and peripheral blood leucocytes from allergic asthmatic subjects (Mita *et al.*, 1986) have been shown to release leukotrienes after specific antigen challenge *in vitro*.

Asthmatic airways are capable of generating leukotrienes after exposure to inhaled antigen (Miadonna *et al.*, 1990; Wenzel *et al.*, 1990) and during acute asthmatic attacks

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(Taylor *et al.*, 1989). In view of these findings, a large number of studies have been carried out in human volunteers using a range of drugs interfering with the synthesis or actions of leukotrienes to modify airway responses to antigen (Britton *et al.*, 1987; Hui *et al.*, 1991; Taylor *et al.*, 1991; O'Shaughnessy *et al.*, 1993) and nasal responses to antigen (Knapp, 1990). To date however, there is little information on the influence such drugs have on antigen-induced airways inflammation.

In the present study we have investigated the ability of a specific leukotriene synthesis inhibitor and LTD₄ antagonist, PF 5901 (2-[3(1-hydroxyhexyl)phenoxyethyl]quinoline hydrochloride) (Van Inwegen *et al.*, 1987; Evans *et al.*, 1991) to influence acute bronchoconstriction, LTB₄ production, pulmonary cell infiltration and airway hyperresponsiveness to inhaled histamine induced by antigen in spontaneously breathing rabbits. Preliminary accounts of this work have been presented to the American Thoracic Society (Herd & Page, 1993) and to the British Pharmacological Society (Herd *et al.*, 1994).

Methods

Animals

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hampshire) of either sex were used throughout the study. The immunization protocol of neonatal rabbits was that described previously (Minshall *et al.*, 1993). Rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract in aluminium hydroxide (Al(OH)₃) moist gel and saline in the ratio of 2:1:1. Antigen and adjuvant administration was repeated weekly for the first month and then biweekly for the following two months. The methods described in this study were subject to Home Office approval and performed under the Animals (Scientific Procedures) Act, 1986.

Pulmonary function measurements

Immunized, adult rabbits (2.0–3.75 kg) were pre-medicated with diazepam (2.5 mg kg⁻¹, i.p.) and were subsequently administered Hypnorm (0.4 ml kg⁻¹, intramuscularly), a regime which produces neuroleptanalgesia and is recommended for recovery experiments in laboratory rabbits (Flecknall, 1987). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of 0.2–0.3 ml Hypnorm i.m. approximately every 30 min (Flecknall, 1987). Animals were intubated with a cuffed endotracheal tube (3.0 mm internal diameter; Mallinckrodt Laboratories, Athlone, Ireland), which was connected to a heated (37°C) Fleisch pneumotachograph (size 00). Measurements of flow, pleural pressure, transpulmonary pressure (the difference between thoracic and pleural pressure) and tidal volume were made according to methods previously described (Minshall *et al.*, 1993). Measurements of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated by an online respiratory analyser (Pulmonary Monitoring System PMS Version 5.1 Mumed Ltd., London).

Experimental protocol

On Day 1, airway responsiveness to aerosolized histamine was determined as a measure of lung function, as previously described (Herd *et al.*, 1992). Following each 2 min aerosol of histamine, animals were disconnected from the nebulizer and attached to the Fleisch tube. The following ten breaths were recorded and the mean value calculated. Cumulative dose-response curves were established and the provocation concentration (PC) of histamine which produced 50% increase in R_L (PC₅₀) and 35% decrease in C_{dyn} (PC₃₅) was

determined for each rabbit. These calculated parameters were used as indices of airway responsiveness.

On day 2, a suspension of PF 5901 (10 mg in a volume of 0.5 ml (40% polyethylene glycol (PEG-400) and 60% saline)) or vehicle alone (40% PEG-400 and 60% saline) was instilled directly into the lung via a cannula passed into the airway to the point of the bifurcation (via the endotracheal tube) 1 h prior to the commencement of the antigen challenge. Each antigen challenge consisted of a 2 min aerosol of saline followed by 5 consecutive aerosols of 2 min duration of *Alternaria tenuis* (10,000 PNU ml⁻¹), after which time respiratory function was recorded as described above.

On Days 3 and 5, airway responsiveness to histamine was determined as on Day 1.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately following histamine challenge on Days 1, 3 and 5. Saline (5 ml) was injected into the lungs through a polyethylene catheter (via the endotracheal tube) and then immediately aspirated and collected. Both total and differential cell counts were enumerated from BAL fluid as previously described (Herd *et al.*, 1992; Minshall *et al.*, 1993).

LTB₄ measurement

On two occasions, immediately preceding both drug instillation and antigen challenge (i.e. 1 h apart), 2 ml blood was sampled from the marginal ear vein and collected into heparin sodium-containing tubes. Duplicate 500 µl aliquots were equilibrated for 10 min at 37°C. Calcium ionophore A23187 (10 µg) was added to each duplicate to provide a final concentration of 15 µg ml⁻¹ and incubated for a further 30 min at 37°C. The samples were then centrifuged (10,000 g, 2 min, 4°C) and the resultant plasma frozen at –20°C for subsequent analysis of the concentration of LTB₄. Two × 1 ml of BAL fluid were aliquoted following each lavage and each similarly frozen at –20°C for subsequent analysis. Using the anti-LTB₄ antiserum described in the Drugs and Chemicals section, LTB₄ was quantified by radioimmunoassay (RIA) as previously described (Salmon *et al.*, 1982).

Analysis of results

For the lung function studies, statistical analysis was performed on log₁₀ transformed data (PC₅₀ and PC₃₅) in order to normalize the distribution of the data and to allow the application of parametric statistics. Two-way analyses of variance for repeated measures were employed to analyze histamine potency data and LTB₄ levels in BAL. Student's *t* test was used to analyze the bronchoconstriction data (R_L and C_{dyn}) (expressed as percentage change) and paired *t* test was used for the ionophore-stimulated whole blood LTB₄ generation. The total and differential cell counts were analyzed by Kruskal-Wallis one-way analysis of variance. Tukey's HSD test was used to determine differences in means when multiple comparisons were made for the parametric data (with analysis of variance) and distribution-free multiple comparisons were used to determine differences in means when multiple comparisons were made for the non-parametric data (Kruskal-Wallis one-way analysis of variance). Results were considered significant if *P* < 0.05.

Drugs and chemicals

The drugs and chemicals used were: *Alternaria tenuis* extract (Batch No. M1-152-7P16; 40,000 PNU ml⁻¹, 1 mg ml⁻¹; Greer Laboratories Inc. Lenoir, NC, U.S.A.); aluminium hydroxide (Al(OH)₃) moist gel (FSA Laboratory Supplies, Loughborough, Leicestershire); histamine diphosphate, calcium ionophore A23187, chromotrope 2R (Sigma Chemical Co., Poole, Dorset); haematoxylin (BDH Chemicals, Poole, Dor-

set); diazepam (Valium 5 mg ml⁻¹; Roche Products Ltd., Welwyn Garden City, Hertfordshire); Hypnorm (a mixture of fentanyl citrate 0.315 mg ml⁻¹ and fluanisone 10 mg ml⁻¹; Janssen Pharmaceutical Ltd., Grove, Oxfordshire); PF 5901 (2-[3(1-hydroxyhexyl)phenoxyethyl]quinoline hydrochloride, a gift from The Purdue Frederick Company, Norwalk, CT, U.S.A.); polyethylene glycol 400 (Fisons Scientific Equipment, Loughborough, Leicestershire); sterile pyrogen-free 0.9% sodium chloride solution (saline; Baxter Healthcare Ltd., Thetford, Norfolk). All reagents were of analytical grade. For *in vivo* studies, all solutions were prepared in saline.

Drugs and chemicals for LTB₄ assay

Rabbit anti-LTB₄ antiserum (Advanced Magnetics Inc., Metachem Diagnostics Ltd., Northampton, Northamptonshire) characterized by cross-reactivity <<1.0% for all leukotrienes and prostanoids tested other than LTB₄ (100%) and the LTB₄ enantiomers 5(S), 12(R)-diHETE (6.7%) and 5(S), 12(S)-diHETE (2.0%); analyte tracer [³H]-LTB₄ (Amersham International plc, Amersham, Buckinghamshire), specific activity 7.5–8.0 TBq mmol⁻¹; unlabelled standard LTB₄, Tris base and hydrochloride (Sigma Chemical Co., Poole, Dorset); gelatin, charcoal and sodium azide (BDH Chemicals, Poole, Dorset); dextran 150 (Fisons Scientific Equipment, Loughborough, Leicestershire).

Results

Airway responsiveness

Baseline absolute values of airway resistance (R_L) or dynamic compliance (C_{dyn}) were not significantly different between vehicle and drug-treated groups on any of the experimental days (Table 1). Furthermore, no significant difference was observed between airway responsiveness (PC₅₀ R_L or PC₃₅ C_{dyn}) to inhaled histamine at 3 months in immunized rabbits pretreated with either vehicle or PF 5901 (10 mg) (Tables 2a and 2b). The combined mean values are PC₅₀ R_L: 21.88 ± 1.33 mg ml⁻¹ (n = 20); PC₃₅ C_{dyn}: 19.50 ± 1.26 mg ml⁻¹ (n = 20).

Acute bronchoconstriction

Bronchoconstriction induced by antigen was not significantly different in groups of immunized rabbits pretreated with vehicle or PF 5901 (10 mg) directly administered into the airway (increases in R_L: vehicle 11.0 ± 5.1% (n = 10), PF 5901 18.0 ± 6.0% (n = 10); falls in C_{dyn}: vehicle -28.3 ± 5.2% (n = 10), PF 5901 -20.5 ± 4.8% (n = 10)).

Airway hyperresponsiveness

Antigen challenge to immunized rabbits pretreated with vehicle caused a significant decrease in histamine PC₅₀ (R_L) and

histamine PC₃₅ (C_{dyn}) values 24 h and 72 h later (Tables 2A and 2B; Figures 1a and 1b). In immunized rabbits pretreated with a single dose of PF 5901 (10 mg) directly instilled into the lungs, histamine PC₅₀ (R_L) and histamine PC₃₅ (C_{dyn}) values were not significantly altered 24 h or 72 h following antigen challenge (Tables 2A and 2B; Figures 1a and 1b). PF 5901 (10 mg) significantly inhibited antigen-induced airway hyperresponsiveness at 24 h following antigen challenge (R_L and C_{dyn}) compared with the vehicle-treated group (P < 0.05) (Tables 2A and 2B; Figures 1a and 1b).

Bronchoalveolar lavage

The mean pretreatment cell counts from immunized rabbits were not significantly different in rabbits administered either vehicle or PF 5901 (10 mg) (Table 3). Total leucocyte counts were significantly elevated in BAL fluid 24 h and 72 h following antigen exposure in rabbits pretreated with vehicle (P < 0.05). In rabbits pretreated with PF 5901 there were no significant differences between total cell numbers prior to or at any of the measured time points following antigen challenge (Table 3). The antigen-induced increase in total cell infiltration in vehicle-treated animals 24 h following challenge was significantly greater than that observed in the drug-treated group at the same time point (P < 0.05, Table 3). The number of neutrophils in BAL fluid was significantly elevated 1 h, 24 h and 72 h following antigen exposure in both treatment groups and there were no significant differences between the groups at any of the time points (Table 3). Similarly, there was a significant increase in the number of eosinophils observed in BAL fluid 24 h and 72 h following antigen in both vehicle- and PF 5901 (10 mg)-treated groups (P < 0.05). There were no significant differences in eosinophil numbers between the groups at any of the measured time points (Table 3); 24 h after antigen challenge mononuclear cell numbers were significantly reduced in rabbits pretreated with PF 5901 (10 mg), which was significantly different from that observed in the vehicle control group at 24 h (P < 0.05, Table 3).

LTB₄ measurements

There was no significant difference in *ex vivo* ionophore-stimulated LTB₄ production between rabbits pretreated with either PF 5901 (10 mg) or vehicle (Table 4a). In contrast, the antigen-induced increase in LTB₄ levels detected in BAL fluid 24 h following challenge was significantly inhibited by PF 5901 (10 mg) (Table 4b).

Discussion

Allergen exposure to allergic asthmatics often results in transient exacerbation of airway responsiveness to inhaled histamine (Cockcroft *et al.*, 1977; Hargreave *et al.*, 1986), which has been suggested as a model of asthma. The current study

Table 1 Baseline lung function prior to, 24 h and 72 h following antigen challenge in immunized rabbits pretreated with vehicle or PF 5901 (10 mg): (A) total lung resistance (R_L) and (B) dynamic compliance (C_{dyn})

A	Pre	Baseline R _L (cmH ₂ O l ⁻¹ s ⁻¹)	
		24 h	72 h
Vehicle	39.2 ± 2.8 (10)	35.3 ± 3.8 (10)	35.6 ± 4.8 (8)
PF 5901	36.8 ± 3.6 (10)	32.4 ± 3.4 (10)	34.0 ± 3.3 (8)
B	Pre	Baseline C _{dyn} (ml cmH ₂ O ⁻¹)	
		24 h	72 h
Vehicle	6.2 ± 0.8 (10)	6.4 ± 0.4 (10)	6.4 ± 0.7 (8)
PF 5901	5.8 ± 0.4 (10)	6.1 ± 0.8 (10)	5.4 ± 0.5 (8)

Values represent mean ± s.e.mean for the number of replicates shown in parentheses.

Table 2 Effect of vehicle or PF 5901 (10 mg) on airway responsiveness to inhaled histamine prior to, 24 h and 72 h following antigen challenge in immunized rabbits: (A) total lung resistance (PC_{50}) and (B) dynamic compliance (PC_{35})

A		Histamine PC_{50} (mg ml ⁻¹)		
PF 5901	Pre	24 h	72 h	
Vehicle	23.71 ± 1.48 (10)	9.34 ± 1.34 (10)*‡	8.85 ± 1.66 (9)*	
10 mg	20.18 ± 1.53 (10)	17.02 ± 1.44 (10)	13.96 ± 1.49 (9)	
B		Histamine PC_{35} (mg ml ⁻¹)		
PF 5901	Pre	24 h	72 h	
Vehicle	21.43 ± 1.34 (10)	4.29 ± 1.09 (10)*‡	5.83 ± 1.26 (8)*	
10 mg	17.70 ± 1.44 (10)	12.25 ± 1.32 (10)	8.20 ± 1.37 (9)	

Values represent mean ± s.e.mean for the number of replicates shown in parentheses in both (A) and (B).

* $P < 0.05$ compared with Pre control group.

‡ $P < 0.05$ compared with drug-treated group.

Table 3 Total and differential cell numbers recovered from bronchoalveolar lavage fluid (BAL), Pre, 1 h, 24 h and 72 h following antigen exposure in immunized rabbits pretreated with PF 5901 (vehicle and 10 mg)

		× 10 ⁵ cells ml ⁻¹			
		Total	Neutrophils	Eosinophils	Mononuclear cells
Vehicle	Pre (n = 9)	2.04 (0.30–5.65)	0.156 (0–0.914)	0.0080 (0–0.0638)	1.88 (0.30–5.65)
	1 h (n = 6)	2.40 (0.50–4.80)	0.743 (0.063–1.608)*	0.0550 (0–0.2880)	1.60 (0.43–2.90)
	24 h (n = 8)	5.71 (1.31–16.25)*‡	3.397 (0.572–11.050)*	0.4070 (0.0520–1.3810)*	1.91 (0.65–4.18)‡
	72 h (n = 6)	6.33 (1.20–25.90)*	3.474 (0.150–17.094)*	0.0635 (0–0.1900)*	2.79 (0.94–8.81)
PF 5901	Pre (n = 9)	2.99 (0.30–7.35)	0.247 (0–0.765)	0.0020 (0–0.0213)	2.75 (0.30–7.13)
	1 h (n = 8)	3.23 (0.50–8.95)	0.808 (0.095–2.026)*	0.0770 (0–0.3190)	2.34 (0.40–7.38)
	24 h (n = 8)	3.69 (0.45–8.95)	2.234 (0.248–5.281)*	0.2130 (0.0225–0.5700)*	1.25 (0.18–3.49)*
	72 h (n = 7)	3.86 (0.50–5.90)	1.339 (0.035–3.885)*	0.1463 (0–0.4640)*	2.38 (0.45–3.89)

Values represent mean with range in parentheses.

* $P < 0.05$ compared with Pre control

‡ $P < 0.05$ compared with drug-treated group.

Figure 1 Effect of vehicle (○, ●) ($n = 10$) and PF 5901 (10 mg) (□, ■) ($n = 10$) on antigen-induced airway hyperresponsiveness in immunized rabbits 24 h following antigen challenge. Closed symbols represent individual animal data and open symbols represent mean ± s.e.mean. (a) Histamine PC_{50} is the concentration of histamine required to cause a 50% increase in airway resistance (R_L); (b) Histamine PC_{35} is the concentration of histamine required to cause a 35% decrease in dynamic compliance (C_{dyn}). * $P < 0.05$ compared with pre-antigen control.

has demonstrated that exposure of allergic rabbits to an aerosol of antigen results in airway hyperresponsiveness to inhaled histamine 24 h later as described previously by others (Shampain *et al.*, 1982; Coyle *et al.*, 1989; 1990) which persisted at 72 h. Such changes in airway responsiveness were associated with an influx of inflammatory cells into the airways as assessed by BAL and accompanied by an increase in the levels of LTB_4 measured in BAL fluid 24 h later. This extends previous observations showing that the lipoxygenase pathway of arachidonic acid metabolism is activated secondary to antigen/antibody interactions and that products of the lipoxygenase pathway contribute to various facets of the allergic response.

The acute antigen-induced bronchoconstriction in the rabbit appears not to be mediated via the release of 5-lipoxygenase products since no significant effect was achieved with prior treatment with PF 5901. Alternatively, a pretreatment time of 1 h prior to antigen exposure may be insufficient for access by PF 5901 to the leukotriene-generating cells in the lung. Similar findings have recently been reported in monkeys and conscious sheep (Wegner *et al.*, 1993). These results differ from observations in man where peptido-leukotriene antagonists or leukotriene synthesis inhibitors attenuate the bronchoconstriction induced by allergen (Britton *et al.*, 1987; Taylor *et al.*, 1991; O'Shaughnessy *et al.*, 1993). Nonetheless, the magnitude of antigen-induced bronchoconstriction in the rabbit model is minimal and it is possible that lipoxygenase products could contribute to bronchoconstriction following a greater antigen burden.

The ability of antigen to induce airway hyperresponsiveness in the neonatally immunized rabbit was significantly attenuated by prior treatment with PF 5901. There was a trend toward airway hyperresponsiveness with respect to the compliance component in the presence of PF 5901. This observation may reflect differences in the contribution of lipoxygenase products in the upper and lower airways. It is

Table 4 Leukotriene B₄ (LTB₄) levels in (A) A23187-stimulated whole blood prior to, and 1 h post antigen challenge and (B) bronchoalveolar lavage (BAL) fluid prior to, and 1 h, 24 h and 72 h post antigen challenge, in immunized rabbits pretreated with vehicle or PF 5901 (10 mg)

A		LTB ₄ level (ng ml ⁻¹)			
		Pre	1 h post		
Vehicle (n = 8)		27.71 ± 5.29	32.47 ± 7.49		
PF 5901 (n = 8)		34.09 ± 7.55	35.32 ± 6.85		
B		LTB ₄ level (ng ml ⁻¹)			
		Pre	1 h post	24 h post	72 h post
Vehicle	0.256 ± 0.08	1.009 ± 0.35*	1.264 ± 0.33	2.339 ± 1.18	
n	8	8	8	8	
PF 5901	0.22 ± 0.1	0.33 ± 0.09	0.945 ± 0.35	0.994 ± 0.35	
n	8	8	8	6	

Values represent mean ± s.e.mean.

**P* < 0.05 compared with drug-treated group at the same time point.

possible that with a higher dose of the drug more complete inhibition of airway hyperresponsiveness could have been achieved. As this drug has been shown to have no effect on airway responsiveness to histamine following saline challenge, this inhibitory action of PF 5901 is not attributable to histamine H₁ antagonism or via some non-specific effect on airway responsiveness (Herd *et al.*, 1992). PF 5901 was originally described as a 5-lipoxygenase inhibitor (Van Inwegen *et al.*, 1987) and is now recognised as being a 5-lipoxygenase-activating protein (FLAP) inhibitor similar to the prototype FLAP inhibitor MK 886 (Evans *et al.*, 1991). Following cell stimulation, leucocyte 5-lipoxygenase is translocated from its cytosolic location to a cell membrane site (Rouzer & Kargman, 1988) and it has been reported that the involvement of FLAP is a requirement for the activation of 5-lipoxygenase (Dixon *et al.*, 1990; Miller *et al.*, 1990). However, recent findings in guinea-pig airways *in vitro* imply that activation of 5-lipoxygenase by antigen can occur with a lesser requirement for FLAP involvement (Yeadon *et al.*, 1993b), possibly by inducing an increase in intracellular calcium of smaller magnitude than that induced by calcium ionophores. In addition, the potency of some leukotriene synthesis inhibitors differs between cell types and in the same cell type against different stimuli (Noonan *et al.*, 1992). This implies that it is not axiomatic that inhibition of leukotriene generation in one cell type by PF 5901 will be accompanied by inhibition of leukotriene generation in all cells in the lungs. PF 5901 is also a moderately potent antagonist of LTD₄ (Van Inwegen *et al.*, 1987). Which of the actions of PF 5901 is responsible for the inhibition of antigen-induced airway hyperresponsiveness in the present study has not yet been determined. In atopic subjects the LTD₄ receptor antagonist ICI 204,219 was found to inhibit significantly allergen-induced airway hyperresponsiveness in response to inhaled histamine (Taylor *et al.*, 1991) and a recent study reports that the selective leukotriene antagonist, ONO-1078 reduces airway responsiveness to methacholine in asthmatic volunteers (Fujimura *et al.*, 1993). In contrast, the oral FLAP inhibitor MK-886 was without effect on allergen-induced increases in airway responsiveness to histamine (Friedman *et al.*, 1993).

The present study shows that PF 5901 is acting to inhibit the 5-lipoxygenase pathway in the cells responsible for the generation of LTB₄, as the increased levels of LTB₄ in BAL following antigen exposure were significantly reduced in animals pretreated with topical PF 5901. Given that LTB₄ has been found to induce airway hyperresponsiveness in allergic dogs (O'Byrne *et al.*, 1985) and allergic guinea-pigs (Pretolani *et al.*, 1993) and the related state of hyperalgesia (Levine *et al.*, 1984), it is plausible that this action of PF 5901 contributes to its ability to inhibit antigen-induced airway hyperresponsiveness. However, the precise cellular source of LTB₄ in the airways remains to be determined. No inhibition by

PF 5901 of ionophore-stimulated release of LTB₄ from peripheral blood leucocytes was noted *ex vivo*, consistent with the poor systemic bioavailability of this drug (Serajuddin *et al.*, 1988). The lower limit of detectability of LTB₄ by this RIA is 0.5 pg ml⁻¹, thus values of 1 ng ml⁻¹ in BAL of immunoreactive LTB₄ represent highly significant amounts of analyte. No signal in the RIA was obtained from either BAL taken prior to administration of PF 5901 or its vehicle, or the components of the vehicle when added to standard curves in the RIA. Since the specificity of the antiserum is extremely high for LTB₄, it is likely that the measurements accurately reflect the concentration of LTB₄ in BAL, or another related material whose cross-reactivity with the antiserum has not been determined.

PF 5901 did not inhibit the influx of eosinophils into the airways induced by antigen despite inhibiting the associated airway hyperresponsiveness. This observation is in accord with the suggestion above that PF 5901 may block the development of airway hyperresponsiveness by inhibiting generation of LTB₄ in BAL, without significant inhibition of peptidoleukotriene generation. Each of the cells involved in the process of airway inflammation release a distinct profile of lipid mediators following cell stimulation. For example, human eosinophils form predominantly peptido-leukotrienes (Henderson *et al.*, 1984) in contrast to neutrophils which produce mainly LTB₄ (Henderson & Klebanoff, 1983). It is possible that access of the drug was limited to the neutrophil as it is recruited to the lung at an earlier time point than the eosinophil.

The direct acting 5-lipoxygenase inhibitor, BW B70C, prevents pulmonary eosinophilia following antigen inhalation in guinea-pigs (Yeadon *et al.*, 1993a), and it has recently been shown that inhalation of LTE₄ can induce eosinophil accumulation in the lungs of asthmatics (Laitinen *et al.*, 1993). Thus, the inhibition by PF 5901 of both LTB₄ generation in BAL and airways hyperresponsiveness, but not of eosinophil accumulation secondary to antigen challenge, suggests that in the rabbit, the peptido-leukotrienes may be important for cell recruitment and LTB₄ for airway hyperresponsiveness. To date no such studies have reported the effect in man of 5-lipoxygenase inhibitors or LTD₄ antagonists on pulmonary cell recruitment. Furthermore, these observations support the idea that cell infiltration and airway hyperresponsiveness may be dissociated events as has been suggested by others. In guinea-pigs, capsaicin will inhibit antigen-induced airway hyperresponsiveness without modifying eosinophil infiltration (Ladenius & Biggs, 1989; Matsuse *et al.*, 1991) and cytokines can cause pulmonary eosinophilia without airway hyperresponsiveness (Kings *et al.*, 1990). Low dose antigen in guinea-pigs has been reported to induce eosinophil accumulation in BAL in the absence of any alteration in airways responsiveness, and at a higher dose of antigen, ketotifen, AH

21132, dexamethasone and aminophylline were shown to inhibit the pulmonary eosinophilia and not the associated airway hyperresponsiveness (Sanjar *et al.*, 1990). Furthermore, a recent study reports that eosinophil recruitment into the airways of mice is not sufficient to induce airway hyperresponsiveness (Vianna *et al.*, 1993). Together these results suggest that the presence of eosinophils within the airway lumen may not be the sole prerequisite for the development of airway hyperresponsiveness. Such an interpretation is consistent with clinical observations that airway hyperresponsiveness may be present in asthmatics without an observed eosinophil infiltrate (Lundgren *et al.*, 1988) and conversely, chronic eosinophilic bronchitis is not always associated with airway hyperresponsiveness (Gibson *et al.*, 1989). Further-

more, a recent study reports no correlation between baseline airway inflammation and increased airway responsiveness in mild asthmatics (Boulet *et al.*, 1993). However, it is possible that eosinophil numbers could be similar in both control and drug-treated groups and yet activation state different. Therefore, without measuring various markers of eosinophil priming in both groups, we cannot rule out the participation of this cell type.

In conclusion, antigen-induced airway hyperresponsiveness in neonatally immunized rabbits can be inhibited by topical airway administration of PF 5901, an effect that may not be related to the associated pulmonary infiltration of eosinophils.

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