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Effect of the glucocorticosteroid budesonide and a novel phosphodiesterase type 4 inhibitor CDP840 on antigen-induced airway responses in neonatally immunised rabbits

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1 The effects of the inhaled corticosteroid budesonide and a novel PDE 4 inhibitor CDP840 given systemically, were evaluated in a model of antigen-induced airway inflammation in the rabbit.

2 Adult litter-matched NZW rabbits (2.4-3.5 kg) immunised within 24 h of birth with *Alternaria tenuis* antigen were pretreated with budesonide (total dose 100 μg, inhaled over 2 days) or CDP840 (total dose 7 mg kg^{-1}, i.p. over 3 days), before antigen challenge. For each drug-treated group a parallel group of rabbits was pretreated with the appropriate vehicle. In all groups airway responsiveness to inhaled histamine was assessed and bronchoalveolar lavage (BAL) performed 24 h before and after antigen challenge.

3 Basal lung function in terms of total lung resistance (R_L; $cmH_2O\ l^{-1}s^{-1}$) and dynamic compliance (C_{dyn}; ml cmH_2O^{-1}) were unaltered by pretreatment with budesonide or CDP840 compared to their respective vehicles 24 h before or after antigen challenge.

4 The R_L component of the acute bronchoconstriction induced by inhaled *Alternaria tenuis* aerosol was unaffected by pretreatment with budesonide. However, budesonide prevented the fall in C_{dyn} due to antigen. Treatment with CDP840 significantly reduced antigen-induced acute bronchoconstriction in terms of both R_L and C_{dyn}.

5 Airway hyperresponsiveness (AHR) to inhaled histamine was indicated by reduced R_L PC_{50} (2.4-4.5 fold) and C_{dyn} PC_{35} (2.1-3.9 fold) values 24 h after antigen challenge. Treatment with either budesonide or CDP840 abolished the antigen-induced increase in responsiveness to inhaled histamine.

6 Total cells recovered per ml of BAL fluid increased 24 h after antigen challenge. Antigen-induced pulmonary eosinophilia was reduced (93%) in budesonide and (85%) in CDP840 treated rabbits. Antigen-induced increases in neutrophil numbers were reduced (76%) with budesonide but not CDP840 pretreatment.

7 Inhalation of *Alternaria tenuis* aerosol elicited an acute bronchoconstriction, followed 24 hours later by an increased responsiveness to inhaled histamine and pulmonary neutrophil and eosinophil recruitment. CDP840 was more effective than budesonide in preventing the antigen-induced increase in total lung resistance (R_L); however, both drugs prevented the antigen-induced reduction in dynamic compliance (C_{dyn}). CDP840 and budesonide also prevented antigen-induced AHR and eosinophilia in the immunised rabbit.

Keywords: Glucocorticosteroid; phosphodiesterase type 4 inhibitor; inflammation; airway hyperresponsiveness

Introduction

Asthma is characterized by reversible airway obstruction, inflammation and airway hyperresponsiveness (Barnes, 1989). Treatment with anti-inflammatory agents is currently recommended in all but the mildest cases of asthma (British Thoracic Society Guidelines, 1993). The anti-inflammatory corticosteroids are considered an important part of asthma management. However, there is increasing concern over the side effect profile of these drugs when given orally, and whilst the introduction of inhaled corticosteroids has reduced the problem, effects on growth have been observed in long term trials in children even with low dose inhaled corticosteroids (Tinkelman *et al.*, 1993). There is therefore a genuine need for an orally active safe alternative to corticosteroids in asthma management.

One such class of drugs is the phosphodiesterase type 4 (PDE 4) inhibitors. The cyclic adenosine monophosphate (cyclic AMP)-specific PDE 4 isoenzyme is prevalent in inflammatory cells including eosinophils, lymphocytes, mast cells, neutrophils, basophils and monocytes and also airway smooth muscle (Torphy & Undem, 1991; Giembycz & Dent, 1992). Agents which elevate cyclic AMP, including inhibitors of PDE 4, can suppress the activation of a variety of inflammatory cells and induce airway smooth muscle relaxation (Torphy & Undem, 1991).

CDP840 (**R**-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenylethyl] pyridine) is a novel potent and selective inhibitor of PDE 4 (IC_{50} 4 nM against human recombinant PDE 4A) with over a 10,000 fold selectivity for PDE 4 compared to other known classes of PDE (Hughes *et al.*, 1995; 1996). Furthermore, CDP840 has been shown to possess anti-inflammatory activity in the guinea-pig (Hughes *et al.*, 1995; 1996).

Neonatal immunisation of rabbits with subsequent repeated exposure to allergen over the first three months of life leads to allergic adult rabbits (Larsen *et al.*, 1987; Minshall *et al.*, 1993). Inhaled antigen in adult rabbits can elicit a similar sequence of events as seen in atopic asthmatics, including an acute bronchospasm and late phase obstruction (Larsen *et al.*, 1987), airway hyperresponsiveness (AHR) (Bloom *et al.*, 1988; Herd *et al.*, 1994) and pulmonary eosinophil recruitment (Marsh *et al.*, 1985; Herd *et al.*, 1994).

We have previously shown that a selective PDE 4 inhibitor,

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rolipram, prevents antigen-induced airway hyperresponsiveness and pulmonary eosinophilia in the immunised rabbit (Gozzard *et al.*, 1995). In the present study we have evaluated the effect of CDP840 and the clinically used steroid budesonide on antigen-induced airway responses in the immunised rabbit.

Methods

Immunisation

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hampshire) of either sex were used throughout the study. The neonatal immunisation of rabbits has been described previously (Minshall *et al.*, 1993). Rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract (40,000 PNU ml^{-1}) in aluminium hydroxide ($Al(OH)_3$) moist gel adjuvant and saline in the ratio of 2:1:1 v/v/v. Antigen and adjuvant administration was repeated weekly for the first month of life 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).

Measurement of lung function

Immunised, adult rabbits (2.4–3.5 kg) were premedicated with diazepam (2.5 mg kg^{-1}, i.p.) followed by Hypnorm (0.4 ml kg^{-1}, i.m.). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of Hypnorm (0.2 ml kg^{-1}, i.m.) every 15–30 min (Flecknall, 1987). Spontaneously breathing rabbits were intubated with a cuffed endotracheal tube (3.0 mm internal diameter; Mallinckrodt Laboratories, Athlone, Ireland), connected to a thermoregulated (37°C) Fleisch pneumotachograph (size 00) to allow measurement of tidal air flow. An oesophageal balloon catheter was inserted to provide a measure of intra-pleural pressure which approximates to transpulmonary pressure (the difference between thoracic and pleural pressure). Measurements were made as previously described (Minshall *et al.*, 1993). Total lung resistance (R_L; $cmH_2O\ l^{-1}s^{-1}$) and dynamic compliance (C_{dyn}; ml cmH_2O^{-1}) values were calculated by an on-line respiratory analyser (Pulmonary Monitoring System, version 5.1 Mumed Ltd., London) according to the method of Von Neergaard & Wirtz (1927).

Airway responsiveness

Airway responsiveness was determined in rabbits under neuroleptanalgesia by performing a cumulative dose-response curve to inhaled aerosolized histamine (1.25–80 mg ml^{-1}; aerosols generated by a De Vilbiss ultrasonic nebuliser; particle size 0.5–5 μm). Following each 2 min aerosol of histamine, 10 breaths were recorded and the mean values of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated.

Antigen challenge

Antigen challenge was performed with rabbits maintained under neuroleptanalgesia and instrumented for lung function measurement. Antigen challenge with inhaled aerosolized *Alternaria tenuis* extract in 0.9% saline (20,000 PNU ml^{-1}; De Vilbiss ultrasonic nebuliser), consisted of a 4 min aerosol of saline, followed by two 2 min antigen aerosols, then 4 consecutive antigen aerosols of 4 min duration (total 20 min with a 2 min dose cycle). After each challenge respiratory function was recorded as described above. A further 2 recordings at 15 and 30 min after antigen were also recorded.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately following histamine dose-response curves. Saline (5 ml) was injected into the lungs through a polyethylene catheter (positioned at the carina via the endotracheal tube) and then immediately aspirated and collected on ice. Total cell counts were enumerated under light microscopy using an improved Neubauer haemocytometer. For differential cell counts cytospin preparations were made. Aliquots of BAL fluid (75 μl) were centrifuged at 1300 r.p.m. for 1 min using a Shandon Cytospin 2 (Shandon Southern Instruments, Sewickley, PA, U.S.A.) at room temperature. Cells were then stained with a combination of haemotoxylin and chromotrope 2R according to the method of Lendrum (1944).

Experimental protocol

Budesonide study On day 1, rabbits were prepared and dose-response curves to inhaled histamine and BAL were performed. Budesonide (25 μg ml^{-1}, 50% ethanol in saline) or vehicle was given 10 min after the lavage by inhalation for 10 min to give a total dose of approximately 50 μg per rabbit. On day 2, rabbits were given a second identical dose of budesonide 30 min before the inhaled antigen challenge. On day 3, histamine responsiveness and BAL were performed as on day 1. Additionally, a group of 6 vehicle-treated rabbits were subjected to the above protocol but challenged with saline in place of antigen on day 2.

CDP840 study Rabbits were treated with CDP840 (1 mg kg^{-1}) or vehicle (saline acidified to pH 4.5 with HCl) given twice daily intraperitoneally (2 ml kg^{-1}) on days 1–3. On day 3, dose-response curves to inhaled histamine and BAL were performed. On day 4, rabbits were given a final dose of CDP840 or vehicle 1 h before the inhaled antigen challenge. On day 5, dose-response curves to inhaled histamine and BAL were performed as on day 3. Additionally, a group of 6 vehicle-treated rabbits were subjected to the above protocol but challenged with saline in place of antigen on day 4.

Expression and analysis of results

All values are means ± s.e.mean and statistical comparisons were considered significant if $P<0.05$.

Acute bronchoconstriction

Acute bronchoconstriction is expressed as the maximum percentage change in R_L and C_{dyn} from baseline during the antigen challenge and 30 min post challenge. Unpaired Student's *t* test was used to compare differences between treatment groups.

Airway responsiveness

Airway responsiveness to inhaled histamine is expressed as the percentage change in R_L and C_{dyn} from baseline values in response to increasing doses of inhaled histamine. The dose of histamine required to provoke a 50% increase (PC_{50}) in R_L and a 35% decrease (PC_{35}) in C_{dyn} were determined. The maximum percentage increase in R_L and decrease in C_{dyn} within the dose range of 1.25–80 mg ml^{-1} were also recorded, with both parameters used as indices of airway responsiveness. Paired Student's *t* tests were used to compare histamine responsiveness data before and after antigen challenge by use of the geometric mean of log_{10} transformed PC_{50} and PC_{35} values or percentage maximum R_L and minimum C_{dyn} values. Unpaired Student's *t* tests were used to compare drug and vehicle treated rabbits.

Bronchoalveolar lavage

At least 300 cells were differentiated as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria and expressed as absolute cell counts per ml of lavage fluid. Cell counts were performed blind with respect to the observer and compared by Wilcoxon rank analysis.

Drugs and chemicals

All reagents were of analytical grade. Drugs and chemicals used were: CDP840, (Celltech Therapeutics Ltd); *Alternaria tenuis* extract (40,000 PNU ml^{-1}, $\cong 1$ mg ml^{-1}; Greer Laboratories Inc. Lenoir, NC, U.S.A.); aluminium hydroxide ($Al(OH)_3$) moist gel (FSA Laboratory Supplies, Loughborough, Leicestershire). Budesonide, chromotrope 2R, histamine diphosphate, ethanol, (Sigma Chemical Co., Poole, Dorset); haematoxylin (BDH Chemicals, Poole, Dorset); diazepam (valium 5 mg ml^{-1}; Roche Products Ltd., Welwyn Garden City, Herts); hypnorm (fentanyl citrate 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1}; Janssen Pharmaceutical Ltd, Grove, Oxon.); sterile pyrogen-free 0.9% sodium chloride solution (saline; Baxter Healthcare Ltd., Thetford, Norfolk).

Results

Basal lung function

Basal airway resistance (R_L) and dynamic compliance (C_{dyn}) were not significantly different between budesonide (Table 1A) and CDP840 (Table 1B) treated rabbits and their respective vehicle-treated groups, either 24 h before challenge, immediately before challenge, or 24 h post challenge with either antigen or saline.

Acute bronchoconstriction

Effect of budesonide (Figure 1a) In vehicle-treated rabbits, antigen challenge caused an approximate 3 fold greater increase in R_L and decrease in C_{dyn} than observed in saline challenged rabbits. This antigen-induced acute bronchoconstriction was reduced (72.9±10.4%) in budesonide-treated rabbits in terms of C_{dyn} only ($P<0.05$). Antigen-induced increases in R_L were unaffected by budesonide treatment.

Effect of CDP840 (Figure 1b) In vehicle-treated rabbits, inhaled antigen caused an acute bronchoconstriction with an approximately 3 fold greater increase in R_L and decrease in C_{dyn} than observed in saline challenged rabbits. Pretreatment with CDP840 significantly inhibited the antigen-induced acute bronchoconstriction in terms of both R_L (58.0±3.7%) and C_{dyn} (43.0±2.2%) ($P<0.05$).

Airway responsiveness to histamine

Effect of budesonide In vehicle-treated rabbits no difference in histamine responsiveness was observed 24 h before or after

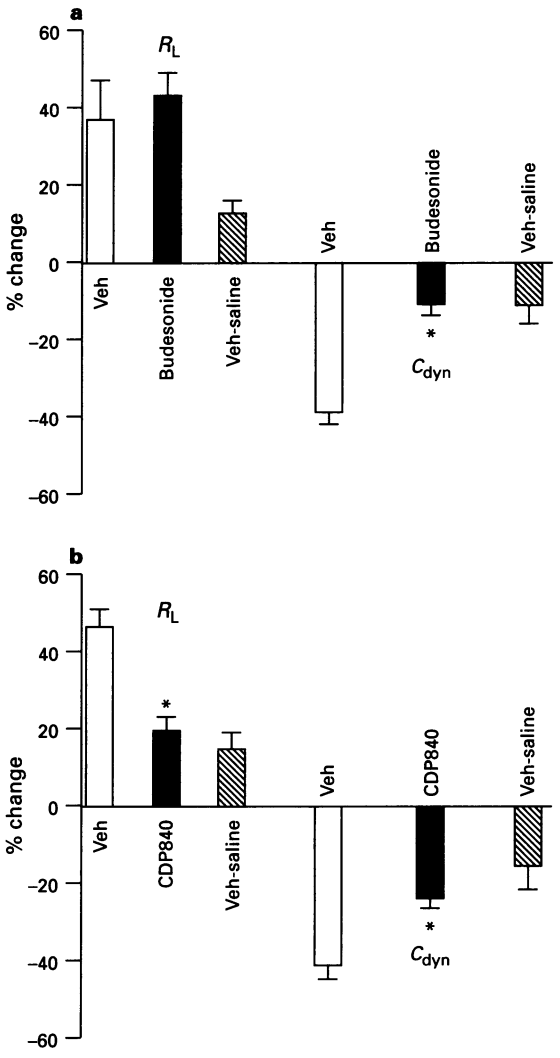

Figure 1 Antigen-induced acute bronchoconstriction is shown as percentage increase in total lung resistance (R_L) and decrease in dynamic compliance (C_{dyn}) in (a) budesonide vehicle ($n=10$) and budesonide ($n=10$) and (b) CDP840 vehicle ($n=9$) and CDP840 ($n=9$). Also shown is the response to inhaled saline in (a) budesonide vehicle ($n=6$) and (b) CDP840 vehicle ($n=6$). Vertical bars show s.e.mean. *Indicates $P<0.05$ vs vehicle-treated antigen-challenged group.

Table 1 Basal lung function (R_L and C_{dyn}) 24 h prior to challenge, immediately before challenge and 24 h post challenge with (a) saline and (b) antigen in (A) vehicle and budesonide pretreated rabbits and (B) vehicle and CDP840 pretreated rabbits

	R_L ($cmH_2O\ l^{-1}\ s^{-1}$)			C_{dyn} (ml cmH_2O^{-1})		
Treatment	*Pre*	*Challenge*	*Post*	*Pre*	*Challenge*	*Post*
(A)						
(a) Budesonide vehicle ($n=6$)	32.2±2.1	32.7±3.1	33.7±3.2	4.7±0.3	4.6±0.3	4.7±0.3
(b) Budesonide vehicle ($n=10$)	32.9±1.2	34.0±2.6	33.2±2.3	4.7±0.2	4.4±0.2	5.1±0.2
(b) Budesonide ($n=10$)	33.5±1.8	31.3±2.3	36.6±2.2	4.6±0.2	4.2±0.2	4.6±0.2
(B)						
(a) CDP840 vehicle ($n=6$)	38.6±4.1	35.2±4.1	37.0±2.0	4.3±0.3	4.6±0.3	4.6±0.5
(b) CDP840 vehicle ($n=9$)	33.5±1.8	35.4±2.0	31.8±1.5	4.4±0.1	4.1±0.2	4.6±0.3
(b) CDP840 ($n=9$)	33.3±2.5	37.5±2.2	34.7±2.3	4.5±0.2	4.4±0.2	4.8±0.2

saline challenge (Table 2A). Prior to antigen challenge histamine responsiveness was similar in terms of R_L PC_{50} and C_{dyn} PC_{35} in both vehicle- and budesonide-treated rabbits (Figures 2a and b).

In vehicle-treated rabbits, antigen-induced airway hyperresponsiveness was shown by a 4.5 fold reduction in R_L PC_{50} and a 3.9 fold reduction in C_{dyn} PC_{35} values. In budesonide-treated rabbits there was no reduction in R_L PC_{50} and C_{dyn} PC_{35} 24 h after antigen challenge ($P<0.05$). In vehicle-treated rabbits the maximum percentage increase in R_L to inhaled histamine ($R_{L,max}$) tended to be increased (48.8±35.7%) 24 h after antigen challenge (Figure 2a). Only a slight increase was observed in budesonide-treated rabbits with significantly reduced $R_{L,max}$ values compared to vehicle-treated rabbits ($P<0.05$). There was no change in the minimum compliance ($C_{dyn,min}$) values before or after antigen challenge in either budesonide or vehicle groups (Figure 2b). In vehicle-treated rabbits R_L PC_{50} values before challenge with saline were lower compared to the group challenged with antigen.

Effect of CDP840 In vehicle-treated rabbits challenged with saline (Table 2B) there was no difference in histamine responsiveness 24 hour before or after saline. Prior to antigen challenge (Table 2B, Figures 3a and b), histamine responsiveness was similar in terms of R_L PC_{50} in both vehicle- and CDP840-treated rabbits. However, CDP840 reduced histamine responsiveness in terms of C_{dyn} PC_{35} ($P<0.05$).

Antigen-induced airway hyperresponsiveness is shown in vehicle-treated rabbits by a 2.4 fold reduction in R_L PC_{50} and a 2.1 fold reduction in C_{dyn} PC_{35} values. In CDP840-treated rabbits there was no reduction in R_L PC_{50} and C_{dyn} PC_{35} 24 h after antigen challenge ($P<0.05$).

In vehicle-treated rabbits an increase in $R_{L,max}$ (45.0±20.4%) to inhaled histamine was observed 24 h after antigen challenge (Figure 3a). No increase was observed in CDP840-treated rabbits and $R_{L,max}$ was significantly lower than in vehicle-treated rabbits ($P<0.05$). There was no change in the minimum compliance ($C_{dyn,min}$) values before or after antigen challenge in either CDP840 or vehicle groups (Figure 3b).

Analysis of bronchoalveolar lavage (BAL) fluid

The volume of fluid recovered from BAL was not significantly different between drug and vehicle-treated rabbits before or after antigen challenge (2.0–3.0 ml, 40–60% recovery, data not shown).

Effect of budesonide In vehicle-treated rabbits challenged with saline there was no change in total cells, mononuclear cells or eosinophils. Neutrophils, however, were increased (3.0 fold) 24 h after saline challenge (Table 3A). Total leucocytes recovered in BAL fluid 24 h after antigen challenge were elevated (87.5%) in vehicle-treated rabbits whilst no increase was observed in budesonide-treated rabbits ($P<0.05$). This increase was due to the recruitment of eosinophil, neutrophil and mononuclear cell types.

Neutrophil numbers were significantly elevated 8.2 fold 24 h after antigen challenge in vehicle-treated rabbits with only a 1.2 fold increase observed in budesonide-treated rabbits ($P<0.05$). Eosinophils were virtually undetectable before antigen challenge and increased 120 fold 24 h after antigen challenge in vehicle-treated rabbits with only an 8 fold increase observed in budesonide-treated rabbits ($P<0.05$). Mononuclear cells tended to increase in vehicle-treated rabbits after antigen challenge whereas in budesonide-treated rabbits mononuclear cells tended to be reduced.

Effect of CDP840 In vehicle-treated rabbits challenged with saline there was no change in total cells, mononuclear cells or eosinophils. However, a 7.0 fold increase in neutrophils was observed 24 h after saline challenge (Table 3B). Total leucocytes increased significantly (22%) 24 h after antigen challenge in vehicle-treated rabbits only. This increase was largely due to the recruitment of neutrophils and eosinophils. Total leucocytes tended to be reduced (26.1%) in CDP840-treated rabbits before antigen challenge and remained unchanged after antigen challenge ($P<0.05$).

Neutrophils increased 9.5 fold 24 h after antigen challenge in the vehicle group with a similar 6.1 fold increase observed in the CDP840 group. Eosinophil recruitment due to antigen was shown in vehicle-treated rabbits by a 100 fold increase in eosinophil counts. A significantly reduced 30 fold increase in eosinophils was observed in CDP840-treated rabbits 24 h after antigen challenge ($P<0.05$).

Discussion

The inhaled glucocorticosteroids are effective anti-inflammatory agents and are used widely in asthma management (British Thoracic Society Guidelines, 1993). The present study demonstrates that budesonide exhibits marked anti-inflammatory activity and inhibits antigen-induced airway hy-

Table 2 The dose of inhaled aerosolized histamine (mg ml^{-1}) required to provoke a 50% (PC_{50}) increase in R_L and 35% (PC_{35}) decrease in C_{dyn} in (A) vehicle and budesonide and (B) vehicle and CDP840 pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

Treatment	R_L PC_{50} (mg ml^{-1}) Pre	R_L PC_{50} (mg ml^{-1}) Post	C_{dyn} PC_{35} (mg ml^{-1}) Pre	C_{dyn} PC_{35} (mg ml^{-1}) Post
A				
(a) Budesonide vehicle ($n=6$)	12.6±1.6	9.2±1.2	9.1±1.3	14.7±1.2
(b) Budesonide vehicle ($n=10$)	18.5±1.2	4.1±1.2†	10.1±4.2	2.6±1.3†
(b) Budesonide ($n=10$)	17.3±1.3	17.7±1.2$^{\#}$	7.3±1.1	9.5±1.2$^{\#}$
B				
(a) CDP840 vehicle ($n=6$)	22.8±1.1	19.9±1.0	20.4±1.4	18.7±1.2
(b) CDP840 vehicle ($n=9$)	21.5±1.3	9.1±1.2†	14.6±1.2	7.0±1.1†
(b) CDP840 ($n=9$)	23.3±1.2	21.7±1.2$^{\#}$	20.3±1.3$^{\#}$	20.4±1.2$^{\#}$

†Indicates $P<0.05$ compared to pre-antigen value (paired t test); $^{\#}$indicates $P<0.05$ compared to vehicle treated group (unpaired t test).

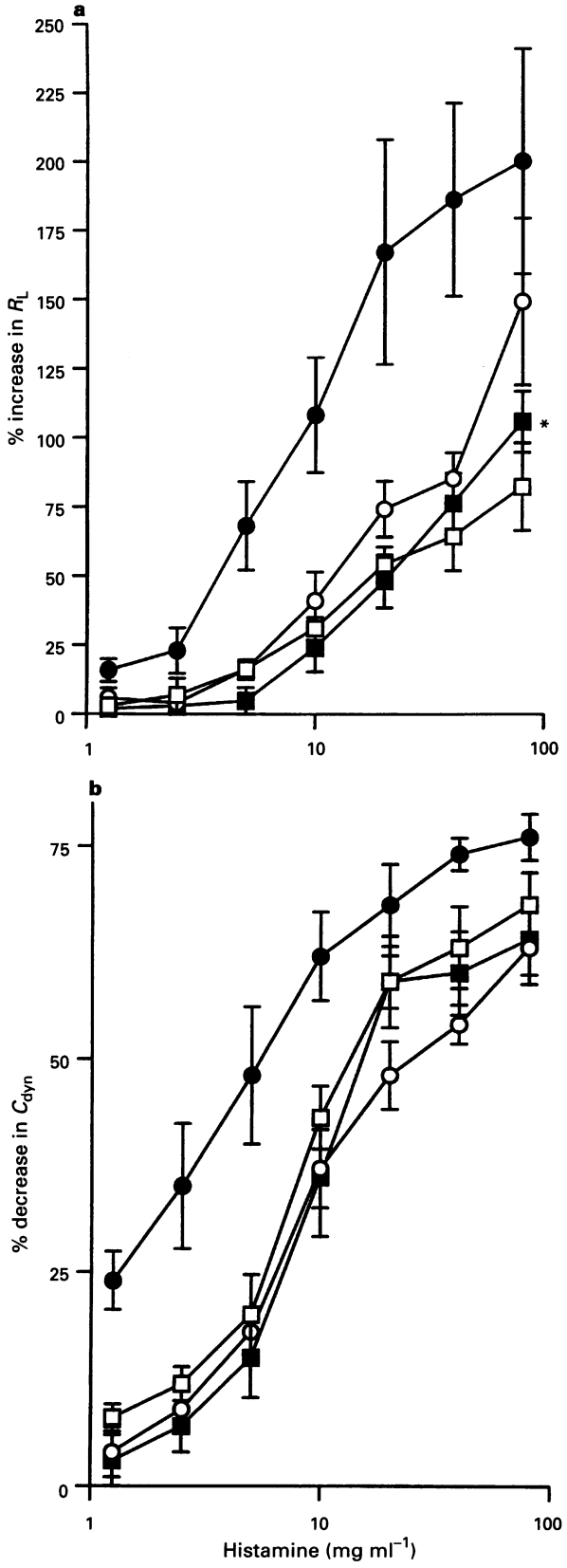

Figure 2 Percentage change in (a) total lung resistance (R_L) and (b) dynamic compliance (C_{dyn}) in response to inhaled histamine 24 h prior to or 24 h post antigen (Ag) challenge in vehicle (pre Ag (○); post Ag (●); $n=10$) and budesonide (pre Ag (□); post Ag (■); $n=10$)-treated rabbits. Vertical lines show s.e.mean. *$P<0.05$ vs vehicle group 24 h post antigen.

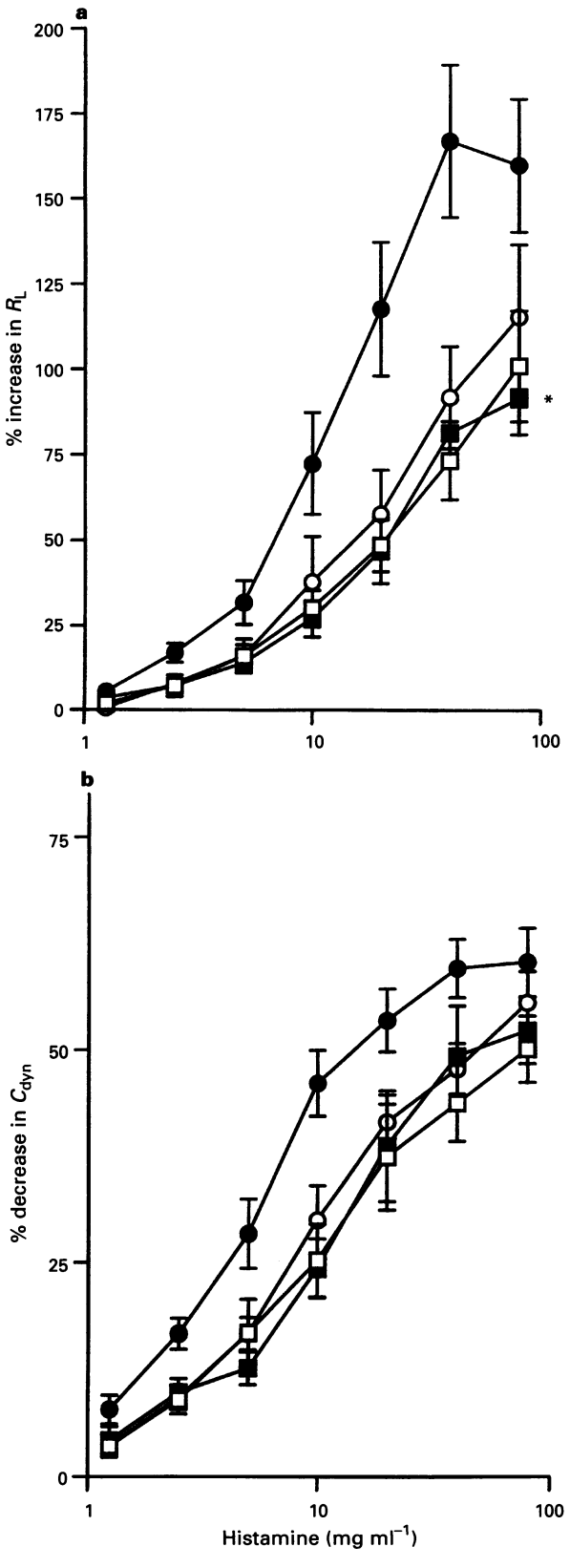

Figure 3 Percentage change in (a) total lung resistance (R_L) and (b) dynamic compliance (C_{dyn}) in response to inhaled histamine 24 h prior to or 24 h post antigen (Ag) challenge in vehicle (pre Ag (○); post Ag (●); $n=9$) and CDP840 (pre Ag (□); post Ag (■); $n=9$)-treated rabbits. Vertical lines show s.e.mean. *$P<0.05$ vs vehicle group 24 h post antigen.

Table 3 Total and differential cell counts ($\times 10^4$ cells ml^{-1}) recovered in BAL fluid from (A) vehicle and budesonide, and (B) vehicle and CDP840 pretreated rabbits 24 h prior to and 24 h post challenge with (a) saline and (b) antigen

	Total		*Mononuclear cells*		*Neutrophils*		*Eosinophils*	
Treatment	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
A								
(a) Budesonide vehicle ($n=6$)	26.3±3.3	21.6±2.4	25.9±3.3	20.5±2.0	0.38±0.02	1.1±0.4†	0.0±0.0	0.0±0.0
(b) Budesonide vehicle ($n=10$)	17.7±2.3	33.2±5.9†	17.2±1.9	25.0±4.4	0.5±0.2	4.1±1.1†	0.01±0.01	1.2±0.22†
(b) Budesonide ($n=10$)	22.8±3.0	17.1±2.3#	21.9±2.7	15.8±2.1	0.9±0.4	1.0±0.4#	0.01±0.01	0.08±0.04†#
B								
(a) CDP840 vehicle ($n=6$)	36.6±6.0	40.0±5.1	35.6±5.5	32.4±6.3	0.9±1.1	7.4±2.9†	0.03±0.07	0.1±0.02
(b) CDP840 vehicle ($n=9$)	34.1±3.6	41.4±3.2†	33.4±3.5	33.6±3.4	0.6±0.2	5.7±1.5†	0.02±0.02	2.0±0.3†
(b) CDP840 ($n=9$)	25.2±3.0	25.2±3.0#	23.9±2.8	18.8±2.7#	1.0±0.7	6.1±2.2†	0.01±0.01	0.3±0.1†#

† Indicates $P<0.05$ compared to pre-antigen value; # indicates $P<0.05$ compared to vehicle treated group (Wilcoxon analysis).

perresponsiveness in the neonatally immunised rabbit model. This demonstrates that the rabbit model is sensitive to a clinically proven anti-inflammatory steroid when administered by inhalation.

In animal models, PDE 4 inhibitors have been found to exhibit pulmonary anti-inflammatory and bronchodilator activity (Torphy & Undem, 1991; Nicholson & Shahid., 1994; Banner & Page, 1995; Hughes *et al.*, 1995; 1996).

We show here that the novel PDE 4 inhibitor CDP840 can attenuate antigen-induced airway hyperresponsiveness and pulmonary eosinophilia to a similar extent as that observed with inhaled budesonide and is more effective than budesonide in reducing the antigen-induced acute bronchoconstriction.

CDP840 was given at a dose of 1 mg kg^{-1} twice a day for three days before antigen challenge. With this dosing regime, we have previously shown that the PDE 4 inhibitor rolipram prevents antigen-induced eosinophil recruitment and airway hyperresponsiveness in the rabbit (Gozzard *et al.*, 1996).

Pretreatment with either budesonide or CDP840 had no effect on basal airway tone suggesting that neither agent, at the doses used, exhibited bronchodilator activity. CDP840 therefore exhibited anti-allergic and anti-inflammatory effects when using a non-bronchodilator dosing regime. Although PDE 4 inhibitors possess some bronchodilator activity, most studies show bronchodilatation of pre-constricted airways rather than prevention of bronchoconstriction or reversal of basal airway tone (Turner *et al.*, 1994).

In both vehicle groups, antigen challenge caused an immediate acute bronchoconstriction followed 24 h later by increased responsiveness to inhaled histamine (AHR) and pulmonary neutrophil and eosinophil recruitment. However, differences in airway responses were observed with the different vehicles used and with their different routes of adminstration. Inhalation of the budesonide vehicle (50% EtOH in saline) caused a greater percentage increase in total cells recovered in BAL fluid and an increased leftward shift in the histamine dose-response curve 24 h after antigen-challenge when compared to the CDP840 vehicle. Due to these disparities between vehicle groups, the effects of budesonide and CDP840 were compared against their respective vehicle groups only and no direct comparison between the two drugs was made.

The antigen-induced acute bronchoconstriction was similar in magnitude in both budesonide and CDP840 vehicle groups. CDP840 inhibited antigen-induced acute bronchoconstriction in terms of R_L and C_{dyn}, whereas budesonide only inhibited the C_{dyn} component of the response. Inhibition of antigen-induced bronchoconstriction with CDP840 is consistent with findings in guinea-pigs (Hughes *et al.*, 1995; 1996) and with the PDE 4 inhibitor rolipram (Howell *et al.*, 1992; Underwood *et al.*, 1994). Furthermore, Heaslip (1992) found that rolipram inhibits antigen-induced leukotriene release from homogenized-sensitized guinea-pig lung. Thus, inhibition of mediator release is a plausible explanation for the inhibition of the acute bronchoconstriction seen with CDP840 in the rabbit.

Airway hyperresponsiveness to inhaled aerosolized histamine was evident in both vehicle-treated groups 24 h post antigen, but not saline challenge. AHR was shown by increased sensitivity (reduction in PC_{50} and P_{35} values) and degree of bronchoconstriction (increased $R_{L,\ max}$ values). This hyperresponsiveness was abolished with CDP840 pretreatment, suggesting PDE 4 to be functional at sites relevant for the generation of airway hyperresponsiveness in the immunised rabbit.

Since capsaicin (Herd *et al.*, 1995) and the mixed 5-lipoxygenase/leukotriene D_4 antagonist PF5901 (Herd *et al.*, 1994) inhibited the development of airway hyperresponsiveness following antigen challenge in the rabbit, it has been suggested that a leukotriene-mediated activation of sensory C-fibres may contribute to antigen-induced airway hyperresponsiveness in this model. Interestingly, a range of PDE 4 inhibitors have been shown to inhibit electrical field stimulated excitatory non-adrenergic, non-cholinergic (eNANC) contraction of guinea-pig isolated bronchi *in vitro* whilst having no effect on contraction due to exogenous substance P (Qian *et al.*, 1994; Spina *et al.*, 1995). Also, CDP840 inhibits vagally mediated non-cholinergic bronchoconstriction in the guinea-pig with no effect on bronchoconstriction due to exogenous substance P (Holbrook *et al.*, 1996). Therefore, the efficacy of the PDE 4 inhibitors may, in addition to reducing leukotriene release, be due to attenuation of tachykinin release from C-fibres. Indeed this activity may explain the ability of both rolipram (Holbrook & Hughes, 1992) and CDP840 (Holbrook *et al.*, 1996) to reduce ozone-induced AHR to inhaled histamine, where the AHR is capsaicin-sensitive and thought to be due to increased tachykinin effects resulting from oxidant damage of luminal neutral endopeptidase (Yeadon *et al.*, 1992).

Budesonide also prevented the development of AHR after antigen challenge presumably via its anti-inflammatory actions, a finding consistent with numerous studies which show that corticosteroids can attenuate AHR in provoked and non-provoked individuals (Cockcroft & Murdoch, 1987; Wiebicke *et al.*, 1990).

It is not known as yet whether the AHR in this model is dependent upon the recruitment of inflammatory cells; however, both CDP840 and budesonide significantly reduced eosinophil recruitment detected in BAL fluid 24 h post antigen challenge.

Total cells, principally neutrophils and mononuclear cells, recovered in BAL fluid 24 h prior to antigen challenge were reduced though not significantly in CDP840-treated rabbits.

This effect on basal lung cellularity has been observed with rolipram in *Ascaris*-sensitive cynomolgous monkeys (Turner *et al.*, 1994) and rabbit (Gozzard *et al.*, 1996). The PDE 4 inhibitors may therefore affect basal trafficking of leucocytes into the airways.

In both vehicle groups, saline challenge did not elicit eosinophil recruitment but caused a similar degree of neutrophil recruitment as that observed after antigen challenge, which suggests that eosinophil recruitment is antigen-dependent in this model. CDP840 significantly inhibited antigen-induced eosinophil recruitment in the rabbit, consistent with findings of PDE 4 inhibitors in the guinea-pig (Underwood *et al.*, 1993; Hughes 1995; 1996) and primate (Turner *et al.*, 1994). Surprisingly, CDP840 had no effect on neutrophil recruitment and this may reflect selective inhibition of antigen-dependent cell recruitment. In contrast, inhibition of antigen-induced neutrophil recruitment with rolipram has been observed in primates (Turner *et al.*, 1994) and guinea-pigs (Underwood *et al.*, 1994).

Budesonide inhibited both neutrophil and eosinophil recruitment as shown in a range of studies in animals and man (Brattsand & Selroos, 1994). The mechanisms by which budesonide exerts its anti-inflammatory actions are numerous and include inhibition of cytokines, lipid derived mediators, cytotoxic proteins and reactive oxygen species from T-lymphocytes, monocyte/macrophages, neutrophils and eosinophils.

As with budesonide the precise mechanisms by which CDP840 inhibits leucocyte recruitment in this model is unclear. The principle mechanism by which CDP840 acts is probably through elevation of cyclic AMP. Chan & Hanifin (1993) showed elevated PDE 4 in T-lymphocytes and monocytes from atopic individuals with the atopic isoenzyme more sensitive to the PDE 4 inhibitor Ro 20-1724 than the PDE 4 from normals. Such findings have recently been confirmed with a range of PDE 4 inhibitors using mononuclear cells from subjects with atopic dermatitis (Banner *et al.*, 1995).

A number of processes required for the recruitment of eosinophils into the airways may be susceptible to PDE 4 inhibition. These include, reduced release of chemoattractive mediators in response to antigen, inhibition of the diapedesis process or a reduced ability of eosinophils to respond to chemoattractants. Turner *et al.* (1994) found that rolipram inhibited tumour necrosis factor-α (TNFα) levels in BAL fluid 4 h after antigen challenge in *Ascaris*-sensitive cynomolgous monkeys. Rolipram has also been shown to inhibit TNFα release from human T-lymphocytes (Schudt *et al.*, 1992) and monocytes (Endres *et al.*, 1991). TNFα induces the expression of a variety of adhesion molecules involved in diapedesis process (Pober & Cotran, 1990) and it is therefore possible that PDE 4 inhibitors may act to inhibit leucocyte migration processes by reducing TNFα-mediated adhesion molecule expression or adhesion molecule expression directly (Wellicome *et al.*, 1990; Pober *et al.*, 1993). CDP840 has been shown to inhibit interleukin-5-mediated eosinophil recruitment into the pleural cavity of rats (Hughes *et al.*, 1995; 1996) indicating a reduced responsiveness of the eosinophil to chemoattractive mediators or inhibition of the diapedesis process. However, due to the ubiquitous distribution of the PDE 4 isoenzyme in the airways CDP840 may act at a number of cellular sites.

In conclusion, with the dosing regimes used, the novel PDE 4 inhibitor CDP840 inhibited antigen-induced airway hyperresponsiveness and eosinophilia, to a similar degree as that achieved with a clinically used steroid budesonide, but is more effective in preventing antigen-induced acute bronchoconstriction in the immunised rabbit.

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