British Journal of Pharmacology (1997) 122, 439–446

RPR 106541, a novel, airways-selective glucocorticoid: effects against antigen-induced CD4$^+$ T lymphocyte accumulation and cytokine gene expression in the Brown Norway rat lung

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1 The effects of a novel 17-thiosteroid, RPR 106541, were investigated in a rat model of allergic airway inflammation.

2 In sensitized Brown Norway rats, challenge with inhaled antigen (ovalbumin) caused an influx of eosinophils and neutrophils into the lung tissue and airway lumen. In the lung tissue there was also an accumulation of CD4$^+$ T lymphocytes and increased expression of mRNA for interleukin-4 (IL-4) and IL-5, but not interferon-γ (IFN-γ). These findings are consistent with an eosinophilia orchestrated by activated Th2-type cells.

3 RPR 106541 (10–300 μg kg^{-1}), administered by intratracheal instillation into the airways 24 h and 1 h before antigen challenge, dose-dependently inhibited cell influx into the airway lumen. RPR 106541 (100 μg kg^{-1}) caused a significant ($P<0.01$) (98%) inhibition of eosinophil influx and a significant ($P<0.01$) (100%) inhibition of neutrophil influx. RPR 106541 was approximately 7 times and 4 times more potent than budesonide and fluticasone propionate, respectively.

4 When tested at a single dose (300 μg kg^{-1}), RPR 106541 and fluticasone each caused a significant ($P<0.01$) (100%) inhibition of CD4$^+$ T cell accumulation in lung tissue. Budesonide (300 μg kg^{-1}) had no significant effect. RPR 106541 and fluticasone (300 μg kg^{-1}), but not budesonide (300 μg kg^{-1}), significantly ($P<0.05$) inhibited the expression within lung tissue of mRNA for IL-4. RPR 106541 (300 μg kg^{-1}) also significantly ($P<0.05$) inhibited expression of mRNA for IL-5.

5 The high topical potency of RPR 106541 in this model, which mimics important aspects of airway inflammation in human allergic asthmatics, suggests that this glucocorticoid may be useful in the treatment of bronchial asthma.

Keywords: Asthma; airway inflammation; T-lymphocytes; eosinophils; interleukin-4; interleukin-5; glucocorticoids

Introduction

In bronchial asthma there is a characteristic chronic inflammation of the airway mucosa. Mediators released by infiltrating cells such as eosinophils are implicated in pathological changes which lead to a decline in lung function and the development of airway hyperreactivity. There is now increasing evidence that antigen-specific CD4$^+$ T lymphocytes and the cytokines that they secrete play a central role in the pathogenesis of allergic asthma. Currently of great interest are the effects of interleukin-4 (IL-4) which plays a critical role in IgE production by influencing class switching by B cells (Pène *et al*., 1988) and IL-5 which promotes the terminal differentiation, migration, activation and survival of eosinophils (Sanderson, 1992).

Glucocorticoids are currently the most effective and widely-used agents available to treat asthmatic airway inflammation. Indeed, their under-use has been associated with high asthma mortality and morbidity (British Thoracic Society, 1993). However, because of the uniformity of glucocorticoid receptors throughout the body, chronic use carries a risk of systemic adverse reactions. Much effort has therefore focused on developing glucocorticoids such as budesonide and fluticasone propionate which have high topical potency in the airways after inhalation but have a reduced capacity to produce systemic effects (Fuller *et al*., 1995).

In the current study we have investigated the contribution of T lymphocytes and the pro-inflammatory cytokines IL-4 and IL-5 in a Brown Norway rat model of airway inflammation and have studied the anti-inflammatory potential of a new glucocorticoid, RPR 106541 ((20**R**)-16α.17α-butylidenedioxy-6α,9α-difluoro-11β-hydroxy-17β-(methylthio)androst-4-en-3-one). We have compared its efficacy with those of budesonide and fluticasone propionate.

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Methods

Animals

Brown Norway rats (male) were purchased from Harlan-Olac (Bicester, U.K.) at 7–9 weeks of age (125–150 g) and housed for 2 weeks before being sensitized. Food and water were supplied *ad libitum*. U.K. Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) Act 1986 were strictly observed.

Sensitization, treatment and challenge regimens

Rats were immunized on days 0, 12 and 21 with ovalbumin (100 μg, intraperitoneal, i.p.) administered together with aluminium hydroxide (100 mg) in saline (1 ml, i.p.). Unsensitized animals received vehicle alone at the same time points.

Twenty four hours and 1 h before antigen challenge, rats were anaesthetized with halothane (4% in O_2 for 2 min) and the head inclined up at an angle of 45°. A blunt-ended dosing needle was gently inserted through the larynx into the trachea as described previously (Raeburn *et al*., 1992) to allow intratracheal (i.t.) instillation of a glucocorticoid or vehicle (saline, 0.5 ml kg^{-1}). Suspensions of glucocorticoids were prepared by grinding and sonicating the solid drug in saline to give a concentration of 2 mg ml^{-1}. Dilutions were made as required in saline.

On day 30, rats were placed individually in a 3.5 l chamber and challenged by exposure for 20 min to an aerosol of ovalbumin generated from a 5 mg ml^{-1} solution by a nebulizer (deVilbiss Pulmosonic, deVilbiss Healthcare, London, U.K.) and delivered into the chamber by an air pump (300 ml min^{-1}). Control (unchallenged) animals were similarly exposed to an aerosol of saline. Treatment groups contained either 5 or 6 animals.

Cell recovery

Airway lumen Twenty four hours after antigen challenge, rats were killed (sodium pentobarbitone, 200 mg kg^{-1}, i.p.) and the trachea was cannulated immediately. Cell influx into the airway lumen was quantified by counting cells recovered in bronchoalveolar lavage (BAL) fluid. BAL was performed by flushing the airways with 2 aliquots (2×10 ml kg^{-1}) of RPMI 1640 medium containing 10% foetal calf serum (FCS) delivered through the tracheal cannula. The 2 aliquots were pooled and centrifuged ($700 \times g$, 10 min), the supernatant removed and the cells resuspended in 1 ml of RPMI (+10% FCS). Eosinophils and neutrophils were counted by means of standard morphological criteria from cytocentrifuge preparations stained with Wright-Giemsa stain. Other cell types were not counted. T cells were not counted in BAL fluid because, in common with others (Holt & Schon-Hegrad, 1987), we have found that the airway lumen contains very low numbers of these cells.

Lung tissue

Twenty four hours after antigen challenge, rats (separate groups from those used for BAL) were killed (sodium pentobarbitone, 200 mg kg^{-1}, i.p.). Cell influx into lung tissue was quantified by counting cells disaggregated from the tissue by collagenase incubation. The thorax was opened and the lungs and heart were removed intact. A cannula was inserted through the right ventricle to allow the pulmonary vasculature to be flushed at low pressure with Hank's balanced salt solution (4°C) to remove the blood pool of cells. The left lung lobes together with associated main stem bronchus were removed, immediately frozen in liquid nitrogen and stored at −80°C until required for mRNA assay (below).

The right lung lobes with associated main stem bronchus were removed and cut into 0.5 mm pieces. To disaggregate cells, 200 mg of lung tissue were incubated (37°C) in 45 ml RPMI/10% FCS containing collagenase (20 u ml^{-1} for 2 h, then 60 u ml^{-1} for 1 h). The recovered cells were filtered (mesh size 70 μm), washed and resuspended in 1 ml RPMI/10% FCS. Eosinophils, neutrophils and monocytes/macrophages were counted as described above. $CD2^+$, $CD4^+$ and $CD8^+$ lymphocytes were counted by use of flow cytometry as described below. Other cell types were not counted. In preliminary experiments, collagenase incubation did not significantly change expression of CD2, CD4 or CD8 on lymphocytes (data not shown).

Flow cytometry

Cells (1×10^5) isolated from lung tissue were incubated in separate tubes in the presence of fluorophore-labelled monoclonal antibodies. An anti-CD2 antibody was used as a pan-T cell marker as it is expressed on approximately 80% of these cells in the rat lung (Strickland *et al*., 1996). Cells were incubated with either anti-CD2 antibody plus anti-CD4 antibody, or anti-CD4 antibody plus anti-CD8 antibody. Incubations were carried out in the dark for 30 min at 4°C with saturating levels of antibody, typically 1 μg in the final reaction volume (100 μl). Following incubation, erythrocytes were lyzed with FACS lyzing solution and the remaining cells were stabilized with a fixing reagent (Immunoprep). Unlabelled cells and matched IgG isotype controls were used to control autofluorescence and non-specific binding, respectively. Flow cytometry was performed by use of an EPICS XL flow cytometer and software (Coulter Electronics, U.K.). Preliminary gating was achieved with forward- and side-scatter characteristics to define the total lymphocyte population. These cells were then gated according to $CD2^+$ fluorescence and the $CD4^+$ population was defined within this population. Alternatively, $CD4^+$ and $CD8^+$ cells were similarly gated within the total lymphocyte population and defined by QUADSTAT gating.

Semiquantitative RT-PCR

RNA extraction RNA was extracted from the left lung lobes (RNA isolation kit, Fluka, Gillingham, U.K.). Chopped (0.5 mm) lung tissue (500 mg) was homogenized in denaturing solution (10 ml containing 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, 70 μl β-mercaptoethanol, 4°C). After addition of sodium acetate (1 ml of a 2 M solution), water-saturated phenol (10 ml) and chloroform/isoamylalcohol (49:1, 2 ml), the mixture was centrifuged ($10000 \times g$, 20 min, 4°C). The aqueous phase, containing RNA, was mixed with an equal volume of isopropanol. The RNA was allowed to precipitate (−20°C, 1 h), recovered by centrifugation ($10000 \times g$, 20 min, 4°C) and dissolved in denaturing solution (3 ml). Isopropanol (3 ml) was added and the RNA was precipitated and recovered as before. RNA was dissolved in water (1 ml) containing dithiothrietol (5 mM) and RNA Guard (50 u ml^{-1}). The RNA yield was determined by means of ultraviolet spectrophotometry (260 nm) and the quality confirmed by agarose gel electrophoresis.

Reverse transcription and polymerase chain reaction (RT-PCR) RNA (4 μg) was reverse transcribed to cDNA in 40 μl reverse transcriptase buffer (50 mM Tris-HCl pH 8.0, 50 mM KCl, 5 mM $MgCl_2$) containing RNA Guard (40 u), dithiothrietol (10 mM), oligo dT (5 μg), 0.5 mM each dNTP and AMV reverse transcriptase (20 u). Tubes were incubated at 20°C for 10 min, then 37°C for 60 min. Reverse transcriptase was inactivated by incubation at 72°C for 10 min.

PCR was carried out to amplify target fragments of IL-4, IL-5, IFNγ and β-actin (included as a control gene) cDNA. PCR mixtures consisted of 5 μl of cDNA in 40 μl polymerase buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 2.5 mM $MgCl_2$) containing 1.25 μM of each primer pair for a particular target sequence, 0.25 mM of each dNTP and 2 u Taq polymerase. The mixture was overlaid with mineral oil. PCR was carried out in a thermal cycler (Mastercycler 5330, Eppendorf). Each cycle consisted of 3 steps: denature (94°C for 1 min or 5 min for the first cycle), primer anneal (60°C for 2 min), primer extension (72°C for 2 min). Preliminary PCR runs were carried out to determine the numbers of cycles necessary to ensure linear amplification of each target sequence (data not shown). From these results it was determined that the numbers of cycles needed was 20 for β-actin and 30 for IL-4, IL-5 and interferon γ (IFNγ). Negative controls (PCR mixtures without reverse transcribed RNA) and positive controls (a standard cDNA sample) were included in all PCR runs.

PCR products together with molecular size markers (100 base-pair DNA ladder), stained with ethidium bromide, were separated by 2% agarose gel electrophoresis. Bands were visualized by u.v. transillumination and scanned. Integrated optical densities (OD × mm) were calculated by image analysis software (Pharmacia Imagemaster, Pharmacia Biotech, Uppsala, Sweden).

Data analysis

Cell numbers in BAL fluid are expressed as cells ml^{-1}. Cell numbers in lung tissue are expressed as cells mg^{-1}. Results are presented as mean ± s.e.mean. Statistical significance of the differences between group means was determined by the Kruskal Wallis multiple comparison test with $P<0.05$ being accepted as significant. For each glucocorticoid, the ID_{50} value (the dose causing 50% inhibition) against eosinophil and

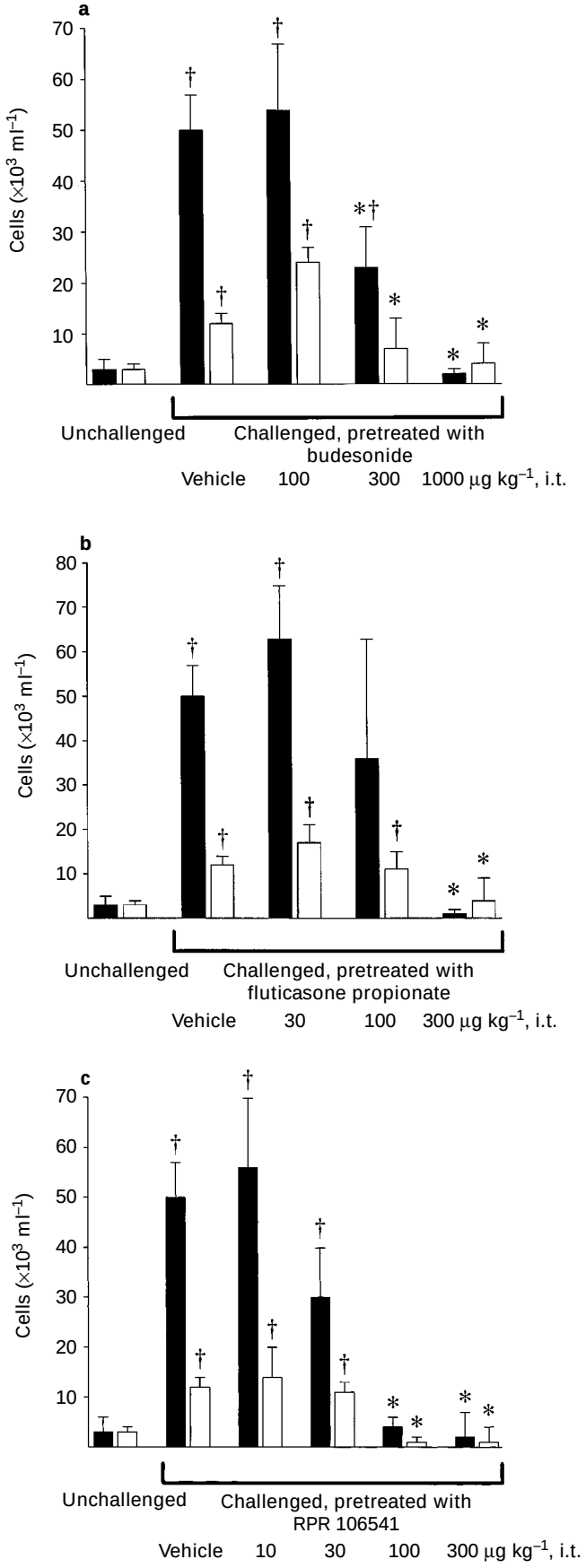

Figure 1 The effects of budesonide (a), fluticasone propionate (b) and RPR 106541 (c) on antigen-induced eosinophil (solid columns) and neutrophil (open columns) influx into the airway lumen of Brown Norway rats. Compounds were administered by intratracheal (i.t.) instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. Cells were recovered by bronchoalveolar lavage 24 h after challenge. Group size was 6. Results represent mean ± s.e.mean. †$P < 0.05$ compared to unchallenged group. *$P < 0.05$ compared to challenged group pretreated with vehicle.

neutrophil influx into the airway lumen was calculated from group means by logarithmic regression analysis.

Drugs and materials

RPR 106541 and fluticasone propionate were synthesized by Rhône-Poulenc Rorer (Dagenham Research Centre, U.K.). Budesonide and other materials were purchased from Sigma (Poole, U.K.) except for: aluminium hydroxide from Prolabo (Fontenay, France); sodium pentobarbitone (Sagatal) from Rhône Mérieux (Harlow, U.K.); Roswell Park Memorial Institute (RPMI) 1640 medium, Hank's balanced salt solution and foetal calf serum (FCS) from Gibco (Paisley, U.K.); fluorophore-labelled monoclonal antibodies for flow cytometry (anti-CD2: MRC OX34 labelled with fluorescein isothiocyanate; anti-CD4: W3/25 labelled with phycoerythrin; anti-CD8: MRC OX8 labelled with fluorescein isothiocyanate) from Serotec (Kidlington, U.K.); Immunoprep from Coulter Electronics (U.K.); fluorescence-activated cell sorting (FACS) lyzing solution from Becton-Dickinson (London, U.K.); reagents for RNA isolation from Fluka (Gillingham, U.K.); dithiothreitol, RNA Guard and reagents for reverse-transcription polymerase chain reaction (RT-PCR) from Pharmacia Biotech (St. Albans, U.K.); PCR primers from R&D Systems (Abingdon, U.K.).

The PCR primers were designed from rat cDNA sequences. The primer sequences were: IL-4, 5′ACCTTGCTGTCA CCCTGTTCTGC3′ and 5′GTTGTGAGCGTGGACTCATT CACG3′ which amplify a 352 base-pair cDNA fragment; IL-5, 5′TGCTTCTGTGCTTGAACGTTCTAAC′3 and 5′TTCT CTTTTTGTCCGTCAATGTATTTC3′ which amplify a 298 base-pair cDNA fragment; β-actin, 5′AGAAGAGCTATGAG CTGCCTGACG3′ and 5′CTTCTGCATCCTGTCAGCC-TACG3′ which amplify a 236 base-pair cDNA fragment; IFN-γ, 5′ACACTCATTGAAAGCCTAGAAAGTCTG3′ and 5′ATTCTTCTTATTGGCACACTCTCTACC3′ which amplify a 432 base-pair cDNA fragment. IL-4, IL-5 and β-actin primer sequences have been published elsewhere (Noble *et al.*, 1993). IFNγ primer sequences were provided in a personal communication (A. Noble, Department of Pathology, Harvard Medical School, U.S.A. and D.M. Kemeny, Department of Immunology, King's College School of Medicine and Dentistry, London, U.K.).

Results

Effects of antigen challenge

Cell influx into the airway lumen Sensitization with antigen (ovalbumin) had no significant effect on the numbers of eosinophils or neutrophils recovered from the airway lumen by BAL (data for unsensitized animals are not shown). Twenty four hours after antigen challenge there was a significant influx of eosinophils and neutrophils into the airway lumen (Figure 1).

Cell influx and mRNA expression in the lung tissue Sensitization with antigen had no significant effect on the numbers of eosinophils, neutrophils, monocytes/macrophages or lymphocytes recovered from the lung tissue (data for unsensitized animals are not shown). Twenty-four hours after antigen challenge there was a significant accumulation of eosinophils, neutrophils and $CD2^+$ lymphocytes (Figures 2 and 3). The lymphocyte accumulation included a significant increase in the number of $CD4^+$ but not $CD8^+$ T cells (Figure 3). The number of monocytes/macrophages in the lung tissue was also higher in challenged animals but the difference was not significant (Figure 2).

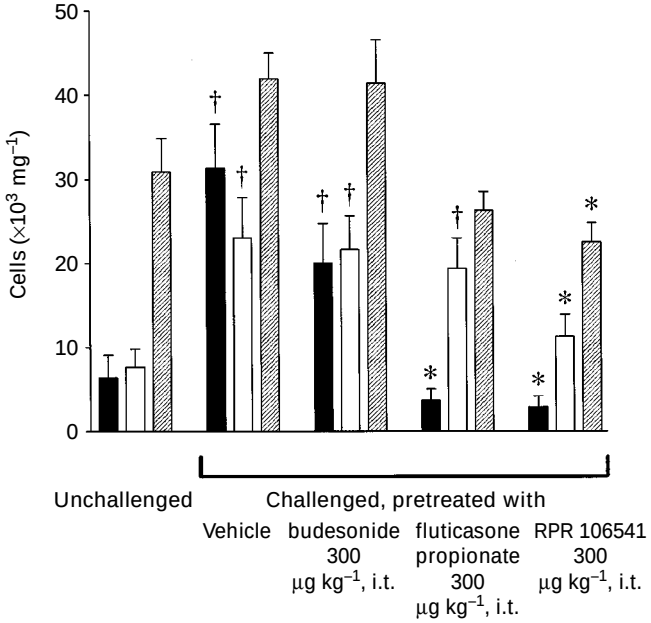

Figure 2 The effects of budesonide, fluticasone propionate and RPR 106541 on antigen-induced eosinophil (solid columns) and neutrophil (open columns) influx, and the number of monocytes/macrophages (hatched columns), in lung tissue of Brown Norway rats. Compounds were administered (300 μg kg^{-1}) by intratracheal instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. Cells were disaggregated from lung tissue 24 h after challenge. Group size was 6. Results represent mean$\pm$s.e.mean $\dagger P<0.05$ compared to unchallenged group. $*P<0.05$ compared to challenged group pretreated with vehicle.

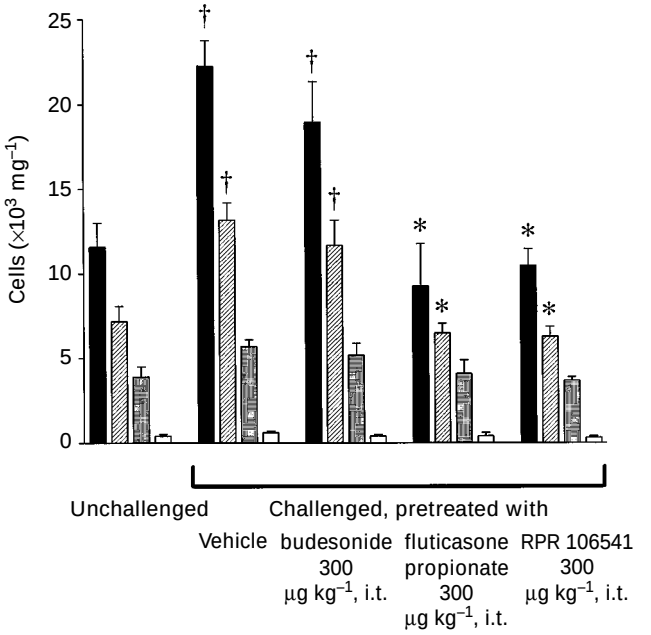

Figure 3 The effects of budesonide, fluticasone propionate and RPR 106541 on the antigen-induced influx of CD2$^+$ (solid columns) and CD4$^+$ (hatched columns) lymphocytes, and the numbers of CD8$^+$ (stippled columns), and CD4$^+$ CD8$^+$ (open columns) lymphocytes in lung tissue of Brown Norway rats. Compounds (300 μg kg^{-1}) were administered by intratracheal instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. Cells were disaggregated from lung tissue 24 h after challenge. Group size was 6. Results represent mean$\pm$s.e.mean, $^{\dagger}P<0.05$ compared to unchallenged group. $*P<0.05$ compared to challenged group pretreated with vehicle.

Sensitization with antigen had no significant effect on the expression within lung tissue of mRNA for any of the proteins studied (data for unsensitized animals are not shown). Twenty four hours after antigen challenge there was a significant increase in expression of mRNA for IL-4 and IL-5, but not IFN-γ (Figures 4 and 5). There was no significant difference between groups in expression of mRNA for the control protein β-actin (data not shown).

Effects of glucocorticoids

Cell influx into the airway lumen In antigen (ovalbumin)-challenged rats, intratracheal instillation of steroid vehicle had no significant effect on cell accumulation (data for untreated animals are not shown). Intratracheal instillation (24 h and 1 h before challenge) of budesonide, fluticasone propionate and RPR 106541 caused a dose-related, significant inhibition of eosinophil and neutrophil influx (Table 1, Figure 1). RPR 106541 was about 7 times and 4 times more potent than budesonide and fluticasone propionate, respectively, as an inhibitor of eosinophil and neutrophil influx.

Cell influx and mRNA expression in the lung tissue In antigen (ovalbumin)-challenged rats, intratracheal instillation of steroid vehicle had no significant effect on cell accumulation or levels of mRNA expression (data for untreated animals are not shown). RPR 106541 (300 μg kg^{-1}, i.t.) significantly inhibited the accumulation of eosinophils, neutrophils, CD2$^+$ cells and CD4$^+$ T cells (Figures 2 and 3). The number of monocytes/macrophages was also significantly reduced by this glucocorticoid (Figure 2). Fluticasone propionate (300 μg kg^{-1}, i.t.) also significantly inhibited the accumulation of eosinophils, CD2$^+$ cells and CD4$^+$ T cells but had no significant effect on neutrophils or monocytes/macrophages (Figures 2 and 3). Budesonide (300 μg kg^{-1}, i.t.) had no significant effect on the numbers of any cell type in the lung tissue (Figures 2 and 3).

RPR 106541 (300 μg kg^{-1}, i.t.) significantly inhibited the increased expression within lung tissue of mRNA for IL-4 and IL-5 (Figures 4 and 5). Fluticasone (300 μg kg^{-1}, i.t.) also significantly inhibited the increased expression of mRNA for IL-4. Expression of IL-5 mRNA was also lower, although the difference was not statistically significant. At the dose studied, budesonide (300 μg kg^{-1}, i.t.) had no effect on expression of mRNA for IL-4 or IL-5. None of the glucocorticoids had any effect on the expression of mRNA for IFNγ (Figure 4), or the control protein β-actin (data not shown).

Discussion

We demonstrated previously that antigen-challenge in the Brown Norway rat results in an influx of eosinophils and neutrophils into the airways (Underwood *et al*., 1995). We have now investigated the contribution of T lymphocytes and the pro-inflammatory cytokines IL-4 and IL-5 to the inflammatory process in this model. Further, we have used the model to study the anti-inflammatory effects of a novel glucocorticoid, RPR 106541 and have compared its efficacy with that of the reference glucocorticoids budesonide and fluticasone propionate.

After antigen challenge, an accumulation of T cells in the lung tissue was demonstrated by a significant increase in the number of CD2$^+$ cells recovered. An interesting observation was that this increase included a significant accumulation of CD4$^+$ (helper) T cells, since an increased number of activated (IL-2 receptor expressing, CD25$^+$) CD4$^+$ T cells within the airways is a feature of human asthma (Bradley *et al*., 1991; Wilson *et al*., 1992; Robinson *et al*., 1993a; Walker *et al*., 1994). In contrast to the accumulation of CD4$^+$ T cells, we saw no significant change in the number of CD8$^+$ T cells.

We counted cells recovered from a homogeneous sample of the lung tissue to allow us to determine the overall recruitment

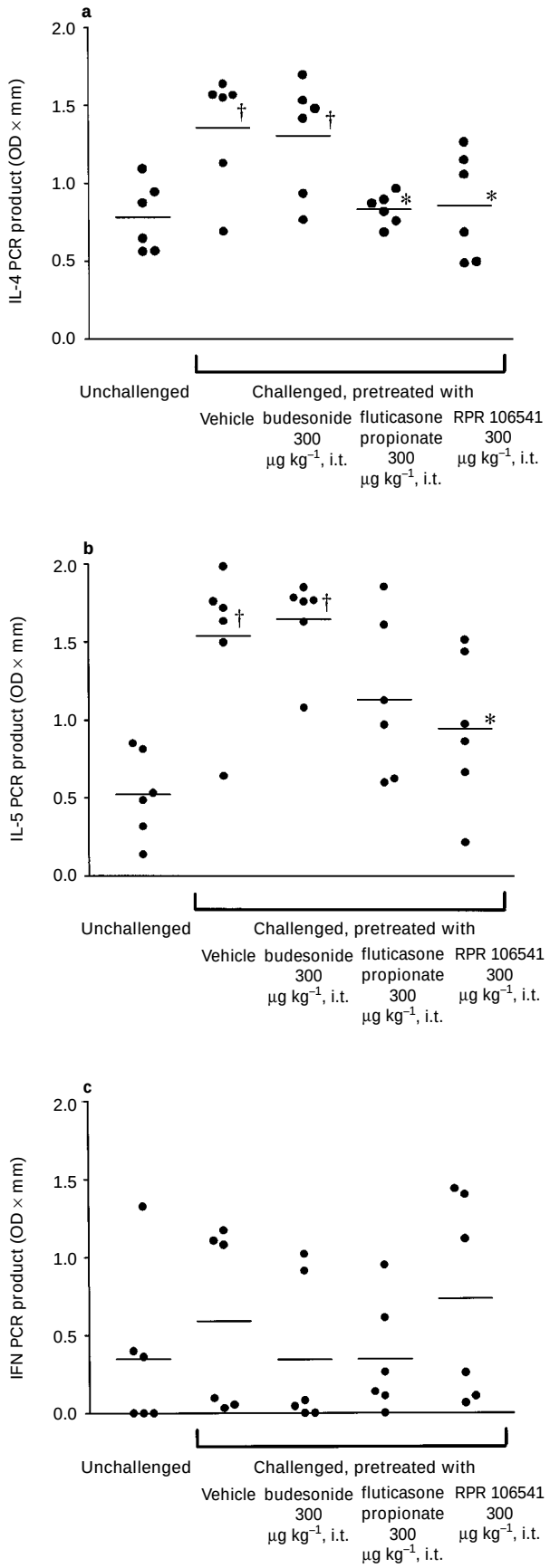

Figure 4 The effects of budesonide, fluticasone propionate and RPR 106541 on the antigen-induced increase in the expression of (a) IL-4 and (b) IL-5 mRNA, and the expression of (c) IFN-γ mRNA, in lung tissue of Brown Norway rats. Compounds (300 μg kg^{-1}) were administered by intratracheal instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. RNA was extracted from lung tissue 24 h after challenge and mRNA measured by RT-PCR. PCR products, stained with ethidium bromide, were separated by agarose gel electrophoresis, visualized by u.v. transillumination and scanned. Integrated optical densities (OD × mm) were calculated by image analysis software. Group size was 6. Horizontal lines represent group means. †$P<0.05$ compared to unchallenged group. *$P<0.05$ compared to challenged group pretreated with vehicle.

of T cells and other cell types from the pulmonary vasculature into the tissue. Within the rat lung, accumulations of T cells are found in the airway mucosa, the bronchus-associated lymphoid tissue, the alveolar septa and in peribronchial and perivascular regions (Holt & Schon-Hegrad, 1987). It is likely that different areas will hold a localized accumulation of specific T cell subsets, and that changes in these populations will occur as a result of both migration of resident cells within the lung tissue and recruitment from the vasculature. Indeed, although we have demonstrated a recruitment of CD4$^+$, but not CD8$^+$, T cells from the vasculature, others (Haczku *et al*., 1996) have found that CD8$^+$, but not CD4$^+$, T cells accumulate within the airway mucosa 24 h after antigen challenge in the Brown Norway rat.

Studies with murine T cells *in vitro* have demonstrated that CD4$^+$ T cells can be classified into T helper 1 (Th1) and Th2 subsets, on the basis of the combination of cytokines which they produce. Among other cytokines, activated Th1 cells secrete IFN-γ but little IL-4 and IL-5. In contrast, activated Th2 cells secrete IL-4 and IL-5 but little IFN-γ. Both cell types produce IL-3 and granulocyte macrophage-colony-stimulating factor (GM-CSF; Mosmann & Coffman, 1989). Although the validity of the Th1/Th2 classification to the human situation has generated much debate, it is becoming clear that there is an increase in the numbers of cells expressing mRNA for IL-4 and IL-5, but not IFN-γ, in the airways of human allergic asthmatics after an antigen challenge and in symptomatic asthmatics when compared with asymptomatic controls (Robinson *et al*., 1993b,d). Furthermore, biopsy studies have revealed increased expression of mRNA for IL-5 in the bronchial mucosa of human asthmatics compared with that of non-asthmatic controls (Hamid *et al*., 1991; Fukuda *et al*., 1994). Bronchial lavage fluid from human asthmatics contains a higher concentration of IL-4 and IL-5, but not IFN-γ, than that from non-asthmatic controls, demonstrating that increased IL-4 and IL-5 gene expression does lead to increased protein synthesis (Walker *et al*., 1994). These findings suggest that an increase in the number of functional Th2-type CD4$^+$ T cells is implicated in the pathogenesis of human allergic asthma.

In our rat model, the antigen-induced accumulation of CD4$^+$ T cells in the lung was accompanied by increased expression within the tissue of mRNA for IL-4 and IL-5 but, importantly, not IFN-γ. These changes in cytokine gene expression in the lung tissue of the antigen-challenged Brown Norway rat resemble those observed in studies of human asthmatics and are similarly consistent with a Th2-type profile of cytokine gene expression by CD4$^+$ T cells. In human asthmatic airways, mRNA for IL-4 and IL-5 is expressed predominantly by cells which are CD2$^+$ or CD3$^+$, indicating that T cells are the main source of these cytokines (Robinson *et al*., 1993b; Ying *et al*., 1995). However, there is evidence that other cell types, including eosinophils and mast cells, may contribute to the production of these cytokines, at least in human asthmatics (Broide *et al*., 1992; Bradding *et al*., 1994; Moqbel *et al*., 1995). We have yet to confirm the cellular source of cytokine mRNA in the rat lung.

In our study, the recruitment of CD4$^+$ T cells into the lung, and the increased expression of mRNA for IL-5, was accompanied by a marked infiltration of eosinophils. This finding reflects the airway eosinophilia which is a well-established characteristic of human allergic asthma and which correlates with both the number of CD4$^+$ CD25$^+$ T cells and the number of cells expressing mRNA for IL-5 in the airways (Bradley *et al*., 1991; Walker *et al*., 1991; Robinson *et al*., 1993b).

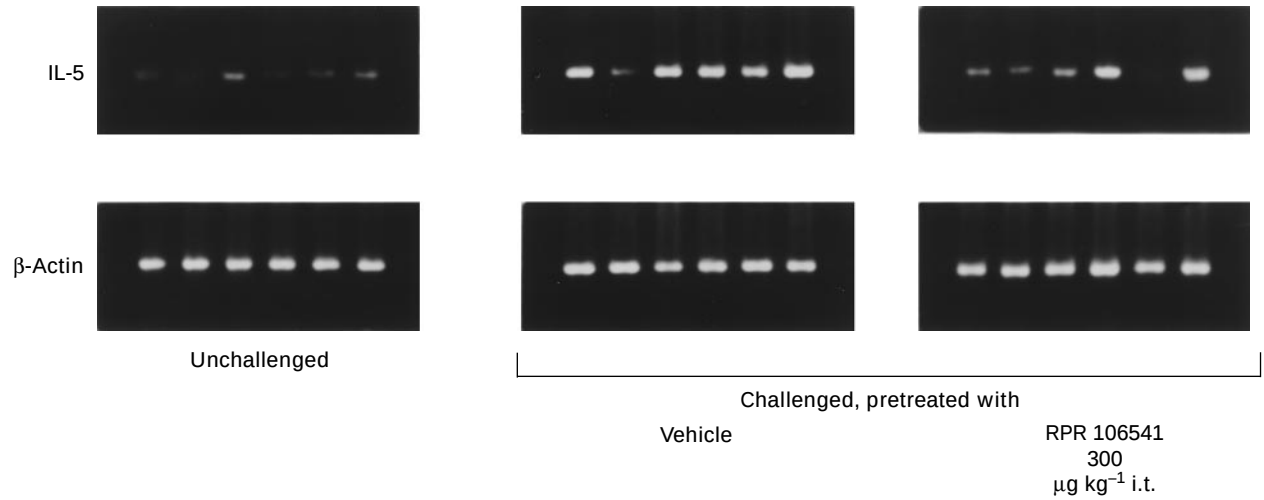

Figure 5 Agarose gels of PCR-amplified cDNA prepared by reverse transcription of RNA extracted from Brown Norway rat whole lung. PCR was performed with primers for IL-5 and β-actin (control gene) cDNA sequences. RPR 106541 (300 μg kg^{-1}) or vehicle were administered by intratracheal instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. RNA was extracted from lung tissue 24 h after challenge. Group size was 6.

Table 1 The effects of budesonide, fluticasone propionate and RPR 106541 on antigen-induced eosinophil and neutrophil influx into the airway lumen of Brown Norway rats

Treatment	*ID_{50} (μg kg^{-1}, i.t.) against antigen-induced influx of cells*	
	Eosinophils	*Neutrophils*
Budesonide	267	448
Fluticasone propionate	141	219
RPR 106541	38	55

Compounds were administered by intratracheal (i.t.) instillation into the airways 24 h and 1 h before challenge by exposure to inhaled antigen. Cells were recovered by bronchoalveolar lavage 24 h after challenge. Group size was 6.

We have shown that glucocorticoids inhibited the antigen-induced influx of CD4$^+$ T cells and expression of mRNA for IL-4 and IL-5 in our rat model. The value of glucocorticoids in suppressing asthmatic airway inflammation is well documented. These compounds reduce the number of T cells (Burke *et al*., 1992; Djukanovic *et al*., 1992), CD25 expression on T cells (Corrigan *et al*., 1993; Wilson *et al*., 1994) and the number of cells expressing mRNA for IL-4 and IL-5 (Robinson *et al*., 1993c; Leung *et al*., 1995) in asthmatic airways. It has been demonstrated in human cultured peripheral blood mononuclear cells and T cell lines that glucocorticoids can suppress the transcription/translation of genes for cytokines such as IL-4 and IL-5, and the secretion of these proteins (Doi *et al*., 1994; Okayama *et al*., 1994; Schmidt *et al*., 1994; Mori *et al*., 1995). The overall effects of glucocorticoids on the expression of cytokine mRNA in the airways of human asthmatics as well as in the present study are likely to be due to both suppression of gene processing and to the inhibition of the recruitment and activation of those cell types, such as T cells, which express these genes.

As would be expected from the effects of glucocorticoids on IL-5 production by T cells, treatment with these compounds reduces airway eosinophilia in both our rat model and in human asthmatics (Djukanovic *et al*., 1992; Laitinen *et al*., 1992; Robinson *et al*., 1993c). In this respect, RPR 106541 was more potent in the rat than the reference glucocorticoids fluticasone propionate and budesonide. It is interesting to note that fluticasone propionate was approximately 2 fold more potent than budesonide, reflecting the relative clinical potencies of these compounds (Fuller *et al*., 1995). Although it is reasonable to assume that inhibition of eosinophilia is at least in part due to abrogated IL-5-mediated eosinophil chemotaxis, it is likely that pro-inflammatory actions of other mediators are also inhibited. Culture supernatants from isolated human CD4$^+$ T cells prolong eosinophil survival, an effect which is reduced in T cells recovered from patients treated with glucocorticoids (Corrigan *et al*., 1995), probably because secretion of GM-CSF and IL-5 is inhibited. Glucocorticoids inhibit the expression of genes for GM-CSF and RANTES (a potent chemokine for eosinophils and lymphocytes), and the secretion of these proteins by human bronchial epithelial cells (Sousa *et al*., 1993; Raeburn & Webber, 1994; Davies *et al*., 1995; Kwon *et al*., 1995). GM-CSF release from human monocytes and alveolar macrophages is also inhibited (Linden & Brattsand, 1994). Glucocorticoids also suppress the function of eosinophils themselves, as demonstrated by their ability to inhibit the pro-survival actions of IL-5 (Wallen *et al*., 1991; Hallsworth *et al*., 1992). Taken together these findings suggest that, while suppression of IL-5 secretion by CD4$^+$ T cells is an important mechanism by which glucocorticoids inhibit eosinophilia, it is likely that these agents simultaneously act on a number of other cell types to inhibit eosinophil recruitment, survival and function further. In asthma patients, these agents may also inhibit eosinophilia by suppressing eosinophil differentiation and maturation, a process orchestrated largely by IL-3, IL-5 and GM-CSF. However, in our study the short-term dosing is likely to have precluded this effect. The multiplicity of inflammation-suppressing actions of glucocorticoids was further demonstrated in our model by the finding that these agents inhibited the antigen-induced neutrophil influx and reduced the number of monocytes/macrophages in the lung.

In conclusion, we have demonstrated that RPR 106541 is a potent, topically-active glucocorticoid which inhibits the antigen-induced influx of pro-inflammatory cell types (CD4$^+$ T cells and eosinophils) and the expression of mRNA for pro-inflammatory cytokines (IL-4 and IL-5) in the lung. RPR 106541 exhibited greater topical potency than the reference glucocorticoids, budesonide and fluticasone propionate. Together with observations that RPR 106541 has lower oral bioavailability and systemic activity than the reference compounds (Foster *et al*., 1996; Lawrence *et al*., 1996), our findings suggest that RPR 106541 may offer distinct advantages over currently prescribed glucocorticoids in the treatment of bronchial asthma.

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(Received January 31, 1997
Revised June 16, 1997
Accepted June 26, 1997)