



Dose-dependent inhibition of allergic inflammation and bronchial hyperresponsiveness by budesonide in ovalbumin-sensitised Brown-Norway rats

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Abstract

Corticosteroids are known to inhibit bronchial hyperresponsiveness (BHR) and allergic inflammation but there is little information on its dose-dependence. We examined the effect of different doses of the glucocorticosteroid budesonide in an allergic model. Brown-Norway rats were sensitised to ovalbumin (OVA) and pretreated with an intra-gastric dose of budesonide (0.1, 1.0, or 10 mg kg⁻¹). Exposure to OVA induced BHR, accumulation of eosinophils in the bronchoalveolar lavage (BAL) fluid and in the airways submucosa. Budesonide dose-dependently inhibited BAL fluid influx of lymphocytes, eosinophils and neutrophils, tissue eosinophils and lymphocytes and BHR. At 0.1 mg kg⁻¹, budesonide did not inhibit these parameters but at 1 mg kg⁻¹, BAL fluid eosinophils and T-cells, and submucosal T-cells were significantly reduced. At 10 mg kg⁻¹, budesonide suppressed BHR, BAL fluid inflammatory cells numbers and tissue eosinophilia. T-cell numbers were more related to BHR than eosinophil numbers. Budesonide inhibited both airway inflammation and BHR, but BAL fluid eosinophil cell counts may be dissociated from BHR.

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1. Introduction

Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma, and has been linked to the inflammatory processes described within the submucosal tissues of the airways of patients with asthma [1,2]. In order to study this link between airway inflammation and BHR, several animal models have been developed [3–5]. In actively ovalbumin-sensitised Brown-Norway (BN)-rats, an increase in airway responsiveness to inhaled acetylcholine after ovalbumin exposure was induced in association with an increase in eosinophil and lymphocyte counts in bronchoalveolar lavage (BAL) fluid and in airway submucosa [6]. This temporal association of lung inflammation with BHR has led to a hypothesis that inflammation or factors related to inflammation may be the cause of BHR.

However, dissociation between inflammation and change in BHR has been noted previously, particularly in asthmatics using agents to suppress inflammation. For example, BHR may persist despite inhibition of allergen-induced inflammation by immunosuppressive agents, such as cyclosporin A [7]. Studies in animal models of allergic inflammation have shown that systemic or topical corticosteroids inhibit both allergen-induced BHR and eosinophilic inflammation [8–10]. In these studies, usually a single high dose of corticosteroid has been studied, but in studies where more than one dose was used, similar results were observed, in that there was both inhibition of BHR and allergic airway inflammation [11,12]. However, the study of Birrell et al. [11] suggested that allergen-induced eosinophilia was more sensitive to inhibition by dexamethasone than BHR. The main aim of this study was to examine the differential sensitivity of BHR and of inflammatory cells to a corticosteroid administered systemically over a dose range. We show that BAL eosinophilia was inhibited at a

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lower dose of corticosteroids than that needed to inhibit airway eosinophilia and BHR.

2. Methods

2.1. Animals, sensitisation procedures and allergen exposure

Pathogen-free inbred male Brown-Norway rats (Harlan Olac Ltd., Bicester, UK) (200–250 g, 9–13 weeks old) were injected with 1 ml of 1 mg ovalbumin (OVA) in 100 mg $\text{Al}(\text{OH})_3$ suspension in 0.9% (wt/vol) saline intraperitoneally (i.p.) on 3 consecutive days. Aerosol exposure (15 min; 1% OVA or 0.9% saline) to rats was performed in a 6.5 l Plexiglas chamber connected to a DeVilbiss PulmonSonic nebuliser (model No. 2512, DeVilbiss Health Care, UK Ltd., Middlesex, UK) that generated an aerosol mist pumped into the exposure chamber by the airflow supplied by a small animal ventilator (Harvard Apparatus Ltd., Kent, UK) set at 60 strokes min^{-1} with a pumping volume of 10 ml. On Day 22, measurement of bronchial responsiveness to ACh was performed 18–24 h after the final challenge.

2.2. Protocol

On Day 21, OVA-sensitised rats ($n = 8$ animals per group) were dosed with drug-vehicle (1 ml per dose of 0.5% carboxymethylcellulose:0.25% Tween in sterile water) or budesonide (0.1, 1.0 or 10.0 mg kg^{-1}) by intragastric gavage 1 h prior to the OVA aerosol exposure. Sham-treated, OVA-sensitised rats ($n = 6$ animals) were administered drug-vehicle by intragastric gavage 1 h prior to the saline aerosol exposure.

2.3. Measurement of bronchial responsiveness

Animals were anaesthetised with 0.3 ml kg^{-1} Hypnorm (i.m.) consisting of fentanyl 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1} and 1.5 mg kg^{-1} Hypnovel (i.p.) consisting of midazolam and ventilated (10 ml kg^{-1} tidal volume; 60 min^{-1} rate). Rats were monitored for airflow by whole body plethysmography with a pneumotachograph (EMMS, Hants, UK) connected to a transducer (EMMS, Hants, UK). Pleural pressure was measured using an oesophageal catheter to access pleural pressure, and transpulmonary pressure was measured by a pressure transducer access the airway opening and the oesophageal catheter (EMMS, Hants, UK). The signals from the transducers were digitised with an analogue-digital board connected to a Microsoft computer and analysed with EMMS software, which is programmed to instantaneously calculate pulmonary resistance (R_L). Aerosol generated from increasing half log 10 concentrations of acetylcholine chloride (ACh) was administered by inhalation (45 breaths of 10 ml kg^{-1} stroke volume) with the initial concentration of $10^{-3.5} \text{ mol l}^{-1}$ and the maximal concentration of $10^{-1} \text{ mol l}^{-1}$. The concentration of ACh needed to increase

R_L 200% above baseline (PC_{200}) was calculated by interpolation of the log concentration-lung resistance curve.

2.4. Bronchoalveolar lavage and cell counting

This is described in detail elsewhere [13]. Briefly, after an overdose of anaesthetic, rats were lavaged with a total of 20 ml 0.9% sterile saline via the endotracheal tube. Total cell counts, viability and differential cell counts from cytospin preparations stained by May-Grünwald-Giemsa stain were determined under an optical microscope (Olympus BH2, Olympus Optical Company Ltd., Tokyo, Japan). At least 500 cells were counted and identified as macrophages, eosinophils, lymphocytes and neutrophils according to standard morphology under $\times 400$ magnification.

2.5. Collection of lung tissues

After opening of the thoracic cavity and removal of the lungs, the right lung without major vascular and connective tissues was cut into pieces and snap-frozen in liquid nitrogen, and then stored at -80°C for later assays for mRNA expression. The left lung was inflated with 3 ml saline/O.C.T. tissue embedding medium (1:1). Two blocks of half cm^3 were cut from left lung around the major bronchus, embedded in O.C.T. medium, and snap-frozen in melting isopentane and liquid nitrogen. Cryostat sections (6 μm) of the tissues were cut, air-dried, fixed in acetone, and then air-dried again, wrapped in aluminium foil and stored at -80°C for later immunohistochemical studies.

2.6. Immunohistochemistry

For detection of eosinophils, we used a mouse IgG1 monoclonal antibody against human MBP, clone BMK-13, which has been shown to be both sensitive and specific for staining rat eosinophils in frozen sections [14]. The cryostat sections were incubated with BMK-13 at a dilution of 1:50 for 30 min at room temperature. After labelling with the second antibody, rabbit anti-mouse IgG, positively stained cells were visualised with alkaline phosphatase-anti-alkaline phosphatase method.

For all tissue sections, alkaline phosphatase was developed as a red stain after incubation with Naphthol AS-MX phosphate in 0.1 M trimethylamine-HCl buffer (pH 8.2) containing levamisole to inhibit endogenous alkaline phosphatase and 1 mg ml^{-1} Fast Red-TR salt. Sections were counterstained with Harris Hematoxylin and mounted in Glycerol. System and specificity controls were carried out for all staining. Slides were read in a coded randomised blinded fashion, under a microscope. Cells within 100 μm beneath the basement membrane were counted in all airways. Submucosal area was quantified with the aid of a computer-assisted graphic tablet. Counts

were expressed as cells per mm of basement membrane length.

2.7. Drugs and reagents

Budesonide, OVA (Grade V, salt-free), CMC, Tween, ACh, Naphthol AS-MX phosphate in 0.1 M trimethylamine-HCl buffer (pH 8.2) containing levamisole, Fast Red-TR salt, EDTA, Al(OH)₃ were from Sigma, Poole, UK; Al(OH)₃, isopentane, Harris Hematoxylin from BDH, Dorset, UK; Hypnorm (fentanyl 0.315 mg ml⁻¹/fluanisone 10 mg ml⁻¹) and protease inhibitor cocktail from Roche Chemicals, UK; Hypnovel (midazolam) from Janssen Animal Health, UK; EZ-Link Sulfo-NHS-LC-Biotinylation Kit was a product of Pierce & Warriner, Chester, UK; Liquid nitrogen was supplied by BOC, Luton, U; O.C.T. medium was from Raymond A Lamb, London, UK; mouse IgG1 monoclonal antibody against human MBP, clone BMK-13, from Monosan[®], Bradsore Biologicals Ltd., Leicestershire, UK; MARE-1 mouse anti-rat IgE (mouse anti-rat IgE heavy chain) and mouse anti-rat (T-cell markers) monoclonal antibodies, and biotin goat anti-mouse antibody were products from Pharmingen, Cambridge Bioscience, Cambridge, UK; Avidin phosphatase, Horseradish peroxidase and Glycergel were from DAKO Ltd., High Wycombe, UK.

2.8. Data analysis

Data are presented as the mean $\pm$ SEM. For multiple comparisons of different groups, Kruskal-Wallis test for analysis of variance was used. If the Kruskal-Wallis test for analysis of variance was significant, we applied Dunns test for comparison between individual groups. Linear regression between inflammatory cell numbers and bronchial responsiveness measured as $-\log PC_{200}$ was assessed using the Pearson correlation coefficient. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Bronchial responsiveness to ACh

There was no significant difference in baseline lung resistance between the groups (data not shown). Sensitised, sham-treated and OVA-exposed rats had a significant increase in mean $-\log PC_{200}$ (2.55 ± 0.17), compared to sensitised saline-exposed rats (1.98 ± 0.12 , $p < 0.05$, Fig. 1). BHR was significantly inhibited at the dose of budesonide 10 mg kg⁻¹ ($-\log PC_{200}$: 1.80 ± 0.14 , $p < 0.01$, Fig. 1) but not at doses of 0.1 and 1 mg kg⁻¹ compared to sensitised OVA-exposed rats.

3.2. Inflammatory cell responses

There was a significant increase in the numbers of cells, expressed as $\times 10^4$ ml⁻¹ of BAL fluid: total cell

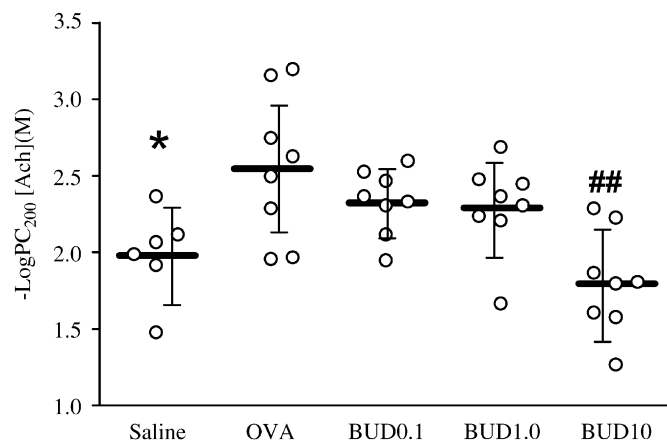


Fig. 1. Mean $-\log PC_{200}$, which is the negative logarithm of the provocative concentration of acetylcholine (ACh) needed to increase baseline lung resistance by 200%, for 5 experimental groups of rats. Treatment with 10 mg kg⁻¹ budesonide significantly inhibited the allergen-induced increase in $-\log PC_{200}$. * $p < 0.05$ compared to OVA; ## $p < 0.01$ compared to OVA. BUD0.1, BUD1.0 and BUD10 are abbreviations for doses of budesonide of 0.1, 1.0 and 10 mg kg⁻¹ respectively used in sensitised rats exposed to ovalbumin; Saline indicates sensitised rats exposed to saline; OVA indicates sensitised rats exposed to ovalbumin. Data shown as mean $\pm$ SEM.

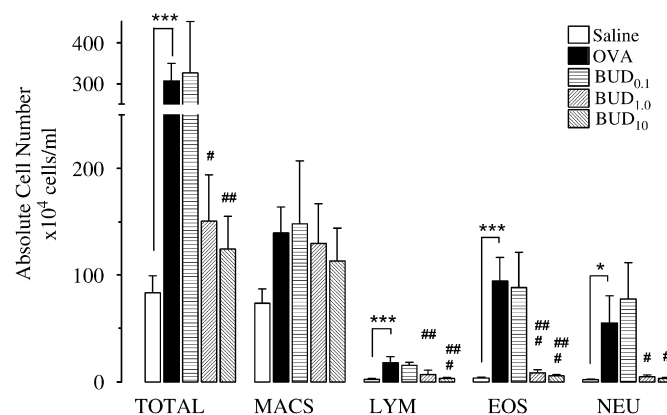


Fig. 2. Mean numbers of total cells (TOT), macrophages (MACS) eosinophils (EOS), lymphocytes (LYM) and neutrophils (NEU) in BAL fluid in experimental groups of rats as specified in Fig. 1. The counts of total cells, eosinophils, lymphocytes, and neutrophils were significantly increased in sensitised rats exposed to OVA aerosol. Budesonide treatment dose-dependently suppressed the increase in counts of lymphocytes, eosinophils and neutrophils. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to saline group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ as compared to OVA group.

(307.4 ± 42.4 , $p < 0.001$), eosinophils (94.4 ± 22.2 , $p < 0.001$), lymphocytes (18.0 ± 5.7 , $p < 0.001$) and neutrophils (55.3 ± 25.2 , $p < 0.05$) in sensitised rats exposed to OVA compared to sensitised rats exposed to saline (total: 83.3 ± 16.2 ; eosinophil: 3.4 ± 1.1 ; lymphocyte: 2.4 ± 0.7 ; neutrophil: 1.9 ± 0.9 , Fig. 2). Budesonide 1.0 mg kg⁻¹ inhibited the OVA-induced increase in total cell (150.5 ± 43.6 , $p < 0.05$), eosinophil (8.5 ± 2.8 , $p < 0.001$), lymphocyte (6.9 ± 3.9 , $p < 0.01$) and neutrophil (4.9 ± 1.6 , $p < 0.05$) counts compared to sham-treated, OVA-exposed rats. Budesonide 10 mg kg⁻¹ inhibited the OVA-induced increase

in total cell (124.0 ± 31.1 , $p < 0.01$), eosinophil (5.7 ± 1.0 , $p < 0.001$), lymphocyte (3.1 ± 0.8 , $p < 0.001$) and neutrophil (3.1 ± 0.7 , $p < 0.05$) counts compared to sham-treated, OVA-exposed rats but not at the dose of 0.1 mg kg^{-1} (Fig. 2).

There was a significant increase in tissue eosinophil infiltration (37.3 ± 9.0 , $p < 0.05$) in the submucosa of rat airways, expressed as numbers per mm basement membrane, after sensitised and repeated OVA-exposure compared to sensitised and repeated saline exposure rats (12.9 ± 3.5 ; Fig. 3a). There was a significant increase in the number of tissue eosinophils (48.0 ± 11.8 , $p < 0.05$) in

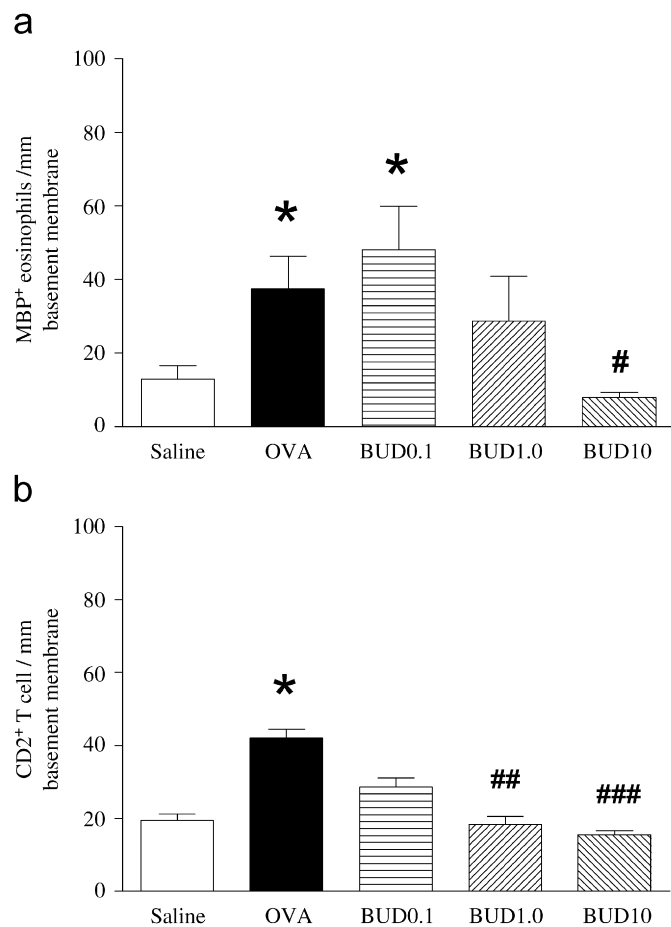


Fig. 3. (a) Mean numbers of eosinophil counts in airway submucosa expressed as cell number per mm of basement membrane for groups as detailed in Fig. 1. Allergen challenge caused a significant increase in number of MBP⁺ eosinophil in airway tissues (* $p < 0.05$ compared to Saline). Budesonide (0.1 mg kg^{-1}) did not have an effect on the increase in tissue eosinophilia as compared to untreated saline-challenge group (* $p < 0.05$). At the dose of 10 mg kg^{-1} , budesonide significantly inhibited the increase in tissue eosinophil induced by OVA exposure compared to OVA-exposed, sham-pretreated rats (## $p < 0.05$). (b) Mean numbers of T-cell counts in airway submucosa expressed per mm of basement membrane. Allergen challenge caused a significant increase in number of CD2⁺ T-cell in airway tissues (* $p < 0.05$) as compared to saline-challenge group. Budesonide (0.1 mg kg^{-1}) did not abolish the increase in tissue T-cell infiltration. At doses of 1 and 10 mg kg^{-1} , budesonide significantly inhibited the increase in tissue eosinophils induced by OVA exposure compared to OVA-exposed, sham-pretreated rats (##, ### $p < 0.01$, 0.001 , respectively).

sensitised, OVA-exposed and budesonide 0.1 mg kg^{-1} pretreated rats compared to sensitised and repeated saline-exposed rats. At dose of 10 mg kg^{-1} , budesonide significantly inhibited tissue eosinophilia (7.9 ± 1.4 , $p < 0.05$) compared to sensitised, OVA-exposed and sham-treated rats, that was not demonstrated at doses of 0.1 and 1.0 mg kg^{-1} (Fig. 3a). There was a significant increase in tissue CD2⁺ T-cell infiltration (42.0 ± 2.5 , $p < 0.05$) in the submucosa of rat airways, expressed as numbers per mm basement membrane, after sensitised and repeated OVA-exposure compared to sensitised and repeated saline exposure rats (19.4 ± 1.80 ; Fig. 3b). At doses of 1.0 and 10 mg kg^{-1} , budesonide significantly inhibited tissue T-cells (18.3 ± 2.3 , $p < 0.01$ and 15.5 ± 1.0 , $p < 0.001$, respectively) compared to sensitised, OVA-exposed and sham-treated rats, but not at the dose of 0.1 mg kg^{-1} (Fig. 3b).

Budesonide suppressed airway inflammation and BHR in a dose-dependents manner. At 0.1 mg kg^{-1} , budesonide did not suppress airway inflammation and BHR. At 1.0 mg kg^{-1} , budesonide inhibited BAL fluid inflammatory cells, eosinophil and T-cell counts ($p < 0.01$) and bronchial T-cell numbers ($p < 0.01$). At the dose of 10 mg kg^{-1} , budesonide inhibited BHR ($p < 0.01$), bronchial eosinophilia ($p < 0.05$), BAL fluid eosinophil and T-cell counts ($p < 0.001$), and bronchial T-cell influx ($p < 0.001$).

3.3. Correlation between bronchial responsiveness and inflammatory cells

Considering that corticosteroid therapy could modulate airway inflammation and BHR, we determined the relationships between the (1) number of bronchial submucosal eosinophils; (2) number of BAL fluid eosinophils; (3) bronchial submucosal T-cells number and (4) BAL fluid T-cells number and BHR for the different concentrations of budesonide. We found that all four parameters were positively correlated with BHR measured as $-\log \text{PC}_{200}[\text{ACh}](\text{M})$ with the number of inflammatory cells. BAL T-cells showed the best correlation with BHR ($r^2 = 0.65$, $p < 0.0001$) (Fig. 4a and b).

4. Discussion

We studied the effect of varying doses of budesonide administered by gavage and examined the relationship between airway inflammation and BHR in a rat model of allergic asthma. For the first time, we showed the dose-response relationship of orally administered budesonide on the suppression of BHR and airway inflammation. This dose-response relationship was different for the various parameters used and we showed that at the lowest dose of 0.1 mg kg^{-1} , there was no inhibition of BHR and inflammatory cell numbers. At the dose of 1.0 mg kg^{-1} , there was inhibition of inflammatory cell numbers but not of BHR. At the highest dose of 10 mg kg^{-1} , all parameters were inhibited. Pre-treatment with inhaled budesonide has been

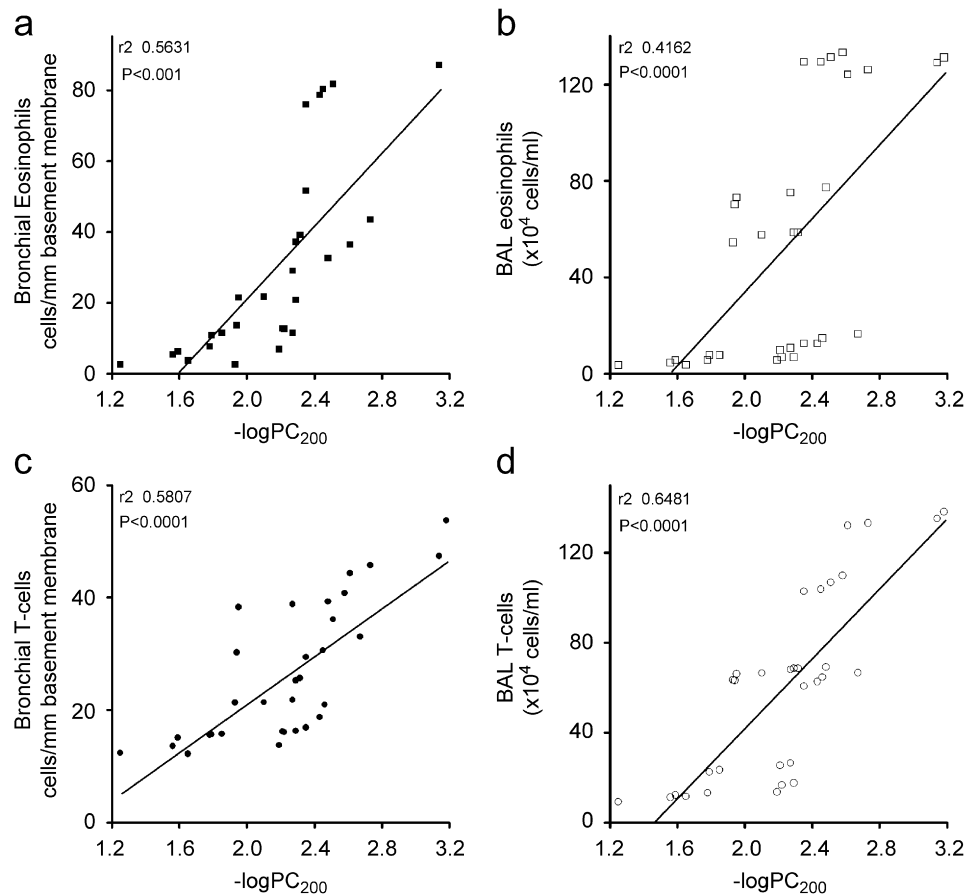


Fig. 4. Relationship of inflammatory markers to bronchial hyperresponsiveness. The 4 panels show the correlation of $-\log PC_{200}[ACh](M)$ used as a measure of bronchial responsiveness with (a) bronchial eosinophils (b) bronchoalveolar lavage (BAL) eosinophils, (c) bronchial T-cells and (d) BAL T-cells. r is the Pearson's correlation coefficient.

reported to attenuate BAL eosinophil, neutrophil and lymphocyte accumulation in asthmatic patients [15,16]. Using this rat model, we have previously demonstrated that topical budesonide by intratracheal instillation 1 h prior to allergen challenge abolished airway inflammatory cell (eosinophils, $CD2^+$, $CD4^+$ and $CD8^+$ T-cells) recruitment, suppressed mRNA expression for interleukin-2, interleukin-4 and interleukin-5, and BHR [17].

Budesonide is a synthetic corticosteroid and is reported to be esterified intracellularly in the airways that greatly increases its lipophilicity, which is hydrolysed back to active drug over a period of several hours, resulting in its characteristic slow release of active budesonide. In rat central airways, $\sim 70\%$ – 80% of budesonide was found in an esterified form within 20 min of administration. In human nasal and lung samples, 30–50% of tissue budesonide was esterified several hours after inhalation [18]. In rat model, budesonide at 1 mg kg^{-1} , administered intraperitoneally, reduced antigen-induced bronchial anaphylactic reactions significantly. At 50 mg kg^{-1} , it reduced these reactions by 50% or more [19]. Similar results have been reported in sheep, in guinea pig and in dog [20–22]. In other studies in the rat, budesonide 1 mg kg^{-1} abolished airway inflammation and suppressed cytokine mRNA expression for interleukin-2, interleukin-4, and interleu-

kin-5 in rat model of allergic airway inflammation, the dose of 2.5 mg kg^{-1} abolished the late response to OVA and strongly inhibited the *in vivo* synthesis of N-acetyl-leukotriene E₄, a major metabolite of cysteinyl-leukotrienes, and the dose of 30 mg kg^{-1} inhibited Sephadex-induced eosinophilia and Th2 cytokine gene [17,23,24]. Based on these previous studies in rat, we chose doses of 0.1, 1.0 and 10 mg kg^{-1} budesonide for our current study to evaluate the dose-dependent relationship of airway inflammatory cell numbers and BHR induced by allergen exposure. Our results showed that at the dose of 1 mg kg^{-1} , budesonide significantly reduced bronchial tissue T-cell, BALF T-cells and eosinophils. At dose 10 mg kg^{-1} , budesonide inhibited BHR, bronchial tissue eosinophil and T-cell influx, and BALF eosinophil and T-cell numbers, following single allergen exposure of sensitised rats.

As previously demonstrated in this model, we showed an increase in airway inflammatory cell infiltration, with eosinophils and T-cells, following single allergen exposure to rat airways. Budesonide inhibits airway eosinophilia in a dose-dependent manner, which is consistent with data obtained in other studies [25]. Corticosteroids suppress the influx of inflammatory cells and cytokine IL-2, IL-4 and IL-5 mRNA expression [7,26]; the latter effect may account

for the reduced inflammatory cell recruitment following allergen exposure. In our results, inhibition of submucosal inflammatory cell influx was more effective for T-cells than for eosinophils where a lower dose of budesonide (1 mg kg^{-1}) is required compared to the dose of 10 mg kg^{-1} for inhibiting eosinophil infiltration. We show that budesonide had inhibitory effects on the number of inflammatory cells that migrated into the lumen. It caused significant reduction in luminal eosinophil and T-cell at both 1 and 10 mg kg^{-1} . At the lower dose (1 mg kg^{-1}), budesonide selectively inhibited the recruitment of inflammatory cells into the BAL fluid but did not affect the number of inflammatory cells in the airway submucosa.

Inhibition of BHR with corticosteroids has been reported in many studies [7,17], and this study demonstrated that budesonide attenuated allergen-induced BHR in a dose-dependent manner. However, we found that there was dissociation in the effects of budesonide in inhibiting BHR and inflammatory cell numbers. At 1.0 mg kg^{-1} , budesonide inhibited T-cells in both BALF and in tissues but had no effect on BHR. At the higher dose of 10 mg kg^{-1} , budesonide inhibited both airway inflammatory cell numbers and BHR. Our results demonstrated that BHR is inhibited at the highest dose of budesonide but inflammatory cell numbers were inhibited at the lower dose. Dissociation between airway inflammatory cell numbers and BHR were also reported in other studies [11,27,28]. Mechanisms of altered BHR in asthma remain unclear. Factors such as airway inflammation and airway wall remodelling may determine the sensitivity and the slope of the airway response to contractile agonists, and the maximal response [29]. Structural changes such as airway wall remodelling, oedema and mucus secretion, which are promoted by inflammatory mediators, may amplify airway narrowing during airway smooth muscle contraction [30]. During airway inflammation, inflammatory cells are recruited into the airways, with release of cytokines, lymphokines, chemokines and mediators from inflammatory cells and structural components may contribute to the change in contractility of airway smooth muscle either directly or indirectly [30]. Tigani et al. [12] demonstrated that the inhibition of the augmented bronchoconstrictor response to adenosine by budesonide after allergen exposure is also associated with the inhibition of eosinophils by the corticosteroid; this effect was shown using the highest concentration of budesonide used (1 mg kg^{-1}). This is similar to our study in which we observed a dose-dependent inhibition of BHR and of inflammatory cells in BAL fluid and bronchial tissue following allergen exposure. We demonstrated that eosinophil and T-cell numbers in BAL fluid were reduced at a lower dose of budesonide treatment whereas BHR and bronchial eosinophilia were only suppressed at the higher doses.

Neutrophil numbers recovered from BAL were increased following allergen exposure in our study, which were suppressed with budesonide treatment in a dose-dependent manner. Birrell and colleagues [11] also reported an

inhibitory effect of dexamethasone on BAL neutrophilia but not on airway neutrophilia induced by allergic response. Neutrophils may not play a role in allergen-induced BHR, which is backed by the fact that neutralizing anti-IL-1 and anti-IL-18 antibodies which prevent neutrophil accumulation did not decrease BHR induced by allergen exposure [31]. The early and transient neutrophil response seen after allergen challenge may not play a direct role in the development of allergen-induced BHR.

Interestingly, our results demonstrate that inhibition of tissue eosinophilia is not proportionate to the inhibition of BAL fluid eosinophil count, whereas inhibition in T-cell numbers is paralleled in both samples. The lesser extent of inhibition induced by budesonide of eosinophilic infiltration than of T-cell in tissues may have been expected to result in a greater number of eosinophils than T-cells in BAL fluid. However, more BAL fluid T-cells were observed than eosinophils at 1 mg kg^{-1} budesonide administration. This discrepancy suggests that migration of inflammatory cells into airway lumen is not solely due to passive vascular leakage, and is associated with active migration, which is inhibited by corticosteroids to differential extent. This is also supported by the results of a study comparing the inhibitory effects of monoclonal antibodies (mAbs) to adhesion molecules and of corticosteroid treatment in a rat model of allergen-induced airway inflammation indicating that eosinophil accumulation in alveoli occur independently of increases in microvascular permeability and plasma leakage [32]. Budesonide at the same dose inhibited eosinophil migration more effectively than T-cell migration.

In summary, this study demonstrated that allergen exposure induces airway inflammation, as measured by increased eosinophils and T-cells in bronchial tissues and in BAL fluid; this is effectively reduced by budesonide treatment. Lower doses of budesonide are required to reducing the number of inflammatory cells in BAL fluid than the number of inflammatory cells in bronchial tissues and BHR. T-cell numbers in the airway mucosa may be a better marker of inflammation than eosinophil numbers.

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