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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.

Keywords: Asthma; Budesonide; Airway inflammation; Bronchial hyperresponsiveness

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 $Al(OH)_3$ 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 $10^{-3.5}$ mol l^{-1} and the maximal concentration of 10^{-1} 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 $-80\,^{\circ}C$ 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 $-80\,^{\circ}C$ 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 trismethylamine-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