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Effect of inhaled roflumilast on the prevention and resolution of allergen-induced late phase airflow obstruction in Brown Norway rats

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Abstract

Orally active phosphodiesterase 4 (PDE4) inhibitors have been developed for the treatment of asthma and chronic obstructive pulmonary disorders (COPD) although their full development has been limited by adverse side effects. Administration of PDE4 inhibitors by inhalation may improve their therapeutic index, but limited information exists on the efficacy of inhaled PDE4 inhibitors to improve lung function. In this study in ovalbumin-sensitized Brown Norway rats, roflumilast was given either intratracheally or by nose-only inhalation and changes in lung function (forced vital capacity, FVC; peak expiratory flow, PEF) and inflammatory cell influx (total cells, eosinophils and neutrophils) into the bronchoalveolar lavage (BAL) fluid were evaluated 24 h after allergen challenge. Intratracheal roflumilast, given 5 h before antigen challenge, inhibited the antigen-induced reductions in FVC (ED_{50}= 140 μg/kg, i.t.) and total cells appearing in the bronchoalveolar lavage fluid (ED_{50}= 50 μg/kg, i.t.). By the nose-only inhalation route, roflumilast reduced the bronchoalveolar lavage fluid total cells (ED_{50}= 10 μg/kg, estimated pulmonary deposition). Intratracheal roflumilast (600 μg/kg, i.t.) was also given to rats 24 h after the antigen challenge and reversed the antigen-induced reductions of FVC by 38% at 1 h, 54% at 5 h and 71% by 16 h. Intratracheal roflumilast also reduced the number of inflammatory cells in the bronchoalveolar lavage fluid and reduced the interstitial airway edema caused by the antigen challenge. These results support the development of inhaled PDE4 inhibitors for the treatment of asthma and COPD, particularly for the improvement of lung function.

Keywords: Roflumilast; Inhaled; Lung function; Inflammation; Dry powder aerosol

1. Introduction

Phosphodiesterase 4 (PDE4) is expressed by a variety of different inflammatory cells that are associated with the acute and chronic lung inflammation in diseases such as asthma and chronic obstructive lung disease (COPD) (Spina, 2003; Chung, 2006). PDE4 is also found in many different effector cells that control lung functions such as airway smooth muscle, airway epithelium, vascular endothelium and airway sensory nerves (Torphy,1998; Spina, 2003). Inhibitors of PDE4 suppress lung inflammation, reduce airway hyperresponsiveness and improve lung function in animal models of asthma and COPD (Bundshuh et al., 2001; Billah et al., 2002; Kumar et al., 2003; Kuss et al., 2003) and several orally active PDE4 inhibitors, notably cilomilast and roflumilast, have been progressed into clinical trials (Rabe et al., 2005; Lipworth 2005; Bousquet et al., 2006; Giembycz, 2006; Rennard et al., 2006). However, the full clinical development of orally active PDE4 inhibitors has been limited by their mechanism-based side effects that include nausea and emesis in humans and vasculitis in the gastrointestinal tract, mesenteric blood vessels and heart of rodents (Larson et al., 1996; Dietsch et al., 2006) and cardiac tissue of primates (Losco et al., 2004).

In an attempt to improve the therapeutic index with PDE4 inhibitors, several companies have taken the approach of giving the compounds by inhalation (Kuss et al., 2003). This method of delivery deposits locally high concentrations of the drug directly to the target organ and minimizes systemic absorption through the oral route. Some of these inhaled products have entered into clinical trials, but there is a paucity of preclinical

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data with inhaled PDE4 inhibitors, particularly for their effects on lung function. AWD 12-281 is an inhaled PDE4 inhibitor that was efficacious for the suppression of cellular lung inflammation in the allergen-challenged Brown Norway rat and this compound was also active against the acute phase bronchoconstriction induced by allergen challenge in sensitized guinea pigs (Kuss et al., 2003).

Roflumilast is a potent and selective PDE4 inhibitor that is orally active in a variety of animal models of asthma and COPD (Kumar et al., 2003; Bundshuh et al., 2001). In this study in ovalbumin-sensitized Brown Norway rats, roflumilast was given by the intratracheal and by nose-only inhalation routes of administration to assess its effects on the late phase reductions in lung function and inflammatory cell influx into the bronchoalveolar lavage fluid induced by antigen challenge. Two different dosing paradigms were used that involved administration of roflumilast before the antigen challenge and a second paradigm that involved administration of roflumilast 24 h after the antigen challenge which is a time at which the reductions in lung function and lung inflammation are already established.

2. Materials and methods

2.1. Sensitization and challenge with antigen

Brown Norway rats, ranging in weight from 200 to 300 g were obtained from Charles River Laboratory (Wilmington, MA). The rats were sensitized by an intra-peritoneal injection of 1 ml alum-precipitated antigen containing 20 μg of ovalbumin (grade III; Sigma chemical Co., St Louis, MO) and 8 mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum–ovalbumin mixture was given 7 days later. Non-sensitized animals were injected with alum only. Seven days after the second injection, animals were exposed to aerosolized ovalbumin (1%) for 30 min which was performed by placing the rats into a closed plexiglass chamber (21 l) and filling the chamber with aerosolized ovalbumin which was generated by an ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA; Model Ultra-Neb 99) and circulated through the chamber at a flow rate of approximately 8 l/min. The experiments performed in this study had been given the prior approval from the Animal Care and Use Committee of Schering-Plough Research Institute, which is a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.2. Measurement of lung function

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a tracheal catheter was surgically implanted. The rats were placed inside a whole body plethysmograph and the tracheal port was connected to an outlet port at the front of the plethysmograph which in turn was connected to a specially designed breathing valve that facilitated the forced inspiratory and expiratory maneuvers. Pulmonary airflow was measured with the aid of a differential pressure transducer (± 2 cmH_2O, Validyne Corp. California, USA) which measured the pressure drop across a wire mesh screen inserted in a 1-inch hole at the top of the plethysmograph. Volume was measured by electronic integration of the airflow signal and a volume calibration was performed before each experiment using a 3-ml syringe. Airway opening pressure was measured directly from the breathing valve using a differential pressure transducer (±70 cmH_2O, Motorola Inc., California, USA). The plethysmograph, breathing valve and software used to operate the system are part of a commercially available forced expiratory maneuvers system for rodents (Buxco Electronics, Troy, NY, USA).

The lungs were inflated to total lung capacity defined by an increase in airway inflation pressure to 20 cmH_2O. This was followed by a rapid deflation (over 0.2 s) of the lungs to a deflation pressure of −40 cmH_2O. The details of this forced expiratory maneuver have been described in detail elsewhere (Celly et al., 2006). From this procedure, forced vital capacity (FVC) and peak expiratory flow (PEF) were measured. This procedure was performed in triplicate and the results expressed as the mean of these three values.

2.3. Bronchoalveolar lavage and lung histology

The rats were sacrificed at the end of each study and a tracheal catheter was inserted. Bronchoalveolar lavage fluid was collected by lavaging the lungs with 2 aliquots of 5 ml of 0.9% sodium chloride solution. Total recovery volume per rat was approximately 8 ml. The total cell count in the bronchoalveolar lavage was performed using a hemocytometer. For the differential white cell count, cytospins were prepared with 200 μl of bronchoalveolar lavage fluid using a cytocentrifuge (Shandon Inc., Pittsburgh, PA, USA) at 250 revolutions per min for 10 min. The air-dried cytospins were stained with Leukostat stain (Fisher Scientific, Pittsburgh, PA, USA). Cells were identified as either eosinophils, neutrophils or mononuclear cells by standard morphology and 200 cells counted under 400× magnification. For the histological evaluation of lung tissue, the lungs were fixed in formalin and embedded in paraffin wax. Sections of lung tissue were cut at 5 μm thickness, mounted on glass slides and stained with hematoxylin and eosin (H × E) to assess lung histopathology including the presence of interstitial airway edema (Celly et al., 2006).

2.4. Intratracheal and nose-only inhalation procedures

Intratracheal administration of roflumilast was performed by placing 3 mg of the micronized drug, admixed with lactose into a fine-tipped dry powder microspray needle (Penn Century, Philadelphia, PA, USA). The microspray needle was inserted into the trachea of lightly anesthetized rats (5% isoflurane at a flow rate of 1 ml/min, supplemented with oxygen) to a position just above the carina and injected into the trachea and lungs with 3 ml of air. We have found that approximately 20% of the injected material deposits into the trachea and lungs with this intratracheal technique.

Roflumilast was also given by nose-only inhalation to conscious rats using a system that has been previously described in detail but adapted for the delivery of drugs by dry