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Inhibition of airway hyperresponsiveness and pulmonary inflammation by roflumilast and other PDE4 inhibitors

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Abstract

Roflumilast is an oral, once-daily phosphodiesterase 4 (PDE4) inhibitor with anti-inflammatory activity. We compared the anti-inflammatory effects of roflumilast with those of PDE4 inhibitors rolipram, piclamilast, and cilomilast in ovalbumin (OVA)-sensitized and challenged Brown-Norway rats. Animals were treated orally 1 h before OVA challenge with roflumilast (0.3, 1.0, and 3.0 mg/kg), rolipram (0.8, 2.8, and 8.3 mg/kg), piclamilast (10.0, 20.0, and 30.0 mg/kg), or cilomilast (10.3, 34.3, and 103.0 mg/kg). Airway hyperresponsiveness (AHR) against adenosine was investigated by measuring airway resistance 200 min after OVA challenge. Subsequently, neutrophil influx and tumor necrosis factor-alpha (TNF-α) release in the lungs were determined by bronchoalveolar lavage. Direct bronchodilation at the time point of AHR assessment by PDE4 inhibitors was examined in serotonin-challenged animals. Evaluation of neutropenic animals or treatment with anti-TNF-α antibody revealed that AHR was independent of neutrophil accumulation or TNF-α release. Roflumilast (50% inhibitory dose [ID_{50}] = 1.5 mg/kg) inhibited AHR 3-, 16-, and 27-fold more potently than rolipram, piclamilast, and cilomilast, respectively. Likewise, roflumilast was a more potent inhibitor of neutrophil influx (ID_{50} = 0.9 mg/kg) than rolipram (ID_{50} = 6.9 mg/kg), piclamilast (ID_{50} = 28.1 mg/kg), or cilomilast (ID_{50} = 37.7 mg/kg). Roflumilast, rolipram, and piclamilast—but not cilomilast—suppressed OVA-induced TNF-α release in a dose-dependent manner. Roflumilast (ID_{50} = 0.9 mg/kg) exhibited 9- and 23-fold more potent inhibition of TNF-α release than rolipram and piclamilast, respectively. Roflumilast did not inhibit serotonin-induced bronchoconstriction 4.5 h after administration, suggesting that inhibition of AHR by roflumilast results from anti-inflammatory, not bronchodilatory, effects. This study suggests that roflumilast has anti-inflammatory action and provides rationale for the investigation of roflumilast in asthmatic patients.

Keywords: Airway hyperresponsiveness; AHR; Neutrophil; Tumor necrosis factor-alpha; Asthma; PDE4 inhibitor

1. Introduction

Asthma is characterized by airway hyperresponsiveness (AHR) and inflammation, resulting in remodeling of the bronchial wall, airway obstruction, and recurrent episodes of wheezing, breathlessness, chest tightness, and coughing [1,2]. Pulmonary airflow limitation and inflammation, in the form of inflammatory cell infiltration and increased secretion of inflammatory mediators in both the large and small airways, are common pathologic features of chronic asthma.

AHR is found in virtually all patients with asthma and has been linked to ongoing inflammatory processes in general, and to hyperreactivity of airway smooth muscles, mucus hypersecretion, and airway wall remodeling in particular [3,4]. Airway responsiveness to various stimuli is accompanied by a suite of immunological and physiological changes in the airways, including production of immunoglobulin (Ig) E antibody, upregulation of proinflammatory cytokines and mediators such as tumor necrosis factor-alpha (TNF-α), and influx of proinflammatory cells such as neutrophils and eosinophils. TNF-α is an important mediator of tissue injury and airway inflammation [5]. TNF-α regulates the transcription of a host of proinflammatory cytokines, chemokines, and adhesion molecules in various leukocytes through activation of the

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nuclear transcription factor nuclear factor-κB. This leads to cell chemotaxis and increased accumulation of proinflammatory cells in the airways as well as activation of cells and release of cytokines and mediators into the airways.

AHR is an important diagnostic parameter used in clinical practice to verify asthma diagnosis. It can be measured using a variety of stimuli, but recent studies indicate that AHR provocation tests with adenosine reflect allergic airway inflammation more accurately than AHR induced by other commonly used stimuli such as methacholine or histamine [3]. Prevention of AHR is a goal of asthma treatment, and the ability of novel therapies to inhibit AHR is often assessed in preclinical models. Additionally, proinflammatory cells and mediators are obvious targets for the development of novel therapies for respiratory diseases because of the chronic inflammation characterizing respiratory diseases such as asthma.

Inhibitors of phosphodiesterase 4 (PDE4) isoenzymes have been investigated in several preclinical airway disease models of inflammation. PDE4 enzymes catalyze the hydrolysis of cyclic adenosine monophosphate (cAMP) in many proinflammatory cells, including neutrophils, basophils, eosinophils, T lymphocytes, macrophages, monocytes, and mast cells [6–8]. PDE4 inhibitors block the hydrolysis of cAMP, leading to elevated intracellular cAMP levels and, thus, suppression of the proinflammatory activity of these cells, including cytokine, chemokine, and IgE production, enabling selective targeting of inflammation. PDE4 inhibitors are under development as potential therapeutic agents for patients with asthma or chronic obstructive pulmonary disease [9].

Roflumilast is an oral, once-daily investigational PDE4 inhibitor that has demonstrated anti-inflammatory effects both in vitro and in animal models [10,11]. Roflumilast inhibited chemotaxis and cytokine secretion in inflammatory cells, including neutrophils, eosinophils, monocytes, and $CD4^+$ T lymphocytes, and reduced the release of TNF-α, an important mediator in the pathophysiology of asthma [10]. In preclinical studies, roflumilast attenuated allergen-induced bronchoconstriction and early airway reactions, inhibited eosinophilia, and reduced levels of total protein and TNF-α [11], indicating a potential therapeutic role for roflumilast in the treatment of asthma. Recently, clinical studies have demonstrated that roflumilast attenuates AHR to histamine and the early and late asthmatic reactions following allergen challenge in patients with mild asthma [12,13].

This report assesses the inhibitory effect of roflumilast on early, antigen-induced AHR against adenosine and pulmonary inflammation in ovalbumin (OVA)-sensitized and OVA-challenged Brown-Norway rats [14]. Because in humans AHR in response to adenosine is correlated with airway inflammation [15] adenosine challenge was used in this model to evaluate the causal relationship between inflammatory markers (e.g., neutrophil influx and TNF-α levels) and AHR. Furthermore, the inhibitory effects of roflumilast on AHR and pulmonary inflammation were compared with the effects of other PDE4 inhibitors (i.e., rolipram [ZK 62711], piclamilast [RP 73401], and cilomilast [Ariflo®, SB 207499]). The effect of PDE4 inhibitors on serotonin-induced bronchoconstriction was also examined in Brown-Norway rats to elucidate whether PDE4 inhibition of AHR results from bronchodilatory or anti-inflammatory effects.

2. Materials and methods

2.1. Animals

Male Brown-Norway rats (weight, 200–300 g), obtained from the department of animal welfare at ALTANA Pharma (Konstanz, Germany) or from Charles River/Wiga (Sulzfeld, Germany) were used for these experiments. Rats were housed in groups of 3–5 animals per Macrolon cage (type IV) at 20–22 °C with a 12-h light–dark cycle, and had free access to water and food (Maintenance Diet 9439 25 W10 for Rats; NAFAG, Gossau, Switzerland). All animals were housed in the department of Animal Welfare at ALTANA in accordance with national guidelines and legal regulations.

2.2. Pretreatment and test compounds

Test compounds included roflumilast (mol. wt. = 403.2; ALTANA Pharma, Konstanz, Germany), piclamilast (mol. wt. = 381.3; Neo Micro, Buergelen, Switzerland), cilomilast (mol. wt. = 343.4; Syncom, Groningen, The Netherlands), and racemic *R*,*S*-rolipram (mol. wt. = 275.4; Sigma-Aldrich, Taufkirchen, Germany). These compounds were dissolved in a 4% aqueous methocel solution containing 20% polyethylene glycol 400 and administered orally 1 h before OVA challenge via gavage at a volume of 10 mL/kg. The doses tested for each test compound were as follows: roflumilast 0.3, 1.0, and 3.0 mg/kg; rolipram 0.8, 2.8, and 8.3 mg/kg; piclamilast 10.0, 20.0, and 30.0 mg/kg; and cilomilast 10.3, 34.3, and 103.0 mg/kg. The solvent methocel/polyethylene glycol 400 solution was administered to non-drug-treated animals (i.e., negative and positive controls).

2.3. Experimental protocol

Animals were sensitized on days 1, 14, and 21 with a subcutaneous injection of OVA solution (0.5 mL/animal) into the skin of the neck and an intraperitoneal injection of *Bordetella pertussis* (4×10^8 heat-killed bacilli/mL; Behringwerke AG, Marburg, Germany) suspended in saline (0.25 mL/animal). The OVA solution for sensitization contained 20 μg/mL OVA (egg albumin, grade V; Fluka, Buchs, Switzerland) and 40 mg/mL aluminum hydroxide gel (Merck, Darmstadt, Germany) suspended in saline (0.9% NaCl).

OVA challenge was performed 28 days after the beginning of sensitization. The animals were anesthetized