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Therapeutic efficacy of AM156, a novel prostanoid DP_2 receptor antagonist, in murine models of allergic rhinitis and house dust mite-induced pulmonary inflammation

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ABSTRACT

Prostaglandin D_2 (PGD_2) is derived from arachidonic acid and binds with high affinity to the G protein coupled receptors prostanoid DP_1 and DP_2. Interaction with DP_2 results in cell chemotaxis, eosinophil degranulation, eosinophil shape change, adhesion molecule upregulation and Th2 cytokine production. In allergic rhinitis and allergic asthma PGD_2 is released from mast cells in response to allergen challenge and may trigger symptoms such as sneezing, rhinorrhea, pruritus, mucus hypersecretion and pulmonary inflammation. In Japan, ramatroban, a dual prostanoid DP_2/prostanoid TP receptor antagonist, is marketed for allergic rhinitis while selective DP_2 antagonists are currently under investigation as therapeutics for asthma and allergic rhinitis. In the studies described herein, we investigated the efficacy of AM156, a novel selective prostanoid DP_2 receptor antagonist, in murine models of allergic rhinitis and asthma. AM156 inhibited sneezing and nasal rubs in a model of allergic rhinitis. AM156 inhibited pulmonary inflammation and mucus hypersecretion induced by chronic inhalation of house dust mite. These results suggest that selective prostanoid DP_2 receptor antagonists such as AM156 may provide beneficial effects for the clinical treatment of diseases such as allergic rhinitis and asthma.

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1. Introduction

Prostaglandin D_2 (PGD_2) is derived from arachidonic acid via the action of COX-1, COX-2 and PGD_2 synthases. Upon release from mast cells and to a lesser extent, antigen presenting cells and Th2 cells (Tanaka et al., 2000; Urade et al., 1990, 1989) PGD_2 binds primarily to two G protein coupled receptors, namely prostanoid DP_1 and DP_2, the latter also known as chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). DP_2 is predominantly expressed on eosinophils, Th2 cells and basophils. Interaction of PGD_2 with DP_2 results in cell chemotaxis, eosinophil shape change, eosinophil degranulation, upregulation of adhesion molecules and Th2 cytokine production.

Allergic rhinitis is a common immune-mediated disease of the upper airways with increasing global prevalence (Bousquet et al., 2008; Savage and Roy, 2005). Many of the symptoms, which include sneezing, rhinorrhea, pruritus and nasal obstruction, are caused by the release of histamine, leukotrienes, prostaglandins, thromboxane A_2 and cytokines, from mast cells. Allergic rhinitis can predispose for asthma and vice versa (Dixon, 2009; Nathan, 2009).

Allergic asthma is a chronic condition characterized by pulmonary inflammation, elevated serum IgE, airway remodeling, goblet cell hypersecretion and airway hyperreactivity. Chronic exposure to environmental allergens such as house dust mite has been implicated in persistent allergic asthma. In the mouse, chronic exposure to inhaled house dust mite extract produces many of these same features (Johnson et al., 2004).

PGD_2 and its receptor prostanoid DP_2 have been associated with both allergic rhinitis and asthma. In patients with allergic rhinitis, for example, nasal lavage fluid PGD_2 is elevated following allergen provocation and correlates with sneezing (Naclerio et al., 1983). In certain populations, DP_2 SNPs are linked to asthma severity and associated with increased risk of atopy, including allergic rhinitis and wheeze (Hsu et al., 2002; Huang et al., 2004; Seibert et al., 2007). An accumulation of CRTH2-positive leukocytes is present in the nasal mucosa of allergic patients (Shirasaki et al., 2009).

The studies described herein examine the efficacy of AM156 ({2′-[(Cyclopropanecarbonyl-ethyl-amino)-methyl]-6-methoxy-4′-trifluoro-methyl-biphenyl-3-yl}-acetic acid), a novel potent and selective prostanoid DP_2 receptor antagonist, in murine models of allergic rhinitis and allergic asthma. *In vitro* selectivity data and *in vivo* pharmacokinetics of this molecule have been previously reported (Stebbins et al., 2010). Briefly, in [^{3}H]-PGD_2 radioligand membrane binding assays conducted in the presence of species-specific serum albumin, the IC_{50s} for AM156 are 24 nM and 61 nM against human and mouse DP_2, respectively. AM156 is selective vs. related prostanoid receptors DP_1 and TP as well as the enzymes

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COX-1 and COX-2 at concentrations up to 10 μM. Allergic rhinitis was modeled in mice using an ovalbumin priming and challenge procedure. We further profiled AM156 in a model of chronic house dust mite (HDM) exposure in the absence of adjuvant. Allergen exposure in the absence of systemic adjuvant more closely mimics the clinical situation in which, over time, repeated exposure to environmental aeroallergens produce an allergic response in susceptible individuals. To our knowledge, this is the first report of efficacy of prostanoid DP_2 receptor selective antagonists in an adjuvant-free allergic system.

2. Materials and methods

2.1. Animals

Female BALB/c mice (Harlan, 8–10 weeks at study initiation) were used throughout. Animals were housed in accordance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. Animal protocols were approved by the Amira Pharmaceuticals Institutional Animal Care and Use Committee.

2.2. Materials

Ovalbumin (Grade V), dexamethasone and methacholine chloride were obtained from Sigma-Aldrich (St. Louis, MO, US). Imject Alum was purchased from Pierce (Rockford, IL, US). House dust mite extract (*Dermatophagoides pteronyssinus*) was purchased from Greer Laboratories (Lenoir, NC, US). AM156 ({2′-[(Cyclopropanecarbonyl-ethyl-amino)-methyl]-6-methoxy-4′-trifluoro-methyl-biphenyl-3-yl}-acetic acid, sodium salt) was synthesized at Amira Pharmaceuticals.

2.3. Allergic rhinitis model

Methods were adapted from Nakaya et al. (2006). Mice were sensitized by intraperitoneal (i.p.) administration of 2 μg of ovalbumin complexed with Imject Alum on days 0 and 7. On days 21 through 25 mice were challenged intranasally with 20 μl of saline ($n=2$ to 4) or a 10 mg/ml solution of ovalbumin ($n=6$ per treatment group). Mice were not anesthetized during the challenge to prevent ovalbumin from reaching the lower airways (McCusker et al., 2002; Nakaya et al., 2006). The number of sneezes and nasal rubs was counted by an independent blind observer during a period of 8 min immediately following ovalbumin challenge on days 21, 23 and 25.

For the priming experiment (Fig. 1A), mice ($n=6$ per treatment group) were randomly assigned on day 0 to receive prophylactic treatment with either AM156 or vehicle and were treated by oral gavage 2 h prior to the first ovalbumin/alum administration and continued through day 11. In a separate experiment to examine efficacy when AM156 was dosed during the challenge phase (Fig. 1B), mice ($n=6$ per treatment group) were randomly assigned on day 21 to receive therapeutic treatment of either AM156 or vehicle and were treated by oral gavage 2 h prior to each intranasal challenge (20 μl of a 10 mg/ml solution of ovalbumin or saline).

After the final sneeze and rub measurements on day 25, mice were euthanized with CO_2 inhalation and blood was collected into EDTA-containing tubes. Blood was centrifuged at 1450×*g* to obtain plasma. IgE and ovalbumin-specific IgE concentrations were determined by ELISA (BD OptiEIA, BD Biosciences, San Jose, CA; MD Biosciences, St. Paul, MN).

2.4. House dust mite sensititization model

Mice ($n=6$ per treatment group) were sensitized to house dust mite according to the methods described by Johnson (Johnson et al., 2004). Briefly, house dust mite extract (*D. pteronyssinus*, 25 μg/mouse, Greer Laboratories, Lenoir, NC) or sterile saline (10 μl/mouse) was administered intranasally under light isoflurane anesthesia 5 days per week for 5 weeks (Fig. 2). Mice were challenged for 7 consecutive days on the final week (week 5). Vehicle, AM156 (10 mg/kg) or dexamethasone (1 mg/kg) were dosed orally once daily during the final 2 weeks. The vehicle for AM156 was 0.5% methylcellulose while that for dexamethasone was 25% hydroxypropyl-β-cyclodextrin. On the days which house dust mite was administered, dosing occurred 1 h prior to house dust mite. 24 h after the last house dust mite challenge, airway hyperreactivity to aerosolized methacholine was determined using whole body plethysmography (Buxco, Sharon, CT). Following pulmonary function measurements, mice were returned to their cages and were dosed with vehicle or drug for 2 additional days. 72 h after the final house dust mite challenge, mice were euthanized with isoflurane inhalation, blood was collected into EDTA-containing tubes and bronchoalveolar lavage was performed as described below.

2.5. Bronchoalveolar lavage

Mice were euthanized with isoflurane inhalation. The trachea was exposed and cannulated with a 20 g luer. Bronchoalveolar lavage was performed by twice infusing and withdrawing 0.5 ml PBS. Cell suspensions were kept on ice until centrifugation (700×*g*, 10 min). Supernatant was collected, aliquoted and stored at −80 °C until further analyses. Cell pellets were resuspended in 300 μl 0.1% BSA/PBS. Total leukocyte counts were determined on a Hemavet FS950. 100 μl cell suspensions were used for preparation of cytospin slides (Thermo-Shandon, Waltham, MA). Slides were stained with 3 Step Stain (Richard Allen Scientific, Kalamazoo, MI). Differential cell counts were performed on 300 cells using standard morphological features.

2.6. Bronchoalveolar lavage fluid supernatant analysis

Cytokines (IL-4, IL-5, IL-13 and IL-17) in the lavage fluid and lung homogenates were analyzed by ELISA (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. The detection limits for these assays were 2 pg/ml, 7 pg/ml, 1.5 pg/ml and 5 pg/ml, respectively.

Mucin was analyzed by enzyme-linked lectin assay (ELLA). Briefly, 50 μl of bronchoalveolar lavage fluid or mucin standard was added to a 96 well plate and dried overnight at 40 °C. Following washing, plates were blocked with 3% BSA/PBS for 2 h. Plates were washed again followed by incubation with UEA-1 lectin-HRP conjugate for 2 h. After washing, TMB substrate was added and color was allowed to develop for 30 min. The reaction was stopped with H_2SO_4 and the resulting

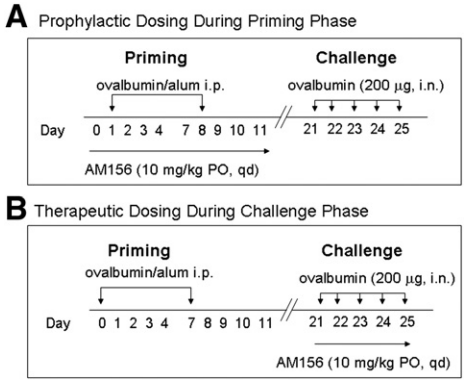

Fig. 1. Experimental design for allergic rhinitis experiments. Mice were sensitized to ovalbumin and dosed with AM156 either during the sensitization (priming) phase (A) or the during the challenge (effector) phase (B). On days 21–25, mice were intranasally challenged with either saline ($n=4$) or ovalbumin ($n=6$ per treatment group). Sneezes and nasal rubs were monitored for 8 min following challenge on days 21, 23 and 25.

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