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The effect of tiotropium in combination with olodaterol on house dust mite-induced allergic airway disease☆

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ABSTRACT

One of the major goals of asthma therapy is to maintain asthma control and prevent acute exacerbations. Long-acting bronchodilators are regularly used for the treatment of asthma patients and in clinical studies the anti-cholinergic tiotropium has recently been shown to reduce exacerbations in patients with asthma. So far it is unclear how tiotropium exerts this effect. For this purpose, we designed an allergen-driven rechallenge model of allergic airway inflammation in mice, to assess the effectiveness of tiotropium and the long-acting β-2 adrenoceptor agonist olodaterol on allergen-induced exacerbations of airway disease.

Female C57BL/6J mice were sensitized intranasally (i.n.) with 1 μg of house dust mite (HDM) extract followed by a challenge regime (5 consecutive days 10 μg HDM extract i.n.) after one week. Mice were exposed to a secondary challenge five weeks after sensitization and were treated i.n. with different concentrations of tiotropium or olodaterol (1, 10 and 100 μg/kg) or a combination thereof (10 μg/kg each) prior to and during the secondary challenge period. Three days after the last challenge, bronchoalveolar lavage (BAL) fluid and lung tissue were collected for flow cytometry and histologic analysis, respectively.

Secondary challenge with HDM extract strongly induced allergic airway disease reflected by inflammatory cell infiltration and goblet cell metaplasia. Treatment with tiotropium, but not with olodaterol reduced tissue inflammation and goblet cell metaplasia in a dose-dependent manner. The combination of tiotropium and olodaterol was more effective in significantly reducing tissue inflammation compared to tiotropium treatment alone, and also led to a decrease in BAL cell counts.

These data suggest that in a model of relapsing allergic airway disease tiotropium directly prevents exacerbations by reducing inflammation and mucus production in the airways. In addition, the combination of tiotropium and olodaterol exerts synergistic effects.

1. Introduction

Asthma is a chronic respiratory disease most commonly caused by hypersensitivity to a variety of allergens and is one of the leading causes of chronic disease worldwide [1]. The disease pathogenesis is characterized by complex airway inflammatory and remodeling processes that lead to airway hyperresponsiveness and various degrees of reversible airflow limitation. Current therapies preferentially consist of a combination of inhaled corticosteroids and inhaled long-acting β-2 adrenoceptor agonists (LABA) and are

Abbreviations: HDM, house dust mite; OVA, ovalbumin; BAL, bronchoalveolar lavage; LABA, long-acting β-2 adrenoceptor agonist.

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beneficial in controlling symptoms and airway inflammation, but have little effect on airway remodeling. However, the majority of asthma morbidity occurs in patients suffering from a severe form of the disease (up to 10% of all asthmatics) with recurring symptoms and exacerbations that are poorly controlled despite the use of standard combination therapy [2].

Recent clinical studies have focused on the option of adding a second long-acting bronchodilator, the anticholinergic agent tiotropium that has been used in the (maintenance and exacerbation) treatment of chronic obstructive pulmonary disease (COPD) for the past years [3–5]. Tiotropium is a muscarinic receptor antagonist that is kinetically selective for M3 receptors [6]. Muscarinic receptor signaling in the airways is primarily induced via the parasympathetic neurotransmitter acetylcholine released by both neuronal and non-neuronal cells including lung structural and inflammatory cells [7]. Signaling induces bronchoconstriction and mucus production by acting on smooth muscles and mucus-secreting cells in the central airways [8–11]. Blocking the receptor using therapeutic muscarinic receptor antagonists results in smooth muscle relaxation and reduced mucus production [12].

Besides its function as a bronchodilator, tiotropium has been demonstrated to reduce signs of allergen-induced airway inflammation in animal models [13–15], and interestingly also in combination with a novel LABA, olodaterol [16]. However, most of these studies were performed with allergens irrelevant to the human situation and so far not in secondary challenge models, in which airway inflammation has been established. Therefore, the aim of the current study was to determine the effect of treatment with tiotropium or olodaterol on allergen-induced exacerbation of airway inflammation and remodeling. To achieve this, a rechallenge model of allergic airway inflammation in mice mimicking allergen-induced exacerbations of the disease was utilized. In addition, a potential synergistic effect of a combination treatment of tiotropium and olodaterol was explored in this model.

2. Material and methods

2.1. Animals and maintenance

8-10 weeks old pathogen-free female C57BL/6J mice (Charles River, 's-Hertogenbosch, The Netherlands) were housed in rooms maintained at constant temperature and humidity with a 12-h light cycle. Animals were allowed food and water *ad libitum*.

All animal procedures were approved by the local animal ethics committee of the Leiden University Medical Center (license number 13185, Dierexperimentencommissie Academisch Ziekenhuis Leiden) and were conducted under strict governmental and international guidelines in accordance with EU Directive 2010/63/EU.

2.2. Treatment protocol

Mice were sensitized by intranasal administration of 1 μg house dust mite extract (HDM; Greer, Lenoir, NC, USA) in 50 μl PBS on day 0 (week 1) and challenged intranasally with 10 μg HDM extract in 50 μl PBS once daily on days 7–11 (week 2). After a recovery period of three weeks, animals were rechallenged intranasally with 10 μg HDM extract once daily on days 35–39 (week 6). Prior to and during the second allergen challenge period, animals were treated daily by intranasal administration of 1, 10 and 100 μg/kg bodyweight (BW) tiotropium (Boehringer Ingelheim; dissolved in PBS), olodaterol (Boehringer Ingelheim; dissolved in PBS) or a combination thereof (both compounds at 10 μg/kg each) starting on day 34, 24 h before the first rechallenge and on days 35–39, 1 h before the challenge. Intranasal administration was performed under isoflurane anesthesia (3%, 0.6 L/min). Control animals received 50 μl PBS intranasally during sensitization, challenge and rechallenge. For a detailed protocol outline, see Fig. 1.

Three days after the last challenge, mice were euthanized with sodium pentobarbital and tracheostomized. All animals were lavaged, the left lung was removed for flow cytometry analysis, and the right lung was fixed in paraformaldehyde (PFA; see below).

2.3. Bronchoalveolar lavage (BAL) and lung single cell suspension

BAL fluid and lung single cell suspensions were obtained to determine lung inflammatory cell recruitment. BAL was performed by instilling the lungs with 3 × 1 ml aliquots of sterile PBS (Braun). Afterwards, cells were spun down at 400 g and resuspended in 0.5% (w/v) BSA (Sigma)/2 mM EDTA (Invitrogen) FACS buffer. Total cell counts were determined in a hemocytometer. Remaining cells were subjected to flow cytometry analysis (see below).

For single-cell suspensions of whole lung tissue, lungs were perfused with sterile PBS via the right ventricle to clear leukocytes and erythrocytes from the pulmonary circulation. Lung homogenization was performed via enzymatic digestion and mechanical dissociation steps using collagenase (1 mg/ml; Calbiochem)/DNase (20 U/ml; Sigma) and 70 μm cell strainers (Corning) followed by red blood cell lysis. Single-cell suspensions were subjected to flow cytometry analysis (see below).

2.4. Flow cytometry

For flow cytometry, cells were stained with live/dead stain Aqua (Invitrogen) and fixed in 1.9% w/v formaldehyde (Merck). The following antibodies were used to distinguish different inflammatory cell populations (neutrophils, macrophages, eosinophils, DCs, B and T cells) in the BAL fluid: Ly-6G/Ly-6C (Gr-1)-FITC (RB6-8C5,

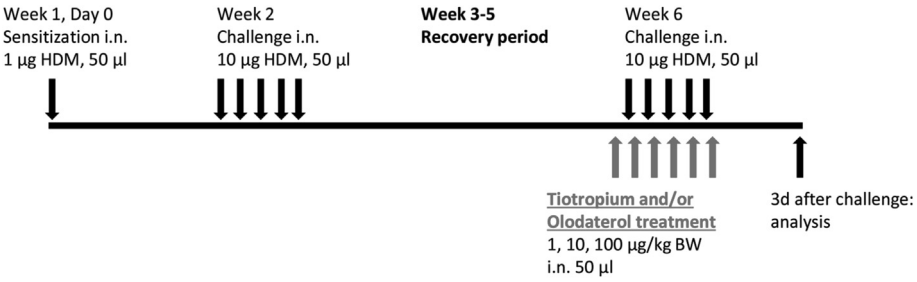

Fig. 1. Rechallenge model of allergic airway inflammation. Mice were sensitized by intranasal administration of 1 μg house dust mite extract (HDM; Greer, Lenoir, NC, USA) in 50 μl PBS on day 0 (week 1) and challenged intranasally with 10 μg HDM extract in 50 μl PBS once daily on days 7–11 (week 2). After a recovery period of three weeks, animals were rechallenged intranasally with 10 μg HDM extract once daily on days 35–39 (week 6). Prior to and during the second allergen period, animals were treated daily by intranasal administration of 1, 10 and 100 μg/kg bodyweight (BW) tiotropium (Boehringer Ingelheim; dissolved in PBS), olodaterol (Boehringer Ingelheim; dissolved in PBS) or a combination thereof (both compounds at 10 μg/kg each) starting on day 34, 24 h before the first rechallenge and on days 35–39, 1 h before the challenge.