



Tiotropium bromide inhibits relapsing allergic asthma in BALB/c mice[☆]



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ABSTRACT

Recurrent relapses of allergic lung inflammation in asthmatics may lead to airway remodeling and lung damage. We tested the efficacy of tiotropium bromide, a selective long-acting, muscarinic receptor antagonist as an adjunct therapy in relapses of allergic asthma in mice. We compared the effectiveness of local intranasal administration of tiotropium and dexamethasone in acute and relapsing allergic asthma in BALB/c mice. Although tiotropium at low doses is a potent bronchodilator, we tested higher doses to determine effectiveness on inflammation and mucus hypersecretion. A 5-day course of twice daily intranasal tiotropium or dexamethasone (1 mg/kg (b.w.)) suppressed airway eosinophils by over 87% during disease initiation and 88% at relapse compared to vehicle alone. Both drugs were comparable in their capacity to suppress airway and parenchymal inflammation and mucus hypersecretion, though tiotropium was better than dexamethasone at reducing mucus secretion during disease relapse. Despite treatment with either drug, serum antigen-specific IgE or IgG1 antibody titres remained unchanged. Our study indicates that tiotropium at higher doses than required for bronchodilation, effectively suppresses inflammation and mucus hypersecretion in the lungs and airways of mice during the initiation and relapse of asthma. Tiotropium is currently not approved for use in asthma. Clinical studies have to demonstrate the efficacy of tiotropium in this respiratory disease.

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1. Introduction

The importance of eosinophilic inflammation in the lungs of individuals with allergic asthma is underscored by their essential role in allergen-induced airway hyperresponsiveness (AHR) and ultimately airway remodeling in mouse models [12,20]. T helper (Th) 2 cytokines IL-4, IL-5, and IL-13 produced by T lymphocytes are important for eosinophil accumulation and activation [3,6,32]. Once present in the lung, eosinophils produce factors such as cytokines that lead to mucus hypersecretion and airway

Abbreviations: AHR, airway hyperresponsiveness; AUC, area under curve; BAL, bronchoalveolar lavage fluid; OVA, ovalbumin; PBS, phosphate buffered saline; Th, T helper.

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remodeling [7,28]. Although it is important to address the long-term adverse effects of chronic eosinophilic lung inflammation in asthmatics, we argue that it is also crucial to recognize the role of eosinophils in recurrent allergen-induced relapses common in individuals with seasonal allergic asthma. Asymptomatic patients between relapses of allergic asthma have lung pathology [25,30,33]. Additionally, mice recovered from one episode of acute allergic asthma have chronic lung inflammation containing allergen-specific Th2 cells without eosinophils and respond vigorously to allergen rechallenge which induces disease relapses [24]. Chronic inflammation, even without eosinophils during asymptomatic remissions and undoubtedly recurrent relapses may lead to airway remodeling.

Anticholinergic drugs, such as tiotropium bromide are important for the treatment of chronic obstructive pulmonary disease, but are generally not used to treat asthma. However, there is clinical evidence revealing that tiotropium, a selective long-acting, muscarinic receptor antagonist treatment reduces relapses in severe asthmatics [27]. The effects of tiotropium on bronchoconstriction are well known [10]. However, its properties

on inflammation and immunity have not been extensively studied [15,26]. Acetylcholine appears to play a role in the regulation of immune responses [8,11] and the release of pro-inflammatory mediators from airway smooth muscle and epithelial cells [14,18,19]. Moreover, lymphocytes, Th2 cells, eosinophils, alveolar macrophages and mast cells all synthesize and contain acetylcholine (reviewed in Ref. [34]). Furthermore, muscarinic receptors are expressed on T cells, neutrophils, macrophages and mast cells [11] and the muscarinic receptor antagonist, atropine inhibits antibody production, T cell proliferation and leukocyte migration [31]. Additional evidence comes from animal models showing that tiotropium effectively suppresses inflammation in chronic asthma in mice and guinea pig [16,21,22].

In this study, we sought to further analyze the anti-inflammatory properties of tiotropium in a mouse model of relapsing allergic asthma that mimics seasonal allergic disease. Our data suggest that higher doses of tiotropium suppress inflammation and have clinical implications for the prevention of allergic relapses.

2. Materials and methods

2.1. Mice

Eight week old female BALB/c mice (Charles River, Germany) provided ovalbumin (OVA)-free food (SSNIF, Soest, Germany) and water *ad libitum* were used in all experiments. All experimental protocols complied with the requirements of the Animal Care Committee of the Austrian Ministry of Science and the ethics committee at Boehringer-Ingelheim, Biberach.

2.2. Induction of allergic asthma

Immunization and challenge of mice in models of acute allergic asthma and disease relapse were done as previously described [24]. Briefly, naïve BALB/c mice were compared with mice immunized with 10 µg of OVA (Sigma Chemical Co., St. Louis, MO, USA) intraperitoneally (i.p.) on days 0 and 21 and then challenged 1 week later (or as indicated) with nebulized 1% OVA in phosphate buffered saline

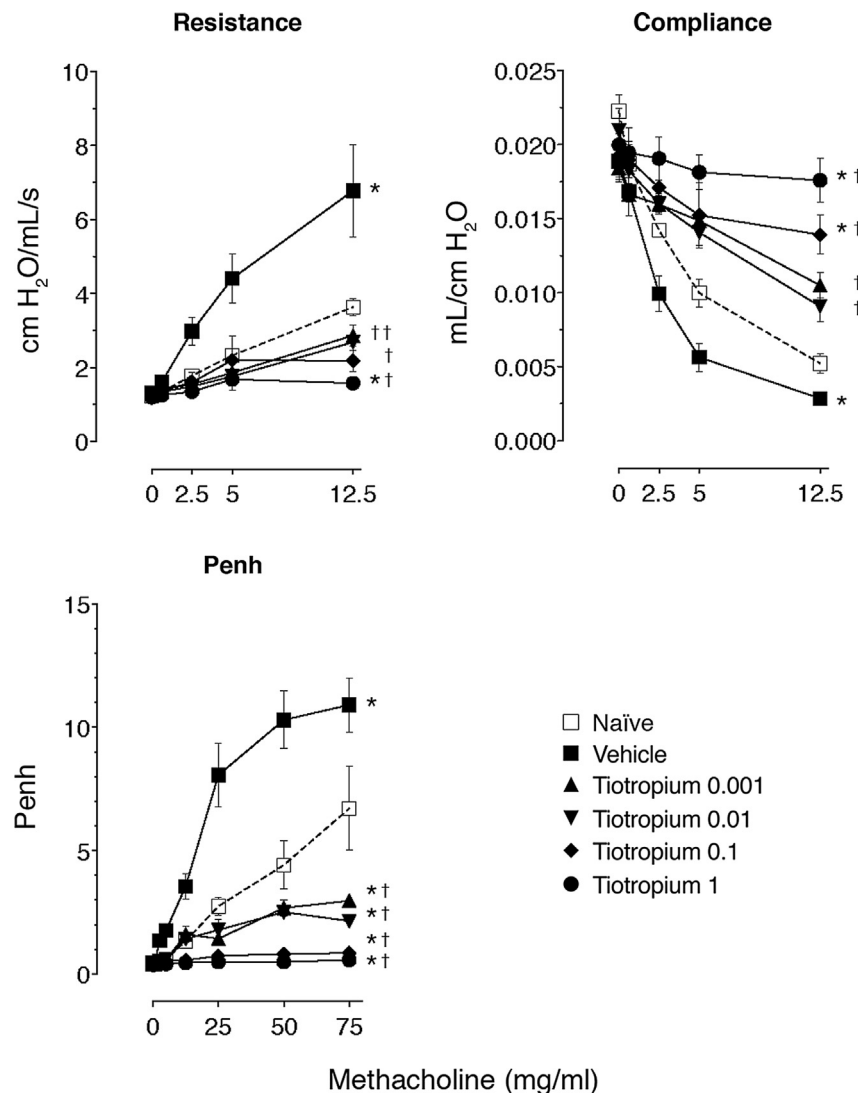


Fig. 1. Effect of tiotropium on methacholine-induced airway hyperresponsiveness. Titrated doses of aerosolized methacholine-induced resistance and compliance measured with resistance and compliance system, as well as enhanced pause measured with whole-body plethysmography. Naïve mice, vehicle-treated, and mice treated with titrated doses of tiotropium from 0.001 to 1 mg/kg were compared 72 h after aerosol challenge. Data are expressed as mean $\pm$ SEM from two independent experiments ($n = 6-8$). One-way ANOVA followed by Dunnett's multiple comparison test of mean AUC; * $p < 0.05$ is significant for all groups compared to the naïve group and † $p < 0.05$ is significant when compared to the vehicle-treated group.

(PBS) in a Plexiglas chamber by an ultrasonic nebulizer (Aerodyne, Kendall, Neustadt, Germany) for 60 min twice daily on 2 consecutive days (days 28, 29 unless indicated) to induce acute onset disease (acute). For disease relapse, mice with acute disease were rested for at least 90 days and then rechallenged with a 1% OVA nebulization for 60 min twice daily on 2 consecutive days as indicated.

2.3. Treatment protocols

For the treatment of AHR, we administered dexamethasone (Sigma Chemical Company, St. Louis, USA) at the dose of 1 mg/kg or tiotropium (Boehringer-Ingelheim Pharma, Biberach, Germany) at the doses of 1, 0.1, 0.01 or 0.001 mg/kg for 5 days, starting 3 days before nebulized OVA challenges in the acute model. For the treatment of other disease parameters before the onset of acute disease and before inducing disease relapse, mice received twice daily intranasal instillations of dexamethasone or tiotropium at the dose of 1 mg/kg for 5 days, starting 3 days before nebulized OVA challenges. For all treatments, we administered the drugs 30 min before aerosol challenges. For each application of 1 mg/kg dose, dexamethasone and tiotropium were dissolved in PBS for a final concentration of 0.5 mg/ml. For 0.1, 0.01 and 0.001 mg/kg doses, tiotropium was dissolved in PBS for a final concentration of 50, 5 and 0.5 µg/ml, respectively. All solutions were administered to the mice in 50 µl.

2.4. Airway hyperresponsiveness

We measured airway hyperresponsiveness (AHR) as a change in airway function at 72 h after the last challenge with aerosolized methacholine (Sigma) using two different techniques. In conscious, unrestrained mice, we measured AHR by whole-body plethysmography (Buxco Electronics Ltd., NY, USA). PBS, followed by increasing concentrations of methacholine, was nebulized for 3 min and lung function was recorded and calculated as enhanced pause (Penh), which is a dimensionless unit that correlates with pulmonary resistance. In separate experiments, airway resistance and compliance were measured in anesthetized and ventilated animals by Resistance and Compliance System (Buxco Electronics Ltd.). As for whole-body plethysmography, PBS followed by increasing concentrations of methacholine was nebulized for 3 min and lung function was recorded and calculated using FinePoint software (Buxco Electronics Ltd.).

2.5. Airway inflammation

Seventy-two hours after last challenge, mice were terminally anesthetized, tracheostomy was performed and a plastic catheter was clamped into the trachea. The lungs were washed 3 times with PBS in a total volume of 1 ml (0.4, 0.3 and 0.3 ml) to collect bronchoalveolar lavage fluid (BAL). The total number of cells in BAL was enumerated in a Neubauer hemocytometer. The percentage of inflammatory cells was determined by morphological examination of at least 300 cells in cytocentrifuged preparations (Cytospin-4, Shandon Instruments, UK), stained with the Kwik-Diff (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA).

2.6. Lung inflammation and mucus hypersecretion

After bronchoalveolar lavage, lungs were dissected and fixed by immersion in 4% paraformaldehyde. Paraplast-embedded lung sections of 3 µm were stained with hematoxylin and eosin (H&E) for morphological evaluation, with Luna stain for eosinophil enumeration [23] and with periodic acid-Schiff stain (PAS) for mucopolysaccharide staining intensity of lung. For scoring of inflammatory cell infiltration, sections containing main stem bronchi from each lung specimen stained with H&E were used. An expert respiratory

pathologist blinded to the treatment groups graded the extent of inflammation in the lungs according to a semi-quantitative scoring system: Grade 0 – no inflammatory infiltrates; Grade 1 – inflammatory infiltrates in central airways; Grade 2 – inflammatory infiltrates extending to middle third of lung parenchyma; and Grade 3 – inflammatory infiltrates extending to the periphery of the lung [24]. For quantification of eosinophil infiltration, eosinophils were counted on 5 random fields in Luna stained sections (400× magnification) containing major airways and vessels (which were selected from low power magnification) and averaged for each lung. For detection of mucus-containing cells in formalin-fixed lung tissue, 4 µm sections containing main stem bronchi from each lung specimen were stained with PAS and counter stained with hematoxylin. The number of mucus-containing cells per millimeter of basement membrane was determined with CellB software (Olympus Europa Holding GmbH, Hamburg, Germany).

2.7. Serum OVA-specific immunoglobulin

For the measurement of OVA-specific IgG1 and IgE, ELISA plates were coated with OVA at 10 µg/ml overnight at 4 °C. The plates were washed and blocked with 2% bovine serum albumin in PBS with 0.05% Tween 20 for 2 h at room temperature. Then sera were titrated onto the plates and incubated for 24 h at 4 °C before washing. Plates were incubated for an additional 2 h at 4 °C with biotinylated anti-IgG1 (Southern Biotechnology Associates Inc., Birmingham, AL, USA) or anti-IgE (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) detection antibodies, followed by incubation with streptavidin horseradish peroxidase (Southern Biotechnology) for 1 h at room temperature. Plates were washed and incubated with TMB substrate solution (100 µl/well, BD OptEIA™,

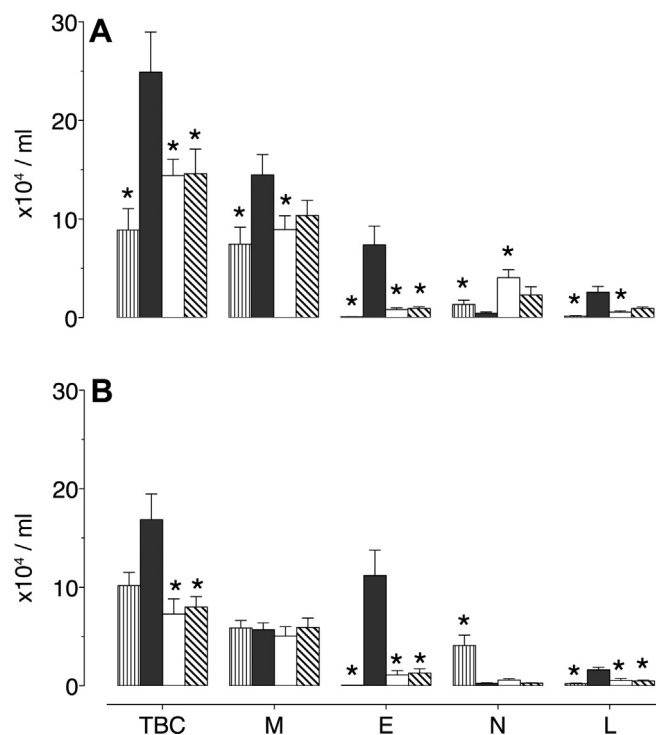


Fig. 2. Effect of tiotropium on airway inflammation. Total inflammatory cell count and differential counts in BAL of naïve, vehicle, dexamethasone and tiotropium treated mice 72 h after the last aerosol challenge at (A) disease initiation and at (B) disease relapse. Results are expressed as mean cell counts $\pm$ SEM from two independent experiments ($n = 6-10$). One-way ANOVA followed by Dunnett's multiple comparison test; * $p < 0.05$ is significant for all groups compared to the naïve group and † $p < 0.05$ is significant when compared to the vehicle-treated group. TBC: Total BAL cell count, M: Macrophages, E: Eosinophils, N: Neutrophils, L: Lymphocytes.

Becton Dickinson Biosciences) for 10 min at room temperature. The reaction was stopped with 100 μ l of 0.18 M H₂SO₄ and the plates were measured at 450 nm.

2.8. Statistical analysis

To compare AHR data, area under curve (AUC) was calculated for each experimental animal on the graph of resistance, compliance or Penh (y axis) vs. the methacholine concentration (mg/ml; x axis). Calculated AUC values from AHR data, airway inflammation data, as well as mucus-positive cell and eosinophil counts in the lungs were log-transformed to equalize variances and analyzed with one-way

ANOVA followed by Dunnett's multiple comparison test. Histology scores for inflammation were analyzed with Chi-squared test for trend. All analyses were done using GraphPad Instat v.5.0 (GraphPad Software Inc.) and *p* values were considered significant at <0.05.

3. Results

3.1. Tiotropium inhibits airway hyperresponsiveness in allergic asthma

Tiotropium as a muscarinic receptor antagonist in chronic obstructive pulmonary disease inhibits bronchoconstriction. To test

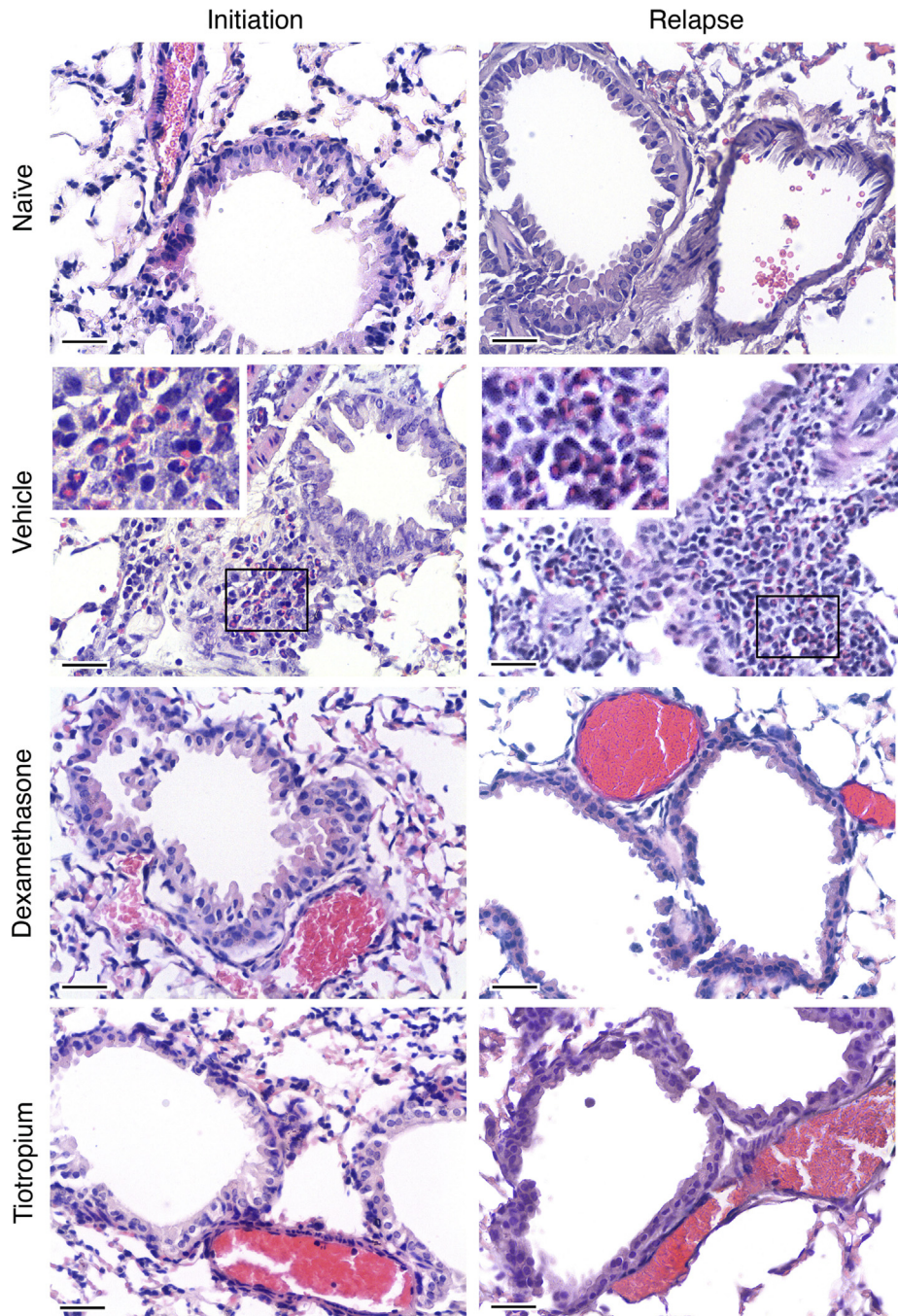


Fig. 3. Effect of tiotropium on lung inflammation. Representative photomicrographs of lung tissue on H&E stained sections (bar 100 μ m) from naïve, vehicle, dexamethasone and tiotropium treated mice 72 h after the last aerosol challenge (*n* = 6–10). Naïve mice have normal tissue without evidence of inflammation. At initiation and relapse, inflammatory infiltrates containing eosinophils (insets) are observed, but are reduced in the lungs of dexamethasone and tiotropium treated mice.

bronchodilator efficacy in acute allergic asthma, we instilled mice with the drugs intranasally for 5 days starting 3 days before the first challenge and then measured methacholine-induced AHR by unrestrained plethysmography. Tiotropium significantly reduced AHR at doses starting at 0.001 mg/kg (Fig. 1). Tiotropium treatment continued to inhibit AHR for 72 h after treatment at all doses, showing a long-acting effect on AHR even at low doses. However, at higher doses of 1 mg/kg, AHR was inhibited for over 1 week and airway eosinophils were suppressed (data not shown). We also measured airway resistance and compliance in anesthetized and ventilated allergen-treated animals and found that tiotropium at the same doses effectively and dose-dependently inhibited airway resistance and compliance (Fig. 1) an effect that was also seen when the mice were treated with dexamethasone (data not shown). Furthermore, in naïve mice tiotropium also reduced methacholine-induced AHR, but not as strongly as seen in the allergen treated mice (data not shown).

3.2. Tiotropium has anti-inflammatory effects in allergic asthma

Dexamethasone is one of the most effective anti-inflammatory drugs used in allergic asthma. To address whether tiotropium exhibited similar anti-inflammatory properties, we compared the effect of dexamethasone and tiotropium on inflammatory cell infiltrates in the airways and lungs of mice. At the initiation of allergic asthma, tiotropium and dexamethasone significantly inhibited overall airway inflammation by 41.1% and 42.1%, respectively compared to vehicle controls (Fig. 2). Both treatment regimens significantly inhibited airway eosinophilia from $7.4 \pm 1.9 \times 10^4$ cells/ml in the vehicle, to $0.84 \pm 0.17 \times 10^4$ and $0.9 \pm 0.18 \times 10^4$ cells/ml in dexamethasone and tiotropium treated mice, respectively. Both drugs also significantly reduced the number of lymphocytes. An additional effect of dexamethasone treatment was the reduction of macrophages and increase in neutrophils, a feature not observed with tiotropium treatment. To evaluate the drug efficacy during a relapse of allergic asthma, mice were treated with either tiotropium or dexamethasone just before and at the time of OVA-aerosol rechallenges (Fig. 2). We found that, similar to the effect on acute initiation of disease, tiotropium and dexamethasone inhibited the extent of inflammation in the airways by 52.6% and 56.8%, respectively, and specifically reduced the number of infiltrating eosinophils by 88.4% and 90.2% and lymphocytes by 69.0% and 66.0%, respectively.

Further evaluation of inflammation was achieved by examining H&E stained lung sections (Fig. 3). These lung sections were graded for the severity of inflammation and eosinophils were enumerated in the tissues (Fig. 4). At disease initiation, drug treatment suppressed overall inflammation in the parenchyma by up to 43.2% and eosinophils were reduced by tiotropium by 82.0% and dexamethasone by 85.2% compared to vehicle alone treatment. Tiotropium and dexamethasone also prevented eosinophilic lung inflammation upon OVA-induced disease relapse (Fig. 4). Taken together, these data demonstrate that tiotropium at higher doses than required to inhibit AHR, has anti-inflammatory properties comparable to dexamethasone.

3.3. Tiotropium effectively prevents mucus hypersecretion in acute and relapsing allergic asthma

To determine whether tiotropium inhibits mucus production in allergic disease, we evaluated the extent of mucus secretion of goblet cells in bronchial epithelium (Fig. 5). We compared the efficacy of tiotropium with dexamethasone and vehicle treatments and observed that both drugs markedly suppressed mucus overproduction at the initiation of allergic asthma compared to vehicle-treated control mice (Fig. 6). Naïve mice have the occasional mucus

producing goblet cell whereas vehicle-treated mice have their airway epithelia filled with mucus producing cells. In mice with acute allergic asthma, tiotropium reduced the number of mucus-positive cells by 50.3% compared to 56.7% by dexamethasone, indicating that tiotropium is as good as dexamethasone at suppressing mucus production. In experiments in which mice were treated before disease relapse, tiotropium treatment decreased the number of mucus-positive cells by 33.7%, which was greater than dexamethasone which suppressed mucus-positive cell number by 16.6%. These data demonstrate that tiotropium inhibits mucus hypersecretion as well as and even better than dexamethasone in acute and relapsing allergic asthma.

3.4. Tiotropium effect on OVA-specific IgE and IgG1

To determine whether B cell immune responses were influenced by treatment, we tested serum OVA-specific antibody titers 5 days after the last aerosol challenge (Fig. 7). We did not detect OVA-specific antibodies in naïve mice, however, vehicle-treated animals had high titres of OVA-specific IgE and IgG1 antibodies. Neither treatment with dexamethasone nor tiotropium reduced antibody titres.

4. Discussion

Here, we compared the potential of tiotropium in the prevention of the onset of acute asthma and a subsequent disease

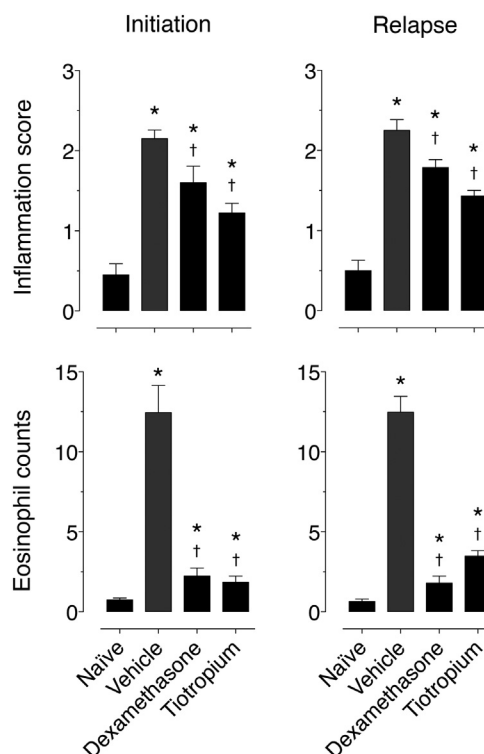


Fig. 4. Effect of tiotropium on inflammation and eosinophila in lung parenchyma. Inflammation scores and eosinophil counts in lungs of naïve control mice, vehicle, dexamethasone and tiotropium treated mice before initiation and relapse of asthma. Luna stained lung sections (per 400 $\times$) sampled at 72 h after final challenge. Results are expressed as mean inflammation score and eosinophil count per high power field $\pm$ SEM from two independent experiments ($n = 6-10$). One-way ANOVA followed by Dunnett's multiple comparison test for eosinophil counts and a Chi-square test for trend for inflammation scores. * $p < 0.05$ compared with naïve group; † $p < 0.05$ compared with vehicle-treated group.

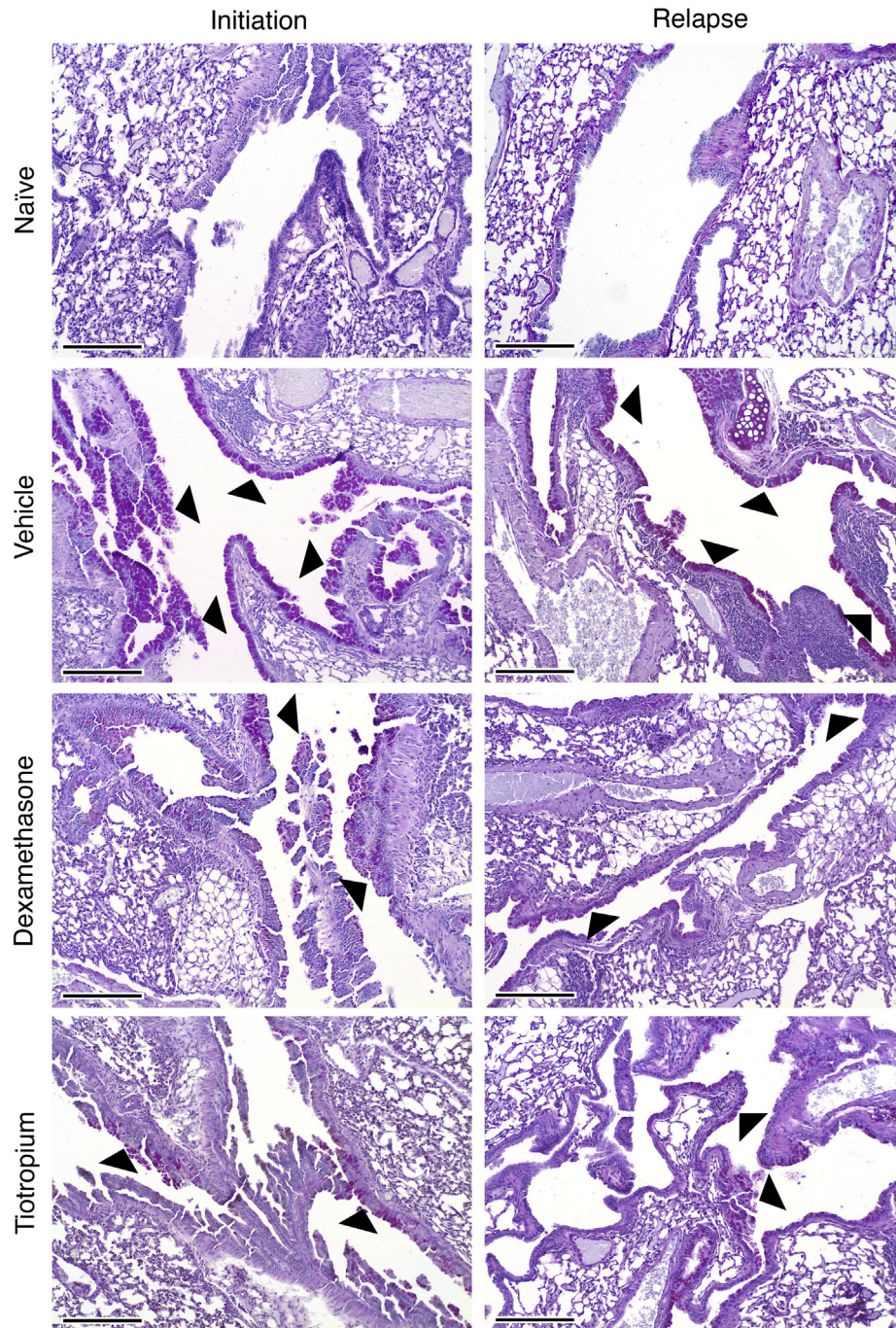


Fig. 5. Effect of tiotropium on mucus hypersecretion. Representative photomicrographs of lung tissue on PAS stained sections (bar 200 μ m) from naïve, vehicle, dexamethasone and tiotropium treated mice 72 h after the last aerosol challenge ($n = 6–10$). Naïve mice have normal tissue with very rare mucus-positive cells. At initiation of disease and relapse in the vehicle-treated sections, almost all cells in the epithelium are positive for mucus. At initiation and relapse, mucus-positive cells are observed, but are reduced in the lungs of dexamethasone and tiotropium treated mice. Arrowheads point to the areas of the epithelium with mucus-positive cells.

relapse. We observed a powerful effect on AHR at low doses and at higher doses tiotropium was as effective as dexamethasone at suppressing mucus hypersecretion and eosinophilic lung inflammation. Tiotropium was as effective at preventing the onset of acute disease as it was at preventing disease relapse, suggesting the potential use of tiotropium in patients with seasonal allergic asthma. Our findings support the use of tiotropium as a bronchodilator, mucolytic and anti-inflammatory in the treatment of relapses of allergic asthma.

Tiotropium successfully reduced AHR at very low doses by either decreasing the reactivity of airway smooth muscle or blocking the binding of methacholine to its receptor. There is evidence that non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea pig model of allergic asthma [4]. Antigen-induced hyperreactivity was completely blocked by tiotropium treatment and partially blocked with atropine treatment and that tiotropium blocked bronchoconstriction induced by intravenous acetylcholine but did not inhibit vagally-induced

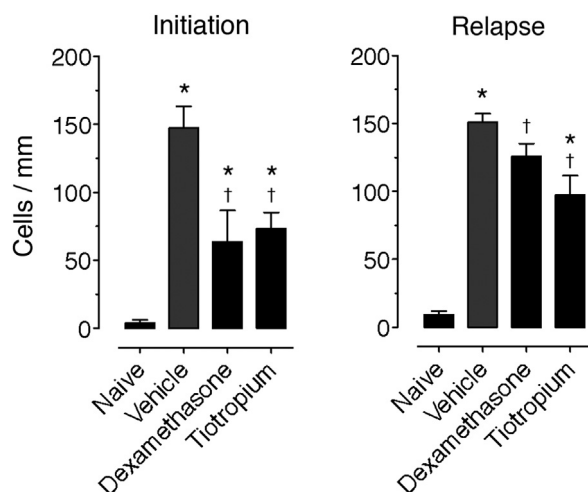


Fig. 6. Effect of tiotropium on the extent of mucus hypersecretion. Number of mucus-positive cells per millimeter of basement membrane in naïve control mice, vehicle, dexamethasone and tiotropium treated mice before initiation and relapse. Results are expressed as mean $\pm$ SEM from two independent experiments ($n = 6-10$). * $p < 0.05$ compared with naïve group; † $p < 0.05$ compared with vehicle-treated group (Chi-square test for trend).

bronchoconstriction in sensitized controls. These data suggest that tiotropium does not block hyperreactivity by blocking receptors for vagally-released acetylcholine and suggests that tiotropium may work via anti-inflammatory mechanisms because it also inhibited eosinophilia in the lungs and around nerves.

Our findings illustrate that at high doses, tiotropium is as effective an anti-inflammatory as dexamethasone in the initiation and relapse of allergic asthma. Our data support previous reports showing that 4 daily doses of 50 μ l of 0.1 mM tiotropium given by aerosol over 3 min had anti-inflammatory effects in the airways of mice with acute onset allergic asthma [26] and a study in guinea pigs revealing that tiotropium (1 μ g/kg) suppresses lung eosinophilia in acute allergic asthma [4]. In contrast, low doses of 50 μ l of 0.1 nM tiotropium (corresponding to a dose of $\sim$ 0.1 μ g/kg) given intranasally for 5 days (on the same days that the animals were challenged with allergen) actually increased airway inflammation with an increase in macrophages and eosinophils [15]. Not only do our results

with high doses illustrate that acute initiation of eosinophilic airway inflammation is suppressed by tiotropium, but that parenchymal lung inflammation is also significantly suppressed.

Tiotropium and dexamethasone almost completely prevent eosinophil infiltration of the airways at disease relapse. Previous mouse studies reveal that mice recovered from allergic asthma have long-lived memory Th2 cells, macrophages, B cells and dendritic cells residing in chronic infiltrates, but lack eosinophils and neutrophils [24]. These memory cells respond rapidly to allergen rechallenge with recruitment of eosinophils that treatment with both tiotropium and dexamethasone prevent. Although, acute onset eosinophilic airway inflammation and eosinophilia in the parenchyma were prevented, we observed that inflammatory infiltrates in the lungs remain, i.e. the lungs are not like those from naïve mice. Unfortunately, these infiltrates are difficult to eliminate and if maintained, the cells within the infiltrates will continue to respond to allergen rechallenges leading to relapses (M. Epstein, unpublished data). Generally, parenchymal infiltration and airway inflammation correlate. However, we have encountered other situations in which they do not [13]. In this study, parenchymal infiltrates were eliminated while airway inflammation persisted after treatment with dexamethasone. In contrast, tiotropium appears to reduce airway inflammation more rapidly than parenchymal inflammation and seems to target eosinophils earlier than macrophages and lymphocytes, which are the predominant cells within the infiltrates. It is possible that for complete elimination of lung infiltrates, chronic treatment is necessary, even in asymptomatic individuals with infrequent relapses. This is especially relevant in lieu of the studies showing that a longer duration of treatment with low dose tiotropium was effective at reducing inflammation and reduced airway remodeling in chronic models in mice [15,26] and in guinea pigs [2,9].

Clinical evidence of tiotropium treatment reducing the number of relapses in severe asthmatics [27] suggests that tiotropium is beneficial in allergic asthma, yet the mechanisms underlying a potential anti-inflammatory effect remain unclear. There is evidence for a role of muscarinic receptors on immune and inflammatory cells [8,11,31], however, our studies suggest that it is necessary to increase the dose of tiotropium to increase efficacy, at least in mice. Having determined that inflammation is suppressed by treatment with tiotropium, that Th2 cytokines are concomitantly reduced [15,26], and being aware that B cells express muscarinic receptors [34], we anticipated that high doses of tiotropium would inhibit B cell responses resulting in reduced allergen-specific antibody titres. However, we observed no effect of either tiotropium or dexamethasone treatment on antibody production. It is not clear why titres remained unaffected but a possible explanation is that the duration of treatment was too short to observe a change in the titres. In a previous study, no changes in allergen-specific antibody titres were observed in a chronic model with chronic tiotropium treatment [26].

The observed anti-inflammatory mechanism may be related to the bronchodilatory properties of tiotropium. However, preclinical evidence suggests anti-inflammatory properties of tiotropium beyond bronchodilation. Cholinergic stimulation was shown to trigger the release of chemotactic factors in bronchial epithelial cells *in vitro* [5,19]. Supernatant taken from epithelial cells stimulated with acetylcholine induced chemotaxis in primary human eosinophils [19] or neutrophils [5]. This migratory response of neutrophils was absent in experiments where the epithelial cells were pretreated with tiotropium [5]. Neutrophil migration was inhibited directly by an LTB4 antagonist suggesting that this mediator may play a role in cholinergic inflammation. Taken together, these data suggest that tiotropium may also have direct anti-inflammatory effects in our mouse model of allergic

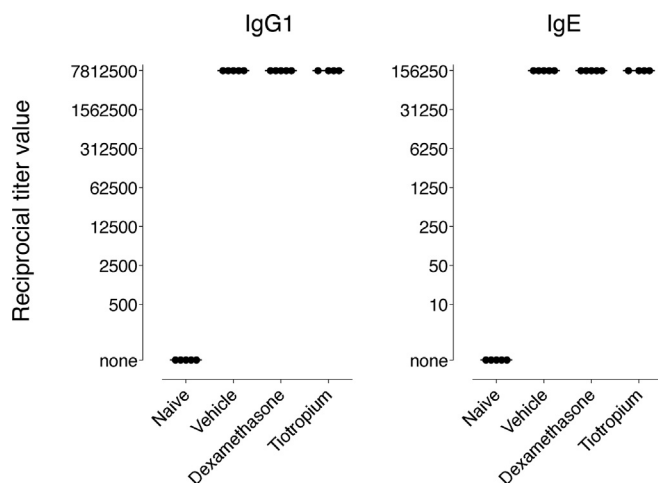


Fig. 7. Tiotropium effect on serum OVA-specific antibody at disease initiation. Analysis of serum OVA-specific IgG1 and IgE antibody titres from naïve, vehicle, dexamethasone and tiotropium treated mice 5 days after the last aerosol challenge for the initiation of allergic asthma. Results are shown as reciprocal titres for individual mice (dots) and group mean (line) from two independent experiments ($n = 4-5$). * $p < 0.05$ compared with naïve group by one-way ANOVA.

inflammation. Further research is needed to elucidate if and by which mechanism tiotropium causes direct anti-inflammatory effects.

Tiotropium and dexamethasone treatment suppressed mucus secretion by over 50% at the initiation of disease, but they were less effective during a relapse and dexamethasone reduced mucus production half as well as tiotropium. Our results demonstrate that both treatments do not abolish mucus production and this may relate to duration of therapy, however, long-term experiments in mice with chronic treatment reduced mucus from approximately 60 to 40%, but this might be because mucus metaplasia is more severe in chronic disease and therefore, more difficult to treat [26].

Our study indicates that tiotropium at higher doses than required for bronchodilation effectively suppresses inflammation and mucus hypersecretion in the lungs and airways of mice during the initiation and relapse of asthma. Recent studies show that tiotropium is effective as an adjunct in uncontrolled asthma at doses of 5–50 µg daily [1,17,29]. The doses used in the studies are not as high as the doses used in mice. It is likely that in this dose level, the effect is on airway smooth muscle. It is not yet known whether higher doses would further increase effectiveness by reducing inflammation. These studies have not been done. Taken together, our studies in addition to others indicate that tiotropium has anti-inflammatory activity at higher doses and that tiotropium is effective in patients. More studies are needed to understand the mechanism underlying the tiotropium effect in asthma.

Authorship contributions

Participated in research design: Bosnjak, Erb, Pieper, Epstein.

Contributed new reagents or analytic tools: none.

Conducted experiments: Bosnjak, Tilp, Tomsic, Dekan.

Performed data analysis: Bosnjak, Erb, Tilp, Tomsic, Dekan, Epstein.

Wrote or contributed to the writing of the manuscript: Bosnjak, Dekan, Pieper, Erb, Epstein.

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