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Tiotropium reduction of lung inflammation in a model of chronic gastro-oesophageal reflux

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ABSTRACT: Gastro-oesophageal reflux is frequent in chronic airway diseases and is considered a trigger for symptoms. In animal models, bilateral vagotomy or muscarinic antagonists prevent the increase in airway resistance and the microvascular leakage induced by acute oesophageal acid instillation.

The present study investigates lung inflammation and remodelling in an animal model of chronic gastro-oesophageal reflux disease (GORD), and the effectiveness of pretreatments with tiotropium, atropine and dexamethasone.

Mice were exposed to twice-daily intra-oesophageal HCl instillations for 21 days. Exposure to HCl causes: marked infiltration by inflammatory cells of the airways and of peribronchial areas; an increase in epithelial thickness; histological features of interstitial pneumonitis; an increase in cell numbers and in the levels of interleukin-8; and soluble intercellular adhesion molecule in bronchoalveolar lavage fluids, as well as of *in vitro* tracheal contractility. The administration of nebulised tiotropium or intraperitoneal atropine prior to each instillation of HCl, considerably inhibited all these changes.

These results indicate a major role of acetylcholine in airway inflammation and remodelling in a GORD model, and demonstrate that tiotropium and atropine can prevent lung inflammation with an effectiveness similar to intraperitoneal dexamethasone, providing additional evidence that anticholinergics might contribute to the control of inflammatory processes in airway diseases.

KEYWORDS: Airway inflammation, airway smooth muscle, anticholinergics, gastro-oesophageal reflux, glucocorticosteroids, mouse

Patients with severe chronic obstructive pulmonary disease (COPD) or asthma have a high prevalence of gastro-oesophageal reflux disease (GORD), which is considered a potential trigger for airway symptoms and exacerbations [1–4]. Medical and surgical treatments for reflux improve wheezing and coughing in ~66% of asthmatic patients, reduce the use of on-demand inhalers in more than half, and improve lung function in nearly 25% of them [1]. Recent studies, however, have challenged the role of GORD as a cause of poorly controlled asthma, at least in patients with asymptomatic GORD [3, 4].

The parasympathetic nervous system provides the dominant autonomic innervations from the larynx down to the smallest airways and alveoli. The efferent cholinergic pathways that travel in the vagus nerve and synapse in airway parasympathetic ganglia cause broncho-constriction [5]. The blockade of muscarinic receptors (M1 and M3) with atropine or inhaled anticholinergics improves airway obstruction, and indirect evidence suggests that parasympathetic activity is increased in COPD and asthma [5].

In asthmatic patients, the reduction in forced expiratory flow at 50% of vital capacity induced by HCl instillation into the oesophagus has been eliminated by atropine pretreatment [6]. In animal models, we and others have previously reported that bilateral vagotomy or atropine pretreatment inhibit the increase in airway resistance and the microvascular leakage induced by acute oesophageal acid instillation [7, 8]. We have also shown that airway microvascular leakage caused by single HCl intra-oesophageal instillation in guinea pigs involved both M1 and M3 receptors [9]. These results indicate that the release of acetylcholine, acting through the activation of muscarinic receptors, contributes

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to the airway responses caused by oesophageal stimulation with HCl.

Acetylcholine is traditionally involved in airway smooth muscle contraction, microvascular leakage and mucus secretion. Recent evidence has also indicated that acetylcholine may contribute to various aspects of lung inflammation and airway remodelling [5]. Through the activation of muscarinic receptors, acetylcholine can stimulate the proliferation of lung fibroblasts and smooth muscle cells, as well as collagen synthesis in lung fibroblasts [5, 10]. Acetylcholine can also promote chemotactic activities in epithelial cells, macrophages and airway smooth muscle [5]. The instillation of diesel particulates into the airways of anesthetised rats evokes neutrophilia in the lung, which is abrogated by atropine pretreatment or bilateral vagotomy [11]. In a guinea pig model of allergic asthma, a prominent role for acetylcholine has also been shown in the increase of airway smooth muscle thickening and contractility, as well as mucus gland hypertrophy, goblet cell number and eosinophilia induced by repeated exposure to allergens [12, 13]. Treatment with tiotropium bromide, a long-acting muscarinic receptor antagonist, prevented these different aspects of allergen-induced airway remodelling [12, 13]. The anti-remodelling effects of tiotropium were comparable to those of budesonide [13], suggesting that the therapeutic effects of anticholinergics may contribute to the reduction in airway remodelling and lung inflammation, in addition to their bronchodilatory effects.

In the present study, we established an experimental model of chronic GORD-induced lung inflammation by daily HCl intra-oesophageal instillation in mice for 21 days and compared the effectiveness of atropine, tiotropium and dexamethasone in preventing various aspects of lung inflammation, including airway remodelling, cellular accumulation into the lung, and airway smooth muscle contractility.

MATERIALS AND METHODS

Animals

Male BALB/c mice from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China), that were 8 weeks old and weighed 18–24 g, were used. All experiments were conducted in accordance with the guidelines for the care and use of laboratory animals from Shanghai Jiao Tong University (Shanghai, China).

A novel mouse model of chronic airway inflammation induced by GORD

Mice were exposed to the instillation an acidic solution (0.3 mL; 0.1 N HCl and 0.5% pepsin) into the distal part of the oesophagus twice daily for 21 consecutive days using a calibrated feeding tube. Control animals were exposed to oesophageal instillations of saline. 1 h prior to each instillation of the acidic solution, mice were treated either with dexamethasone (1 $mg \cdot kg^{-1}$) or atropine (1 $mg \cdot kg^{-1}$) administered intraperitoneally, or with tiotropium (5 μg or 25 $\mu g \cdot kg^{-1}$) delivered by nebulisation for 20 min in a chamber using an Ultrasonic Nebulizer 4021 (Shanghai Siling Medical Apparatus Factory, Shanghai, China). The doses of dexamethasone, atropine and tiotropium were chosen according to doses previously tested [10, 12, 14].

Evaluation of lung inflammation: bronchoalveolar lavage measurements and lung histology

Animals were euthanised with urethane (100 $mg \cdot kg^{-1}$ *i.p.*) 24 h after the last exposure to the acidic solution (day 22). The chest wall was then opened and the animals were exsanguinated by cardiac puncture.

Immediately after cardiac puncture, bronchoalveolar lavage (BAL) was performed by infusion and extraction of 1 mL of PBS *via* a tracheal cannula. This was repeated twice, and the recovered BAL fluids (BALF) were pooled. The BALF was centrifuged at 1,500 × g for 5 min at 4°C, and the supernatant was stored at -70°C and used for interleukin (IL)-8 and soluble intercellular adhesion molecule (ICAM)-1 detection. IL-8 and ICAM-1 measurements were performed using ELISA kits (Rapidbio Lab, Langka Trade Co. Ltd, Shanghai, China). The cell pellet was resuspended in 1 mL of PBS, and the total number of cells was counted with a haemocytometer. Differential counts of macrophages, lymphocytes, neutrophils and eosinophils were determined on cytospin smears of BAL samples from individual mice, and stained with Wright–Giemsa after counting 400 cells. Results are expressed as cell number $\times 10^6 \cdot L^{-1}$ of BALF.

Immediately after BALF recovery, the oesophagus and the right lung were removed, rinsed with PBS, instilled with 10% phosphate-buffered formalin and fixed with the same solution for 18–24 h. Parasagittal sections through the lung were then cut, embedded in paraffin and sectioned at 8 μm in thickness. The slides were stained with haematoxylin and eosin (H&E) and examined by two histologists in a blinded fashion. The thickness of the bronchial epithelial and smooth muscle layers was measured in H&E-stained sections at 10 random non-overlapping locations using Image-Pro Plus 6.0 software (Olympus, Shanghai, China). The mean thickness was calculated on seven medium-sized bronchi (measuring 250–400 μm in luminal diameter), for which the ratio of the maximum and minimum diameters was <2 to ensure that the airways were not obliquely cut. Segments of the oesophagus were treated as described for lung tissues.

Airway function: isometric tension measurements

Immediately after BALF recovery, the trachea was removed and prepared free of serosal connective tissue. Single ring preparations were mounted for isometric recordings in organ baths containing modified Krebs–Henseleit (KH) solution (composition in mM: NaCl 118.0; KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25.0; $MgSO_4$ 1.2; KH_2PO_4 1.2; glucose 11.0; $EDTA \cdot Na_2$ 0.5), maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2. Each ring preparation was connected vertically to a force-displacement transducer under a resting tension of 0.5 g. Preparations were allowed to equilibrate for $\geqslant$1 h, during which the buffer solution was renewed every 15 mins. Resting tension was re-adjusted to 0.5 g and tracheal rings were precontracted twice with 40 mM KCl. Isometric contractions were recorded using a PowerLab 8sp life analysis system (ADInstruments Shanghai Trading Co., Shanghai, China). Following a thorough washing of the tissues over a period of $\geqslant$30 mins, cumulative concentration–response curves were constructed to acetylcholine (1 nM–3 mM) using 0.5 log increments. The changes in airway isometric tension were expressed as force generated (g) and

pEC50, *i.e.* -$\log_{10}$ of the concentration of acetylcholine causing 50% of the maximal force generated (EC_{50}). The EC_{50} was calculated from the logarithmic regression of each concentration–response curve.

Drugs

Tiotropium was purchased from Phoesher Chemical Co. Ltd (Jinan, China), dexamethasone was purchased from Tongyong Pharmaceuticals Company (Shanghai, China), and atropine and acetylcholine were purchased from Sigma Chemical (St Louis, MO, USA). All other chemicals were of analytical grade.

Data analysis

All data are presented as mean±SEM. Differences between groups were analysed using ANOVA, followed by a Dunnett t-test for selected pairs if appropriate. Differences between means were considered to be significant at a p-value of <0.05. All statistical analyses were performed using Prism version 5.0 (Graph-Pad Software, San Diego, CA, USA).

RESULTS

Repeated oesophageal instillation of HCl caused oesophagitis and lung inflammation

Mice exposed to HCl exhibited consistent erosion of the oesophageal stratified squamous epithelium associated with an impressive infiltration of inflammatory cells in the muscularis mucosa. The thickness of the muscularis mucosa was not significantly altered (data not shown).

Lung histopathology revealed that HCl exposure resulted in marked infiltrations by polynuclear phagocytes, lymphoplasmocytes and macrophages of the airway walls and of peribronchial and peribronchiolar areas. The formation of lymphoid follicles containing germinal centres was observed in certain areas (fig. 1a and b *versus* c and d). The accumulation of inflammatory mucus exudates containing polynuclear phagocytes, lymphoplasmocytes and macrophages was also observed in the lumen of airways. In addition, histological features of interstitial pneumonitis were noted, with a thickening of alveolar walls with infiltration by polynuclear phagocytes and lymphoplasmocytes associated with congested capillaries. Morphometric analysis of airway wall remodelling showed a significant epithelial thickening associated with a weak but significant smooth muscle thickening (fig. 2). The analysis of cells retrieved from the airways by BAL revealed a significant increase (1.8-fold) in the total cell number. Differential cell counts also revealed significantly increased numbers of macrophages, lymphocytes and neutrophils, which rose 1.7-, 2.0- and 3.1-fold, respectively, compared with saline control (fig. 3). There was no increase in BALF eosinophil numbers. Furthermore, the concentrations of IL-8 and ICAM-1 were significantly elevated in BALF (fig. 4).

Reduced lung inflammation in mice treated with tiotropium and atropine

Mice treated either with nebulised tiotropium (25 $\mu g \cdot kg^{-1}$) or intraperitoneal atropine (1 $mg \cdot kg^{-1}$) throughout exposure to HCl exhibited marked reductions in inflammatory cell infiltration and in the presence of congested capillaries in the alveolar walls (fig. 1e and f, and g and h, respectively). A nonsignificant decrease of the epithelium thickening was observed in the group of mice treated with 5 $\mu g \cdot kg^{-1}$ of tiotropium. However, no significant reduction of the smooth muscle thickening was observed in the three groups of mice treated with anticholinergics, but the smooth muscle thickness was not significantly different from those in control mice not exposed to HCl (fig. 2).

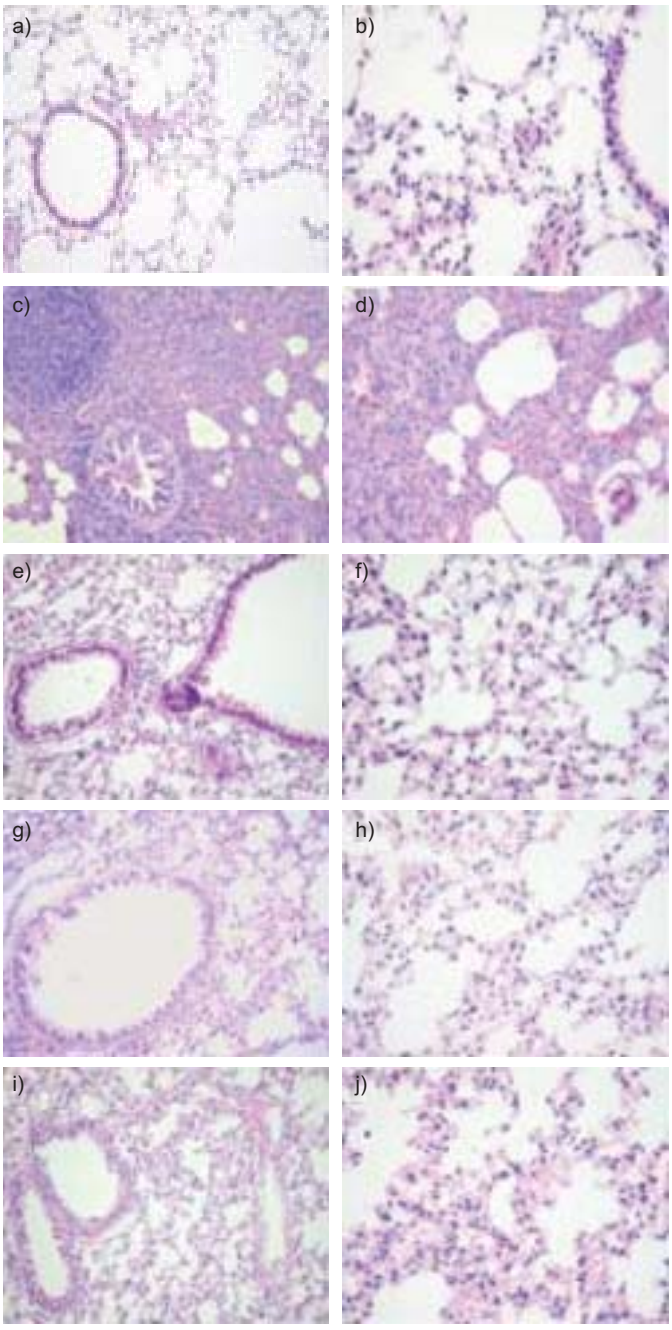

FIGURE 1. Representative photomicrographs of lung sections stained with haematoxylin and eosin. a and b) Saline-exposed animals, c and d) untreated HCl-exposed animals, e and f) HCl-exposed animals treated with nebulised tiotropium (25 μg) or g and h) treated with intraperitoneal injection of atropine (1 $mg \cdot kg^{-1}$), or i and j) dexamethasone (1 $mg \cdot kg^{-1}$). Photographs were taken at a, c, e, g and i) 100× magnification and at b, d, f, h and j) 200× magnification.

The number of cells in BALF was also decreased in mice treated either with nebulised tiotropium or intraperitoneal

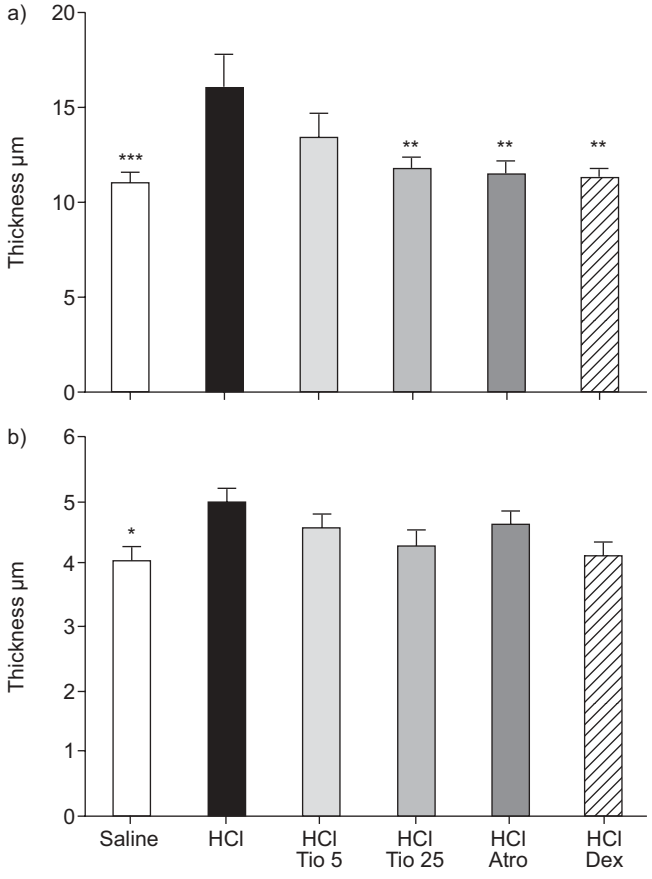

FIGURE 2. Effects on a) epithelial and b) smooth muscle thickness in medium-sized bronchi, of twice-daily exposure to HCl for 21 days and treatment by nebulisation of tiotropium 5 µg (Tio 5) or 25 µg (Tio 25), or by intraperitoneal injection of atropine (Atro) 1 mg·kg^{-1} or dexamethasone (Dex) 1 mg·kg^{-1}. Data are presented as mean±SEM (seven animals per group) *: $p<0.05$ when compared to untreated HCl-exposed animals; **: $p<0.01$ when compared to untreated HCl-exposed animals; ***: $p<0.001$ when compared to untreated HCl-exposed animals.

atropine (fig. 3). In mice treated with tiotropium at 5 µg·kg^{-1} and 25 µg·kg^{-1}, the reduction in the HCl-induced increase in BALF cell numbers reached 76 and 89% (total cells), 79 and 91% (macrophages), 83 and 89% (lymphocytes), and 70 and 77%, (neutrophils), respectively. In mice treated with atropine, the reduction in the HCl-induced increase in BALF cell numbers reached 63% (total cells), 71% (macrophages), 65% (lymphocytes) and 54% (neutrophils). There were no significant differences in the BALF cell numbers between the mice treated with tiotropium and atropine. IL-8 and ICAM-1 concentrations in BALF were reduced by atropine and the highest dose of tiotropium (fig. 4).

Reduced lung inflammation in mice treated with dexamethasone

Mice treated with dexamethasone (1 mg·kg^{-1} *i.p.*) throughout exposure to HCl exhibited a reduction in lung inflammation (fig. 1i and j), with a significant decrease of the epithelium thickening and no alteration of the smooth muscle thickening (fig. 2). Dexamethasone also markedly inhibited the recruitment of bronchoalveolar inflammatory cells (fig. 3). Reductions in the increase in BALF cell numbers reached 93% (total cells), 98% (macrophages), 95% (lymphocytes) and 81% (neutrophils). IL-8 and ICAM-1 concentrations in BALF were both reduced by dexamethasone (fig. 4).

Inhibition by tiotropium, atropine and dexamethasone of the enhanced acetylcholine-induced contraction of mouse trachea

3 weeks of twice-daily instillations of HCl into the distal part of the oesophagus enhanced contraction of mouse tracheal rings in response to the cumulative addition of acetylcholine (fig. 5). The maximal contractile response to acetylcholine increased from 0.35±0.02 g in saline-exposed mice to 0.55±0.06 g in HCl-exposed mice ($p<0.001$). Sensitivity to acetylcholine, however, was not affected (pEC_{50} 5.7±0.3 g and 6.0±0.7 g for saline- and HCl-exposed mice, respectively). Treatments with tiotropium (25 µg·kg^{-1}) and atropine completely prevented the increase in maximal contraction induced by HCl exposure (25 µg·kg^{-1}tiotropium 0.32±0.04 g and atropine 0.41±0.02 g (fig. 5)). The sensitivity to acetylcholine was also not altered (25 µg·kg^{-1} tiotropium pEC_{50} 5.4±0.4 g and atropine pEC_{50} 5.4±0.2 g). Similarly, pretreatment with dexamethasone completely prevented the increase in contractility caused by HCl exposure (maximal response 0.37±0.23 g) with no alteration of the sensitivity to acetylcholine (pEC_{50} 5.5±0.3 g).

DISCUSSION

The main findings of the present study are that airway remodelling, inflammatory cellular infiltration of the lung and the increase in airway reactivity to acetylcholine caused by a twice-daily instillation of HCl into the oesophagus of mice for 21 days, can be partially or even considerably prevented by muscarinic receptor antagonists, including tiotropium, a long-lasting anticholinergic widely prescribed in COPD patients. We have previously shown in guinea pigs that the airway vascular leakage induced by acute intra-oesophageal HCl instillation involving acetylcholine release from vagus nerve terminals, was blocked by atropine and was mediated through the activation of M1 and M3 receptors [9]. In this previous study, the upper portion of the oesophagus was ligated to prevent stimulation of the larynx by HCl and to ensure an oesophageal origin of the cholinergic reflex-induced airway leakage [9]. In these previous experimental conditions, however, we used a concentration of HCL (1 N) far above the physiological range to trigger a cholinergic reflex. In the present study, it was not possible to ligate the upper part of the oesophagus since instillation of HCl took place twice daily for 21 days, but we used a concentration of HCl (0.1 N) in the physiological range [15]. It is likely that oesophago-laryngeal reflux as well as microaspirations of HCl occurred in this chronic model of GORD as well as microaspiration of gastric content, as suggested by the observation of microscopic food fragments in the airways of some HCl-exposed untreated mice (data not shown). Pulmonary microaspiration of gastric content has also been demonstrated in children with chronic respiratory symptoms [16, 17]. Stimulation of the larynx or the trachea with HCl causes a much greater bronchoconstriction than oesophageal stimulation [18–20], suggesting that oesophago-laryngeal reflux is a more likely mechanism for the bronchoconstriction and airway inflammation associated

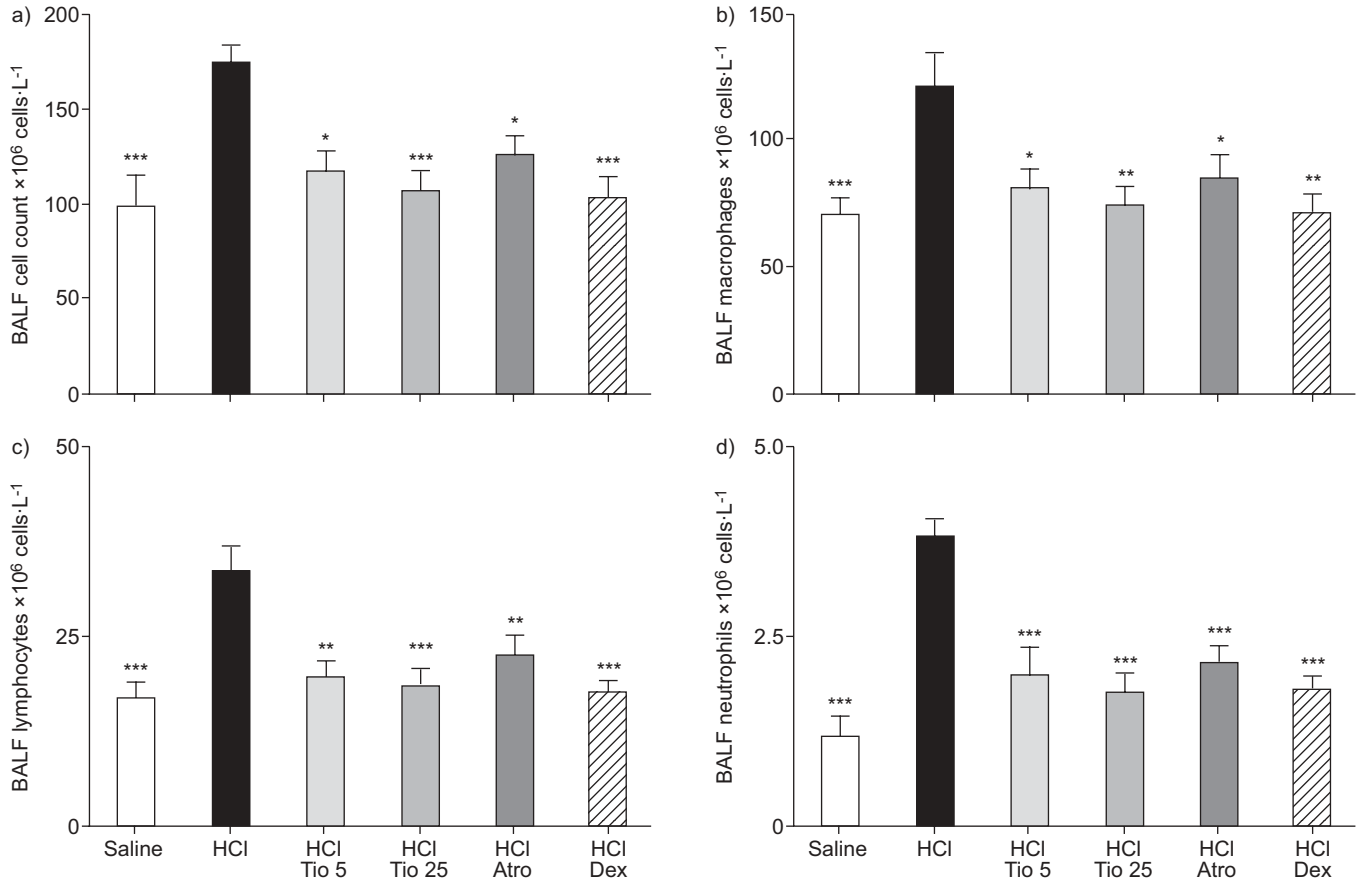

FIGURE 3. Effects on a) total and differential cell counts of b) macrophages, c) lymphocytes and d) neutrophils in bronchoalveolar lavage fluid (BALF), of twice-daily exposure to HCl for 21 days and treatment by nebulisation of tiotropium 5 μg (Tio 5, n=9) or 25 μg (Tio 25, n=12), or by intraperitoneal injection of atropine (Atro, n=12) 1 mg·kg^{-1} or dexamethasone (Dex, n=11) 1 mg·kg^{-1}. Data are presented as mean±SEM. *: $p<0.05$ when compared to untreated HCl-exposed animals (n=17); **: $p<0.01$ when compared to untreated HCl-exposed animals (n=17); ***: $p<0.001$ when compared to untreated HCl-exposed animals (n=17). 16 control animals were exposed to saline for 21 days.

with GORD than an oesophageal-bronchial reflex. Results of studies in animals indicate that bronchoconstriction caused by activation of tracheal irritant receptors involved a vagally mediated reflex [21]. Apart from microaspiration, acetylcholine therefore appears to be involved in the pulmonary changes associated with GORD through cholinergic reflex originating either from the oesophagus or from the upper airways. In addition, non-neuronal acetylcholine release from inflammatory and epithelial cells [5] might also contribute to the development of airway inflammation and hyperresponsiveness in the present model of chronic GORD.

The present results show that the efficacy of either *i.p.* administered atropine or tiotropium administered by nebulisation in preventing airway remodelling and inflammatory cellular infiltration of the lung is very similar to that of *i.p.* administered dexamethasone. In our model of GORD, the increase in the epithelial and smooth muscle thickness of the airways was rather weak in comparison to the increases previously reported after chronic exposure of mice to allergen [22]. The high doses of tiotropium, atropine and dexamethasone fully prevented this moderate increase in epithelial thickening. Acetylcholine stimulates the proliferation of rat airway epithelial cells through the activation of M1 muscarinic receptors and nicotinic receptors [4], an observation that may help explain the inhibitory effects of atropine and tiotropium on the epithelium thickening. A nonsignificant increase in the smooth muscle thickness was observed in mice treated with anticholinergics or dexamethasone but the weak increase in untreated mice precludes any firm conclusion on the preventive effect of these drugs. However, in sensitised guinea pigs exposed to allergen, tiotropium was previously shown to markedly prevent the increase in airway smooth muscle thickening but was less effective than budesonide [13]. In the present study, the marked increase in the contractility of isolated trachea from untreated mice exposed to HCl was considerably inhibited by tiotropium, atropine and dexamethasone. The inhibitory effect of tiotropium is unlikely to be due to a residual blockade of the muscarinic receptors caused by the persistence of this long-acting muscarinic antagonist in the trachea (>24 h after the last administration), since no residual effect on methacholine-induced guinea pig tracheal contraction has been previously shown 24 h after *in vivo* exposure [12], and since a similar inhibitory effect was observed with atropine, a short-acting anticholinergic [23]. We did not study the smooth muscle thickness of the trachea but the weak increase in thickness of the peripheral airways did not support a relationship between the increase in contractility and the

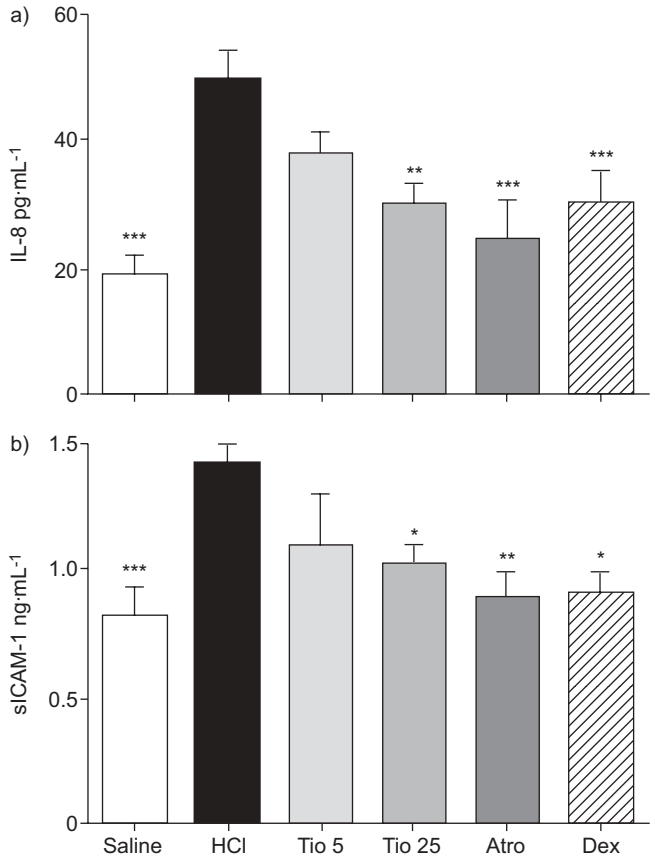

FIGURE 4. Effects on bronchoalveolar lavage fluid levels of a) interleukin (IL)-8 and b) soluble intercellular adhesion molecule (sICAM)-1, of twice-daily exposure to HCl for 21 days and treatment by nebulisation of tiotropium 5 μg (Tio 5, n=7) or 25 μg (Tio 25, n=11), or by intraperitoneal injection of atropine (Atro, n=7) 1 mg·kg^{-1} or dexamethasone (Dex, n=7) 1 mg·kg^{-1}. Data are presented as mean±SEM. *: p<0.05 when compared to untreated HCl-exposed animals (HCl, n=11); **: p<0.01 when compared to untreated HCl-exposed animals (HCl, n=11); ***: p<0.001 when compared to untreated HCl-exposed animals (HCl, n=11). Control animals exposed to saline for 21 days (n=11).

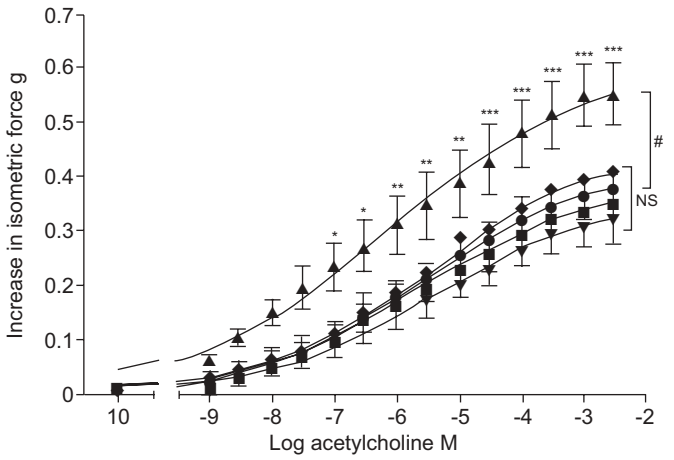

FIGURE 5. Effects on acetylcholine-induced isometric contraction of tracheal preparations, of twice-daily exposure to HCl for 21 days and treatment by nebulisation of tiotropium (25 μg), or by intraperitoneal injection of atropine (1 mg·kg^{-1}) or dexamethasone (1 mg·kg^{-1}). Data are presented as mean±SEM (six animals per group). ▲: HCl; ◆: atropine; ■: saline; ▼: tiotropium; ●: dexamethasone. *: p<0.05 when compared to untreated HCl-exposed animals (HCl, ANOVA for repeated measures); **: p<0.01 when compared to untreated HCl-exposed animals (HCl, ANOVA for repeated measures); ***: p<0.001 when compared to untreated HCl-exposed animals (HCl, ANOVA for repeated measures); NS: nonsignificant; #: p<0.05 when compared to HCl.

increase in smooth muscle mass. In a previous study by BOS *et al.* [13], the tracheal contractility to methacholine was increased without concomitant changes in airway smooth muscle content in the large airways, in contrast with the increase in peripheral airways, suggesting that the central airways have acquired a hypercontractile phenotype after repeated allergen challenge. As observed in the present study, tiotropium completely prevented the increase in contractility in this model of allergen-driven lung inflammation.

Treatment of mice with tiotropium, atropine and dexamethasone markedly prevented the infiltration by polynuclear phagocytes, lymphoplasmocytes and macrophages of the airway walls and of peribronchial and peribronchiolar areas caused by repeated oesophageal instillation of HCl. This cellular infiltration of the lung tissues was associated with an enhanced number of macrophages, lymphocytes and neutrophils in BALF and an increase in BALF levels of IL-8 and ICAM-1. Activated neutrophils are known to play a key role in pulmonary inflammation and oxidative damage. IL-8 (a major chemoattractant for neutrophils that is produced by various pulmonary cells, including macrophages and epithelial cells, and sICAM-1, which is mainly produced by alveolar epithelial cells in the alveolar lining fluid) can activate leukocytes [24, 25]. In children with GORD and asthma-like symptoms, neutrophilia have previously been shown to positively correlate with IL-8 levels in BALF [26], and both neutrophilia and IL-8 levels were demonstrated to correlate with the number of proximal reflux events [16]. *In vitro* studies have revealed that muscarinic receptor stimulation, particularly the M3 receptor subtype, triggers the release of pro-inflammatory mediators involved in neutrophil recruitment, such as IL-8 and leukotriene (LT)B_4, from airway smooth muscle, epithelial cells and alveolar macrophages [27–29]. IL-8 and LTB_4 are also involved in monocyte chemoattraction [24, 30]. In the three most recent studies [27–29], tiotropium suppressed the release of IL-8 and LTB_4, at least partly explaining the preventive efficacy of blocking muscarinic receptors with tiotropium or atropine on the increased number of neutrophils and macrophages in the airways in our model in the present study.

In conclusion, the results of the present study demonstrate that muscarinic receptor blockade by atropine or tiotropium prevents airway inflammation and remodelling in an animal model of chronic GORD. The preventive efficacy of the two muscarinic antagonists is similar to those of dexamethasone. The present study extends the beneficial effects of tiotropium in allergen-induced airway inflammation and remodelling to a different model of pulmonary inflammation, providing further evidence that the beneficial effects of anticholinergics might exceed those of bronchodilation both in asthma and in COPD, particularly in patients suffering from GORD.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

A statement of interest for P. Devillier can be found at www.erj.ersjournals.com/misc/statements.dtl

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