Glycopyrrolate Causes Prolonged Bronchoprotection and Bronchodilatation in Patients With Asthma*

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**Introduction:** Inhaled anticholinergic drugs are effective bronchodilators in the treatment of COPD, and tiotropium bromide has recently been introduced as a once-daily bronchodilator for use as a maintenance treatment. Racemic glycopyrrolate is an anticholinergic drug that has been used orally to control gastric acidity, parenterally as an antisialogogue and to reverse neuromuscular blockade, and has been studied by inhalation for asthma and COPD.

**Design and objective:** We investigated the duration of protection against the constrictor effects of inhaled methacholine of a single dose of inhaled nebulized racemic glycopyrrolate (0.5, 1.0, and 2.0 mg) compared with ipratropium bromide (0.5 mg) and placebo in 10 atopic asthmatic volunteers in a double-blind, five-way, crossover study.

**Results:** Protection against methacholine-induced bronchospasm after administering glycopyrrolate was maintained to 30 h, the last time point measured. Both bronchodilatation and bronchoprotection were significantly longer with glycopyrrolate than after ipratropium bromide, and bronchoprotection was significant at all time points from 2 to 30 h compared to placebo. Dryness of the mouth and nose was described in 18% of patients after the highest dose of glycopyrrolate.

**Conclusions:** The prolonged bronchodilator response and the protection against methacholine-induced bronchospasm demonstrated in asthma suggests that inhaled racemic glycopyrrolate would be superior to ipratropium bromide for treatment of stable COPD.


**Key words:** anticholinergic; bronchodilator; glycopyrrolate; muscarinic receptor

**Abbreviations:** M₁ = muscarinic subtype 1; M₂ = muscarinic subtype 2; M₃ = muscarinic subtype 3; PC₂₀ = provocative concentration causing a 20% decrease in FEV₁

The parasympathetic division of the autonomic nervous system provides cholinergic or vagal tone in the airways, and this is the major reversible component of airflow resistance in COPD.¹ Blockade of muscarinic receptors can be obtained with the inhaled short-acting anticholinergic agents ipratropium bromide and oxitropium bromide, and these drugs have been extensively used as bronchodilators in COPD since the 1970s.² Tiotropium bromide is a novel, long-acting, once-daily anticholinergic drug that has recently been licensed in the United States and Europe³ and is a first-line maintenance therapy for COPD in the updated Global Initiative for Chronic Obstructive Lung Disease guidelines of 2003.⁴

Acetylcholine appears in primitive life forms early in evolution, where it has distinct nonneuronal cholinergic properties distinct to its role as a neurotransmitter.⁵ The synthesizing enzyme choline acetyl transferase has been demonstrated in the epithelium...
of human airways. Five distinct muscarinic receptors are present in man, of which muscarinic subtype 1 (M1), muscarinic subtype 2 (M2), and muscarinic subtype 3 (M3) receptors have been identified in human lung, and muscarinic subtype 4 receptors have been identified in rabbit lung. Acetylcholine release from parasympathetic nerves activates muscarinic receptors on airway smooth muscle, submucosal glands, and blood vessels to cause bronchoconstriction, mucus production, and vasodilation. M1 receptors are localized to parasympathetic ganglia in the bronchial plexus that continues into the smaller airways. M2 receptors are autoreceptors found on the parasympathetic nerve endings and inhibit the release of acetylcholine, while M3 receptors on airway smooth-muscle cells mediate bronchoconstriction. Since acetylcholine causes not only airway smooth-muscle contraction but also proliferation, it could mediate some aspects of airway remodeling in asthma and COPD. In addition, cholinergic receptors are found outside the neuromuscular system, with muscarinic receptors on lymphocytes and neutrophils, and on a variety of other airway cells.

Glycopyrrolate is a quaternary ammonium derivative with minimal mucosal absorption and systemic toxicity when inhaled. A study in human and guinea pig airways has demonstrated that glycopyrrolate binds with high affinity to muscarinic receptors and has a prolonged dissociation profile. In normal subjects, IV glycopyrrolate has been shown to cause bronchodilatation, while nebulized administration caused a long duration of bronchodilatation without the systemic anticholinergic effects of inhaled atropine. Inhaled glycopyrrolate has been shown to cause bronchodilatation for at least 12 h in patients with asthma and has been successfully employed in the treatment of exercise-induced asthma and acute exacerbations of asthma. In addition, nebulized glycopyrrolate has been found to be an effective bronchodilator in COPD and to have utility when combined with albuterol in treating acute exacerbations of COPD.

Anticholinergic therapy is important in the management of patients with COPD, but most of these patients have airway obstruction with limited reversibility. In this study, we assessed the effects of single doses of inhaled glycopyrrolate in comparison to ipratropium bromide and placebo in subjects with mild stable asthma and airway reversibility, since this population is more sensitive to the effects of methacholine in causing bronchospasm. Assessment of the length of time that glycopyrrolate causes bronchoprotection is a more sensitive way to assess duration of action than measuring direct bronchodilatory effects and may be of greater clinical significance.

**Materials and Methods**

This study was reviewed by the Royal Brompton & Harefield NHS Trust Research Ethics Committee. All patients were informed of the nature of the study and risks of the procedures before being invited to sign a consent form.

**Study Design**

This was a five-way, double-blind, placebo-controlled, crossover, randomized study. All subjects received three single doses of glycopyrrolate (0.5 mg, 1 mg, and 2 mg), one dose of ipratropium bromide (0.5 mg), and one dose of placebo. Because of the potentially long duration of action of glycopyrrolate, each treatment day was separated by a washout period of at least 7 days.

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**Figure 1. Study design**

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measurements of FEV1 at 1-min intervals, the best of which was laboratory, each subject rested quietly for 15 min prior to three protocol was used for all provocation tests. On arrival in the spirometer (Vitalograph; Buckingham, UK). A standard challenge to inhaled methacholine with a provocative concentration causing (Tables 1, 2). All subjects had documented hyperresponsiveness to inhaled methacholine with a provocative concentration causing a 20% fall in FEV1 (PC20) of <4 mg/mL. Subjects were nonsmokers and did not have COPD or other significant pulmonary or systemic diseases. None had suffered an exacerbation of wheeze or respiratory infection in the preceding 6 weeks.

Patient Demographics

Twelve subjects with mild-to-moderate asthma were studied (6 men and 6 women; 10 whites and 2 blacks) [Table 1]. Subjects had a baseline FEV1 > 80% of their predicted normal value.

Materials and Drug Delivery

On each study day, fresh solutions of methacholine were made up to produce a range of concentrations in 0.9% saline solution from 0.0625 to 128 mg/mL. Study medication was prepared by a designated unblinded person in the hospital pharmacy, who ensured that all clinical staff remained blinded throughout the study. The unblinded staff member did not participate in study management or study evaluations. Methacholine solutions and study treatments were administered using a nebulizer (DeVilbiss Pulmomate compressor; DeVilbiss; Somerset, PA).

Bronchial Provocation and Measurement of Pulmonary Function

Pulmonary function was measured as FEV1 with a dry wedge spirometer (Vitalograph; Buckingham, UK). A standard challenge protocol was used for all provocation tests. On arrival in the laboratory, each subject rested quietly for 15 min prior to three measurements of FEV1 at 1-min intervals, the best of which was used as the baseline value. At 2, 12, 24, and 30 h after administration of study drug, subjects underwent three further measurements of FEV1 and then inhaled five breaths of doubling increments of methacholine at 3-min intervals until a > 20% fall in FEV1 from the post-saline solution value was achieved. A log dose-response curve was constructed, and the PC20 value was calculated by linear interpolation.

Statistical Analysis

For continuous and ordinal variables, descriptive statistics (number, mean, SD, median, and minimum and maximum) were calculated. For categorical variables, frequencies and percentages were calculated. The intent-to-treat set included all patients who received at least one dose of study medication. The “evaluable for efficacy” or “per protocol” set was only used to analyze the primary efficacy variable and only included those subjects who completed all treatment visits and were not major protocol violators. Efficacy analyses included log PC20, PC20, FEV1; FVC; forced expiratory flow, midexpiratory phase; and peak expiratory flow. The primary efficacy variable was to assess the duration of protective effect of glycopyrrolate doses as compared to placebo, expressed as methacholine log PC20/log 2. The pairwise comparisons of the differences of log PC20/log 2 at 2, 12, 24, and 30 h after dose between glycopyrrolate and placebo were performed by constructing an analysis of variance model for a 5 × 5 crossover (Latin squares) design. A significance level of 0.05 was used in declaring statistical significance of the pairwise differences at each time point.

RESULTS

Twelve patients were randomized, but 2 patients discontinued prematurely and were replaced by

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<th>Patient No.</th>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Baseline FEV1, L</th>
<th>Baseline FEV1, % Predicted</th>
<th>Baseline Visit 1 Methacholine PC20, mg/mL</th>
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</tbody>
</table>

*Premature discontinuations: patient 102 (incorrect/implausible PC20) and patient 107 (restricted medication, amoxicillin).
patients who received the same treatment sequence. Therefore, 12 patients received at least one dose of study medication and were included in the intent-to-treat and safety analyses. Only the 10 patients who completed all five study visits were evaluable for efficacy.

Pairwise analyses of the primary efficacy variable (log PC$_{20}$/log 2) showed that there were no statistically significant differences among the active-treatment study arms and placebo prior to dosing. After treatment, each glycopyrrolate dose provided significantly more protection against methacholine challenge than placebo at all time points, and against ipratropium bromide at 12, 24, and 30 h (but not 2 h) [Fig 2]. The peak protective effect was observed 2 h after dosing, and the duration of the protective effect was sustained for up to 30 h, with some evidence of a dose response to glycopyrrolate in terms of bronchoprotection. Results observed in the analyses of the PC$_{20}$ values and the lung function parameters were consistent with those of the primary efficacy variable. In addition, glycopyrrolate caused bronchodilation at up to 30 h (Fig 3).

Inhalation of glycopyrrolate was found to be generally well tolerated. There were two transient severe reactions: headache on placebo and menstrual cramp on glycopyrrolate (2.0 mg). All other adverse events were not serious, of mild or moderate severity, and transient. Headache was the most common adverse event for placebo (30%), glycopyrrolate 0.5 mg (30%), and glycopyrrolate at 1.0 mg (18.2%), while dry mouth and nose was the most common adverse event for glycopyrrolate at 2.0 mg (18.2%). No notable changes in vital signs, laboratory findings, or 12-lead ECG were found.

**Discussion**

In this pilot study, 12 patients were enrolled and 10 patients were evaluable for efficacy. Treatment with a single inhalation of racemic glycopyrrolate caused significant and sustained bronchoprotection against methacholine challenge for up to 30 h in subjects with mild asthma. There was not a clear dose response since glycopyrrolate at doses of 0.5, 1.0, or 2.0 mg caused bronchoprotection up to 30 h. This suggests that even the lowest dose of glycopyrrolate exerts the maximally achievable cholinergic blockade, indicating that the applied doses were very high and that a dose reduction might retain efficacy with a lesser frequency of side effects such as dry mouth. Glycopyrrolate at all doses showed significantly superior bronchoprotection compared to ipratropium bromide at 12, 24, and 30 h. These doses of glycopyrrolate are higher than that of tiotropium bromide (administered as 18 μg per actuation from a dry powder inhaler), suggesting that racemic glycopyrrolate is less potent.

Glycopyrrolate also caused a significant bronchodilatation for up to 30 h after each inhalation and revealing a circadian rhythm. The time profile after placebo shows a tendency toward bronchodilation during daytime and toward bronchoconstriction during nighttime in comparison to baseline. This is in line with the known circadian rhythm of endogenous cortisol production. Surprisingly, 2.0 mg of glycopyrrolate had only approximately half the effect of 0.5 mg and 1 mg of glycopyrrolate at 15 min, 1 h, 2 h, and 12 h. The high dose may be causing increased activation of M$_2$ receptors and causing negative feedback. This lesser effect of 2.0 mg does not occur at 24 h and 30 h, when there are lesser amounts of
drug substance, supporting this hypothesis. The bronchodilator effect was small, approximately 10%, but this is because the asthma patients we studied had mild disease with little "room to move," and is similar to the bronchodilator response to tiotropium in a similar patient population.24 It is probable that clinically significant bronchodilatation would be achieved in subjects with more severe reversible airway obstruction. However, due to the small number of patients, definitive conclusions cannot be drawn. A follow-up investigation is recommended for confirmation and for extrapolation of our results observed in patients with mild, stable asthma with more severe airways obstruction, and both asthma and COPD should be studied.

Glycopyrrolate was well tolerated without adverse effects on cardiovascular, respiratory, or laboratory parameters, although dry mouth affected every fifth patient. Since glycopyrrolate contains a quaternary nitrogen, systemic absorption and passage across the blood-brain barrier is minimal, so systemic side effects following inhalation are minimal. However, a theoretical danger with administering anticholinergic agents by nebulizer or metered-dose inhaler is that they could be sprayed into the eye and cause glaucoma by local action. This possibility is minimized by the use of a breath-activated dry powder inhaler.

Pharmacologic characterization of glycopyrrolate in human and guinea pig airways has shown that glycopyrrolate lacks selectivity for binding to M₁, M₂, and M₃ receptors.12 Furthermore, glycopyrrolate dissociates more slowly from human airway smooth-muscle receptors than ipratropium. Tiotropium bromide also has the capacity to bind to M₁, M₂, and M₃ receptors. Furthermore, tiotropium shows kinetic selectivity through rapid dissociation from the M₂ receptor but slow dissociation from M₁ and M₃ receptors.25

In animal models of hyperresponsiveness, increased release of acetylcholine may be due to malfunction of inhibitory M₂ receptors7 or alternatively due to excess acetylcholine production by airway epithelial cells.5 It has been shown that eosinophil major basic protein has the capacity to bind to M₂ receptors and prevent acetylcholine binding. Since the normal feedback of acetylcholine release is lost, acetylcholine release is then increased and bronchoconstriction, airway hyperresponsiveness, and airway remodeling may result.8

Glycopyrrolate has two chiral centers corresponding to four potential different enantiomers (RR-, RS-, SR-, and SS-). However, the drug substance administered in the present study is a racemic mixture of only two of these four enantiomers, namely RS- and SR-glycopyrrolate. All four enantiomers have been demonstrated to be muscarinic receptor blockers in vitro, but they differ regarding their dissociation half times from the human airways M₃ receptor.27 For this reason, enantiomerically pure RR-glycopyrrolate has the potential for greater potency and longer duration than the RS- and SR-glycopyrrolate used in our study.

Racemic glycopyrrolate by inhalation is a long-acting anticholinergic bronchodilator that in the future might be used as an alternative to tiotropium bromide on a once-daily basis for COPD. Glycopyrrolate is a potent but nonselective muscarinic receptor blocker, the RR enantiomer having a relatively slow dissociation rate from the M₃ vs the M₂ recep-
tor, with kinetic M₃ selectivity comparable to tiotropium bromide. Glycopyrrolate could be therapeutically useful when combined with both inhaled β₂-agonists and inhaled corticosteroids, either from separate inhalers or from a single inhaler.²⁸

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