Respiratory Medicine (2011) 105, 900–906

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Effects of formoterol and tiotropium bromide on mucus clearance in patients with COPD

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Received 5 May 2010; accepted 6 February 2011
Available online 11 March 2011

KEYWORDS
Mucus clearance;
Formoterol;
Tiotropium bromide

Summary
Background: Lung mucociliary clearance is impaired in patients with chronic obstructive pulmonary disease (COPD). Treatment guidelines recommend that patients with COPD receive maintenance therapy with long-acting beta-agonists and anticholinergic agents.
Methods: Twenty-four patients with mild to moderate COPD received formoterol (12 μg, twice daily from Turbuhaler® dry powder inhaler (DPI)) or tiotropium (18 μg, once daily from Handihaler® DPI) for 14 days. They also received single doses of formoterol, tiotropium, salbutamol (200 μg) and placebo. A radioaerosol technique was used to assess the effects on mucus clearance of 14 days treatment with formoterol or tiotropium, as well as single doses of these drugs.
Results: The 4 h whole lung retention of radioaerosol was significantly higher after 14 days treatment with tiotropium ($P = 0.016$), but not after 14 days treatment with formoterol. However, patients bronchodilated after 14 days treatment with both drugs, so that the deposited radioaerosol may have had an increased distance to travel in order to be cleared by mucociliary action. A single dose of formoterol enhanced radioaerosol clearance significantly compared to other single dose treatments ($P < 0.05$).
Conclusion: Formoterol (12 μg) enhances mucus clearance in patients with mild to moderate COPD when given as a single dose, and may do so when given for 14 days. Studies of longer

Abbreviations: COPD, Chronic obstructive pulmonary disease; FEV_1, Forced expiratory volume in 1 s; FVC, Forced vital capacity; Kr, Krypton; RV, Residual volume; Tc, Technetium.

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0954-6111/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.rmed.2011.02.007

duration would be needed in order to assess the effects of the study drugs on mucus clearance when they are used for long-term maintenance therapy.

Introduction

Treatment guidelines recommend that patients with moderate or severe chronic obstructive pulmonary disease (COPD) are given maintenance therapy with long-acting inhaled bronchodilators.[1] Both long-acting beta-agonists (e.g. formoterol and salmeterol) and long-acting anticholinergic agents (e.g. tiotropium bromide) are effective in the management of COPD,[2] and can enhance quality of life.

Mucociliary clearance is a natural lung defence mechanism,[3] which helps to maintain a healthy environment in the lungs. Mucociliary clearance is impaired in COPD patients,[4] especially in the larger more central airways of the lungs.[5] Patients may compensate for ineffective mucociliary clearance of pulmonary secretions by increasing the amount of mucus they clear by cough.[6,7] Bronchodilating drugs may influence mucus clearance,[8,9] but the nature of the effects depend on the type of drug and the length of time for which it is administered. While tertiary ammonium compounds such as atropine may slow mucociliary clearance,[9,10] quaternary ammonium compounds such as ipratropium bromide[11] and tiotropium bromide[12] have been reported not do so. Beta-agonists including fenoterol,[13] salbutamol,[14] and formoterol[15] have been reported to enhance mucus clearance in patients, or to increase ciliary beat frequency in animal models.[16]

The effect of a bronchodilator on mucus clearance may differ according to whether it is given continuously over several weeks, or as a single dose. The objectives of this study were to assess the effect on mucus clearance of (a) treatment for 14 days with either formoterol or tiotropium, and (b) single inhalations of formoterol or tiotropium. The latter assessment used the short-acting beta-agonist salbutamol as a comparator, and placebo as a control. Formoterol and tiotropium were given by dry powder inhalers (DPIs). Mucus clearance was assessed from the retention of inhaled radioaerosol particles in the lungs.

Methods

Study population

Patients with an established clinical diagnosis of COPD, having severities I (mild) or II (moderate) according to the guidelines of the Global Initiative for COPD,[1] were included in the study. All patients had a cigarette smoking history, which was defined by current or previous smoking of more than 10 pack-years. An additional inclusion criterion was the requirement for at least 4 weeks' therapy on a stable medication regimen before the screening visit. All patients gave informed consent in writing, and the study was approved by the local ethics committee and by the federal office for radiation protection in Germany.

Unpublished data from patients with COPD who had inhaled a radioaerosol in order to assess mucus clearance were used to estimate the required number of subjects. These data indicated a mean whole lung retention of radiolabeled particles after 2 h of 82% (standard deviation (SD) 8%). Considering a two-sided, two sample *t*-test, a sample size of 18 subjects was required (alpha = 5%, power = 80%). In order to obtain a balanced sample, 24 subjects were to be investigated.

Study design and treatment

The study was open-label, single-centre, randomised, and cross-over in design. It was designed in order to allow the effects of both 14 days therapy with either formoterol or tiotropium, and single doses of the same drugs, to be determined (Figs. 1 and 2). After the initial screening visit (visit 1) and a 2–7 day run-in period, patients were randomly assigned to receive for 14 days either formoterol (metered dose 12 μg, Oxis®, AstraZeneca) inhaled twice daily via Turbuhaler® DPI, or tiotropium (capsule dose 18 μg, Spiriva®, Boehringer Ingelheim) inhaled once daily via HandiHaler® DPI. Each patient was given two devices, such that patients receiving formoterol also inhaled from a Handihaler containing empty capsules, while patients receiving tiotropium also inhaled from an empty Turbuhaler. After a one week wash-out period, each patient crossed over onto the other active treatment. Mucus clearance was measured using a radioaerosol technique before and after each two week treatment period (visits 2, 3, 4 and 5). The whole lung retention of the radioaerosol after 2 h and 4 h was used as a measure of mucus clearance, in order to assess the effects of the 14 day treatments.

The single dose study involved inhalation of either formoterol (12 μg, Oxis®, AstraZeneca), tiotropium (18 μg, Spiriva®, Boehringer Ingelheim), salbutamol (200 μg, Evohaler®, GlaxoSmithKline) or placebo (empty Handihaler capsules) at visits 2, 3, 4 and 5. Administration of the single doses of drug or placebo took place 4 h after inhalation of the radioaerosol, and was block-randomised across the four visits using a Latin square design (Fig. 2). The whole lung retentions of the radioaerosol after 4 h and 6 h were used as measures of mucus clearance, in order to assess the effects of single dose treatments.

Patients were trained in the correct use the Turbuhaler® for inhalation of formoterol, Handihaler® for inhalation of tiotropium, and pressurised metred dose inhaler (pMDI) for inhalation of salbutamol, according to the manufacturer's instructions.

Permitted concomitant therapy during the study period included short-acting theophylline, oral steroids, and salbutamol. Therapies with long-acting anticholinergic agents (other than the study medication), long-acting beta-agonists (other than the study medication), long-acting theophylline, and inhaled corticosteroids were not permitted for at least 48 h before visit 2, until completion of visit 5. During each 6 h mucus clearance measurement,

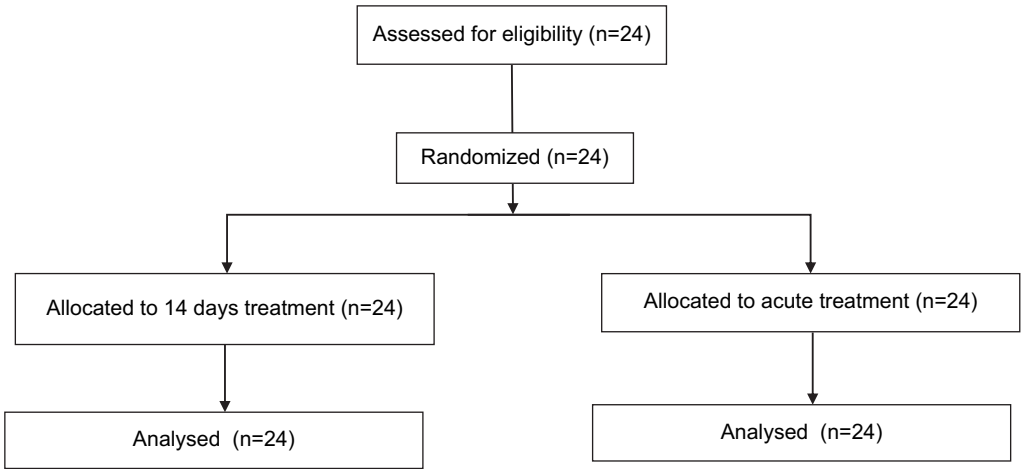

Figure 1 Description of study design, intended to assess the effects of 14 days treatment with formoterol or tiotropium, and single doses of the same drugs.

the use of any bronchodilators, apart from the single dose intervention after 4 h, was not permitted. Smoking was prohibited for the period beginning 2 h before radioaerosol inhalation to 6 h afterwards. Inhalation of formoterol or tiotropium was not permitted during the 12 h immediately preceding visits 3 and 5.

Radioaerosol generation and inhalation

Radiolabeled iron oxide particles used for the clearance measurements were produced by mixing iron (III)-oxide colloid with stannous chloride solution and ^{99m}Tc-pertechnetate.[17] In a Sartorius dialysis cell, the chloride content of this suspension was eliminated and aerosol particles were produced with a spinning top aerosol generator. The aerosol from the spinning top generator was led through a concentrator and the concentrated aerosol was passed through a tube furnace at 800 °C and mixed with reduction gas to reduce iron (III)-oxide into iron (II, III)-oxide particles. Particle size could be adjusted appropriately either by changing the velocity of the spinning disk in the aerosol generator or changing the concentration of iron (III)-oxide colloid in the original suspension. The required mass median aerodynamic diameter (MMAD) was 4.5 μm, and the required geometric standard deviation (GSD) was 1.1.

The aerosol particles were produced immediately before inhalation and were held in a reservoir from which the patient inhaled. The specific radioactivity and particle size in the reservoir was checked by withdrawing particles onto a filter. If sufficient radioactivity was available, and if the MMAD and GSD were within ±10% of the required values, subjects were allowed to inhale the particles. All inhalations of test aerosol were controlled with a flow meter, with a target inhaled flow rate of 15 L/min. The test aerosol was given as an aerosol bolus which was inhaled into a volumetric lung depth of 40% of dead space volume. The quantity of ^{99m}Tc deposited in the lungs was 1.15 ± 0.3 MBq.

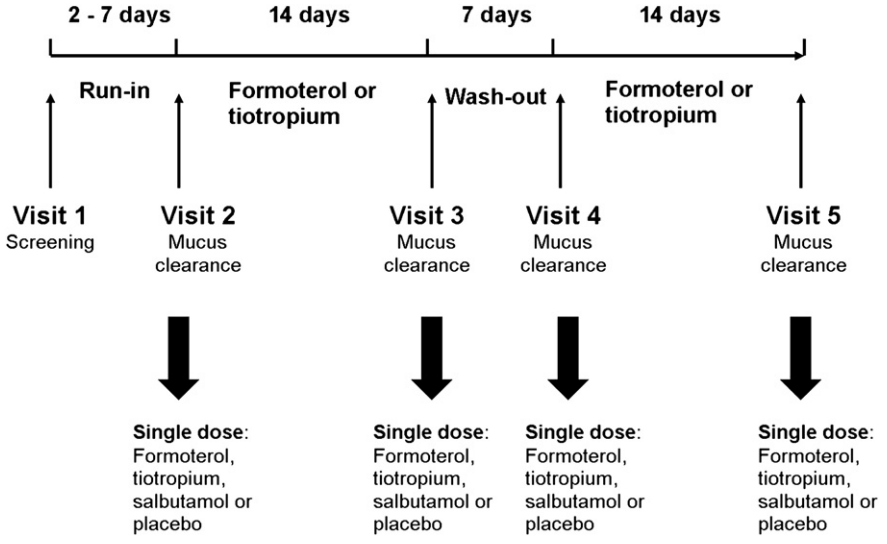

Figure 2 Flow chart of the study.

Clearance measurements

Clearance kinetics of the radiolabeled iron oxide particles were assessed from the background and decay-corrected decline in whole lung radioactivity during 6 h using a single-headed gamma camera (Diacam®, Siemens) with a 40 cm field of view and a low energy parallel-hole collimator. The duration of imaging was 5 min immediately after radioaerosol inhalation, 6 min at 2 h, 7 min at 4 h and 8 min at 6 h. Patients were seated for imaging. In order to assess the lung outlines and hence to define whole lung regions of interest, a ^{81m}Kr gas ventilation scan was performed for each patient.

Percentage whole lung retention of radioaerosol during visits 2, 3, 4 and 5 was quantified at 2 h, 4 h, and 6 h post inhalation (Fig. 3). The lung image taken immediately after radioaerosol inhalation was used as the 100% value. The effects of 14 days treatment with formoterol or tiotropium were assessed by comparing 2 h and 4 h retentions before and after treatment. The effects of single doses of formoterol, tiotropium, salbutamol or placebo were assessed from the change in retention between 4 h and 6 h after each treatment.

During visit 2, the clearance measurement was extended in order to measure 24 h retention of the iron oxide particles (Fig. 2). The 24 h retention was determined from a comparison of the background and decay-corrected counts obtained immediately after inhalation and then 24 h later using a purpose-built human lung counter.[18] This 24 h retention in the lungs was used as an indication of the proportion of particles deposited in the non-ciliated airways and therefore not available for mucociliary clearance.[19]

Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and residual volume (RV) were measured by body plethysmograph (MasterScreen Body®, Jaeger). Statistical differences in whole lung retention and in lung function parameters were assessed from paired *t*-tests using an Excel spreadsheet.

Results

Demographics of study population

Twenty-four patients (20 male, 4 female) with COPD completed the study (Fig. 1, Table 1). The mean (SD) age was 60.2 (8.2) y. FEV_1 was 67.2 (12.8)% predicted, and FEV_1 expressed relative to forced vital capacity (FVC) was 52.6 (9.4)%. RV was 154.5 (36.4)% predicted.

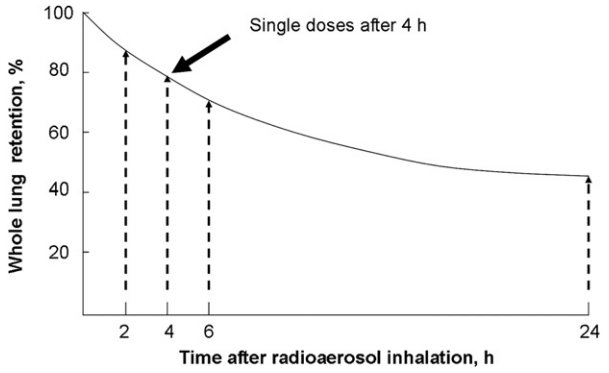

Figure 3 Schematic of mucus clearance measurements. Percentage whole lung retention of radioaerosol was measured at 2 h, 4 h and 6 h at each of visits 2, 3, 4 and 5. At visit 2, the 24 h whole lung retention was also measured.

Table 1 Demographics of 24 COPD patients completing the study. There were 20 males and 4 females.

	Mean	SD	Range
Age (y)	60.2	8.2	(40—76)
Height (m)	1.72	0.08	(1.56—1.88)
Weight (kg)	74.5	11.5	(46—94)
FEV_1 (L)	2.06	0.56	(1.39—3.57)
FEV_1 (% predicted)	67.2	12.8	(50—101)
FVC (L)	3.97	0.99	(2.45—6.23)
FVC (% predicted)	101.7	19.3	(66—139)
FEV_1/FVC (%)	52.6	9.4	(35.2—68.5)
RV (L)	3.59	1.04	(1.88—5.20)
RV (% predicted)	154.5	36.4	(80—216)

14 days treatment with formoterol

As shown in Fig. 4a, there were no significant differences in radioaerosol retention in the whole lung at either 2 h (before treatment 91.9 (7.7)%, after treatment 91.1 (9.8)%), or 4 h (before treatment 85.2 (9.9)%, after treatment 84.8 (12.3)%). After treatment, patients had bronchodilated significantly, with FEV_1 increasing from 1.82 (0.56) L before treatment to 2.10 (0.56) L afterwards ($P < 0.001$, Table 2).

14 days treatment with tiotropium

As shown in Fig. 4b, whole lung retention measured 2 h after radioaerosol inhalation was similar before and after 14 days treatment with tiotropium (92.3 (7.7)% versus 93.7 (8.5)%). However, whole lung retention at 4 h was significantly lower before the 14 day treatment period compared to after (85.9 (11.2)% versus 89.3 (12.4)%, $P = 0.016$). After treatment, patients had bronchodilated significantly, with FEV_1 increasing from 1.92 (0.62) L before treatment to 2.12 (0.60) L afterwards ($P < 0.001$, Table 2).

Single dose treatments

As shown in Fig. 5, whole lung retentions of radioaerosol after 4 h in patients randomised to receive single doses of

Table 2 Mean (SD) lung function data before and after 14 days treatment with either formoterol or tiotropium.

	Before	After	*P*
Formoterol			
FEV_1 (L)	1.82 (0.56)	2.10 (0.56)	<0.001
FVC (L)	3.75 (0.95)	4.02 (0.92)	0.004
Tiotropium			
FEV_1 (L)	1.92 (0.62)	2.12 (0.60)	<0.001
FVC (L)	3.80 (0.97)	4.01 (1.05)	0.023

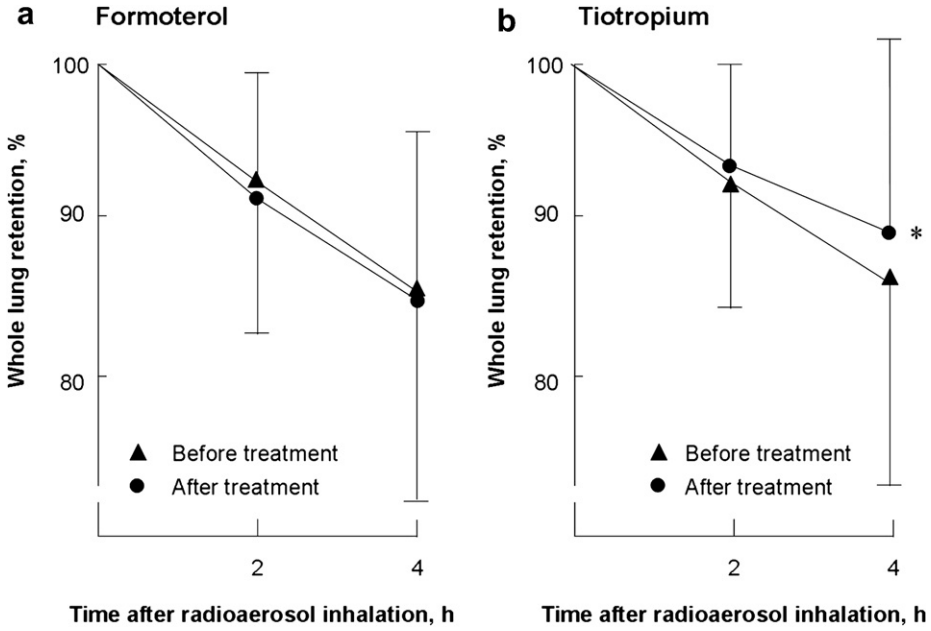

Figure 4 Mean (+SD) whole lung radioaerosol retentions at 2 h and 4 h, before and after 14 day treatment periods with (a) formoterol or (b) tiotropium. * denotes a statistically significant difference compared with retention before treatment.

formoterol, tiotropium, salbutamol and placebo were similar. Whole lung retention at 6 h was lowest after formoterol (73.3 (13.4)%), and the difference was statistically significant compared with placebo (81.2 (10.5)%, $P = 003$). Retentions measured 6 h after single doses of tiotropium (77.6 (14.8)%), salbutamol (78.0 (14.1)%) and placebo were not significantly different. The change in whole lung retention between 4 and 6 h was highest for formoterol (13.6 (7.4)%), and this change was significantly greater than those for tiotropium (8.0 (7.4)%, $P = 0.021$), salbutamol (6.9 (6.5)%, $P = 0.005$), or placebo (6.4 (5.2)%, $P = 0.002$). Lung function was similar prior to administration of each of the four single dose treatments (Table 3).

24 h retention

Mean (SD) radioaerosol retention at 24 h, measured on one study day, was 51.0 (8.8)%, suggesting that approximately half the radioaerosol was deposited on the ciliated airways of the lungs, and approximately half on the non-ciliated airways. Mean (SD) 24 h retention was 47.1 (7.8)% and 53.9 (9.3)%, respectively, in patients who received formoterol or tiotropium at visit 2.

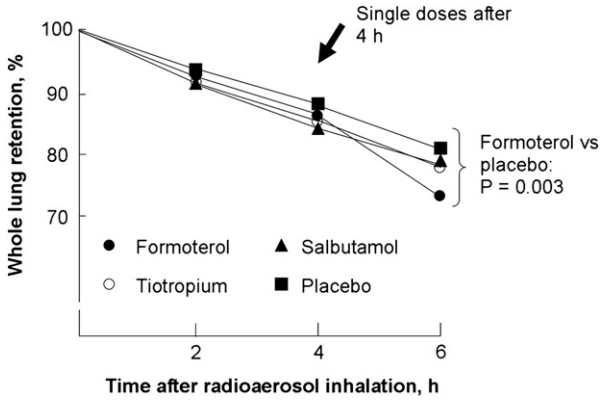

Figure 5 Mean whole lung radioaerosol retentions at 2 h, 4 h and 6 h, for study days on which single doses of formoterol, tiotropium, salbutamol or placebo were administered after 4 h.

Discussion

Clearance of mucus from the human lung is usually measured by radioaerosol methods,[4,6,20,21] in which pulmonary retention of radioaerosol is monitored by gamma camera. This study has shown statistically significant effects of the study medications on pulmonary mucus clearance in patients with mild to moderate COPD.

Mucus clearance data between 4 h and 6 h after radioaerosol inhalation were used to assess the effects of single doses of formoterol, tiotropium, salbutamol and placebo administered after 4 h. The study was balanced in such a way that the lung function parameters (FEV_1 and FVC) were not significantly different before administration of each single dose. The data showed that a single dose of formoterol (12 μg) enhanced mucus clearance in patients with mild to moderate COPD, while a single dose of tiotropium (18 μg) had no effect. Positive effects of single doses of inhaled beta-agonists have been shown in other studies. Administration of single inhaled doses of fenoterol[13] and salbutamol[14] have been shown previously to

Table 3 Mean (SD) lung function before administration of single doses of tiotropium, formoterol, salbutamol and placebo.

	Formoterol	Tiotropium	Salbutamol	Placebo
FEV_1 (L)	2.00 (0.58)	1.99 (0.70)	2.01 (0.58)	2.05 (0.55)
FVC (L)	3.98 (1.00)	3.83 (1.02)	3.94 (1.02)	4.08 (1.01)

enhance mucociliary clearance in COPD patients. While a single dose of 500 μg salbutamol was shown in another study to enhance mucociliary clearance during the next hour,[14] no effect of a single dose of 200 μg salbutamol was observed in this study.

The mucus clearance data up to 4 h after radioaerosol inhalation were used to assess the effects of 14 days treatment with either formoterol or tiotropium using a cross-over study design. After 14 days treatment with formoterol, lung retention of radioaerosol was unchanged compared with that before treatment. Previous studies have shown that terbutaline sulphate given for one week either as tablets[22] or as an aerosol[23] did not increase mucociliary clearance in COPD patients. However, in another study, inhaled salbutamol (5 mg three times daily) or a novel inhaled dopamine receptor/adrenoceptor agonist increased mucociliary clearance when given to COPD patients for 10 days.[24]

When mucus clearance was measured after 14 days treatment with tiotropium, there was a significantly higher retention in the lung after 4 h compared with the measurement before treatment began. This observation suggests a retardation of mucus clearance after 14 days treatment with tiotropium. This finding differs from those of an earlier study[12] which concluded that 21 days treatment with tiotropium (18 μg daily) did not adversely affect mucus transport.

Salbutamol was allowed as rescue medication, in order to maintain patient stability and well-being. Although salbutamol can affect mucus clearance in patients with COPD,[14] we think it unlikely that salbutamol use affected the findings of this study. Of the 24 patients, only 3, 6, 7 and 3 patients took any salbutamol during the 6 h period preceding each of visits 2, 3, 4 and 5. This may reflect the fact that the patients had COPD rather than asthma. Salbutamol would not have influenced the effects of single doses of either formoterol or tiotropium on mucus clearance, assessed from the change in radioaerosol retention between 4 h and 6 h, because no salbutamol was permitted during each of the visits 2 to 5.

In this study, approximately 50% of the radioaerosol particles were deposited on the conducting airways and 50% in the alveoli, as based on the use of the 24 h retention of particles as a measure of alveolar deposition. The fractionation between conducting airways and alveoli was similar for patients subsequently receiving formoterol or tiotropium for 14 days. In theory, it is possible that radioaerosol could have been deposited at a site in the lungs where one or both of the drugs was ineffective in terms of its influence on mucus clearance. However, this seems unlikely because the radioaerosol was distributed fairly uniformly in the lungs, being divided approximately equally between conducting airways and alveoli.

The rate of clearance of inhaled radioaerosol particles depends on their initial deposition site in the lungs.[25] If particles are deposited peripherally, then the transit path they must follow in order to undergo mucociliary clearance is longer compared to that after central deposition. A longer transit path could lead to an apparent reduction of mucus clearance. The aerosol bolus delivery system used in this study was designed to deliver the radioaerosol to a reproducible deposition site in each individual.[26] However, after treatment for 14 days with both formoterol and tiotropium, the COPD patients had bronchodilated, and this may have led to more peripheral radioaerosol deposition. Therefore, while 4 h retention after 14 days treatment with formoterol was identical to that before, this could be consistent with an enhancement of mucus clearance. Similarly, while 4 h retention after 14 days treatment with tiotropium was increased compared with that before treatment, this could be consistent with mucus clearance being unchanged.

Mucus clearance occurs by both mucociliary and cough clearance mechanisms,[6] and the clearances observed in this study may have resulted from a combination of both processes. Mucociliary clearance depends on both ciliary activity and the physical properties of mucus. Therefore, while the data from this study indicate that a single dose of inhaled formoterol may influence mucus clearance in patients with mild to moderate COPD, it is not possible to be specific about the mechanisms by which this effect took place. However, beta-agonists are believed to enhance mucociliary function in COPD patients,[6,24,27,28] suggesting that an enhancement of mucociliary function was responsible for the effects of a single dose of formoterol seen in this study.

It is important that the data from this study are not overinterpreted. Although statistically significant effects of long-acting bronchodilators on lung mucus clearance were observed in this study, the clinical significance of these findings is unclear. The changes observed in mucus clearance, although statistically significant, were small in magnitude. Studies of longer duration would be needed in order to assess the effects of the study drugs on mucus clearance when they are used for long-term maintenance therapy.

Financial support

This study was sponsored by AstraZeneca. AstraZeneca has not influenced the study design, the collection, analysis and interpretation of data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Conflict of interest

All authors are disclosing any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work on this manuscript.

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