Effect of salmeterol on mucociliary and cough clearance in chronic bronchitis

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Summary

The effect of long acting $\beta_2$-adrenergic bronchodilators on impaired mucociliary clearance in chronic bronchitis is unknown. Using a radiolabeled aerosol (technetium-99m-labeled sulfur colloid) and gamma camera analysis, we measured the acute effect of salmeterol vs. placebo on mucociliary and cough clearance in mild-moderate chronic bronchitics ($n=14$) over a 2 h period. During the 1–1(1/2) h period of observation patients performed 60 controlled coughs on each study day. Average whole lung clearance through 1 and 2 h after administration of salmeterol (42 $\mu$g) or placebo via metered dose inhaler (double-blinded, crossover design study) showed no significant difference between treatments. Similarly, for the specific period when cough was added to mucociliary clearance, there was no difference on whole lung clearance between treatments. However, when clearance from the peripheral region of the lung was assessed over the entire 2 h period of observation, salmeterol provided a 30% enhancement of airway clearance compared to placebo, average peripheral 2 h clearance ($\%$) = 22 $\pm$ 9 vs. 17 $\pm$ 10 for salmeterol vs. placebo ($P=0.05$ by paired analysis). Thus, in addition to its bronchodilating effects, salmeterol acutely enhances peripheral airway clearance of secretions in mild-moderate chronic bronchitis.

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1. Introduction

Measurements of mucociliary clearance made by inhalation of radiolabeled particles to label airway surface liquid suggest that clearance of secretions is impaired in chronic smokers and patients with COPD [1,2]. Salmeterol (a long acting $\beta_2$-adrenergic agonist) is widely used to treat bronchoconstriction in patients with COPD and asthma. A number of short acting $\beta$-agonists, e.g. salbutamol, isoproterenol, and albuterol have been shown to acutely enhance the rate of mucociliary clearance from the lung [3]. This enhancement is likely via increases in ciliary activity and increased water and/or mucus secretions onto the airway surface. Less is known about the effects of the new longer acting $\beta$-agonists (e.g. salmeterol and formoterol) on mucociliary clearance. Devalia et al. [4] found that salmeterol had a more potent, faster, and longer duration of action than salbutamol on stimulation of ciliary beat frequency (CBF) in cultured human bronchial epithelium. Yet both agonists produced only slight increases in CBF above baseline ($<20\%$ increase) [4]. A more recent study confirmed the ability of salmeterol to enhance CBF in both normal and COPD nasal epithelium [5] but again only to a modest degree. Piatti et al. [5] also found no changes in rheology for COPD sputum to which salmeterol had been added ex-vivo at a variety of concentrations [5]. But no reports have been made on the in-vivo effect of salmeterol pretreatment on either the quantity or quality of airway secretions in such patients. Hasani et al. [6] recently suggested that 2 week treatment of salmeterol in asthmatics mildly enhanced clearance of secretions from the airways. However, their findings were confounded by changes in the site of radiolabeled particle deposition following the 2-week...
treatment. There have been no assessments of the acute effect of salmeterol on mucociliary clearance in patients with airways disease.

Cough clearance is an important adjunct to mucociliary clearance in chronic bronchitic (CB) patients. We found previously that an anti-cholinergic bronchodilator, ipratropium bromide (IB), inhibited cough clearance in patients with severe COPD, the class of patients who rely most on cough to clear secretions from their airways [7]. It was not clear if the effect we observed with IB resulted from changes in flow dynamics induced by bronchodilation or changes in airway hydration and/or rheology of secretions. The effect of a \( \beta_2 \)-adrenergic bronchodilator on cough clearance in CB patients has not been investigated in the same manner. If the effect of diminished cough clearance by IB therapy [7] were the result of changes in flow dynamics induced by bronchodilation per se, then it might be expected for salmeterol to have a similar effect.

In the present study we compared the acute effect of salmeterol to placebo on mucociliary and cough-enhanced clearance of secretions in patients with mild chronic bronchitis. To accomplish this, we used a radiolabelled aerosol (Tc99m sulfur colloid) and gamma camera analysis to determine rates of clearance on 2 study days (1) a placebo/control day and a (2) a salmeterol treatment day.

2. Methods

2.1. Subjects

Fifteen adult (age 39–70) (7F/8M) subjects with mild-moderate chronic bronchitis (current or ex-smokers with at least 10 pack-years) were studied. All subjects had a clinical diagnosis of chronic bronchitis based on the American Thoracic Society (ATS) definition, i.e. excess mucus production, occurring on most days for at least 3 months of the year for at least two successive years. All were current or ex-smokers (44±26 pack years) with FEV\(_1\)% pred ≥40% (mean 85±21 at time of screening). Subjects had no acute respiratory exacerbation for 4 weeks prior to testing. Only one subject was currently using pulmonary medications, combination serevent and fluticasone, and he refrained from its use for 24 h prior to testing on each study day. All current smokers had to refrain from smoking from 2 h prior to and throughout the study period on each study day. The study was approved by the University of North Carolina Committee on the Protection of the Rights of Human Subjects and informed consent was obtained.

2.2. Protocol

Each subject was studied in a crossover, randomized double blind design requiring two separate visits, i.e. salmeterol and placebo treatment, each separated by at least 5 days but not longer than 2 weeks. A xenon 133 equilibrium lung scan was recorded for each subject on their initial study day [7,8]. On each study day baseline FEV\(_1\), FVC, and FEF\(_{25-75}\)% were measured by spirometry. The patient then inhaled an aerosol produced by jet nebulization (a modified Devilbiss 646) (mass median aerodynamic diameter of 5 μm) of sulfur colloid labeled with Tc99m (CIS sulfur colloid kit from Mallinckrodt Medical) [8–10]. By following a sinusoidal flow signal on an oscilloscope while breathing the radiolabeled aerosol, the subject matched his/her tidal volume and breathing rate at 500 ml and 25 min\(^{-1}\), respectively, in order to match regional deposition in the lung on the two study days.

Following radioaerosol inhalation (less than 2 min), an initial deposition scan was recorded. Then, as the subject remained seated in front of the gamma camera (Elscint Model SP-4 with low energy collimator), continuous two-minute images were recorded for a period of 2 h to monitor clearance of particles from the lung. Following the initial two 2 min deposition scans the patient inhaled one of the aerosolized treatments, salmeterol (42 μg) (Glaxo SmithKline, Inc. RTP, NC) or placebo (also provided by Glaxo SmithKline, Inc, RTP, NC), as two puffs from a metered dose inhaler (MDI). At 1 and 2 h following administration of the treatment drug, FEV\(_1\), FVC, and FEF\(_{25-75}\)% were remeasured by spirometry. During the 1 h of imaging patients were encouraged to suppress spontaneous coughing. Following spirometry at 1 h, subjects voluntarily coughed through a peak flow meter 60 times dispersed evenly over the next 30 min to assess clearance with cough. The peak flow of every fifth cough was recorded and averaged over the cough period. Spontaneous cough frequency was recorded throughout the 2 h period of sequential imaging. For each study visit the subject returned 24 h after the radiolabeled aerosol exposure to obtain a scan of 24 h lung retention.

2.3. Data analysis

Significance was set at \( p \leq 0.05 \) for paired analysis. Unless otherwise specified, data are presented as mean ± SD. Gamma camera analysis of retention (R) vs. time and regional deposition (i.e. central-peripheral (C/P) ratio of deposited particles) has been described in detail previously [7,8,11]. Analysis of regional clearance was performed by constructing a smaller rectangular region (about 25% by area of the whole right lung region) to define the central region [11]. Clearance from the outer peripheral region (lying between the central rectangle and the whole lung rectangle) was then determined. Mean clearance (CI = 100 – R) for both whole lung and peripheral regions was determined by averaging clearance values at each time point over a given period.
Table 1
Spirometric Values for n=14 subjects (mean±SD) (pre- and post-MDI treatment) associated with mucociliary and cough clearance measurements

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Salmeterol</th>
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</thead>
<tbody>
<tr>
<td>FEV1 %pred</td>
<td>91±21</td>
<td>90±20</td>
</tr>
<tr>
<td>Pre-MDI</td>
<td>89±19</td>
<td>92±19</td>
</tr>
<tr>
<td>1 h post-MDI</td>
<td>88±19*</td>
<td>93±18*</td>
</tr>
<tr>
<td>2 h post-MDI</td>
<td>2.32±1.13</td>
<td>2.16±0.92</td>
</tr>
<tr>
<td>FEF 25–75 (L/s)</td>
<td>2.27±1.00</td>
<td>2.44±1.14*</td>
</tr>
<tr>
<td>1 h post-MDI</td>
<td>2.27±0.98</td>
<td>2.45±1.08*</td>
</tr>
</tbody>
</table>

*p<0.05 and ^p<0.01 by paired comparison to pre-MDI.

3. Results

Table 1 shows the spirometric data (FEV1%pred and FEF 25–75 (L/s)) for the two treatment days, in each case pre, 1 and 2 h post-MDI treatment. For the salmeterol treatment day, FEV1%pred and FEF 25–75 were significantly increased at both 1 and 2 h compared to pre-MDI measurements. A decrease in FEV1%pred between pre- and post-MDI was seen for the placebo treatment study day. The mean peak flow rates associated with the controlled coughs (i.e. during 1–1(1/2) h post-MDI treatment) were increased from 6.0 to 6.6 L/s for placebo vs. salmeterol (p<0.05).

Fig. 1 illustrates mean whole lung clearance (retention vs. time through 2 h) of Tc99m-SC for the two study days (n=14), placebo (P) vs. salmeterol (S). One of the 15 patients studied was eliminated from the analysis because she had 43 spontaneous coughs during the first hour of imaging on one of the two study days. On the other study day, she had no coughing during the same period. All other subjects had less than seven coughs during the first hour of imaging on all study days, with most coughing only once or twice during that period. By paired analysis, there were no significant differences in CIP ratios or retention at 24 h (R24) for the two study days, CIP = 1.66±0.29 (P) vs. 1.60±0.40 (S) and R24 0.41±0.14 (P) vs. 0.42±0.14 (S).

Only the right lung was used to analyze both CIP ratios and retention vs. time due to effects of stomach activity on the left side. While there was a tendency for salmeterol to enhance whole lung clearance over the 2 h period, the difference was not statistically significant, average 2 h clearance (Cl0–2) = 22±10% (P) vs. 25±11% (S), NS. With the addition of cough at 1 h (Fig. 1), the slope of retention vs. time increased for both treatments. However when retentions between 1 and 2 h were normalized to R1, the average normalized clearance during the period 1–2 h (norm Cl1–2) was also not different between treatments, norm Cl1–2 16±9% for both. The C/P ratios at the 1 h time point were also not different between the two treatment days, 1.57±0.50 (P) vs. 1.55±0.43 (S).

Fig. 2 shows mean peripheral lung clearance (retention vs. time through 2 h) of Tc99m-SC for the two study days (n=14), placebo (P) vs. salmeterol (S). Even more than observed with whole lung clearance (Fig. 1), there was a tendency for average clearance through the 1 h to be greater with salmeterol vs. placebo, Cl0–1 = 7±9% (P) vs. 11±7% (S) (p=0.12, NS by paired analysis). However, average clearance during the entire 2 h of observation (including cough) was significantly enhanced by salmeterol, Cl0–2 = 17±10% (P) vs. 22±9% (S), p=0.05 by paired analysis. As with whole lung clearance the normalized peripheral clearance during the 1–2 h period where cough was added was not different between the two, norm Cl1–2 = 16±9% (P) vs. 15±11 (S).

For neither whole nor peripheral lung clearance were there significant correlations between the average clearance over any period relative to placebo and either baseline lung function or improvements in lung function (e.g. %increase in FEF 25–75) associated with salmeterol treatment. There was also no relationship between smoking history or medication use and improvement in whole lung or peripheral airway clearance over the 2 h period.

4. Discussion

In the present study we found that in mild chronic bronchitics, there was no acute effect on whole lung...
mucociliary clearance with or without cough as a result of a single administration of salmeterol (a long acting β2-adrenergic agonist). On the other hand, when region of interest analysis was employed we found that salmeterol significantly enhanced clearance from the peripheral lung region (Fig. 2). The fact that clearance during the cough period (1–2 h) was not different between salmeterol and placebo treatment suggests that the salmeterol enhanced clearance over the entire 2 h period was due primarily to its effect on mucociliary clearance during that period. While the mechanism for this effect is unclear, it may be due to salmeterol effects on ciliary beat frequency [4,5] in these airways. Similar improvements in the rheology of airway secretions with salmeterol treatment have not been investigated but cannot be ruled out as contributing to an improvement in mucociliary clearance in these patients.

The ability for salmeterol to enhance peripheral airway clearance but not whole lung clearance (which includes large bronchial airways) is not entirely clear. It may be that by focusing on the peripheral region we eliminated more of the cumulative effect of cough clearance that was not different between treatments. Inclusion of the central region which contains the large bronchial airways where cough is most effective [9,11] might have diminished our ability to observe a salmeterol effect associated with enhanced ciliary beating throughout the entire bronchial tree. In other words, by attempting to assess cough clearance at the 1 h time point we may have reduced our ability to find a significant effect on whole lung mucociliary clearance. Had we not intervened with the coughing protocol the whole lung clearance through the 2 h period (reflecting only mucociliary clearance) may have also differed between salmeterol and placebo. It may also be important that the intermediate and smaller bronchial airways are more compromised in these mildly obstructed patients. Morphologic studies suggest that the initial lesions in smokers occur in the more peripheral bronchi and bronchioles [12,13]. Using region of interest analysis, as we have employed here, Weiss et al. [14] showed that peripheral zone clearance was especially compromised in smokers similar to those in our study. Thus, by using similar analysis to better focus on peripheral airways (i.e. eliminating the larger, central airways from the analysis), we were able to detect an acute improvement in airway clearance with salmeterol.

The acute response on airway clearance we observed with salmeterol appears relatively mild compared to other muco-active agonists studied in similar patients [15]. In a similar group of mild chronic bronchitics we were able to dramatically enhance whole and peripheral lung mucociliary clearance over a 1 h time period with aerosolized UTP (uridine 5′ triphosphate), a P2Y2 agonist [15]. UTP, like salmeterol, enhances ciliary beating but may also improve clearance by hydration of the airway surface liquid [11,15]. Furthermore this previous study [15] shows that our technique is capable of detecting an acute, dramatic enhancement of mucociliary clearance in such patients.

It may be that the slow, but long acting nature of salmeterol compared to faster, short acting β-agonists such as albuterol or terbutaline indicate that longer observation times for mucociliary clearance should be considered than the 2 h studied here. The longer time to onset for peak effects of long acting β-agonists may have also been a factor in our inability to observe an effect on whole lung clearance by 1 h (i.e. before we commenced with the cough protocol). However, it also seems clear from studies of shorter acting β-agonists that the mucociliary enhancing effects of these agents in patients with depressed clearance are much less than those seen in healthy individuals [16–18]. While the dose of salmeterol resulted in significant bronchodilation in our patients (Table 1), higher doses than those administered here may be needed to have maximal effects on mucociliary clearance. There also may be no correlation, as we found here, between the bronchodilator effects of β-agonists and their ability to enhance mucociliary clearance [16].

A recent cross-over study by Hasani et al. [6] also showed no significant enhancement of mucociliary clearance in asthmatics following 2 weeks treatment with salmeterol compared to placebo. Their study was complicated by the fact that improvement in lung function after 2 weeks of salmeterol resulted in a greater penetration of the radiolabeled particles to the lung periphery. Such an effect confounds/biases against seeing an improvement of clearance for salmeterol vs. placebo treatment. Recognizing this bias, these authors suggested that salmeterol had a positive effect on mucociliary clearance based on the fact that they found similar clearance between the two or, on average, slightly (though not significantly) faster tracheo-bronchial clearance (normalized to alveolar deposition) following salmeterol vs. placebo treatment.

Using a similar protocol to that used here, we showed that ipratropium bromide, an anti-cholinergic bronchodilator, tended to slow cough-associated clearance (i.e. when cough was added during the period between 1 and 2 h) in more severe COPD patients compared with placebo [7]. If the effect of diminished clearance by IB therapy were a result of changes in flow dynamics induced by bronchodilation, then salmeterol should have had a similar effect on cough clearance. Our failure to find an enhancement of cough clearance for salmeterol vs. placebo is not likely due to an inability of our technique to detect such changes. Once again, using similar techniques, we recently showed that a single dose of aerosolized UTP enhanced cough clearance in patients with primary ciliary dyskinesia who rely on cough to clear their airways of secretions [19].

Cough clearance becomes more important for clearing secretions as mucociliary function becomes compromised in CB. We have previously used the technique of controlled coughing during measures of particle clearance in such patients and shown significant enhancement of secretions under these conditions compared to clearance without cough [20]. We have shown a mild but significant effect of a single MDI treatment of salmeterol on peripheral airway clearance.
in mild chronic bronchitics. The observed enhanced clearance seems to be primarily via mucociliary as opposed to cough mechanisms. Smokers with mild chronic bronchitis, such as those studied in the present study, have been shown previously to have mildly depressed mucociliary clearance compared to normal. Thus, salmeterol may provide an additional therapeutic benefit to such patients exclusive of its bronchodilatory effects.

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References