



## Chronic inhalation of nebulized levalbuterol does not increase mucociliary clearance in healthy subjects

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### Abstract

Acute inhalations of  $\beta_2$ -adrenergic receptor agonists increase mucociliary clearance (MCC). Less is known about the effect of long-term inhalations of these agents on MCC, or cough clearance (CC). We hypothesized that chronic inhalations of nebulized levalbuterol, the R-isomer of albuterol, would enhance MCC and/or CC in healthy subjects, compared to albuterol or placebo. This was a randomized, double-blind, placebo-controlled trial in ten healthy, adult subjects who inhaled nebulized levalbuterol (1.25 mg), albuterol (2.5 mg), or placebo for 7 days, three times daily. MCC and CC were measured 6–7 h after the last dose of drug on the 7th day of treatment. These were quantified from gamma camera images of the lungs following inhalation of an aerosol containing the isotope  $^{99m}\text{Tc}$ . Levalbuterol did not improve MCC or CC. MCC averaged ( $\pm$ SD)  $12.3 \pm 8.3\%$ ,  $9.2 \pm 4.7\%$  and  $10.0 \pm 9.6\%$  with placebo, albuterol and levalbuterol, respectively. CC averaged  $3.9 \pm 6.8\%$ ,  $4.9 \pm 4.3\%$  and  $3.8 \pm 6.4\%$  with placebo, albuterol and levalbuterol, respectively. These results indicate that chronic inhalations of nebulized levalbuterol for 1 week do not increase MCC or CC in healthy subjects, compared to albuterol or placebo.

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### 1. Introduction

In healthy lungs, inhaled insoluble materials such as bacteria, viruses, antigens, and toxins deposit in the tracheobronchial airway mucus and are removed from the lung in a matter of hours by mucociliary clearance (MCC). When MCC is overwhelmed or impaired, some mucus can be removed by mechanical, or cough clearance (CC). Impairment of MCC typically leads to the accumulation of mucus in the airways, and this in turn is associated with acute infections, chronic bacterial colonization and chronic inflammation [1–3].

Acute doses of inhaled  $\beta$ -adrenergic receptor agonists have been shown to stimulate MCC. For example, several studies in healthy individuals show that MCC is stimulated after inhaling acute doses of terbutaline sulfate [4,5], salbutamol (albuterol) [6], isoproterenol [7–9], isoetharine hydrochloride [8], or fenoterol [10]. Acute doses of inhaled

fenoterol [11] and salbutamol [6] and oral doses of terbutaline [12] also enhance MCC in patients with chronic bronchitis. Based on these results, clinicians often instruct their patients with airway disease to inhale a  $\beta$ -adrenergic receptor agonist several times a day, thinking this therapeutic approach will not only improve lung function, but will also improve MCC throughout the day.

Nevertheless, it is unclear if long-term treatment with a  $\beta$ -adrenergic receptor agonist several times a day improves the removal of mucus. The few studies of the effect of chronic, multiple administrations of these drugs on MCC have produced mixed results. Two studies showed no significant difference in MCC after 1 week of daily, multiple treatments with inhaled or oral terbutaline, compared to placebo, in patients with chronic bronchitis [13,14] and Perry and Smaldone [9] found no increase in MCC in healthy subjects after 1 week of daily, inhalations of albuterol, compared to placebo. In contrast, oral doses of both fenoterol and tulobuterol resulted in a significant improvement in MCC after 1 week of chronic, multiple treatments, in patients with chronic bronchitis [15].

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The racemic form of albuterol has been available for years and contains two optical isomers: levo (R) and dextro (S). Analysis of these two isomers indicates that the S-isomer lacks significant  $\beta_2$ -adrenergic receptor affinity and it is thought that this binding is necessary to stimulate MCC [16]. If this is the case, one might speculate that drug containing only the R-isomer would stimulate MCC to a greater extent than drug containing the S-isomer as well. This hypothesis is supported by *in vitro* data reported by Frohock and colleagues [17]. They quantified ciliary beat frequency, an integral part of MCC, in single ovine airway epithelial cells that were exposed to racemic albuterol, the R-isomer alone, or the S-isomer alone. Results from that study demonstrated that the R-isomer had a greater stimulatory effect on ciliary beat frequency than either racemic albuterol, or the S-isomer alone. This was despite the same amount of R-isomer being present in both the racemic form and the single-isomer form [17]. The hypothesis is also supported by results from another study of mucociliary transport in a calf trachea model, which showed that the R-isomer is a log more potent than its racemate in terms of increasing mucociliary transport velocity (Y. Schwartz, Sepracor, unpublished data).

Another study reported that the S-isomer has a 12-fold slower rate of metabolism compared to the R-isomer, leading to a longer retention time in the lung [18]. This difference in retention may not be important with acute inhalations, but could lead to desensitization to the racemate with daily, multiple dosing and desensitization could be one explanation for the lack of stimulation observed when racemic albuterol was administered four times daily for a week to healthy subjects [9].

Since levalbuterol, the R-isomer of albuterol, is now available for inhalation, we decided to repeat the study by Perry and Smaldone [9] with levalbuterol, hypothesizing that chronic, multiple inhalations of nebulized levalbuterol would stimulate MCC and/or CC to a greater extent than either racemic albuterol, or placebo.

## 2. Material and methods

### 2.1. Study subjects

Ten non-smoking, healthy males and non-pregnant females,  $\geq 18$  years of age completed the protocol. A sample size of ten healthy subjects was calculated to provide 80% or greater power to detect a change in MCC of 12% between visits, if the standard deviation (SD) for the change in MCC between visits was 10% or less. In addition, a previous study in our laboratory in lung transplant patients indicated that significant improvement in MCC could be detected in a sample as small as seven individuals, following an acute inhalation of 180  $\mu\text{g}$  racemic albuterol by metered dose inhaler [19]. Based on these results and our power calculation, we reasoned that a sample size of ten healthy subjects should be sufficient to detect significant differences in MCC following chronic

administration of the more potent R-isomer of albuterol. Altogether 14 patients enrolled in the study. However, four patients did not complete the study for personal reasons.

Although patients with asthma, or chronic obstructive pulmonary disease (COPD), are the target population for this drug, these patients are likely to have damaged epithelial cells, due to the inflammation associated with the disease process, and that damage could mask the inherent effects of the drug on MCC. To eliminate this possibility, we decided to quantify the MCC response in healthy subjects. All subjects gave informed, signed consent, as approved by the Johns Hopkins School of Medicine Institutional Review Board.

### 2.2. Study design

This was a randomized, double-blind, placebo-controlled trial. The CONSORT guidelines [20] were followed (ClinicalTrials.govIdentifier: NCT00325767). There was one screening visit and three study visits. Prior to each study visit, subjects were treated with one of three study drugs for 7 days. There was a washout period (no drug) of at least 1 week after treatment weeks 1 and 2. The primary outcomes of MCC and CC were quantified on the 7th day of each treatment week.

### 2.3. Distribution of study drug

Blinded stocks of albuterol, levalbuterol and placebo were supplied by Sepracor, Inc. (Waterford, MA) and distributed to subjects in coded containers by Johns Hopkins Research Pharmacy personnel. The order of drug administration was also randomized by our research pharmacy, which used the Block Stratified Randomization (version 5.0) computer program. The pseudorandom number generator was a linear congruential algorithm of Park and Miller with Bays-Durham shuffling.

### 2.4. Drug doses

Subjects were instructed to inhale aerosol three times daily for 7 days. Each dose of placebo aerosol consisted of 3 ml of drug vehicle. The dose of racemic albuterol was 2.5 mg/3 ml, which is the standard clinical dose that is routinely administered to patients with asthma to relieve their bronchospasm. The dose of levalbuterol was 1.25 mg/3 ml, which is also a standard clinical dose. In a previous study of daily, multiple administrations in patients with asthma, this dose was well-tolerated and resulted in clinically significant increases in pulmonary function measurements up to 8 h after the last dose of drug was administered [21].

### 2.5. Drug administration

Aerosolized drug was generated by an LC Plus nebulizer (PARI Respiratory Equipment, Inc., Midlothian, VA)

connected to a Pari Duraneb compressor. Subjects inhaled aerosol while breathing continuously and slowly from functional residual capacity, until no more aerosol was being generated. Subjects kept a diary of the time of dose inhalation and recorded any symptoms of nervousness, dizziness, shakiness, or fast heart rate.

## 2.6. Screening visit

To qualify, eligible subjects demonstrated a forced expiratory volume in one second ( $FEV_1$ ) and a forced vital capacity (FVC)  $\geq 80\%$  of predicted values and normal systolic and diastolic blood pressures following a physical exam on a screening visit. FVC and  $FEV_1$  were measured by a computerized 10-liter Survey III spirometer (Warren E. Collins, Inc.; Braintree, MA), in accordance with the American Thoracic Society/European Respiratory Society guidelines [22].

A ventilation image of the lung was also obtained on the screening visit. Patients inhaled  $^{133}\text{Xe}$  gas, using a Pulmonex xenon system (Biodex Medical, Shirley, New York), while sitting with their back to the gamma camera. Lung borders identified on the ventilation image were superimposed on subsequent aerosol images as described previously [23,24].

## 2.7. Study visits 1–3

MCC and CC were measured 6–7 h after the subjects' last dose of study medication on the 7th day of each drug treatment period. By measuring MCC and CC during this time period, we hoped to determine if there was both a sustained effect from the drug (i.e. the effect lasted up to 6–7 h after drug administration) and if the effect was sustained over a prolonged treatment period (i.e. 7 days). On each visit, subjects first underwent a physical exam with interval history and measurement of vital signs and pulmonary function testing. Then, they inhaled an aerosol containing the isotope  $^{99m}\text{Tc}$ -sulfur colloid (radioaerosol). A gamma camera recorded radioactivity deposited in the lungs immediately after inhalation of the radioisotope and every 4 min thereafter for 76 min, as described previously [19,25].

## 2.8. Radioaerosol administration and characteristics

Radioaerosol was generated by a raindrop nebulizer (Nellcor Puritan Bennett, Pleasanton, CA) connected to a compressed air tank with regulator set at 44 psi. The volume median diameter of this aerosol was  $2.9\ \mu\text{m}$ , measured by a Mastersizer<sup>TM</sup> (Malvern Instruments, Ltd., Worcestershire, UK). Radioaerosol was generated by a Spira Elektro-2 Dosimeter (Spira, Hameenlinna, Finland) as described previously [26]. After inhalation, participants rinsed their mouth with water, expectorated the rinse and drank water to wash any remaining radioactivity into the stomach.

## 2.9. Lung imaging and image analysis

Following inhalation of radioaerosol, participants underwent 19 posterior lung scans with a large-field-of-view ZLC gamma camera (Siemens, Munich, Germany). Lung images were stored on computer (GE Healthcare, Waukesha, WI) for analysis.

## 2.10. Quantifying deposition pattern

We quantified deposition of the radioaerosol in an inner and outer zone of the lung image that was acquired immediately after aerosol inhalation (time 0), as described previously [23,24]. Mean counts per picture element in the inner and outer regions of the ventilation scan and the radioaerosol scan were calculated and inner:outer (I:O) ratios derived. Ratios for the aerosol scan were divided by that of the ventilation scan to correct for lung volume differences. The deposition pattern for the radioaerosol was then reported in terms of the corrected I:O ratio.

## 2.11. Percent MCC calculation

To quantify MCC, we measured the total amount of radioactivity ( $^{99m}\text{Tc}$ ) that was detected in the right lung at time 0 and the amount of radioisotope that was retained in the right lung every 4 min thereafter over the 76 min acquisition period. The total amount of radioactivity that was detected in the right lung at each of these time points was corrected for background and decay-corrected to time 0.

Radioactivity detected at time 0 reflected 100% retention of the initially deposited radioactivity. Decay- and background-corrected activity for each of the later time points was expressed as a percentage of the time 0 activity. Percent retention values at each time point were plotted and the best-fit line, determined through appropriate regression analysis, was calculated as described previously [19,23]. Percent retention at 76 min (Y) was then recalculated, based on the regression equation for the best-fit line. MCC at 76 min was expressed on a continuous scale from 0 to 100 as the complement of percent retention and calculated from the equation:

$$\% \text{ MCC at 76 min} = 100\% - Y.$$

## 2.12. Percent CC calculation

CC was measured on each of the study visits, following the 76 min of imaging for MCC. Subjects were asked to cough gently 60 times in 10-cough intervals. CC was calculated as the difference in the amount of radioactivity that was measured in the lung image before coughing (i.e. at 76 min) and after coughing (i.e. at 85–90 min).

### 2.13. Data analysis

Data are presented as mean  $\pm$  standard deviation for I:O ratio, MCC, CC, FEV<sub>1</sub>, and FVC for the ten subjects who completed the entire protocol. The Friedman two-way analysis of variance by ranks test was used to compare these parameters for the 3 weeks of treatment. This non-parametric test was chosen to offset the potential for large changes in one subject biasing a parametric analysis of mean values in such a small sample size. *P*-values  $\leq 0.05$  indicated a statistically significant difference.

## 3. Results

### 3.1. Subject demographics

Table 1 shows the baseline characteristics for the 10 study subjects. These data were obtained on the screening visit. There were four male and six female subjects in the study, with a mean age of  $28.4 \pm 9.5$  yr (range = 18–44 yr). Their baseline FEV<sub>1</sub> averaged  $99.7 \pm 10\%$  of predicted values, and their FVC averaged  $95.5 \pm 7\%$ .

### 3.2. Commencement of MCC study relative to the time of the last dose of drug

The average time from the last dose of drug to commencement of the MCC study was  $7.2 \pm 1.0$  h on the placebo visit,  $6.7 \pm 1.3$  h on the albuterol visit and  $7.2 \pm 1.2$  h on the levalbuterol visit.

### 3.3. Subject symptoms

Overall, the treatments with nebulized levalbuterol, albuterol, and placebo were well tolerated. No subjects withdrew from the study due to treatment side effects. Over half the subjects noted some mild shakiness with both albuterol and levalbuterol, and five subjects reported rapid heart rate with either albuterol, levalbuterol, or

both. One patient described nervousness with both albuterol and levalbuterol, and another patient reported mild dizziness only with albuterol.

### 3.4. Deposition pattern analysis

The I:O ratio averaged  $1.5 \pm 0.4$  for placebo,  $1.4 \pm 0.4$  for albuterol, and  $1.4 \pm 0.4$  for levalbuterol. There were no statistically significant differences in the I:O ratio between treatments, indicating that the radioactive marker initially deposited in similar regions of the lungs on each of the study visits. This means that our measurements of MCC were unaffected by the initial site of deposition of our radioactive marker.

### 3.5. Representative lung images

Fig. 1 is a representative example of the posterior images obtained from one of the study subjects following 1 week of treatment with placebo. Fig. 1A shows radioaerosol deposited in both lungs at time 0 (immediately after radioisotope inhalation). Fig. 1B shows radioactivity remaining in the lung after 76 min. For quantitative analysis, the radioactivity remaining in the lungs after 76 min was corrected for background and decay.

### 3.6. Treatment effects on MCC from right lung

Fig. 2 shows average percent retention (100–%MCC) for the entire right lung for each treatment for all 10 subjects combined. There was no significant effect of levalbuterol or albuterol on retention of the radiolabel within the right lung at any time point. Fig. 3 shows percent whole lung MCC from the right lung after 76 min. MCC averaged  $12.3 \pm 8.3\%$  for placebo,  $9.2 \pm 4.7\%$  for albuterol and  $10.0 \pm 9.6\%$  for levalbuterol, which were not statistically significantly different.

MCC from the smaller airways was quantified by analyzing retention in the outer right lung region over

Table 1  
Baseline characteristics for 10 study subjects on screening visit

Patient no.	Age (years)	Race	Sex	Height (cm)	Weight (kg)	FEV <sub>1</sub> (%)	FVC (%)
1	44	C	M	178.6	86.6	107	98
2	25	C	M	175.1	79.1	87	84
3	18	AA	F	166.9	54.1	104	103
4	19	AA	F	168.7	114.0	100	97
5	26	C	F	174.7	72.0	84	85
6	23	AA	M	178.1	90.0	96	93
7	36	C	F	159.2	66.3	115	103
8	44	C	M	184.8	91.9	96	83
9	24	C	F	175.5	69.2	112	97
10	25	C	F	171.2	68.5	96	102
Mean	28.4			173.3	79.2	99.7	95.5
SD	9.5			7.2	17.0	10.0	7.0

Definition of abbreviations: C: Caucasian; AA: African American; FEV<sub>1</sub>: forced expiratory volume in one second; FVC: forced vital capacity.

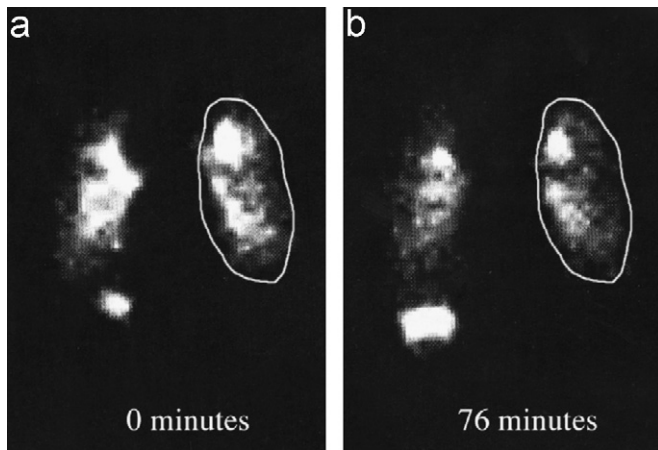


Fig. 1. Gamma camera images showing radioactivity deposited in the lungs immediately after inhalation (a) and radioactivity remaining in the lungs after 76 min (b). The right lung in each image is demarcated by the white border. This border was first identified on the ventilation scan and then superimposed onto the aerosol scan.

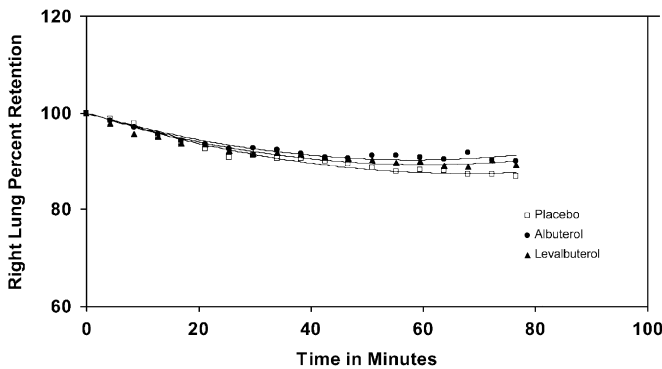


Fig. 2. Percent right whole lung retention throughout the imaging period for each of the three treatments (note: y-axis origin at 60% retention) ( $n = 10$ ). □—placebo, ●—albuterol, ▲—levalbuterol.

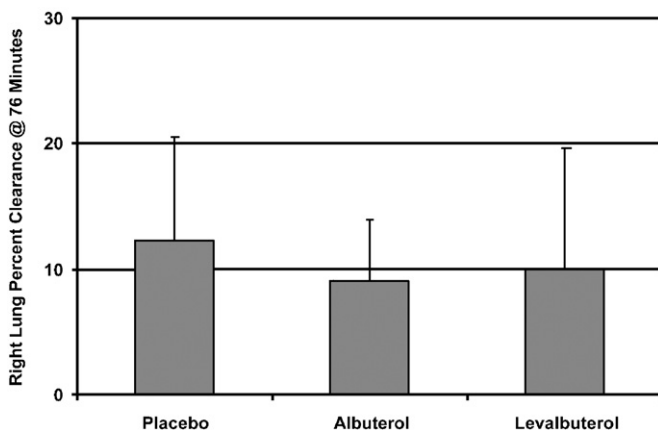


Fig. 3. Mean ( $\pm$  standard deviation) right lung percent MCC at 76 min for each of the three treatments ( $n = 10$ ).

time. Fig. 4 shows average percent retention in the outer lung region for each treatment for all 10 subjects combined. Clearly, there was a trend toward less retention with levalbuterol, compared to placebo or albuterol treatment;

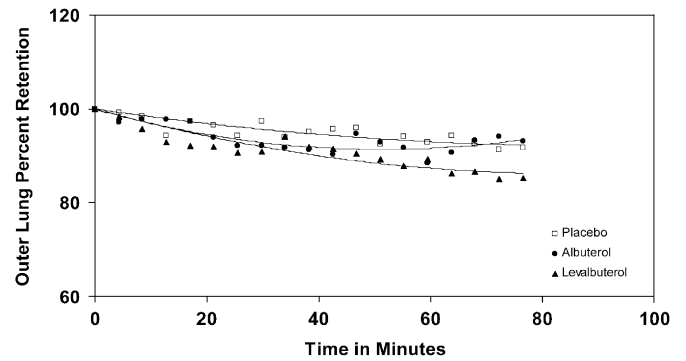


Fig. 4. Percent outer right lung retention throughout the imaging period for each of the three treatments (Note: y-axis origin at 60% retention) ( $n = 10$ ). □—placebo, ●—albuterol, ▲—levalbuterol.

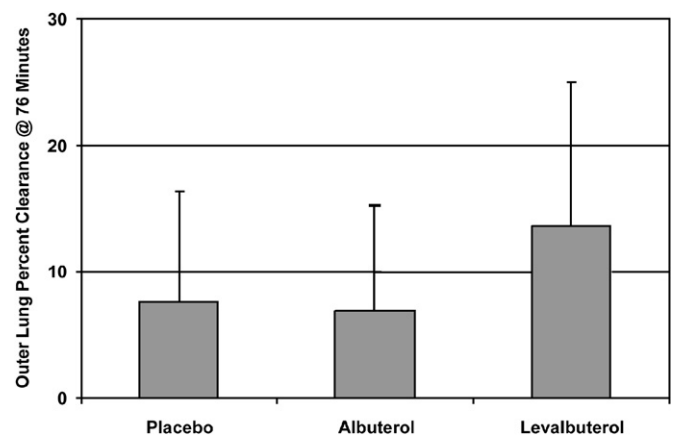


Fig. 5. Mean ( $\pm$  standard deviation) percent MCC from the outer right lung region at 76 min for each treatment ( $n = 10$ ).

however, the three treatments were not statistically significantly different. MCC from the outer zone averaged  $7.7 \pm 8.7\%$  for placebo,  $7.0 \pm 8.2\%$  for albuterol and  $13.6 \pm 11.4\%$  for levalbuterol (Fig. 5).

### 3.7. Treatment effects on CC from right lung

CC was not affected by albuterol or levalbuterol treatment. Percent CC from the right lung after the coughing procedure averaged  $4.0 \pm 6.8\%$  for placebo,  $4.9 \pm 4.3\%$  for albuterol and  $3.8 \pm 6.4\%$  for levalbuterol. These differences were not statistically significant.

### 3.8. Treatment effects on spirometry values

Levalbuterol did not alter lung function, compared to albuterol and placebo, when measured immediately before the MCC procedures. FEV<sub>1</sub> averaged  $102 \pm 10.6\%$  after 1 week of levalbuterol,  $104 \pm 10.9\%$  after albuterol and  $101 \pm 11.6\%$  after placebo. FVC averaged  $97 \pm 5.6\%$  after levalbuterol,  $98 \pm 6.0\%$  after albuterol and  $96 \pm 6.1\%$  after placebo. These differences were not statistically significant.

#### 4. Discussion

We hypothesized that 1 week of chronic, multiple dosing with nebulized levalbuterol aerosol ( $1.25 \text{ mg} \times 3/\text{day}$ ) would enhance MCC and/or CC in healthy subjects, compared to 1 week of multiple treatments with either nebulized racemic albuterol ( $2.5 \text{ mg} \times 3/\text{day}$ ), or placebo. Our results indicate that this was not the case. These results are similar to those reported by Perry and Smaldone [9], who found no significant difference in MCC in healthy subjects after 7 days of multiple treatments with inhaled albuterol ( $180 \mu\text{g} \times 4/\text{day}$ ), delivered by metered dose inhaler (MDI), compared to placebo.

The effect of daily, multiple administrations of  $\beta_2$ -adrenergic receptor agonists on MCC in patients with lung disease have produced mixed results. For example, no change was seen in the clearance of lung secretions, mucociliary transport velocity, or sputum properties with 1 week of multiple treatments with inhaled ( $125 \mu\text{g} \times 4/\text{day}$  by MDI), or oral ( $2.5 \text{ mg} \times 3/\text{day}$ ) terbutaline in studies of patients with chronic bronchitis [13,14].

In contrast, 7 days of multiple oral administrations of tulobuterol ( $2 \text{ mg} \times 2/\text{day}$ ), or fenoterol ( $2.5 \text{ mg} \times 3/\text{day}$ ), showed significant increases in MCC compared to pre-treatment values in patients with chronic obstructive diseases [15]. Similarly, Hasani et al. [27] recently showed that MCC was significantly improved in 15 patients with COPD following 10 days of inhalation of nebulized albuterol ( $5 \text{ mg} \times 3/\text{day}$ ).

Results from the Hasani study [27] suggest that the dose of albuterol required to produce a significant improvement in MCC with daily, multiple inhalation treatments may be higher than the doses that were administered in either the current study, or in the studies by Perry and Smaldone [9], or by Pavia et al. [13]. This leads to speculation that multiple inhalations with higher doses of nebulized levalbuterol (i.e.  $2.5 \text{ mg} \times 3/\text{day}$ ) might increase MCC in healthy subjects following 1 week of treatment. However, higher doses also have the potential for increasing drug side effects and adverse events. Additional studies are needed to test this hypothesis.

Another explanation for our results could be the well-established effect of desensitization of G-protein-coupled receptors, including  $\beta$ -adrenergic receptors, which occurs with prolonged agonist exposure [16]. This notion is supported by results from a study by Hayes et al. [28] who showed that 2 weeks of albuterol therapy resulted in a decrease in  $\beta_2$ -adrenergic receptor density in seven healthy subjects. In that study, albuterol dosing consisted of  $4 \text{ mg}$  orally ( $\times 2/\text{day}$ ) and  $200 \mu\text{g}$  by MDI ( $\times 4/\text{day}$ ) for the first week of treatment and  $8 \text{ mg}$  orally ( $\times 2/\text{day}$ ) with  $400 \mu\text{g}$  of inhaled albuterol ( $\times 4/\text{day}$ ) during the second week. Using positron emission tomography (PET) technology, the authors found that, on average, maximal  $\beta_2$ -adrenergic receptor binding capacity in the lungs decreased by 22% after the 2 weeks of treatment with the combined therapy [28]. In addition, they noted a decrease in the bronchodi-

lator response to additional inhaled albuterol. The authors reported that this decrease in binding capacity was a generalized phenomenon throughout the lung that could be due to a reduction in  $\beta_2$ -adrenergic receptor mRNA expression and a reduction in the relaxation response to  $\beta_2$ -adrenergic receptor agonist. It is not known if the observed decrease in receptor binding capacity in that study was the result of multiple administrations of the inhaled albuterol, the oral albuterol, or a combination of both. It is also not known if a similar decrease in binding capacity occurred over the 7 days of treatment in the current study.

A third explanation for our results could be the timing of the MCC testing relative to the time of administration of the drug. In the current study, MCC was measured 6–7 h after the subjects' last dose of study medication. Perhaps by measuring at this late time point, we missed the effect of drug on MCC. However, a review of other studies that measured MCC at different time points following dosing with  $\beta_2$ -adrenergic receptor agonists indicates that the timing of the MCC measurement may not be critically important. Such a review shows that both positive and negative results have been reported, with no apparent association between the various time points following drug administration. For example, a significant improvement in MCC has been reported when MCC was measured at 8 h after the last dose of tulobuterol tablets [15], 3 h after fenoterol tablets [15], or over a 6-h period after the last dose of salbutamol inhalation [27]. In contrast, no improvement in MCC was reported when MCC was measured as soon as 30 min after the last drug dose of albuterol by inhalation [9], or when measured at approximately 2 h after the last dose of terbutaline inhalation [13], or 3 h after terbutaline tablets [14]. These results suggest that the timing of the MCC testing relative to drug administration may not be as important as the dose of drug, as noted above.

In summary, results from this study do not support the theory that chronic, multiple treatments with nebulized levalbuterol ( $1.25 \text{ mg} \times 3/\text{day}$ ) increases the removal of mucus from the lungs of healthy subjects to a greater extent than racemic albuterol treatment ( $2.5 \text{ mg} \times 3/\text{day}$ ). In addition, daily, multiple treatments with either drug do not appear to increase MCC when compared to placebo. Further research is needed to ascertain whether this observed lack of effect on MCC was the result of too low a dose of levalbuterol,  $\beta_2$ -adrenergic receptor desensitization, or a reduction in receptor number, with repeated dosing.

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