Randomized placebo controlled assessment of airway inflammation due to racemic albuterol and levalbuterol via exhaled nitric oxide testing

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Study Objectives: The S-stereoisomer found in racemic albuterol may have associated proinflammatory properties. We tested the hypothesis that airway inflammation as assessed by exhaled nitric oxide is no different in patients with COPD when using racemic albuterol relative to levalbuterol or placebo.

Measurements: Twelve mild to moderate COPD patients were assigned to five days each of nebulized racemic albuterol, levalbuterol, and saline placebo. Before and after each course of treatment, airway inflammation was assessed via exhaled nitric oxide breath testing. Secondary functional outcomes that were measured included spirometry, a functional assessment utilizing a six-minute walk, and symptoms score using the University of California, San Diego Shortness of Breath Questionnaire.

Results: There was no statistically significant difference in pre and post FeNO levels within and between treatment groups ($p = 0.121$). There were also no significant differences within or between treatment groups for the secondary outcome measurements of FEV_1 ($p = 0.913$), functional assessment utilizing a six-minute walk ($p = 0.838$) and the symptom scores using Shortness of Breath Questionnaire ($p = 0.500$).

Conclusion: We found no difference in mild to moderate COPD patients treated with racemic albuterol, levalbuterol or placebo for measurement of exhaled nitric oxide or the secondary outcomes that were measured.

Keywords: Airway inflammation, albuterol, COPD, exhaled nitric oxide, levalbuterol

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease state characterized by airflow limitation that is not fully reversible and progressive chronic inflammation throughout the airways, parenchyma, and pulmonary vasculature (GOLD 2005). Various bronchodilator medications, to include the β2-agonists, are commonly used in treating COPD. β2-agonists bind to the β2-receptor and relax airway smooth muscle reducing airflow obstruction. They are recommended both on a regular schedule and on an as needed basis (GOLD 2005).

Albuterol, a short-acting β2-agonist, is available as a racemic preparation that is composed of (R) and (S)-isomers in a 50:50 mixture or as a single (R)-isomer (levalbuterol). The (R)-isomer is predominantly responsible for the bronchodilator effect and side effects of tachycardia, tremor, and nervousness (Handley 2001). The (S)-isomer, considered inert due to its weak binding to the β2-adrenoceptor, has been reported to have potentially deleterious effects (Handley 1999, 2001; Page and Morley 1999) to include the promotion of bronchoconstriction and bronchial

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hyperresponsiveness (Mazzoni et al 1994; Wang et al 1994; Gauvreau et al 1997; Templeton et al 1998). In addition, the (S)-isomer has been reported to have various proinflammatory effects including alterations in cytokine production (Gavreau et al 1997; Frieri et al 2000; Cho et al 2001; Baramki et al 2002), enhanced production of histamine (Cho et al 2001), immune cell proliferation/activation (Manolitsas et al 1995; Morley et al 1995; Gauvreau et al 1997; Volcheck et al 1998; Baramki et al 2002), and increased nitric oxide release in stimulated small airway epithelial cells (Frieri et al 2000).

As chronic inflammation can contribute to accelerated loss of lung function, the use of levalbuterol has been considered due to concern about the inflammatory potential of the (S)-isomer (Costello 1999). Although information exists suggesting that the (S)-isomer may induce inflammation in the setting of asthma, to our knowledge, there are no studies investigating the presence of inflammatory response in the setting of COPD. In this study, we tested the hypothesis that airway inflammation, as measured by exhaled nitric oxide, is no different in COPD patients when sequentially comparing nebulized racemic albuterol, levalbuterol, or placebo.

Airway inflammation was assessed by measurement of the fraction of exhaled nitric oxide (FeNO), a marker of airway inflammation. Relative to baseline levels or normal controls, elevated FeNO levels have been documented in the setting of COPD (Agusti et al 1999; Corradi et al 1999; Ansarin et al 2001; Kharitonov and Barnes 2004), chronic bronchitis (Delen et al 2003) and COPD exacerbations (Maziak et al 1998; Agusti et al 1999). Additionally, inhaled corticosteroids have been shown to reduce baseline levels of elevated FeNO in stable COPD (Zietkowski et al 2005), suggesting that FeNO may serve as a surrogate measure of chronic perseverant airway inflammation at baseline that is ameliorated through the use of inhaled corticosteroids.

Methods

The study was conducted at Wilford Hall Medical Center and Brooke Army Medical Center in San Antonio, Texas. Patients 18 years of age and older, who had at least a 10 pack year smoking history with no recent tobacco use within the six months preceding protocol enrollment, and mild to moderate COPD defined by a baseline forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) ratio of less than 0.70 and an FEV_1 greater than 50% predicted were enrolled in the study. The Brooke Army Medical Center Institutional Review Board approved the study, and all patients gave written informed consent.

Twelve patients were enrolled for the study. They were randomized, but not blinded, to complete three separate five-day treatments. The treatment groups consisted of albuterol (2.5 mg four times daily), levalbuterol (0.63 mg three times a day with one saline placebo dose), and 5 ml of saline placebo four times daily. All medications were administered through a nebulizer machine (PARI LC nebulizer, Midlothian, VA) that the patients took home with them. Each patient received instruction in how to properly use the nebulizer device and administer the medication. They were also instructed to complete a diary at home that recorded their use of medication. There was a minimal two day washout period between treatment groups during which the patients abstained from medications intended to treat COPD. All patients were provided with an albuterol metered dose inhaler (MDI) which could be used for "rescue" purposes at any time during the study. However, rescue inhaler use constituted ground for exclusion from further study participation. None of the participants used a rescue treatment throughout the course of the study.

Patients were excluded from the study if they had had an exacerbation of their COPD requiring an adjustment in their COPD medications, the addition of an oral corticosteroid, or the administration of antibiotics, within the 6 months prior to trial enrollment. Patients were also excluded if they had a history of hospitalization due to COPD. Further exclusion criteria included use of an inhaled corticosteroid regimen or phosphodiesterase inhibitor (ie, theophylline) within the previous one month, use of an oral β-blocker medication, long-acting inhaled β-agonist, antihistamine, or nasal corticosteroid within the preceding week of, or during, trial participation. Patients could not have evidence of a concurrent restrictive lung disease on pulmonary function testing, or 12% or greater improvement in FEV_1 following administration of a bronchodilator. All patients who experienced a COPD exacerbation during the study interval were excluded from continuing the study (n = 1).

The primary outcome of the study was the measurement of FeNO. Based on a previous study (Agusti et al 1999) in clinically stable COPD patients following exacerbation, we assumed a mean exhaled nitric oxide level of 15.8 ± 3.8 parts per billion at baseline. An increase of FeNO by 2.4 standard deviations was considered to be significant. Our study was powered at 80% to detect this difference with a level of confidence of 95%. Nine subjects acting as their own control were needed to detect a difference with the desired level of power and confidence.

FeNO measurements were made using a NIOX® nitric oxide monitoring system (Aerocrine AB, Sweden) using a flow rate of 50 ml/sec and reported as parts per billion (ppb). FeNO levels were measured both before and after completion of each treatment group and were carried out in accordance with the guidelines published by the American Thoracic Society (ATS 1999).

Secondary outcomes that were measured included spirometry, a functional assessment utilizing a six-minute walk, and symptom score using the University of California, San Diego Shortness of Breath Questionnaire (SOBQ) (Eakin et al 1998). A Sensorimedics 6200 machine was used for spirometry to record FEV_1, FVC, and FEV_1/FVC ratio. The six-minute walk test (6MWT) was conducted in accordance with the guidelines described by the American Thoracic Society in their 2002 Official Statement (ATS 2002). A MeterMan measuring wheel (Model 1212) was used to measure the distance covered by the patient in the six-minute period. A difference of 54 meters in the six-minute walk distance was considered to be clinically significant based on previous estimates (Redelmeier et al 1997). The SOBQ was used with permission from the University of California, San Diego Medical Center's Pulmonary Rehabilitation Program. Based on previous recommendations a change of 5 units for the SOBQ was considered to be a minimal clinically important difference (Kupferberg et al 2005). Secondary outcome measurements were obtained at the completion of each treatment group and compared with baseline measurements.

Data were analyzed using SPSS Sample Power, Version 2.0 (SPSS Inc, Chicago, IL). The distributions of the FeNO values were not significantly different from normal on the Kolmogorov-Smirnov test ($p > 0.05$) and were therefore analyzed with parametric testing. Paired t-test was used to assess differences in FeNO between times within treatments. Repeated measures of ANOVA using paired t-test on FeNO, FEV_1, and six minute walk by treatment were used for between group comparisons. The Hyun-Feldt statistic was used to analyze the summed SOBQ scores for between group comparisons as the data were not normally distributed.

Results

Patient characteristic data is shown in Table 1. The average age of our patients was seventy years and ranged from fifty-five to eighty. There were nine males and three females. All patients had a baseline obstructive FEV_1/FVC ratio less than 0.70. Baseline FEV_1s were normal in four patients, mild in five, and moderate in three. Ten patients completed the study. One patient withdrew secondary to transportation issues. Another withdrew secondary to a COPD exacerbation that occurred while receiving placebo, which required treatment. No other patients reported use of albuterol for "rescue" purposes during the course of the study.

Table 1 Subject characteristics (n =12)

Average age: years (range)	70 (55–80)
Male	9
Female	3
FEV_1 > 80% predicted	4
FEV_1 66%–79% predicted	5
FEV_1 50%–65% predicted	3

Abbreviations: FEV_1, forced expiratory volume in one second.

The mean pre- and post-treatment FeNO levels for all patients in each treatment group are listed in Table 2. The data are represented in an intention-to-treat format and include the two participants who were eventually excluded from the trial. Due to the constraints of the statistical methods, the remaining calculations only include the 10 subjects who completed the study. In an analysis of those patients, the mean FeNO levels and standard deviation were as follows: levalbuterol (pre- 27.3 ± 19.1 ppb; post- 26.1 ± 20.6 ppb), albuterol (pre- 30.6 ± 24.4 ppb; post- 28.9 ± 22.4 ppb), and placebo (pre- 26.9 ± 20.5 pbb; post- 23.8 ± 15.0 ppb). The between-group comparisons in FeNO are noted in the box plot in Figure 1. There was no statistically significant difference in FeNO between treatment groups ($p = 0.121$). Patient number twelve was an outlier and upon re-review of the patient's medical records and condition we were not able to explain the elevation in FeNO. This patient's data were included in the analysis. Repeated analysis of treatment effect with subject number twelve excluded did not change the significance of our findings.

Results for the secondary outcome measurements are listed in Table 3. There were no differences within or between treatment groups for FEV_1 ($p = 0.913$), functional assessment utilizing the 6MWT ($p = 0.838$) and symptom scores using the SOBQ ($p = 0.500$). Complete SOBQ data were available for eight patients. There was a 6.25 and 4 unit increase in the SOBQ score with levalbuterol and albuterol respectively compared with baseline.

Discussion

Given the associated proinflammatory potential of the (S)-isomer of albuterol, our study was designed to determine if

Table 2 Mean pre- and post-exhaled nitric oxide levels (parts per billion)

	Pre	Post	P value
Levalbuterol	28.5 (± 18.6)	27.4 (± 20.0)	0.581
Albuterol	29.9 (± 23.2)	28.7 (± 21.2)	0.530
Placebo	25.8 (± 18.9)	24.3 (±14.3)	0.609

airway inflammation, as measured by FeNO, was different in patients with COPD treated with albuterol compared with levalbuterol or placebo. We anticipated to measure a rise in FeNO if the (S)-isomer of albuterol was associated with induction of inflammation. In addition, we sought to determine if there were significant differences in racemic albuterol or levalbuterol relative to placebo in terms of improvement in spirometry, functional assessment utilizing a 6MWT, and symptom scores using the SOBQ.

We found no difference within or between treatment groups for measurement of FeNO, spirometry, functional capacity, or symptom scores. However, there was a 6.25 and 4 unit increase in the SOBQ score with levalbuterol and albuterol, respectively, compared to baseline. A five unit increase of the SOBQ score has previously been reported to represent a minimal clinically important difference suggesting levalbuterol may have provided a clinically significant improvement in dyspnea. However, these conclusions are limited by the open-label property of the study.

Of note, the amount of (R)-isomer in 2.5 mg of racemic albuterol is 1.25 mg, which is higher than the equivalent (R)-isomer dose of 0.63 mg used in the levalbuterol arm of the study. This could have limited our ability to find significant differences in the utilized functional secondary outcome measures. However, by using the higher dose of racemic albuterol, we administered a larger amount of (S)-isomer, which should have amplified any (S)-isomer associated difference in attendant airway inflammation, if present. No increase in airway inflammation, as measured by FeNO, was found with this higher dose of albuterol.

Our study had some limitations. Our study subjects' baseline FeNO levels were higher than we initially assumed. This may have limited the sensitivity of the study. In addition, the small sample size may have limited our ability to detect a difference in FeNO levels if one existed. Also, the patients were not blinded to the treatment groups, which may have influenced the reporting of effort or subject-related measures such as walking effort or symptom score reporting. However, this should not have influenced the primary outcome measure of FeNO. A majority of our subjects had relatively mild disease, which may have limited our ability to detect clinically significant differences in the secondary outcomes that were

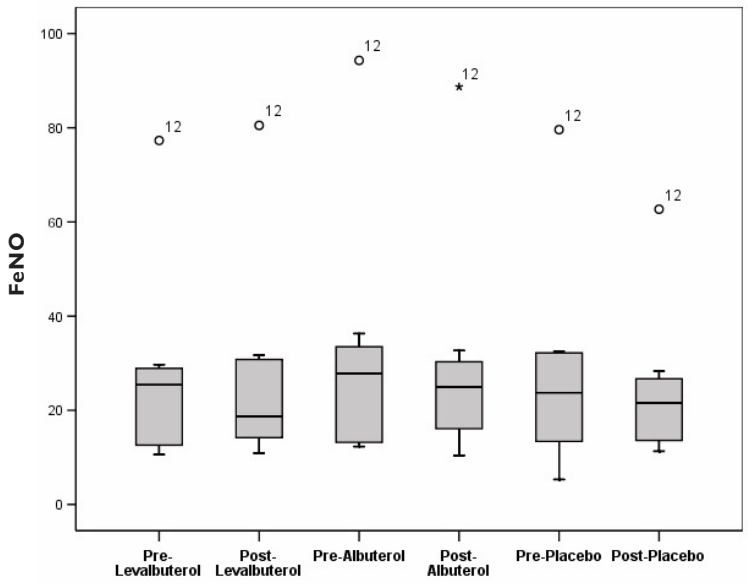

Figure 1 Box-plot of FeNO by treatment and time. The median is the dark line within each box. Each box is defined by the 25th and 75th percentiles so 50% of cases have values within the box. The error flags represent the largest and smallest observed values that are not outliers. No significant differences were noted between groups (p = 0.121).
Notes: °Outliers are values more than 1.5 box-lengths from the quartile; *Extremes are values more than 3 box-lengths from the quartiles.
Abbreviations: FeNO, fraction of exhaled nitric oxide.

Table 3 Secondary outcome measures

	Baseline	Levalbuterol	Albuterol	Placebo	P value
FEV_1 (L/sec)	2.00	1.94	1.96	1.97	0.913
6MWT (meters)	410	411	401	416	0.838
SOBQ	26.63	32.88	30.63	28.25	0.500

Abbreviations: FEV_1, forced expiratory volume in one second; SOBQ, Shortness of Breath Questionnaire; 6MWT, six-minute walk test.

measured. Lastly, the length of time on each therapy may not have been enough to cause a significant change in airway inflammation that could be detected by FeNO.

The prospects for improved therapy in COPD by the use of levalbuterol have been discussed previously (Costello 1999). Despite the theoretical advantages there have been few studies addressing the potential usefulness of levalbuterol in the management of COPD. In a study by Truitt and colleagues (2003), levalbuterol demonstrated a clinical and economic advantage over racemic albuterol in the treatment of hospitalized patients with COPD and asthma, although the significance of this finding has been brought into question (Hendeles 2003). Another study by Datta and colleagues (2003) found no advantage of using levalbuterol over conventional nebulized bronchodilators in single dose for as needed treatment in stable COPD.

In conclusion, our study found no evidence of increased airway inflammation with the use of racemic albuterol compared with levalbuterol as determined by measurement of FeNO. In addition, there were no significant differences between levalbuterol and racemic albuterol in the secondary outcomes that were measured. As a whole, levalbuterol does not appear to be more advantageous than racemic albuterol for the treatment of COPD. Without a clearly derived benefit, the routine use of levalbuterol for the management of COPD is not recommended at this time. Further studies with more subjects and a longer treatment period are needed to confirm these results.

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