Comparison of bronchodilator responses of levosalbutamol and salbutamol given via a pressurized metered dose inhaler: A randomized, double blind, single-dose, crossover study

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KEYWORDS
Asthma; Bronchodilator; Levosalbutamol; pressurized metered dose inhaler; salbutamol

Summary
Background: Salbutamol, the most widely used short-acting β2-agonist, consists of a racemic mixture of equal amounts of two enantiomers, (R)-salbutamol and (S)-salbutamol. The bronchodilator effects of salbutamol are attributed entirely to (R)-salbutamol (levosalbutamol), while (S)-salbutamol has been shown to possess bronchospastic and pro-inflammatory effects both in vitro and in vivo studies. Levosalbutamol, the (R)-enantiomer of salbutamol is currently available only in a liquid formulation for use via a nebulizer. Recently, levosalbutamol to be administered via a pressurized metered dose inhaler (pMDI) has been developed.
Aims: To compare the time-dependent bronchodilator responses of single doses of 100 mcg levosalbutamol and 200 mcg racemic salbutamol administered via a pMDI in subjects with stable mild-to-moderate bronchial asthma over a period of 6 h.
Methods: Single doses of 100 mcg levosalbutamol, 200 mcg salbutamol and placebo were administered with a pMDI in 30 stable asthmatic subjects in a randomized, double-blind, placebo-controlled, three-way cross over study. Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured at baseline, and over 6 h post-study drug administration.

Abbreviations: ANOVA = Analysis of variance; ATS = American Thoracic Society; AUC = Area under curve; CI = Confidence interval; COPD = Chronic obstructive pulmonary disease; FEV₁ = Forced expiratory volume in 1 s; FVC = Forced vital capacity; L = Liters; Min = Minutes; mL = Milliliters; pMDI = pressurized metered dose inhaler
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Introduction

Salbutamol (Albuterol) is the most widely used short-acting β2-agonist in the symptomatic relief of asthma and chronic obstructive pulmonary disease (COPD). In all formulations, salbutamol consists of a racemic mixture of equal amounts (50:50) of (R)- and (S)-isomers. Although these isomers are chemically identical, they differ in conformation, being exact non-superimposable (mirror) images of one another, or stereoisomers. (R)-salbutamol has been shown to have a 2-fold greater binding affinity than racemic salbutamol and a 100-fold greater binding affinity than (S)-salbutamol for the β2-adrenergic receptor. As a result, the bronchodilator property of racemic (R,S)-salbutamol is attributed entirely to (R)-salbutamol. Clinical studies, especially in children have shown that levosalbutamol produces a similar bronchodilator response as racemic salbutamol even when administered at one-half or one-fourth the dose. (S)-salbutamol has no clinically meaningful ability to relax airway smooth muscle. Pre-clinical studies have suggested that (S)-salbutamol might antagonize the smooth muscle relaxing actions of (R)-salbutamol by increasing intracellular Ca2+ levels. (S)-salbutamol given alone has also been reported to enhance airway hyperresponsiveness to spasmodens in vitro, promote eosinophil recruitment and activation, increase the production of histamine, and increase airway smooth muscle contractility. These divergent properties of (R)- and (S)-salbutamol suggest that administration of only (R)-salbutamol would have a better therapeutic index than racemic salbutamol.

Although levosalbutamol [(R)-salbutamol] has been introduced in the management of asthma and COPD since 1999, it is available only in a liquid formulation to be administered by a nebulizer. The pressurized metered dose (pMDI) inhaler is the most widely used device for drug delivery in patients with asthma. It is convenient, cheap, easy to use and effective. Salbutamol administered via pMDI is widely used in the management of symptomatic relief of acute asthma, and when administered via a spacer, is as effective as that administered via a nebulizer in patients with acute severe exacerbations. Recently, levosalbutamol has been developed to be delivered via a pMDI (50 mcg per puff). The aim of this study was to compare the time-dependent bronchodilator effects of single doses of 100 mcg levosalbutamol and 200 mcg racemic salbutamol, both administered by a pMDI in subjects with chronic stable mild-to-moderate asthma.

Study design

Male and female stable asthmatic subjects between the age groups of 18–65 years were recruited into the study. Asthma was defined according to American Thoracic Society (ATS) criteria (history suggestive of asthma, improvement in forced expiratory volume in 1 s (FEV1) of at least 12% and 200 mL with 200 mcg racemic salbutamol). All the study subjects were non-smokers and none of them received oral steroids or had an acute asthma exacerbation 4 weeks prior to the start of the study. Those subjects who were on regular (at least 4 weeks duration) inhaled corticosteroids were allowed to continue with their medication at the same dose throughout the entire duration of the study. All subjects had a baseline FEV1 of at least 60% predicted [calculated as per European Community for Coal and Steel (ECCS) X 0.9] and underwent assessment for the proper use of a pMDI. Pregnant and lactating women were exempted from the study. Peripheral venous blood was analyzed for routine hemogram and biochemistry during the screening visit to rule out other associated disorders. The study was approved by the institutional ethics committee and a written informed consent was obtained from all study subjects.

Single doses of 100 mcg levosalbutamol, 200 mcg racemic salbutamol and placebo (manufactured by Cipla Ltd, India) were administered on three separate study days, at least 3 days apart, in a randomized, double-blinded, crossover manner via a pMDI and a non-static spacer (ZerostatTM spacer, Cipla Ltd., India). The propellant used in this study was CFC (Chlorofluorocarbons) comprising of trichloro-fluoromethane and dichlorofluoromethane. Care was taken to ensure that the subjects had avoided short-acting β2-agonists for at least 8 h and long-acting β2-agonists and oral theophyllines for at least 24 h prior to the start of the study visits. Lung function parameters (FEV1 and forced vital capacity (FVC)) were measured before (baseline values) and 5, 15, 30, 60, 120, 240 and 360 min after the study drug administration using a volume-based bellows Gold Standard Spirometer. The lung function tests were performed by a trained lung function technician using ATS guidelines. The highest FEV1 value at each time point was considered for analysis. The difference of up to 12% in the baseline FEV1 between the two study visits was accepted. Blood was collected for the measurement of serum potassium levels before and 1 h after the administration of the study medications. Pulse rate was measured before, 1 and 6 h after the administration of study drug medications.

Results: Levosalbutamol and salbutamol produced significantly better bronchodilator responses than placebo. Both the drugs showed equivalent time-dependent bronchodilator responses as measured by area under curve for percent change in FEV1, and FVC over 6 h. The time to onset of action, mean maximum bronchodilator response and duration of bronchodilator response were similar between levosalbutamol and salbutamol.

Conclusion: A single dose of 100 mcg levosalbutamol administered by a pMDI produced a similar bronchodilator response as salbutamol when measured over 6 h in subjects with stable, mild-to-moderate bronchial asthma.

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The primary efficacy parameter was the mean difference in area under the curve (AUC) for percent change in FEV1 and FVC from baseline to 6 h. The secondary efficacy parameters were the mean maximum FEV1 and FVC change from baseline, time to onset of response, time to maximum response and duration of response. The time to onset of bronchodilator response was defined as an increase in FEV1 of 12% and 200 mL from the baseline value, while the duration of action was taken as the previous time point when the fall in FEV1 was at least 200 mL from the baseline value. The safety parameters assessed were mean changes in pulse rate and serum potassium levels from baseline values to 1 h after study medications.

Sample size estimation for this study was done using a PS (Power and Sample Size) Software Version 2.1.31 (Vanderbilt, Canada) considering the mean maximum change in FEV1 following salbutamol administration from our previous study. A sample size of 24 was required to detect differences between study medications with 80% power to show equivalence at a significance level of 5%.

Spirometric data (FEV1, FVC) for each study treatment were analyzed using student’s t-test for paired variables. The mean response to all treatments was compared by multi-factorial analyses of variance (ANOVA) to determine the overall effect of interventions. Duncan’s multiple range testing with 95% confidence interval (CI) was used where the differences were significant (P<0.05). The AUC for FEV1 was calculated for each subject during each study treatment using the trapezoidal rule. The mean difference in the AUC for the two treatment groups were analyzed using ANOVA approach for Schuirmann’s two one-sided test of equivalence. Equivalence was accepted if the mean difference with 95% CIs of the study variables were contained within the set limits. An independent statistician who was blinded to the codes for the study medications performed the statistical analysis. The medication codes were broken at the end of the complete analysis.

Results

Forty-two subjects were screened for the study and 30 were randomized (11 subjects did not meet the criteria for either airflow reversibility or percent FEV1 predicted, while one subject withdrew consent). All the subjects completed the study without any significant adverse effects. The mean age of the study population was 44.8 (±11.0) years. The summary of the demographic details is shown in Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>44.8 (11.0)</td>
</tr>
<tr>
<td>Sex</td>
<td>17 males, 13 females</td>
</tr>
<tr>
<td>Mean FEV1 (SD) L</td>
<td>1.70 (0.55)</td>
</tr>
<tr>
<td>Mean FVC (SD) L</td>
<td>2.73 (0.86)</td>
</tr>
<tr>
<td>Mean FEV1/FVC (SD)</td>
<td>70.54 (5.73)</td>
</tr>
<tr>
<td>FEV1 reversibility (SD)</td>
<td>17.61 (4.87)</td>
</tr>
</tbody>
</table>

(Fev1: forced expiratory volume after 1 s, FVC: forced vital capacity, L: litres, so: standard deviation).

Table 1 Demographic details of study subjects.

The mean response to all treatments was compared by multi-factorial analyses of variance (ANOVA) to determine the overall effect of interventions. Duncan’s multiple range testing with 95% confidence interval (CI) was used where the differences were significant (P<0.05). The AUC for FEV1 was calculated for each subject during each study treatment using the trapezoidal rule. The mean difference in the AUC for the two treatment groups were analyzed using ANOVA approach for Schuirmann’s two one-sided test of equivalence. Equivalence was accepted if the mean difference with 95% CIs of the study variables were contained within the set limits. An independent statistician who was blinded to the codes for the study medications performed the statistical analysis. The medication codes were broken at the end of the complete analysis.

![Figure 1](image_url) Mean percentage change in FEV1 values over a period of 6 h following single dose administration of levosalbutamol, racemic salbutamol and placebo.

AUC0–6h: 3814.8 versus 3463.2 for levosalbutamol and salbutamol, respectively; (P>0.05)] (Fig. 1 showing FEV1% change), suggesting that 100 mcg levosalbutamol and 200 mcg racemic salbutamol produced equivalent bronchodilator responses over 6 h.

The maximum difference for FEV1 from baseline was 537 mL with levosalbutamol and 538 mL with racemic salbutamol, while the mean maximum difference for FVC was 456 and 417 mL, respectively (Fig. 2). These differences were not statistically significant, suggesting that 100 mcg levosalbutamol and 200 mcg racemic salbutamol produced an equivalent mean maximum increase in FEV1 and FVC.

Levosalbutamol took 58.3 and 52.6 min to reach the maximum FEV1 and FVC response, while racemic salbutamol took 67.5 and 64.3 min, respectively. These differences were not statistically significant when compared to each other (P = 0.94). The time to onset for the bronchodilator response (increase of at least 200 mL and 12% in FEV1 from baseline) for levosalbutamol was 12 min, while that for racemic salbutamol was 18 min and the differences between the two were not significant. The mean duration of response (difference between onset and termination of effect) for levosalbutamol was 208 min and for racemic salbutamol was 212 min. This difference was not statistically significant (P = 0.75), suggesting that there was no
equally, when measured 1 h after administration. Increased pulse rate and reduced serum potassium levels between the two study medications. Both the drug responses and the duration of responses were similar and FVC, time to reach the mean maximum FEV1 and FVC pMDI produced a similar time-dependent bronchodilator single dose of 100 mcg of levosalbutamol administered via a pMDI. In this randomized, double-blind, placebo-controlled, cross-over study, we have demonstrated for the first time that a single dose of 100 mcg of levosalbutamol administered via a pMDI produced a similar time-dependent bronchodilator response as that of 200 mcg racemic salbutamol in subjects with mild-to-moderate stable asthma. The time to onset of bronchodilator response, mean maximum change in FEV1 and FVC, time to reach the mean maximum FEV1 and FVC responses and the duration of responses were similar between the two study medications. Both the drugs increased pulse rate and reduced serum potassium levels equally, when measured 1 h after administration.

Salbutamol is the most widely used inhaled short-acting \(\beta_2\)-agonist in the symptomatic relief of asthma and COPD. It consists of a racemic mixture of equal amounts of (R)- and (S)-enantiomers. These enantiomers have similar physical and chemical properties, but have different receptor specificity and therefore have different pharmacological and physiological effects. The bronchodilator properties of racemic salbutamol (R,S) have been shown to be attributed entirely to the (R)-enantiomer. Human epinephrine produced by the adrenal gland occurs only in the (R)-isomer form. Clinical studies have demonstrated that the bronchodilator and bronchoprotective effects of racemic salbutamol in subjects with asthma lie entirely with the (R)-isomer, with the (S)-isomer being inert. Moreover, in vitro cellular data have implicated (S)-salbutamol as a possible cause of airway hyperreactivity, bronchoconstriction or inflammation,\(^6\,19\) perhaps induced by stimulating intracellular calcium accumulation and inhibiting adenyl cyclase.\(^5\,6\,19\,20\) These pre-clinical findings have been confirmed in some clinical studies that show a greater bronchodilation with levosalbutamol compared to salbutamol when given either as a long-term therapy\(^6\) (4 weeks) or when given in a single dose.\(^19\) This leads to an increase in the therapeutic index of levosalbutamol when compared to racemic salbutamol. These divergent pharmacologic properties form the scientific rationale for the potential advantages of levosalbutamol over racemic salbutamol in the treatment of asthma and other airway disorders.

### Discussion

In this randomized, double-blind, placebo-controlled, cross-over study, we have demonstrated for the first time that a single dose of 100 mcg of levosalbutamol administered via a pMDI produced a significant decrease in serum potassium level 1 h after study drug administration compared to their respective baseline values, (4.61–4.25; \(P<0.001\) and 4.39–4.13; \(P=0.004\), respectively). The placebo treatment did not show any significant decline in serum potassium levels (4.47–4.37; \(P=0.19\)). Racemic salbutamol and levosalbutamol 60 min post-drug administration both produced a significant increase in pulse rate versus their baseline, (73.9–77.8 beats/min; \(P<0.05\) and 72.4–77.3 beats/min; \(P<0.05\), respectively). There was no significant difference in the pulse rate 6 h post-drug administration compared to baseline values (data not shown).

Our study demonstrates that 100 mcg levosalbutamol administered in a pMDI formulation is as effective as 200 mcg racemic salbutamol, when measured by percentage changes in FEV1 and FVC AUCs from baseline to 6 h, time to onset of bronchodilator response, mean maximum change in FEV1 and FVC and duration of the bronchodilator effect. The results of this study confirm earlier reports that the bronchodilator effects of salbutamol are entirely attributable to its (R)-enantiomer. Although earlier studies\(^19\,20\) had suggested that levosalbutamol might show lesser clinical side effects than the racemic form, we did not find any difference in pulse rates and decreases in serum potassium levels between levosalbutamol and racemic salbutamol. These observations are in accordance with Lotvall et al.\(^5\) who have demonstrated that the adverse effects of racemic salbutamol on heart rate and potassium levels are mediated mainly by the (R)-enantiomer. Cockcroft et al. have reported that nebulized levosalbutamol produced similar effects on heart rate as racemic salbutamol, suggesting that tachycardia caused by salbutamol is attributed to (R)-salbutamol. Pauw et al.\(^21\) have reported that (R)-salbutamol and racemic salbutamol are equally effective in lowering serum potassium levels. This study has therefore shown that the bronchodilator effect and systemic side effects of salbutamol reside with the (R)-enantiomer. We did not assess tremors. Since we used single doses of 100 mcg levosalbutamol and 200 mcg of racemic salbutamol we did not expect to find significant numbers of patients experiencing tremors at this small dose. Also tremor assessment is a subjective observation, which has its own drawbacks.

![Figure 2](image-url)
Bronchodilator effect of pMDI levosalbutamol

In summary, a single dose of levosalbutamol administered in a pMDI formulation produced an equivalent time-dependent bronchodilator response over 6 h as racemic salbutamol at half the dose. Thus, levosalbutamol administered in a pMDI formulation may be used routinely for acute symptomatic relief in subjects with asthma. Further studies involving larger numbers of patients and for longer duration are required to determine long-term safety and efficacy of levosalbutamol administered via a pMDI.

References