Original articles

Effect of single doses of S-salbutamol, R-salbutamol, racemic salbutamol, and placebo on the airway response to methacholine

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Abstract

Background – Commercially available salbutamol is a racemic mixture consisting of equal amounts of the two enantiomers, R-salbutamol and S-salbutamol, felt to be active and inert, respectively.

Methods – A double blind, randomised, four way, crossover study was performed in 12 well controlled asthmatic subjects (forced expiratory volume in one second (FEV₁) >70% predicted, no β_2 agonists for ≥ 4 weeks). Subjects were studied on four days at intervals of 48 hours to seven days. FEV₁ was assessed before and both FEV₁ and methacholine PC₂₀ were measured 20 and 180 minutes after a single dose of nebulised racemic salbutamol 2.5 mg, R-salbutamol 1.25 mg, S-salbutamol 1.25 mg, and placebo.

Results – Equivalent bronchodilation was seen for both R-salbutamol and racemic salbutamol (mean (SE) 12.4 (3.1)% and 12.0 (3.0)%, respectively, at 20 minutes and 5.9 (2.9)% and 5.2 (2.2)% at 180 minutes). The increase in FEV₁ of 5.2 (0.9)% at 20 minutes and the decline in FEV₁ of 2.9 (2.1)% at 180 minutes after S-salbutamol were not significantly different from the placebo response. Compared with placebo the methacholine PC₂₀ after R-salbutamol and racemic salbutamol improved by 3.3 (95% CI 2.5 to 4.1) and 3.4 (95% CI 2.6 to 4.2) doubling doses, respectively, at 20 minutes and 1.2 (95% CI 0.6 to 1.8) and 1.0 (95% CI 0.2 to 1.8) doubling doses at 180 minutes. S-salbutamol resulted in an improvement of 0.9 (95% CI 0.3 to 1.5) doubling doses at 20 minutes and no

change at 180 minutes. Restlessness (n = 11) and increased pulse were seen 20 minutes after racemic and R-salbutamol but not S-salbutamol or placebo, and not at 180 minutes. There were no other adverse events.

Conclusion – A single dose of 1.25 mg nebulised R-salbutamol produced equivalent bronchoprotection, bronchodilation, restlessness, and tachycardia as did 2.5 mg of racemic salbutamol. S-salbutamol 1.25 mg had a weak bronchoprotective effect; this could be because of a small amount of contamination with R-salbutamol or because S-salbutamol is an intrinsically weak β_2 receptor stimulant.

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Keywords: salbutamol, enantiomers, bronchodilation, bronchoprotection.

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Table 1 Characteristics of patients studied

Subject no.	Age	Sex	Height (cm)	Weight (kg)	FEV ₁		Methacholine PC ₂₀ (mg/ml)
					(l)	% Pred	
1	25	F	160	52	2.56	85	0.56
2	24	M	190	84	5.10	96	5.9
3	22	M	174	70	3.73	87	2.2
4	22	F	152	54	2.51	88	0.95
5	22	F	160	48	2.46	80	0.13
6	24	M	172	72	3.55	83	0.46
7	22	M	182	70	4.31	91	0.69
8	23	M	178	71	3.96	88	0.39
9	22	M	178	70	4.12	93	5.4
10	25	F	163	52	3.07	98	1.5
11	23	F	171	68	3.61	103	3.6
12	29	F	157	55	2.60	91	0.75

Salbutamol in clinical use, like many other medications, is a racemic mixture of equal parts of two enantiomers termed R and S.¹ R-salbutamol is the active β_2 receptor stimulant¹ and S-salbutamol has been presumed to be inert. Regular use of inhaled β_2 agonists may adversely influence asthma.² Two direct adverse effects have been identified – namely, tolerance, particularly to the bronchoprotective effect of these agents,³ and an increase in airway responsiveness to allergen.^{4,5} It has been suggested that the S enantiomer may be responsible for one or both of these effects, most probably the allergen effect.¹ Prior to embarking upon studies to assess the effects of regular treatment with S-salbutamol and R-salbutamol, we undertook a double blind, four way, crossover study to compare single doses of nebulised S-salbutamol, R-salbutamol, racemic salbutamol, and placebo and their effect on forced expiratory volume in one second (FEV₁) and airway responsiveness to methacholine in 12 asthmatic subjects.

Methods

SUBJECTS

Twelve well controlled asthmatic subjects were selected from volunteers (table 1). Subjects were studied at a time when their asthma was well controlled and they were able to remain off inhaled β_2 agonists during the study and for

Table 2 Mean (SE) pulse rate, blood pressure and restlessness following the four treatments

	RS-salbutamol	R-salbutamol	S-salbutamol	Placebo	p value
Pulse					
Baseline	72.6 (2.8)	71.3 (2.7)	72.5 (2.4)	73.7 (3.0)	<0.0001
20 min	84.0 (3.2)	84.1 (2.5)	71.1 (2.7)	74.4 (2.8)	
180 min	76.6 (2.7)	75.4 (2.8)	72.3 (2.6)	71.8 (2.7)	
Systolic BP					
Baseline	110 (2.0)	108 (2.9)	110 (2.4)	106 (3.2)	NS
20 min	115 (2.1)	110 (2.7)	105 (2.3)	103 (2.3)	
180 min	107 (1.8)	109 (2.8)	107 (2.5)	105 (2.7)	
Diastolic BP					
Baseline	68 (1.7)	70 (2.6)	70 (1.6)	68 (2.8)	NS
20 min	64 (2.2)	63 (3.8)	69 (2.8)	67 (2.2)	
180 min	67 (2.9)	66 (3.2)	67 (2.4)	70 (2.6)	
Restlessness					
Baseline	n=0	n=0	n=0	n=0	<0.01
20 min	n=11	n=11	n=0	n=1	
180 min	n=2	n=3	n=0	n=1	
FEV ₁ (% Δ)					
Baseline					<0.0001
20 min	12.4 (3.0)	12.0 (3.1)	5.2 (0.9)	1.2 (1.4)	
180 min	5.7 (2.9)	5.2 (2.2)	-2.9 (2.1)	0.9 (2.4)	

four weeks beforehand. FEV₁ was $\geq 70\%$ of expected. There was no exposure to allergens or respiratory tract infections for at least four weeks before and during the study. Inhaled corticosteroids were permitted at a stable dose but were used by only one subject. The study was approved by the University of Saskatchewan ethics committee and written informed consent was obtained.

METHACHOLINE CHALLENGE

Methacholine inhalation tests were carried out by our standardised technique reported elsewhere.^{6,7} Aerosols were generated with a Bennett twin jet nebuliser calibrated to deliver an output of 0.13 ml/min. Aerosols were delivered to the patient via a loose-fitting face mask and inhaled by tidal breathing with the nose clipped. Subjects initially performed FEV₁ manoeuvres in triplicate. Isotonic sterile saline, the diluent, was then inhaled for two minutes and the FEV₁ repeated 30 and 90 seconds later. Following this, doubling concentrations of methacholine from 0.03 to 64.0 mg/ml were inhaled for similar two minute tidal breathing periods at five minute intervals. The FEV₁ was repeated 30 and 90 seconds after each inhalation and the percentage fall calculated from the lowest post-saline to the lowest post-methacholine FEV₁. The study was continued until there was an 18% or greater fall in FEV₁ and the PC₂₀ was extrapolated or interpolated from the log dose-response curve as previously reported.⁸ Starting concentrations for methacholine challenges were not always 0.3 mg/ml but, rather, were based on published guidelines⁷ and our previous knowledge of the subject; however, for all methacholine challenges in a given individual the same starting concentration and dosage regimen were always used.

STUDY DESIGN

Subjects attended the laboratory on five occasions. On the first occasion spirometric parameters were measured and a baseline

methacholine PC₂₀ value obtained. The four subsequent visits were made with a minimum interval of two days and a maximal interval of seven days at the same time of day. Nebulised medications were administered by a Perry LC jet nebuliser in a double blind, randomised fashion. The medications were racemic salbutamol 2.5 mg in 3 ml, S-salbutamol 1.25 mg in 3 ml, R-salbutamol 1.25 mg in 3 ml, and placebo 3 ml. Nebulisation time was approximately five minutes. Spirometric parameters were measured in triplicate prior to the nebulisation and 20 and 180 minutes afterwards. Pulse, blood pressure, respiratory rate, and temperature were measured on the three occasions. Subjects were asked to assess their degree of "restlessness" as none, mild, moderate or severe (scored 0, 1, 2, 3 respectively) at the same three times. Two methacholine PC₂₀ values were measured, one starting at 20 minutes and the other at 180 minutes after completion of the nebulisation. No bronchodilator was administered after the first methacholine challenge; the FEV₁ was allowed to recover spontaneously between the completion of the first methacholine challenge and the measurement of spirometric parameters at 180 minutes. A single dose of ipratropium bromide was occasionally administered following the second methacholine challenge. Subjects refrained from the use of inhaled β_2 agonists throughout the trial but had access to ipratropium bromide to use as needed for relief of symptoms; this was withheld for at least eight hours before all testing.

ANALYSIS OF DATA

The primary end point was the log₁₀ methacholine PC₂₀ for the four treatments at the two time periods. Δ FEV₁ (% change from time 0 to both 20 and 180 minutes) was a secondary end point. These were analysed by a computerised ANOVA. Vital signs and restlessness (present or absent) were also compared at the two times.

Results

All subjects completed the study without any significant adverse events. Changes in pulse rate and blood pressure are shown in table 2. There was a significant increase in the pulse rate from a mean (SE) value of 72.6 (2.8) to 84.0 (3.2) beats/min ($p < 0.0001$) 20 minutes after racemic salbutamol and from 71.3 (2.7) to 84.1 (2.5) beats/min ($p < 0.0001$) 20 minutes after R-salbutamol; there was no significant difference in the pulse rate at 180 minutes ($p = 0.08$). There was no change in blood pressure (table 2), respiratory rate, or temperature. Restlessness was reported by 11 subjects 20 minutes after both R-salbutamol and racemic salbutamol but not after S-salbutamol ($n = 0$) or placebo ($n = 1$) (table 2; $p < 0.01$). The restlessness was frequently graded as severe; the average restlessness score at 20 minutes was 1.75 after R-salbutamol and 1.58 after racemic salbutamol compared with 0.17 after placebo and 0 after S-salbutamol.

Figure 1 Mean (SE) forced expiratory volume in one second (FEV_1) at time 0, 20 minutes, and 180 minutes for the four treatments.

Figure 2 Geometric mean (SE) methacholine PC_{20} at 20 and 180 minutes. The geometric mean methacholine PC_{20} plotted at time 0 is the screening value (and not a repeated true "time 0" measurement).

The FEV_1 data are summarised in table 2 and fig 1. The baseline FEV_1 was similar on the four days. There was a significant treatment effect ($p=0.0001$) on the ΔFEV_1 at 20 minutes, the mean percentage improvements after racemic salbutamol (12.4 (3.0)%) and R-salbutamol (12.0 (3.1)%) being greater than after S-salbutamol or placebo. The improvement in FEV_1 of 5.2 (0.9)% 20 minutes after S-salbutamol was not significantly different from placebo ($p=0.10$). The FEV_1 at 180 minutes also showed a significant treatment effect ($p=0.021$); pairwise comparisons showed a significant difference for % ΔFEV_1 between S-salbutamol (-2.9 (2.1)%) and both racemic (5.7 (2.9)%) and R-salbutamol (5.2 (2.2)%), but none was significantly different from placebo. The absolute values for mean (SE) FEV_1 are shown in fig 1. There was no treatment sequence effect ($p>0.05$).

There was a significant treatment effect on log methacholine PC_{20} at both time periods ($p<0.0001$; fig 2). At 20 minutes the $\log_{10} PC_{20}$ had increased from 0.03 (0.14) to 1.12 (0.16) after racemic salbutamol and 1.10 (0.16) after R-salbutamol - 3.4 (95% CI 2.6 to 4.2) and

3.3 (95% CI 2.5 to 4.1) doubling doses above placebo (0.1 (0.14)), respectively. At 20 minutes after S-salbutamol the $\log_{10} PC_{20}$ was 0.36 (0.15) (0.9 (95% CI 0.3 to 1.5) doubling dose above placebo); this was significantly lower than $\log_{10} PC_{20}$ following racemic salbutamol and R-salbutamol and significantly larger than $\log PC_{20}$ following placebo ($p=0.0049$). At 180 minutes there was still a significant treatment effect with $\log_{10} PC_{20}$ after R-salbutamol (0.59 (0.20)) and racemic salbutamol (0.52 (0.19)) which was significantly higher than following S-salbutamol (0.20 (0.19)) or placebo (0.23 (0.17)). These represent an increase of 1.2 (95% CI 0.6 to 1.8) and 1.0 (95% CI 0.2 to 1.8) doubling doses from placebo for R-salbutamol and racemic salbutamol, respectively. There was no significant difference at 180 minutes between $\log_{10} PC_{20}$ after S-salbutamol and after placebo. There was no treatment sequence effect ($p>0.05$).

Discussion

Nebulised racemic salbutamol (2.5 mg) and R-salbutamol (1.25 mg) provided significant and similar bronchoprotection against methacholine at both 20 minutes and three hours compared with placebo and S-salbutamol (1.25 mg). The S-salbutamol provided slight bronchoprotection at 20 minutes but none at three hours. The study was not designed with bronchodilation as a major end point. However, there was significant bronchodilation at 20 minutes following racemic and R-salbutamol; at three hours the improved FEV_1 following racemic and R-salbutamol was significantly greater than the FEV_1 following S-salbutamol. Restlessness or shakiness and tachycardia were both seen with racemic and R-salbutamol but not with S-salbutamol.

These data confirm that the R enantiomer is the active bronchodilating and broncho-protecting molecule in humans. The R enantiomer also causes the tremor and tachycardia. There was a weak, borderline, and short-lived bronchoprotecting effect of S-salbutamol. At this point, considering that we used a large single dose of nebulised medication, the mechanism of action of S-salbutamol is not clear. It is possible that the small effect seen after this large single dose of S-salbutamol could be either due to a weak intrinsic effect on, or a low affinity for, the β_2 receptor or due to the small (up to 1%) contamination with R-salbutamol.

Perrin-Fayolle recently reported a similar study;⁹ however, differences in study design make comparison with our data difficult. Forty subjects completed a double blind parallel study with 10 subjects in each treatment arm and the doses used were smaller (an estimated 200 μ g racemic salbutamol and 100 μ g of the individual enantiomers). The results were analysed arithmetically rather than geometrically. A significant increase in methacholine PC_{20} was seen 20 minutes after racemic salbutamol and R-salbutamol but not at 180 minutes. The PC_{20} after S-salbutamol at 20 minutes was not significantly different from that following

placebo. However, at 180 minutes the PC₂₀ after S-salbutamol was significantly lower than the PC₂₀ after placebo, but apparently was not significantly lower than baseline PC₂₀ in the S group.

We believe the crossover design used in this study has advantages for several reasons. Previous studies in our laboratory have shown a wide range of acute shift of methacholine PC₂₀ following a single dose of racemic salbutamol (or salmeterol). This inter-individual variability can range from a low of about one to a high of more than five doubling dose improvement. There is also a concern regarding the performance of two methacholine tests within less than three hours. There are two opposing possibilities. The first is that some subjects may demonstrate a tolerance to the effect of methacholine and show an increased PC₂₀ on the second test; this is known to occur in normal subjects and very mild asthmatic patients both with regard to histamine and methacholine.¹⁰ By contrast, there is also the possibility of a cumulative effect of methacholine leading to the opposite result. This is also an area where one might expect some inter-subject variability. We feel that a crossover design offers the advantage of removing these two important areas of inter-subject variability. In addition, since airway responsiveness data are probably log-normally distributed,^{11,12} we feel it important to log transform the data prior to analysis.

Our study employed larger doses. There was a non-significant reduction in FEV₁ at three hours following S-salbutamol while PC₂₀ was virtually identical at three hours after S-salbutamol and placebo. It is possible that we missed a significant adverse effect of S-salbutamol by not following our subjects for a longer time after these larger doses. However, one could argue that, with the larger dose of S-salbutamol used (1250 µg nebulised, approximately 400 µg inhaled, compared with 100 µg in the Perrin-Fayolle study), any adverse effect of S-salbutamol might have been more rather than less evident. In addition, there were no delayed adverse effects reported by our subjects.

Since the use of inhaled β₂ agonists can cause tolerance to their protective effect,³ we felt it important to study this in subjects whose asthma was mild and/or well controlled enough so that they could remain off inhaled β₂ agonists before and throughout the study. This is an ideal population in which to look at broncho-

protection, our primary end point, but is not an ideal population in which to assess bronchodilation. Hence, changes in FEV₁ were a secondary end point. The study has <80% power to detect a Δ FEV₁ of <5% such as that seen 180 minutes after S-salbutamol. This also might not be the ideal population in which to detect an adverse effect of S-salbutamol. However, the data show that S-salbutamol had no adverse effects in these well controlled asthmatic subjects and provide some reassurance prior to embarking upon multiple dose studies.

In conclusion, we have shown that the R enantiomer of salbutamol causes the bronchodilation, bronchoprotection, restlessness, and tachycardia seen following racemic salbutamol. In our crossover study the large dose of S-salbutamol had a minor bronchoprotective effect at 20 minutes but was not significantly different from placebo at three hours. We are more concerned with the effect of regular dosing with racemic salbutamol and the possibility that regular dosing with S-salbutamol might have adverse effects, particularly on the airway response to allergen. These data provide a good background for commencing multiple dosing studies.

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