

Dose equivalence and bronchoprotective effects of salmeterol and salbutamol in asthma

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Abstract

Background – Salbutamol is the most widely prescribed short acting β_2 agonist and salmeterol is the first long acting inhaled β_2 agonist. The dose equivalence of salmeterol and salbutamol is disputed. Estimates of weight-for-weight dose ratio have ranged from 1:2 to 1:16. A study was undertaken to clarify the true dose ratio.

Methods – The bronchoprotection afforded against repeated methacholine challenge by inhaled salmeterol 25 μg and 100 μg and salbutamol 100 μg and 400 μg was compared in a randomised, double blind, placebo controlled, crossover trial. Subjects were 16 stable asthmatics with a baseline forced expiratory volume in one second (FEV_1) of $\geq 65\%$ predicted, screening concentration provoking a fall in FEV_1 of 20% ($\text{PC}_{20}\text{FEV}_1$) of $\leq 8 \text{ mg/ml}$, and a shift in $\text{PC}_{20}\text{FEV}_1$ of more than two doubling concentration steps following inhalation of salbutamol 400 μg . On five separate occasions subjects underwent methacholine challenge before and 30 and 120 minutes after drug administration. $\text{PD}_{20}\text{FEV}_1$ was calculated for each challenge. FEV_1 at 90 minutes after drug administration was also recorded.

Results – Bronchoprotection afforded by salmeterol was increased at 120 minutes compared with 30 minutes and protection by salbutamol was decreased. Protection by both doses of salmeterol was similar to salbutamol 100 μg at 30 minutes but significantly greater at 120 minutes. FEV_1 at 90 minutes was significantly greater after salmeterol 100 μg than after placebo, but there were no other significant differences between treatments. Maximal observed protection was equivalent for salmeterol 100 μg and salbutamol 400 μg . **Conclusions** – The data are compatible with a weight-for-weight dose ratio for salmeterol:salbutamol of $\leq 1:4$.

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Keywords: salmeterol, salbutamol, dose equivalence.

Inhaled β_2 adrenoceptor agonists are the most effective bronchodilators and are widely utilised for the relief and prophylaxis of bronchoconstriction in asthma. Until recently all available β_2 agonists had short durations of action. Salmeterol xinafoate is a recently introduced long acting inhaled selective β_2 adrenoceptor agonist which produces dose-dependent

bronchodilatation,¹ reduction in diurnal variation in peak expiratory flow, improvement in daytime and nocturnal symptoms, reduction in requirement for a short acting bronchodilator,^{2,3} and increased quality of life⁴ in asthmatic patients. Bronchodilatation^{5,6} and protection against non-specific bronchial challenge with histamine⁷ or methacholine⁸ are maintained for at least 12 hours after a single dose of salmeterol compared with 4-6 hours after salbutamol.

Salmeterol and salbutamol have similar β_2 receptor selectivity⁹ but dose equivalence is disputed, estimates of the weight-for-weight dose ratio ranging from 1:2 to 1:16 in single dose studies.^{5,7,10,11} In the light of continuing debate regarding a possible association between the use of β_2 agonists and increasing asthma morbidity and mortality worldwide and concern about relative potencies of different agents,¹² it is important that the relative potencies of these two drugs be defined.

Increased responsiveness to non-specific bronchoconstrictor agents is a characteristic feature of clinical asthma¹³ and inhaled histamine or methacholine bronchoprovocation tests are commonly used in diagnosis in patients who present with vague or atypical symptoms.¹⁴ The aim of this placebo controlled study was to compare the potency of salmeterol and salbutamol in asthmatic subjects by measurement of the protection afforded by salmeterol 25 μg and 100 μg and salbutamol 100 μg and 400 μg against repeated methacholine challenge. The design is a model for multidose comparison of inhaled bronchodilator drugs.

Methods

SUBJECTS

Sixteen stable mild asthmatic subjects (seven men) of mean age 29 (range 22-42) years were studied (table 1). Three were smokers and one was an ex-smoker. Subjects were either members of hospital or medical school staff or patients recruited from outpatient clinics. All had a diagnosis of asthma as defined according to American Thoracic Society criteria.¹⁵ Mean time (range) since diagnosis was 17 (1-38) years and mean (SD) baseline forced expiratory volume in one second (FEV_1) was 101.2 (14.5)% predicted. Nine subjects used inhaled salbutamol as required, one inhaled terbutaline as required, and three inhaled salbutamol as required plus an inhaled glucocorticosteroid regularly. Three subjects were not currently taking any asthma medication. No subject was using salmeterol prior to the study. No subject had had a recent respiratory tract infection nor

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Table 1 Characteristics of subjects

Subject	Sex	Age (years)	Height (cm)	Baseline FEV ₁ (% predicted)	Screening PD ₂₀ (μmol)	Asthma history (years)	Smoking status	Asthma medication
1	F	34	166	90.9	0.68	33	N	SALB, BDP
2	F	24	168	107.3	0.96	2	S	SALB
3	F	22	168	102.9	1.05	8	S	SALB
4	F	26	159	87.9	1.26	1	N	SALB
5	F	23	165	119.6	2.87	10	N	TERB
6	F	31	166	123.3	0.20	5	N	SALB
7	M	25	181	116.1	1.24	15	N	None
8	M	29	185	91.0	0.65	26	N	SALB, BUD
9	F	34	174	93.6	0.22	32	X	SALB
10	M	32	181	80.0	2.80	10	N	SALB
11	M	23	179	94.6	0.99	22	N	SALB
12	M	32	188	117.7	1.75	20	S	None
13	M	37	174	111.0	0.16	30	N	SALB
14	F	21	161	96.6	1.59	8	N	SALB, BDP
15	F	30	154	110.6	0.20	8	N	SALB
16	M	42	178	75.9	0.10	38	N	None

N = non-smoker; S = smoker; X = ex-smoker; SALB = salbutamol; TERB = terbutaline; BDP = beclomethasone 500 μg bd; BUD = budesonide 200 μg bd.

an exacerbation of asthma in the previous four weeks. None had been admitted to hospital for an exacerbation of asthma in the previous 12 months. The study was approved by the Royal Postgraduate Medical School and Hammersmith Hospital Research ethics committee and written informed consent was obtained from each subject prior to entry into the study.

STUDY DESIGN

The study was of a randomised, crossover, double blind, double dummy design. Subjects attended the laboratory for a screening visit and five study visits at the same time of day, with a washout period of at least three days between each attendance. Short acting β₂ agonists were discontinued for at least six hours before each attendance. Subjects were also asked to avoid tea and coffee for the same period because of the possible bronchodilator effects of caffeine.¹⁶ At screening a medical history was taken and methacholine challenge was performed. Subjects participated in the study if they had a provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀FEV₁) of ≤8 mg/ml and a shift in PC₂₀FEV₁ of more than two doubling concentration steps 30 minutes after inhalation of salbutamol 400 μg, while remaining ≤64 mg/ml. Baseline FEV₁ at each study visit was required to be ≥65% of the predicted value and to not deviate by more than 15% from the screening value. Baseline PC₂₀FEV₁ at each visit was required to be within two concentration steps lower or one higher than the screening value, and ≤8 mg/ml. If these criteria were not met an appointment was made for reattendance on another day.

On each study day baseline bronchial challenge with methacholine was performed. One hour after commencing the challenge subjects inhaled the study drug (salmeterol 25 μg or 100 μg, salbutamol 100 μg or 400 μg, or placebo). This was administered in a randomised, double blind, double dummy fashion. Further methacholine challenges were performed 30 and 120 minutes following drug administration. In addition, FEV₁ was measured 90 minutes after dosing. At each visit subjects inhaled salbutamol 400 μg im-

mediately after the final challenge to reverse bronchoconstriction, and were allowed to leave when their FEV₁ had returned to at least 80% of its baseline value.

SPIROMETRY, DRUG DELIVERY AND METHACHOLINE CHALLENGE

Spirometric measurements were made using a dry wedge bellows spirometer (Vitalograph, Vitalograph Ltd, Buckingham, UK) and performed according to American Thoracic Society guidelines.¹⁷ The highest of a minimum of three technically acceptable measurements from a maximum of eight attempts was recorded. The two largest values from each set of measurements were required to be reproducible to within 5%.

Study medication (supplied by Glaxo Group Research, Greenford, Middlesex, UK) was delivered from blinded metered dose inhalers via a large volume spacer (Volumatic, Allen & Hanburys Ltd, Uxbridge, Middlesex, UK). Subjects wore nose clips and were instructed to breathe in and out through the valve in the spacer five times to maximise drug delivery.

Methacholine responsiveness was assessed by a standardised challenge technique similar to that previously described.¹⁸ Methacholine (Sigma, Poole, UK) aerosol was delivered via Mefar dosimeter (Brescia, Italy) driven by compressed air at a pressure of 1.5 kg/m² with a one second actuation time and five seconds breath-hold. Output of the dosimeter was 11 μl per puff. At each challenge subjects inhaled five puffs of diluent (isotonic saline) followed by five puffs of doubling concentrations of methacholine solution (minimum 0.0625 mg/ml, maximum 128 mg/ml) until a 20% reduction in FEV₁ occurred or the highest concentration was administered. Measurements of FEV₁ were made 90 seconds after aerosol inhalation and subsequent doses of methacholine were given at three minute intervals. In order to abbreviate the challenge procedure, and since the algorithm used to calculate the provocative dose causing a 20% fall in FEV₁ (PD₂₀FEV₁) utilises only the concentrations of the two highest doses given,¹⁹ study challenges were started at methacholine concentrations three dose steps lower than that achieved at

Table 2 Mean (SD) FEV₁ in litres at baseline and 30, 90 and 120 minutes after drug administration.

	Baseline	30 min	90 min	120 min
Placebo	3.73(0.88)	3.47(0.89)	3.14(0.80)	3.40(0.82)
Salbutamol 100 µg	3.65(0.90)	3.81(0.98)	3.34(0.74)	3.60(0.82)
Salbutamol 400 µg	3.71(0.83)	3.94(0.93)	3.33(0.74)	3.63(0.81)
Salmeterol 25 µg	3.74(0.88)	3.71(0.94)	3.30(0.76)	3.62(0.79)
Salmeterol 100 µg	3.68(0.84)	3.83(0.98)	3.46(0.75)	3.76(0.87)

screening or at 0.0625 mg/ml, whichever was the higher. As a safety precaution, salbutamol by nebuliser was always immediately available for rapid reversal of bronchoconstriction if required.

DATA ANALYSIS

Means were adjusted for baseline patient and treatment. PD₂₀FEV₁ values for each challenge were obtained by linear interpolation from the cumulative log dose-response curve for that challenge.¹⁹ For PC₂₀FEV₁ values greater than 128 mg/ml a value of 130 mg/ml was assigned. All the data were then logged and analysed parametrically. The pre-dosing PD₂₀FEV₁ and the nature of the crossover design were taken into account in the analysis. Carry over, period and sequence effects were also investigated. The slopes of the FEV₁ changes measured during each challenge were analysed parametrically.

Results

Methacholine challenges were generally well tolerated, although subject no. 6 became uncomfortable and required 2.5 mg nebulised salbutamol for reversal of bronchoconstriction after the 120 minute challenge following salmeterol 25 µg. Adverse events considered to be related to study medication were tremor and headache in two different subjects, both after salbutamol 400 µg. These side effects are consistent with the known pharmacology of β₂ adrenoceptor agonists. Four subjects were required to attend extra study visits because of

changes in baseline methacholine responsiveness – two subjects on one occasion, one on two occasions, and one on four occasions.

FEV₁

The effects of the different treatments on FEV₁ are shown in table 2. Tests for carry over and two-factor interactions with treatment were non-significant.

At 30 minutes after medication, prior to second bronchial challenge, mean FEV₁ was reduced after placebo (due to persistent bronchoconstriction following baseline methacholine challenge and possible diurnal variation) but increased after salmeterol 100 µg, salbutamol 100 µg, and salbutamol 400 µg. The absolute change in FEV₁ from baseline was significant in favour of all active treatments when compared with placebo (p ≤ 0.001). The increase in FEV₁ after salbutamol 400 µg was significantly greater than after salmeterol 25 µg (absolute difference = 0.24 l (95% CI 0.10 to 0.38), p = 0.001). There were no other significant differences between treatments.

The lowest FEV₁ achieved during the 30 minute methacholine challenge did not differ significantly on any of the study days. On all treatment days mean FEV₁ 90 minutes after dosing was reduced compared with the baseline value due to the preceding methacholine challenge, but further recovery occurred by 120 minutes. There was no significant difference between study treatments other than a significantly smaller reduction in FEV₁ after salmeterol 100 µg than after placebo (absolute difference = 0.31 l (95% CI 0.12 to 0.50), p = 0.002).

The mean FEV₁ 120 minutes after salmeterol 100 µg was increased compared with the baseline value whereas it was reduced for all other treatments. However, all active treatments showed a significantly smaller reduction in FEV₁ than placebo (p ≤ 0.011). The effect on FEV₁ was significantly greater after salmeterol 100 µg than after salmeterol 25 µg (absolute difference 0.16 l (95% CI 0.02 to 0.31), p = 0.028), salbutamol 100 µg (absolute difference 0.18 l (95% CI 0.04 to 0.32), p = 0.015), and salbutamol 400 µg (absolute difference 0.16 l (95% CI 0.02 to 0.31), p = 0.027). There were no other significant differences between treatments.

METHACHOLINE CHALLENGE PD₂₀FEV₁

The baseline geometric mean dose of methacholine causing a 20% reduction in FEV₁ (PD₂₀FEV₁) did not differ significantly between different treatment days. PD₂₀FEV₁ 30 and 120 minutes after dosing are shown in fig 1. All active treatments offered significantly better protection against methacholine-induced bronchoconstriction than placebo at 30 minutes (p < 0.001) and at 120 minutes (p < 0.001). Tests for carry over and two-factor interactions with treatment were found to be non-significant for both challenges. The results are shown in fig 2 and table 3 as doubling doses related to placebo response. Statistical analyses of treat-

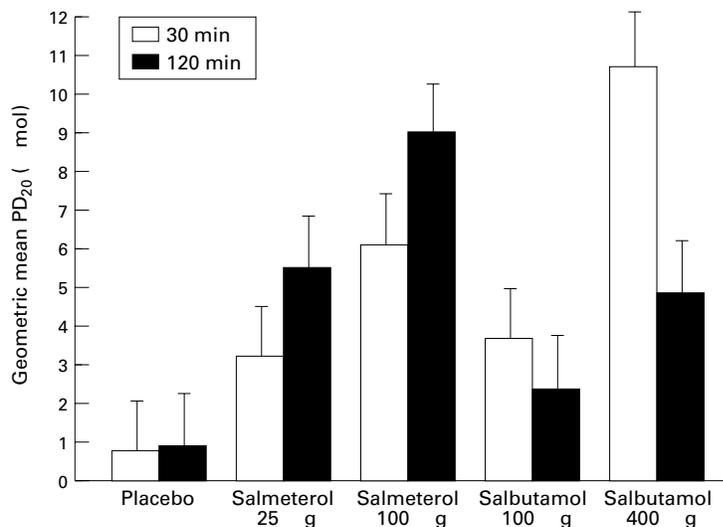


Figure 1 Geometric mean (SE) PD₂₀FEV₁ at 30 and 120 minutes.

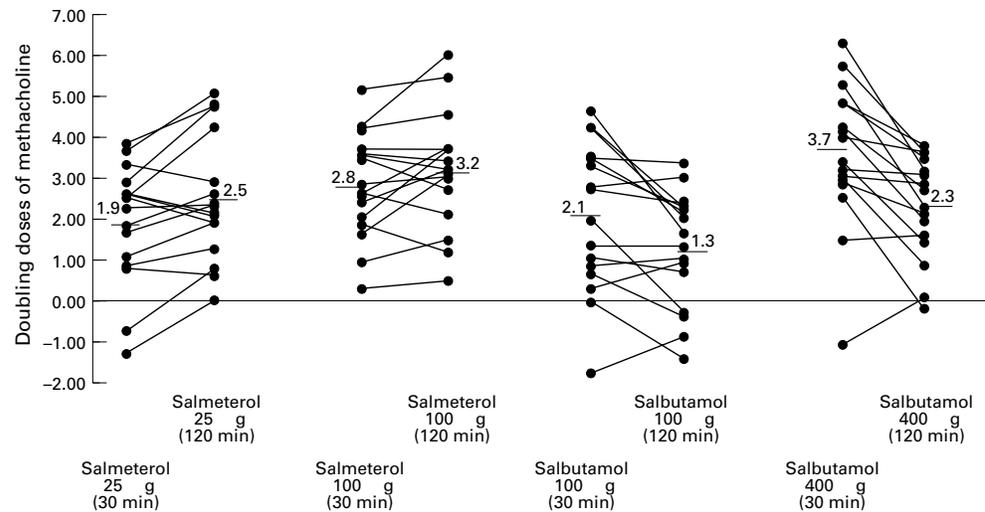


Figure 2 Individual $PD_{20}FEV_1$ data expressed as doubling doses of methacholine related to placebo response.

Table 3 Mean (SE) $PD_{20}FEV_1$ expressed as doubling doses of methacholine related to placebo response

	Salbutamol		Salmeterol	
	100 µg	400 µg	25 µg	100 µg
30 min	2.1(0.45)	3.7(0.44)	1.9(0.37)	2.8(0.32)
120 min	1.3(0.35)	2.3(0.32)	2.5(0.38)	3.2(0.36)

Table 4 Geometric mean $PD_{20}FEV_1$ data at 30 and 120 minutes expressed as ratios between different treatments

	Salmeterol		Salbutamol	
	25 µg	100 µg	100 µg	400 µg
30 minutes				
Treatment/placebo				
Ratio	3.9	6.9	4.4	13.4
p value	<0.001	<0.001	<0.001	<0.001
95% CI	2.5 to 6.2	4.4 to 10.8	2.8 to 7.0	8.5 to 21.1
Treatment/salmeterol 25 µg				
Ratio		1.8	1.1	3.4
p value		0.017	0.59	<0.001
95% CI		1.1 to 2.8	0.7 to 1.8	2.2 to 5.4
Treatment/salmeterol 100 µg				
Ratio			0.6	2.0
p value			0.059	0.005
95% CI			0.4 to 1.0	1.2 to 3.1
Treatment/salbutamol 100 µg				
Ratio				3.0
p value				<0.001
95% CI				1.9 to 4.8
120 minutes				
Treatment/placebo				
Ratio	5.7	9.3	2.5	5.2
p value	<0.001	<0.001	<0.001	<0.001
95% CI	3.8 to 8.6	6.1 to 14.1	1.7 to 3.9	3.4 to 7.9
Treatment/salmeterol 25 µg				
Ratio		1.6	0.4	0.9
p value		0.02	<0.001	0.65
95% CI		1.1 to 2.5	0.3 to 0.7	0.6 to 1.4
Treatment/salmeterol 100 µg				
Ratio			0.3	0.6
p value			<0.001	0.008
95% CI			0.2 to 0.4	0.4 to 0.9
Treatment/salbutamol 100 µg				
Ratio				2.0
p value				0.001
95% CI				1.4 to 3.1

Statistical significance calculated by analysis of covariance.

ment differences in $PD_{20}FEV_1$ values at 30 and 120 minutes are presented in table 4.

At 30 minutes salmeterol 100 µg afforded significantly greater protection than salmeterol 25 µg, and comparison with salbutamol 100 µg approached significance. Salbutamol 400 µg showed a greater protective effect than salbutamol 100 µg, salmeterol 25 µg, and salmeterol 100 µg. In decreasing rank order, potency of the drugs in protection against methacholine challenge at 30 minutes was salbutamol 400 µg > salmeterol 100 µg > salbutamol 100 µg ≥ salmeterol 25 µg.

At 120 minutes salbutamol 400 µg and salmeterol 25 µg offered greater protection than salbutamol 100 µg. Protection due to salmeterol 100 µg was greater than that due to salbutamol 100 µg and salbutamol 400 µg. At 120 minutes the decreasing rank order of potency was salmeterol 100 µg > salmeterol 25 µg ≥ salbutamol 400 µg > salbutamol 100 µg.

SLOPE OF FEV_1 VERSUS LOG METHACHOLINE CONCENTRATION

Analysis of the slope of FEV_1 versus log methacholine concentration was performed. At both post-dose challenges each active treatment had a smaller gradient (afforded greater protection) than placebo. At the 30 minute challenge salbutamol 400 µg had a significantly smaller gradient than both salmeterol 25 µg ($p=0.025$) and salbutamol 100 µg ($p=0.037$). There were no significant differences in other treatment comparisons at either time point. The rank ordering of the adjusted means was identical to those of the $PD_{20}FEV_1$ data.

Discussion

We have compared salmeterol 25 µg and 100 µg and salbutamol 100 µg and 400 µg in terms of bronchoprotective effect against repeated methacholine challenge in order to estimate dose equivalence in subjects with mild stable asthma. Maximal observed bronchoprotection

was equivalent for salmeterol 100 µg and salbutamol 400 µg but was significantly greater for salmeterol 25 µg than salbutamol 100 µg ($p=0.01$). Salmeterol 25 µg was equivalent to salbutamol 100 µg at 30 minutes but significantly more effective at 120 minutes. At 30 minutes salmeterol 100 µg was less effective than salbutamol 400 µg while at 120 minutes it was significantly more effective. The magnitude of protection due to salmeterol increased over the course of 120 minutes, while protection afforded by salbutamol decreased. This is consistent with the known onset and duration of action of these drugs. It is compatible with previous studies showing a slower onset of action of salmeterol and a longer duration of action than salbutamol in human airways *in vivo*^{5,7,8} and *in vitro*.²⁰

Inevitably there are some limitations to a study of this nature. Only a relatively small number of subjects was studied but the study design ensured that the pre-drug methacholine challenge was reproducible by determination of baseline on each treatment day. Subjects were clinically stable throughout the duration of the study. Baseline FEV₁ did not differ significantly between study days and was comparable to screening values, so the observed changes in FEV₁ and PD₂₀FEV₁ can confidently be attributed to drug and dose differences. The study methodology was adequate to distinguish a fourfold difference in dose for salbutamol. Methacholine challenge at 30 minutes and at 120 minutes could have been performed on different days to avoid possible cumulative bronchoconstriction, but such a design would be impractical and has not been adopted by others. It would be similarly onerous to increase the number of doses of each drug studied to construct full log dose-response curves.

No study has previously reported the bronchoprotective effects of salmeterol at exactly these doses and time points but our results are in line with those of other workers. Cheung and coworkers²¹ reported an acute protective effect of salmeterol 50 µg with a 2.3-fold doubling dose increase in PC₂₀FEV₁ at one hour, whereas Derom *et al*⁸ found salmeterol 50 µg equivalent to salbutamol 200 µg with a 1.9-fold doubling dose increase in PC₂₀FEV₁ at one hour. Gongora *et al*⁷ compared the protective effects of salmeterol and salbutamol, delivered by metered dose inhaler, against histamine challenge in a placebo controlled study. They reported an increase of 2.7 doubling doses of histamine one hour after salmeterol 50 µg compared with 1.8 after salbutamol 200 µg. These results are very comparable to our own. Our time points were chosen to obtain comparable bronchodilator effects of the two drugs, bearing in mind their different time courses of action.

It should be noted that bronchoprotective effects may not necessarily be identical to bronchodilator effects. However, it would be difficult to compare bronchodilator effects of the higher doses of the two β₂ agonists used due to the shape of the dose-response curve and the tendency of FEV₁ to plateau below maximal therapeutic dosage so that increasing

doses cannot be discriminated.²² In the present study there was a trend for salmeterol 100 µg to exhibit a greater bronchodilator effect than salmeterol 25 µg after baseline methacholine challenge, as shown by FEV₁ at 90 and 120 minutes, though this was not statistically significant. There was a trend to greater protection after salbutamol 400 µg than after salmeterol 100 µg, although this did not achieve statistical significance. We cannot exclude the possibility of a plateau bronchoprotective effect at the higher dose of salbutamol, suggesting that the relative potency ratio for salmeterol to salbutamol might be slightly less than 1:4. Comparison of the effects of β₂ adrenoceptor agonists on methacholine responsiveness therefore offers a sensitive, convenient and reproducible method for comparing potency of drugs of this class, and has been recommended as a method for determining equivalence of inhaled medications.^{22,23}

In vitro studies suggest a fourfold greater affinity of salmeterol for β₂ receptors and that salmeterol is 2–15 times more potent than salbutamol.^{9,20} Previous single dose studies *in vivo* in humans have suggested a dose equivalence of salmeterol compared with salbutamol of 2–16, but these studies have largely relied on change in FEV₁. As discussed above, this is an insensitive method of estimating relative potency. A more comprehensive study compared cumulative doses of salbutamol with three single doses of salmeterol.¹¹ Heart rate, plasma potassium, QTc interval, and tremor were examined in addition to FEV₁. This study showed a wide range of dose equivalence values (3–13) depending on which parameter was analysed. Our data are compatible with a weight-for-weight dose ratio for salmeterol to salbutamol of ≤1:4 and a molar dose ratio of ≤1:3.8.

This is the first study to compare directly protection afforded by two doses of salmeterol and two doses of salbutamol. It agrees with the large multicentre clinical studies on which the clinically recommended therapeutic doses of salmeterol are based.^{5,6} These data should help to alleviate concerns regarding risks of salmeterol in the treatment of asthma proposed on the basis of previous high estimates of its relative β₂ agonist potency.

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