Pharmacokinetics and extrapulmonary β_2 adrenoceptor activity of nebulised racemic salbutamol and its R and S isomers in healthy volunteers

B J Lipworth, D J Clark, P Koch, C Arbeeny

Abstract

Background – Racemic salbutamol remains one of the most commonly used bronchodilators in the treatment of reversible airways obstruction. Data from animal and human studies suggest that the S-isomer, whilst contributing no bronchodilator activity, may induce increased bronchial hyperreactivity and may explain the adverse effects of regular racemic salbutamol on asthmatic disease control. The purpose of this study was to evaluate the dose-response effects of racemic (+/-) salbutamol and its R(-) and S(+) isomers in terms of pharmacokinetics and pharmacodynamics at extrapulmonary β_2 adrenoceptors when given by the inhaled route to healthy volunteers.

Methods – Twelve healthy volunteers of mean age 20.6 years were studied in a double blind, placebo controlled, cross-over design comparing cumulative doubling doses of nebulised R-salbutamol (R) and S-salbutamol (S) isomers (200 μ g/400 μ g/800 μ g/1600 μ g/3200 μ g) and racemic salbutamol (RS) (400 μ g/800 μ g/1600 μ g/3200 μ g/6400 μ g). Doses were administered at 20 minute intervals ($t_0/t_{20}/t_{40}/t_{60}/t_{80}$) and measurements were made of extrapulmonary β_2 responses as an increase in finger tremor and heart rate and fall in plasma potassium at baseline and each dose level ($t_0/t_{20}/t_{40}/t_{60}/t_{80}/t_{100}$). Plasma levels of salbutamol were measured at 15 minutes after each dose with a further sample at 30 minutes after the last dose (t_{110}).

Results – Pharmacodynamics showed dose related β_2 responses for R-salbutamol and RS-salbutamol but not for the S isomer, and a plateau in response was not reached within the administered dose range. No differences in responses were found between R-salbutamol and RS-salbutamol when compared on a 1:2 microgram basis. The effects of the S isomer were indistinguishable from those of placebo. For all β_2 responses there were differences between R-salbutamol and S-salbutamol (for t_{100} response as change from placebo); tremor (log units): R 0.74 vs S 0.03 (95% CI 0.39 to 1.03); fall in potassium (mmol/l): R 0.35 vs S -0.02 (95% CI 0.03 to 0.71). Pharmacokinetics showed consistently higher levels for S-salbutamol than R-salbutamol at 15 minutes after each dose,

with R-salbutamol already being cleared and S-salbutamol reaching peak levels at 30 minutes after the last dose (at t_{110}). There were higher plasma levels of R-salbutamol and S-salbutamol following administration of the respective isomers alone compared with their levels after administration of the racemate, suggesting an influence of each isomer on the clearance of the opposite isomer when given as a racemate.

Conclusions – The S-isomer of salbutamol has no detectable activity at extrapulmonary β_2 adrenoceptors whilst exhibiting higher plasma levels than the R-isomer, in keeping with greater clearance of R-salbutamol than S-salbutamol. Inhalation of R-salbutamol and RS-salbutamol produced dose-related β_2 responses which were equivalent when compared on a 1:2 microgram basis, despite higher plasma levels of R-salbutamol after administration of the R isomer than after administration of the racemate. Further dose ranging studies are required at steady state to evaluate the pharmacokinetics of R- and S-salbutamol and their relative effects on bronchial hyperreactivity when given on a regular basis to asthmatic subjects.

(Thorax 1997;52:849-852)

Keywords: salbutamol, nebulised, racemic, enantiomers, β_2 adrenoceptors, pharmacokinetics.

Departments of
Clinical
Pharmacology and
Respiratory Medicine,
Ninewells Hospital and
Medical School,
University of Dundee,
Dundee DD1 9SY, UK
B J Lipworth
D J Clark

Sepracor Inc,
Marlborough,
Massachusetts 01752,
USA
P Koch
C Arbeeny

Correspondence to:
Dr B J Lipworth.

Received 13 February 1997
Returned to authors
23 April 1997
Revised version received
8 May 1997
Accepted for publication
12 May 1997

There has been considerable debate as to whether the use of regular β_2 agonist therapy is associated with increased morbidity and mortality in asthmatics.¹ This continues to be relevant, despite a well established move towards earlier use of preventive treatment in view of the fact that large numbers of less stable asthmatic subjects continue to use high doses of regular β_2 agonists.² There is some evidence to suggest that regular β_2 agonists may cause worsening of disease control in asthmatics.^{3,4} In this respect it is known that tolerance develops to the protection of salbutamol against direct and indirect bronchoconstrictor stimuli such as histamine, methacholine, allergen, and adenosine monophosphate,⁵⁻⁷ whilst there is also a rebound increase in the histamine response after treatment with regular terbutaline.⁶ Also, perhaps more importantly, following regular use of in-

Table 1 Mean (SE) baseline values (at t_0) for extrapulmonary β_2 responses

	RS-salbutamol	R-salbutamol	S-salbutamol	Placebo
Tremor (log units)	0.366 (0.014)	0.379 (0.015)	0.397 (0.015)	0.377 (0.017)
Potassium (mmol/l)	4.47 (0.10)	4.45 (0.07)	4.33 (0.08)	4.35 (0.06)
Heart rate (beats/min)	67.7 (2.9)	64.4 (3.0)	63.8 (3.0)	63.5 (1.8)

There were no significant differences between the four treatments for any of the measured parameters.

haled salbutamol there is an increase in both early and late airway responsiveness to allergen.^{5,8} Indeed, concomitant administration of inhaled corticosteroid does not appear to prevent the increased airway responsiveness to allergen induced by regular salbutamol.⁹

The mechanism behind these changes in airway responsiveness has been the topic of much research. The agonist activity of racemic salbutamol is thought to reside solely with the R configuration. However, in sensitised guinea pigs the racemates and S isomers of isoprenaline, terbutaline, and salbutamol have been shown to induce airway hyper-responsiveness.¹⁰⁻¹² The effects of racemic salbutamol and its constituent isomers, R-salbutamol and S-salbutamol, have been evaluated in asthmatic subjects.¹³ This revealed that the S isomer of salbutamol produced hyper-responsiveness to methacholine, whilst the R isomer reduced hyperresponsiveness more effectively when given alone than when administered as the racemate. However, these findings have been criticised in that the analysis was performed on non-transformed skewed data, as well as reporting the final dose of methacholine rather than the shift in PC_{20} value. We have evaluated for the first time the pharmacokinetics and dose-response effects of racemic (+/-) and the R (-) and S (+) isomers of inhaled salbutamol at extrapulmonary β_2 adrenoceptors in man.

Methods

SUBJECTS AND PROTOCOL

Twelve normal subjects of mean (SE) age 20.6 (0.40) years and forced expiratory volume in one second (FEV₁) of 108.9 (2.1)% were

studied in a double blind, crossover study to compare cumulative doubling doses of nebulised placebo, R-salbutamol and S-salbutamol (200 µg/400 µg/800 µg/1600 µg/3200 µg) and racemic (RS) salbutamol (400 µg/800 µg/1600 µg/3200 µg/6400 µg). All treatments were given via a System 22 Sidestream nebuliser (Medicaid, Pagham, UK) with a fill volume of 4 ml driven by compressed air at a flow rate of 8 l/min and nebulised to dryness. Doses were given at 20 minute intervals ($t_0/t_{20}/t_{40}/t_{60}/t_{80}$). Measurements of extrapulmonary β_2 responses were made over a five minute period at baseline and at 15 minutes after each dose administration corresponding to $t_0/t_{20}/t_{40}/t_{60}/t_{80}/t_{100}$ for each dose level. Samples were also taken for measurement of plasma salbutamol levels at 15 minutes after each dose administration, with an extra sample taken at 30 minutes after the last dose (t_{110}). All measurements were made with the subject supine. The subjects were studied on four days, each separated by one week.

MEASUREMENTS

Postural finger tremor was measured as previously described with an accelerometer transducer (Entran, Ealing, UK).¹⁴ Heart rate was measured from standard lead II of an electrocardiogram monitor. Plasma potassium levels were assayed by flame photometry using an IL943 analyser (Instrumentation Laboratory Ltd, Warrington, UK). The intra-assay and interassay values for analytical imprecision were 0.41% and 1.04%, respectively. The assay for determination of plasma levels of R- and S-salbutamol was made by high performance liquid chromatography with fluorescence detection and was fully validated according to published guidelines¹⁵ with a limit of quantitation of 0.25 ng/ml.

STATISTICAL ANALYSIS

The results were analysed using the "Statgraphics" statistical software package (STSC

Table 2 Values for extrapulmonary β_2 responses for R-salbutamol (R), S-salbutamol (S) and racemic salbutamol (RS) at each time point (20–100 minutes) and also for area under curve (AUC)

	20 min	40 min	60 min	80 min	100 min	AUC
Tremor (log units)						
RS	0.24	0.37	0.45	0.61	0.66* (0.47 to 0.86)	0.76* (0.52 to 1.00)
R	0.04	0.39	0.39	0.57	0.74* (0.56 to 0.92)	0.71* (0.48 to 0.93)
S	-0.02	0.003	-0.04	-0.04	0.03 (-0.15 to 0.21)	-0.03 (-0.26 to 0.19)
Potassium (mmol/l)						
RS	-0.13	0.13	0.28	0.30	0.41* (0.22 to 0.61)	0.42* (0.19 to 0.65)
R	0.11	0.07	0.19	0.19	0.35* (0.16 to 0.55)	0.30* (0.07 to 0.53)
S	-0.04	-0.13	-0.06	-0.08	-0.02 (-0.21 to 0.18)	-0.11 (-0.34 to 0.12)
Heart rate (beats/min)						
RS	-0.5	5.9	10.2	9.7	10.5* (4.3 to 16.8)	670* (350 to 990)
R	-2.7	4.6	6.8	8.6	13.4* (7.2 to 19.7)	600* (260 to 900)
S	0.2	1.6	6.0	5.3	0.1 (-6.1 to 6.3)	110 (-230 to 450)

Results are given as mean change from placebo with 95% CI for mean for t_{100} and AUC only.

Units for AUC: tremor (log units.h), potassium (mmol.h/l), heart rate (beats).

* $p < 0.05$ versus placebo for t_{100} and AUC.

Figure 1 (A) Mean (SE) increase in tremor and (B) fall in plasma potassium levels (20–100 minutes) as change from placebo values for R-salbutamol (R), S-salbutamol (S), and racemic salbutamol (RS) given in cumulative doubling doses. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for difference between R or RS compared with S for area under curve.

Software Publishing Group, Rockville, USA). All data were analysed as change from placebo with tremor data being log transformed as they were not normally distributed. Dose-response

data were calculated as area under the curve (AUC) in order to obviate multiple comparisons at each time point. For all parameters comparisons were made by multifactorial analysis of variance (MANOVA) and Duncan's multiple range testing was used to establish where the differences were significant for t_{100} and AUC data only. A probability value of $p < 0.05$ (two tailed) was considered significant. The study was designed with 80% power (beta error = 0.2) in order to detect a 20% difference in tremor between treatments.

Results

EXTRAPULMONARY β_2 RESPONSES

There were no differences in baseline values (t_0) between the four treatments (table 1). A dose-response effect was observed for R-salbutamol and RS-salbutamol but not for S-salbutamol (table 2, fig 1). A plateau in the response was not reached within the administered dose range for R-salbutamol (3200 μg) or RS-salbutamol (6400 μg). No differences were found between R- and RS-salbutamol when compared on a 1:2 microgram basis. There were no differences between S-salbutamol and placebo. For all β_2 responses there were significant differences between R- and S-salbutamol isomers.

PHARMACOKINETICS

Plasma levels 15 minutes after administration of each dose were consistently higher for S-salbutamol than R-salbutamol (fig 2). At 30 minutes after administration of the last dose (t_{110}) the plasma levels of R-salbutamol were beginning to fall, whilst levels of S-salbutamol reached their peak. Plasma levels of R- and S-salbutamol were higher following administration of the respective isomers alone than after administration of the racemate.

Discussion

The results of the present study show that S-salbutamol has no activity at extrapulmonary β_2 adrenoceptors whilst R-salbutamol and RS-salbutamol exhibit equivalent effects on a 1:2 microgram basis. It was noteworthy that plasma levels of S-salbutamol were consistently higher than R-salbutamol owing to greater clearance of the R isomer than the S isomer as was evident at 30 minutes after the last dose. Furthermore, higher plasma levels of R- and S-salbutamol occurred after administration of each isomer compared with levels after administration of the racemate, suggesting a possible influence of each isomer on the clearance of the opposite isomer when given as the racemate. The higher levels of R-salbutamol after administration of the R isomer alone compared with levels of R-salbutamol after administration of racemic salbutamol might partly explain previous observations of greater bronchoprotection with R-salbutamol than RS-salbutamol, although it would not explain the adverse effect of S-salbutamol alone on airway hyperreactivity.¹³ Our pharmacokinetic data are in keeping with

Figure 2 Mean plasma levels of R-salbutamol (R) and S-salbutamol (S) at 15 minutes after administration of cumulative doubling doses of R-, S-, and RS-salbutamol and at 30 minutes after the last dose (t_{110}). The dose levels are shown for administration of each of the R- or S-salbutamol isomers given alone or as a racemic mixture. Arrows denote time of administration for each dose. LOQ = limit of quantitation for assay.

the known stereoselective sulphate conjugation of salbutamol,¹⁶ with data using human lung cytosol showing that the R-isomer is cleared 11 times faster than the S-isomer.¹⁷

The pharmacodynamic results are consistent with animal data that show the S-isomer to be devoid of activity at β_2 adrenoceptors. This in turn suggests that any effects of the S-isomer on airway hyperreactivity must occur via a mechanism unrelated to β_2 adrenoceptor occupancy. The aim of our study was to look at extrapulmonary β_2 responses in normal subjects so we did not evaluate airway effects of salbutamol. However, it has been shown in vitro and in vivo that S-salbutamol causes increased airway responsiveness in guinea pig and human airways.^{11–13,18} This might explain the findings of Cockcroft *et al* that regular use of racemic salbutamol induces an increase in the early and late responses to allergen challenge.^{5,8} It is likely that there would be much greater accumulation of S-salbutamol than R-salbutamol with chronic dosing of racemic salbutamol due to greater clearance of the R-isomer. This emphasises the need to perform further dose ranging studies at steady state to characterise properly the pharmacokinetics and relative effects of R- and S-salbutamol on airway hyperresponsiveness. Studies in man with oral and intravenous terbutaline have shown that the clearance of the S-isomer is greater than the R-isomer,¹⁹ suggesting that it may not be possible to extrapolate findings with salbutamol to other β_2 agonists.

This study was supported by a grant from Sepracor Inc (USA).

- 1 Lipworth BJ. The β -agonist controversy: fact or fiction. *Clin Exp Allergy* 1992;22:659–64.
- 2 Clark DJ, Lipworth BJ. Dose-response with inhaled drugs in asthma. *Clin Pharmacokinet* 1997;32:58–74.
- 3 Sears MR, Taylor DR, Print CG, Lake DC, Li Q, Flannery. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;336:1391–6.
- 4 Spitzer WO, Wuisa E, Ernst P, Horwitz RI, Habbick B, Cockcroft D, *et al*. The use of beta-agonists and risk of death and near death from asthma. *N Engl J Med* 1992;326:501–6.
- 5 Cockcroft DW, McPartland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;342:833–7.
- 6 Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988;i:554–8.
- 7 O'Connor BJ, Aikman S, Barnes PJ. Tolerance to non bronchodilator effects of inhaled β_2 agonists in asthma. *N Engl J Med* 1992;327:1204–8.
- 8 Cockcroft DW, O'Byrne PM, Swystun VA, Bhagat R. Regular use of inhaled albuterol and the allergen-induced late asthmatic response. *J Allergy Clin Immunol* 1995;96:44–9.
- 9 Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled β_2 -agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. *Am J Respir Crit Care Med* 1995;152:1485–9.
- 10 Sanjar S, Kristersson A, Mazoni L, Morley J, Schaublin E. Increased airway reactivity in the guinea-pig follows exposure to intravenous isoprenaline. *J Physiol* 1990;425:43–54.
- 11 Andersson RGG, Johansson F, Aberg G. Effects on bronchial reactivity and histamine release of the isomers of albuterol. *Am J Respir Crit Care Med* 1995;151(Suppl 2):A273.
- 12 Mazzoni L, Naef R, Chapman ID, Morley J. Hyperresponsiveness of the airways following exposure of guinea-pigs to racemic mixtures and distomers of β -selective sympathomimetics. *Pulm Pharmacol* 1994;7:367–76.
- 13 Perrin-Fayolle M. Salbutamol in the treatment of asthma. *Lancet* 1995;346:1101 (letter).
- 14 Lipworth BJ, McDevitt DG. Beta-adrenoceptor responses to inhaled salbutamol in normal subjects. *Eur J Clin Pharmacol* 1989;36:239–45.
- 15 Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yarobi A, *et al*. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *Pharm Res* 1992;9:588–92.
- 16 Walle UK, Persola GR, Walle T. Stereoselective sulphate conjugation of salbutamol in humans: comparison of hepatic, intestinal and platelet activity. *Br J Clin Pharmacol* 1993;35:413–8.
- 17 Eaton EA, Walle UK, Wilson HM, Aberg G, Walle T. Stereoselective sulphate conjugation of salbutamol by human lung and bronchial epithelial cells. *Br J Clin Pharmacol* 1996;41:201–6.
- 18 Blum P, Morley J, Gunawardena KA, Aberg GA. Exposure to S-albuterol changes responsivity of human airways to methacholine. *Am J Respir Crit Care Med* 1995;151(Suppl 2):A273.
- 19 Borgstrom L, Nyberg L, Jonsson S, Lindberg C, Paulson J. Pharmacokinetic evaluation in man of terbutaline given as separate enantiomers and as the racemate. *Br J Clin Pharmacol* 1989;27:49–56.