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Determination of the relative bioavailability of salbutamol to the lung following inhalation

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1 The urinary excretion of salbutamol and its sulphate metabolite was measured following oral (4 mg) and inhaled (4 × 100 μg) administration of salbutamol.
2 Total urinary recovery of salbutamol and its sulphate conjugate indicated a mean (s.d.) relative bioavailability of 92.2 (24.8) % following inhalation compared with oral administration.
3 The mean (s.d.) elimination half-lives of salbutamol and its sulphate conjugate were 5.7 (1.4) and 4.1 (2.1) h, respectively, after oral administration and following inhalation they were 6.1 (2.1) and 5.1 (1.0) h, respectively.
4 Following oral and inhaled administration it was found that in the first 30 min the mean (s.d.) percentage of the dose excreted in the urine as unchanged salbutamol was 0.18 (0.14) and 2.06 (0.80) %, respectively ($P < 0.01$). The drug content of a urine sample taken 30 min after inhalation is, therefore, considered to be representative of the amount of drug delivered to the lungs. It is proposed that this method can be used to evaluate the relative bioavailability of salbutamol to the lung following inhalation by different techniques and devices.

Keywords salbutamol urinary excretion inhalation

Introduction

Salbutamol is used widely for the treatment of asthma and other reversible obstructive airway diseases (Cullum *et al.*, 1969). The administration of bronchodilators, by a metered dose inhaler (MDI), provides a rapid and effective method of delivering drug to the airways.

Methods of assessing the bioavailability of β-adrenoceptor agonists to the lung following inhalation have been limited by analytical problems in measuring low plasma drug concentrations and the lack of a suitable gamma radio-nuclide label. In addition, since 90% of an inhaled dose is swallowed it is difficult to discriminate between the inhaled and swallowed fractions (Newman *et al.*, 1981).

We have developed a simple non-invasive method of measuring the relative bioavailability of salbutamol to the lung following inhalation from a MDI, using an assay with sufficient sensitivity to measure urine concentrations of salbutamol (SAL) and its sulphate conjugate (MET). The chromatographic separation is based on an ion-pair assay for salbutamol in plasma (Jarvie *et al.*, 1987).

Methods

Salbutamol assay

Chemicals Racemic salbutamol BP (free base) was a gift from Glaxo Group Research (Greenford, Middlesex, UK). The internal standard bamethane sulphate (IS) was obtained from Sigma Chemicals Ltd (UK). Analytical grade methanol, ethyl acetate, dichloromethane and acetonitrile were used. Sodium dodecyl sulphate (specially purified for biochemical work) and potassium dihydrogen phosphate were obtained from BDH Chemicals Ltd (Poole, Dorset, UK).

Apparatus and chromatography The h.p.l.c. system comprised an autosampler with a 100 μl loop (Shimadzu SIL 9A), a Gilson Model 302 pump, a fluorescence detector (Waters 470) and a C-R6A Shimadzu integrator. A 25 cm × 4.6 mm Zorbax C18 column was used. The mobile phase consisted of methanol:water (60:40) with sodium dodecyl sulphate 20 mmol l^{-1} and potassium dihydrogen phosphate 10 mmol l^{-1}. The mobile phase was adjusted to pH 3 with 1.0 M phosphoric acid. The flow rate was 1.1 ml min^{-1}. The column temperature

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was ambient and the effluent was monitored at an excitation wavelength of 276 nm with emission set at 609 nm.

Solid phase extraction Solid phase extraction was carried out using Bond Elut Certify LRC columns (Varian, USA). The columns were conditioned using 1 ml methanol followed by 1 ml water. The urine sample (0.5 ml) was placed on the column with 0.2 ml bamethane sulphate (50 mg l^{-1}). The mixture was drawn through the columns over a period of 2–3 min using a Vac Elut system (Varian, USA). The columns were then washed with 2 ml 25% v/v methanol in water, followed by 1 ml dichloromethane, then 0.2 ml ethyl acetate and finally 2 ml acetonitrile. The columns were then dried for 5 min followed by elution using 0.5 ml of 6% v/v ammonia in methanol. The final eluate was evaporated to dryness under a gentle stream of nitrogen, reconstituted in 0.5 ml mobile phase and transferred to microvials for autosample injection.

Measurement of the ester sulphate conjugate Urine samples were extracted before and after hydrolysis with HCl using a method based on that of Hutchings (1986). Hydrochloric acid 0.1 M was used to prevent elution of salbutamol from the solid phase columns.

Urine (0.5 ml) was added to a tube containing 2 ml 0.1 M HCl. The tube was then covered with aluminium foil and placed in a boiling water bath for 30 min. The sample was allowed to cool, 2 ml 0.1 M NaOH was added and it was then extracted as described above.

The concentration of the sulphate conjugate (MET) was calculated from the difference between the pre-(SAL) and post-(SAL + MET) acid hydrolysis salbutamol concentration.

Pharmacokinetic study Ten healthy subjects consented to take part in a study to examine the urinary excretion of salbutamol following oral and inhaled administration. Ethics committee approval was obtained from the University of Bradford. Each subject was given, in random order at an interval of 1 week, an oral dose of 4 mg salbutamol in the form of a syrup (Ventolin, Allen & Hanburys Ltd, UK) and a dose of 4 × 100 μg salbutamol (Ventolin, Allen & Hanburys Ltd, UK) administered from a metered dose inhaler (MDI). Each subject was trained in the inhalation technique to exhale to functional residual capacity (FRC) prior to actuation then to take a slow, deep inhalation to total lung capacity over 5–10 s followed by a 10 s breath hold. Urine collections on each occasion were made at 0, 0.5, 1, 2, 4, 6, 10 and 24 h post dose. The elimination half-life in each individual was calculated from the terminal slopes of the log excretion rate-time data by linear regression analysis. Comparisons were made by Students *t*-test.

Subsequently, each of the 10 subjects was given an oral dose of 400 μg salbutamol to examine possible dose-dependence in absorption. Urine collection intervals were 0–0.5 and 0.5–24 h. Intrasubject variability of the urinary salbutamol excretion in the first 30 min following inhalation was examined in two subjects. 4 × 100 μg salbutamol doses were inhaled from an MDI on five occasions, using the technique described above, with 3 day washout periods. Urine samples were collected after 30 min.

All urine samples were stored at −20° C prior to analysis. The volume of urine passed and its pH were recorded.

Results

Salbutamol assay

Figure 1 shows representative chromatograms obtained from the analysis of blank urine and of a urine sample taken 0.5 h after inhalation of salbutamol. No interfering peaks were observed. During assay development a large peak was seen to co-elute with salbutamol, but the addition of sodium dodecyl sulphate as an ion pairing agent allowed resolution. The retention times of salbutamol (SAL) and bamethane (IS) were 11.7 and 21.3 min, respectively.

The limit of quantification was 50 μg l^{-1}, although by using a larger volume of urine (1 ml) this could be decreased to 25 μg l^{-1}. Typical concentrations measured 30 min after inhalation of salbutamol ranged from 160 μg l^{-1} to 576 μg l^{-1}.

Calibration curves obtained during the analysis of salbutamol samples in urine were linear over the concentration range of interest. The precision and accuracy of the assay are indicated by the data in Table 1. Salbutamol was stable in urine stored at −20° C for up to 1 month. The mean (s.d.) % recovery of salbutamol from spiked urine samples compared with direct injection of standard solution was 100.4% (6.08), (n = 7).

Pharmacokinetic study

Ten (five female) healthy subjects with mean (s.d.) age, weight and height of 29.3 (7.3) years, 68.3 (10.7) kg, and 1.71 (0.12) m respectively, completed the study. Their mean FEV_1 was 103.4% of the predicted values. The pH values of all urine samples collected were in the range 4.5–6.5.

Mean (± s.d.) urinary excretion rates of salbutamol and its sulphate metabolite after oral and inhaled

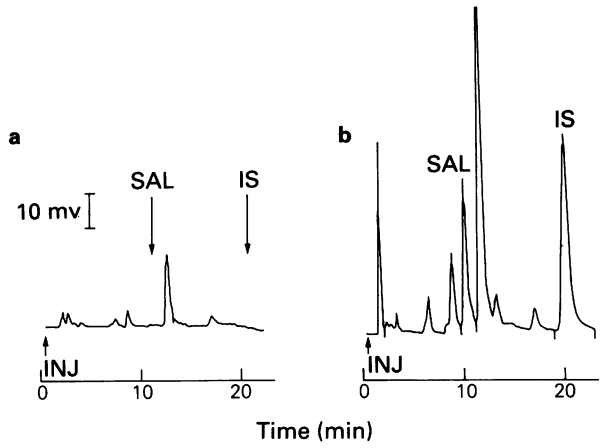

Figure 1 Chromatograms of a blank urine sample (a) and a urine sample obtained 0.5 h after inhalation of 4 × 100 μg salbutamol from an MDI (b). SAL salbutamol, IS bamethane.

Table 1 Precision and accuracy of the assay of salbutamol in urine

	Precision (n = 5)		*Accuracy* (n = 5)		
Nominal concentration (mg l)	*Intra-day CV (%)*	*Inter-day CV (%)*	*Measured concentration (mean ± s.d.) (mg l^{-1})*	*Percentage of nominal concentration (%)*	*CV (%)*
0.05	12.8	11.1	0.05 ± 0.002	104	3.5
0.1	5.6	9.4	0.09 ± 0.005	94	4.9
0.3	2.5	8.2	0.30 ± 0.022	99	7.4
0.6	4.2	3.0	0.57 ± 0.026	95	4.5
1.0	5.6	8.5	0.98 ± 0.048	98	4.9
2.5	7.3	8.4	2.45 ± 0.123	98	5.0

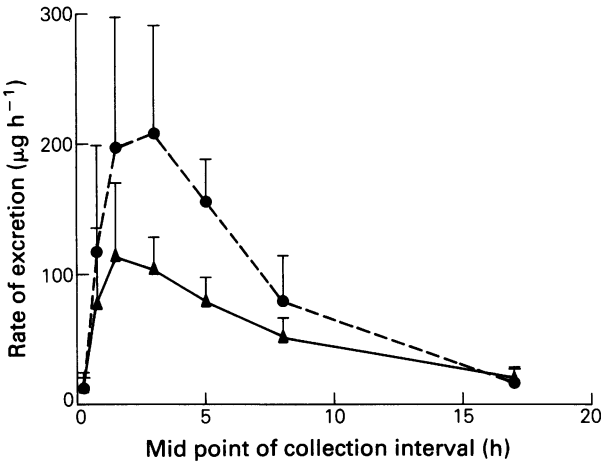

Figure 2 Mean (s.d.) rates of urinary excretion of salbutamol (▲) and its sulphate ester after oral administration of 4 mg salbutamol syrup (Ventolin).

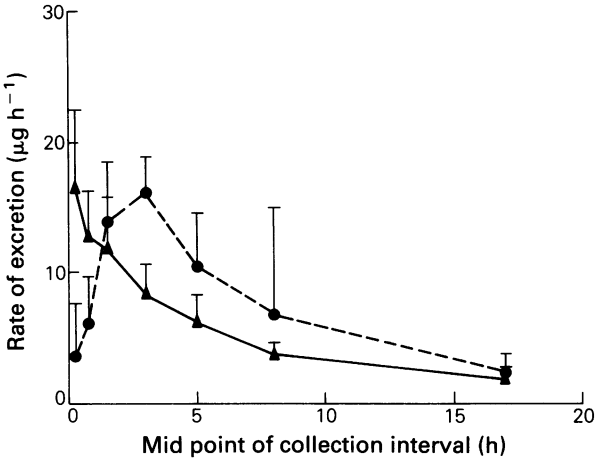

Figure 3 Mean (s.d.) rates of urinary excretion of salbutamol (▲) and salbutamol from an MDI (Ventolin)

administration of salbutamol are shown in Figures 2 and 3. The ten-fold increase in rates of excretion after the syrup compared with inhalation reflects the dosage difference. There was no significant difference in the urinary recovery of either SAL or MET in the 30 min and 24 h urine sample after 4 mg and 400 μg oral doses. The mean (s.d.) unchanged salbutamol recoveries 0.5 h after 4 mg and 400 μg doses were 0.18% (0.13) and 0.23% (0.20) of the dose, respectively. Corresponding values for the total 24 h recovery (SAL + MET) were 63.34% (10.95) and 58.83% (11.07), respectively.

Mean urinary recoveries expressed as a % of dose are shown in Table 2. The data in this table and in Figure 4 indicate that significantly more unchanged salbutamol was excreted in the first 30 min following inhalation compared with oral administration ($P < 0.01$—Mann Whitney test). The mean difference (95% confidence limits) between the 30 min urinary salbutamol excretion following inhaled and oral administration was 1.88% (1.45–2.57), expressed as a % of the doses given. Table 2 shows that the total 24 h recovery of salbutamol was similar following oral and inhaled administration.

Table 3 shows that the mean (s.d.) relative bioavailability of the inhaled dose compared with oral administration, based on the total 24 h recovery (of SAL + MET) was 92.2 (24.8)%. The mean (s.d.) elimination half-lives of salbutamol and its sulphate ester following oral administration were 5.7 (1.4) and 4.1 (2.1) h,

Table 2 Mean (s.d.) urinary recovery of salbutamol (SAL) and its sulphate conjugate (MET) expressed as % of the dose following oral and inhaled administration ($n = 10$)

Urine collection period (h)	*Oral*			*Inhaled*		
	SAL (%)	*MET (%)*	*Total SAL+MET (%)*	*SAL (%)*	*MET (%)*	*Total SAL+MET (%)*
0–0.5	0.18 (0.13)	0.16 (0.11)	0.33 (0.18)	2.06 (0.76)	0.45 (0.51)	2.51 (0.89)
0.5–1.0	0.97 (0.73)	1.47 (0.73)	2.44 (1.65)	1.60 (0.44)	0.77 (0.45)	2.37 (0.79)
1.0–2.0	2.84 (1.42)	4.93 (2.29)	7.77 (3.93)	2.95 (1.00)	3.49 (1.14)	6.43 (1.91)
2.0–4.0	5.17 (1.27)	10.41 (4.14)	15.58 (5.15)	4.19 (1.14)	8.09 (1.37)	12.28 (1.63)
4.0–6.0	3.94 (0.92)	7.79 (1.63)	11.74 (2.26)	3.09 (1.07)	5.25 (2.04)	8.34 (2.54)
6.0–10.0	5.09 (1.54)	7.99 (3.50)	13.08 (4.69)	3.78 (0.94)	6.84 (8.18)	10.62 (8.18)
10.0–24.0	6.75 (2.60)	5.65 (4.25)	12.40 (5.64)	6.25 (3.68)	8.57 (4.92)	14.82 (7.30)
Total	24.95 (5.49)	38.39 (6.36)	63.34 (10.95)	23.91 (5.10)	33.46 (13.10)	57.42 (14.90)

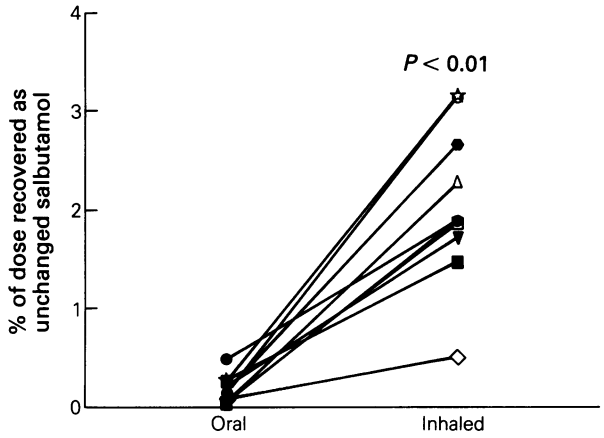

Figure 4 Individual values of urinary unchanged salbutamol recovery 0.5 h after oral and inhaled administration.

Table 3 Individual total (SAL + MET) urinary recovery expressed as a % of the dose administered by oral and inhaled routes

Subject	*Inhaled (%)*	*Oral (%)*	*Ratio (%)*
1	62.5	71.6	87.3
2	66.2	78.9	83.9
3	48.0	43.9	109.3
4	66.7	60.4	108.7
5	93.2	64.3	144.8
6	56.8	66.8	85.0
7	45.5	45.2	100.7
8	37.0	62.7	59.0
9	48.0	63.4	75.7
10	51.3	76.1	67.4
Mean	57.4	63.3	92.2
(s.d.)	14.9	10.9	24.8

respectively. Corresponding values after inhalation were 6.1 (2.1) and 5.1 (1.0) h. There was no statistical significant difference between any of these values.

The coefficient of variation of the inhalation technique in two subjects was 6.4% and 5.8% ($n = 5$). The mean (s.d.) recovery of unchanged salbutamol in the urine 30 min after inhalation in these two subjects was 2.49 (0.2)% and 3.76 (0.2)%.

Discussion

Plasma concentrations of salbutamol are low following administration of the doses used for inhalation therapy. For example a median plasma drug concentration of 7.4 $\mu g\,l^{-1}$ was reported at 1 h after a 5 mg dose of nebulised salbutamol (Lewis *et al.*, 1990). This concentration is close to the limit of detection of the assay used (3 $\mu g\ l^{-1}$). However, concentrations of salbutamol in urine are much higher and offer a much better prospect for assessing bioavailability after inhalation. Urinary excretion is the major route of elimination of both unchanged salbutamol and its sulphate conjugate. Furthermore, the urinary excretion of salbutamol is unaffected by the time interval between micturition (Horn *et al.*, 1990) and, because of its relative polarity and basic properties, salbutamol is unlikely to exhibit significant pH-dependent renal clearance, especially at urine pH values below 6.5.

The proportions of salbutamol and its sulphate ester recovered in the urine are dependent upon the route of administration. Following intravenous administration salbutamol is eliminated mainly unchanged (64%), whereas after oral administration most is excreted as the sulphate ester (48%) with a smaller proportion unchanged (32%) (Morgan *et al.*, 1986). The latter recoveries compare with the values of 38% and 25% observed after oral administration in the present study. After direct bronchial instillation of radiolabelled salbutamol the urine recoveries of salbutamol and its metabolite were similar to those after intravenous administration (Shenfield *et al.*, 1976). Most of the conjugation with sulphate takes place in the gastrointestinal mucosa and since the majority of an inhaled dose is swallowed then the greater proportion of metabolite to salbutamol observed in the present study is as expected.

The renal excretion of salbutamol following inhalation occurs in two phases. Initially there is elimination of unchanged salbutamol representing the fraction of the dose that has been delivered to the lungs. The excretion of the majority of the dose, which is swallowed following impaction in the mouth and throat, as both unchanged drug and the metabolite will commence subsequently. Clearance of salbutamol from the lungs would be either by absorption into the pulmonary capillary network or by mucociliary clearance followed by oral absorption. After oral administration 0.18% ± 0.14 (mean ± s.d.) of the dose was excreted in the urine after 30 min. This low recovery is due to the lag between administration of the dose and the start of absorption. No saturation of salbutamol absorption was demonstrated by the similar recoveries following 4 mg and 400 μg doses. The lag time is not seen when drug is administered directly to the lung via a bronchoscope (Shenfield *et al.*, 1976). Therefore, following inhalation the increased initial recovery of 2.06 ± 0.80 (% of dose) is due to salbutamol which has been delivered to the lungs, rapidly absorbed via the alveoli and then excreted unchanged by the kidneys. After inhalation salbutamol has an onset of action within 5 min and shows a peak effect after 15 min (Hetzel & Clarke, 1976). The fraction of the dose recovered 30 min after inhalation is, therefore, representative of the dose delivered to the site of action and is a measure of the relative bioavailability of salbutamol to the lung.

In theory, the higher proportion of unchanged salbutamol found in the 30 min urine sample following inhalation could be due to buccal absorption. However, buccal absorption of salbutamol has been shown to be negligible; systemic absorption occurs after swallowing of a sublingual dose (Lipworth *et al.*, 1989).

One hour after an inhalation dose the excretion rates of drug and metabolite are seen to resemble those following oral administration, that is after the swallowed fraction of the dose is systemically absorbed and is subjected to first pass metabolism.

Simultaneous administration of charcoal and terbutaline has been shown to prevent oral absorption of the β-adrenoceptor agonist (Burgstrom & Nilsson, 1990; Davies, 1984). Using this method Burgstom & Nilsson (1990) found that 9.1% of the inhaled dose of terbutaline

was deposited into the lungs. However, this method may be more difficult to apply to salbutamol because of its greater oral bioavailability.

Taking a urine sample 30 min after an inhaled dose of salbutamol is reproducible and provides a simple and effective method of assessing its relative bioavailability to the lung. An advantage of this method is that it uses the patient's own inhaler. All other investigations of lung deposition following inhalation from a metered dose inhaler require either the ingestion of charcoal or the use of a radiolabel inhaled marker. The present method may be used together with the measurement of lung function, to demonstrate that improved deposition correlates with improved spirometry. This was not possible in this study as healthy subjects were used. Simultaneous pharmacokinetic and pharmacodynamic studies should allow evaluation of optimal inhalation techniques and devices.

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