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Studies of cyclodextrin inclusion complexes. IV. The pulmonary absorption of salbutamol from a complex with 2-hydroxypropyl-β-cyclodextrin in rabbits

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Summary

The present study details the fate of an HP-β-CYD-salbutamol complex following pulmonary administration in order to evaluate the possibility of obtaining sustained release of salbutamol using HP-β-CYD as a carrier. Using a randomised cross-over design, the absorption and pharmacokinetics of HP-β-CYD-salbutamol complex have been investigated in four healthy New Zealand White male rabbits, after intravenous bolus (i.v.) by means of the marginal ear vein and pulmonary administration via intratracheal instillation (i.t.) at the bifurcation of the trachea. Although the terminal half-life of salbutamol did not change significantly after complexation, the pulmonary absorption of salbutamol as a complex was prolonged, as shown by its maximum plasma concentration which was observed approx. 23 min after instillation compared to approx. 14 min for the uncomplexed salbutamol, and also by the increased absorption time. However, the availability of the complexed salbutamol was reduced to about 80% of that of the free drug compared to that seen after i.v. dosing. The plasma profile for HP-β-CYD was shown to be identical when administered as a complex with salbutamol and in its absence. Complexation did not sufficiently extend the salbutamol release profile to justify its use as a sustained release formulation. However, this approach to achieving prolonged pulmonary drug release profiles may be appropriate for drugs exhibiting stronger complexes with HP-β-CYD or prompt a search for CYD derivatives having a better complexing ability for salbutamol.

Introduction

Cyclodextrins (CYDs) are cyclic oligosaccharides, known for their ability to form inclusion complexes with many drugs, thereby changing their physicopharmaceutical properties. Cabral Marques et al. (1990a,b) have shown that salbutamol forms complexes with β-CYD and certain of its derivatives.

The use of an inhaled β_2-agonist, such as salbutamol, is the first-line treatment for a variety of lung diseases associated with airflow obstruction. Being a selective β_2-adrenergic agonist, salbutamol relaxes the bronchiolar smooth muscle with a relatively low stimulatory effect on the heart, in comparison with older sympathomimetic drugs such as isoproterenol. After inhalation, sig-

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nificant bronchodilatation takes place within 5 min and is sustained for about 4 h (Vaisman et al., 1987).

Cabral Marques et al. (1990c) showed that HP (2-hydroxypropyl)-β-CYD is a possible candidate for pulmonary sustained release applications as it has been shown to have a longer pulmonary residence compared with β- and DM-β-CYDs after intratracheal instillation in rabbits.

In this study, we have determined the pharmacokinetic behaviour of the HP-β-CYD-salbutamol complex in four healthy New Zealand White (NZW) male rabbits after intravenous bolus (i.v.) and intratracheal instillation (i.t.) in order to evaluate the sustained release of salbutamol.

Materials and Methods

Materials

Salbutamol B.P. (base) (batch no. 73267023) and HP-β-CYD (D.S. = 7.0) were generous gifts from Lilly Research Centre Ltd, Surrey, U.K. [^{14}C]HP-β-CYD was purchased from Izinta Isotope Trading Enterprise, Budapest. Salbutamol purity was established by comparison of its melting point (154–155 °C) with literature values (155 °C) (British Pharmacopoeia, 1988), and was used without further purification. HP-β-CYD was used as received. The complex was prepared employing a 1 : 1 molar ratio and using the freeze-drying method as previously reported (Cabral Marques et al., 1990a).

Methods

In a randomised cross-over design, the absorption and kinetics of free and complexed salbutamol have been investigated in New Zealand White male rabbits ($n = 4$; 3.7–4.6 kg), after intravenous bolus (i.v.) (1 mg/kg) by means of the marginal ear vein and pulmonary administration via intratracheal instillation (i.t.) (2 mg/kg) at the bifurcation of the trachea as detailed previously (Cabral Marques et al., 1991). In the case of delivery of the complex, a ratio of 1 : 6.38 mg/kg salbutamol : HP-β-CYD was used.

Approx. 1 ml of blood from the marginal ear vein was collected into tubes containing 10 μl of heparinised saline (100 IU/ml) at specified time intervals for 6 h after dosing. The plasma was separated by centrifugation and stored at −20 °C before HPLC analysis of salbutamol or used immediately for the [^{14}C]HP-β-CYD assay.

Determination of salbutamol

100 μl of water was added to 100 μl of plasma and after shaking for 30 s, 50 μl of a 20% trichloroacetic acid solution was added. Following a 30 s vortex mixing and centrifugation for 5 min at 3000 × *g* (9000 rpm), 200 μl of the upper layer was added to 35 μl of 1 M NaOH. After shaking for 30 s, 200 μl were injected onto the HPLC column (3.9 mm × 25 cm, C18 reversed phase) and assayed by fluorescence detection (Perkin-Elmer LS-5 luminescence spectrometer with LC flow cell) at 273 nm for excitation and 310 nm for emission, using slit widths of 15 and 20 nm, respectively. The flow rate of the eluent (methanol-phosphate buffer (pH 6.0) 35 : 65, containing 10 mM sodium heptanesulphonate as an ion-pair reagent) was 1.5 ml/min. The detection limit was 20 ng/ml of salbutamol.

Peak heights were used to calculate the salbutamol concentration based on calibration curves prepared from spiked plasma samples. Plasma samples ($n = 7$) were spiked with salbutamol (5 μg/ml) and assayed on separate days with samples from the pharmacokinetic studies. The inter-day coefficient of variation for the spiked samples was 8.8%.

Data analysis

Areas under plasma concentration-time curves (AUC) and plasma concentration · time-time (AUMC) profiles were determined by trapezoidal summation. Terminal half-life ($t_{1/2,z}$), used for extrapolating areas to infinity, was determined with the non-linear regression program, Minim (Purves, 1988). Clearance (CL), bioavailable fraction (F) and steady-state volume of distribution (V_{ss}) were calculated from area measurements using standard procedures (Rowland and Tozer, 1989).

Results and Discussion

The i.v. kinetics were similar for free and complexed salbutamol, as demonstrated by the fact that the salbutamol plasma profiles (Fig. 1) in the presence and absence of HP-β-CYD were almost superimposed. Except for the steady-state volume of distribution (V_{ss}), all other parameters were not significantly different (Table 1). Following i.t. dosing (Fig. 2), the time to reach maximum plasma drug concentration (t_{max}), the absorption half-life ($t_{1/2,a}$), the maximum plasma concentration (C_{max}) and the bioavailable fraction (F) were significantly different (Table 2), using Student's paired t-test. Wilcoxon's signed-ranks test was also used for comparing the t_{max} data and gave a statistically ambiguous result ($p = 0.1$). However, it is known that nonparametric tests such as this are not very powerful for analysing small data sets. Complexation of salbutamol with CYD slowed its absorption as demonstrated by a longer $t_{1/2,a}$, which resulted in a lower C_{max} and prolonged t_{max} (23 min for the complex as compared to 14 min for the free drug). The t_{max} for free salbutamol is in agreement with the early peak plasma level after intrabronchial instillation reported by Shenfield et al. (1976). Complexation, however, did not alter the post-peak decline of salbutamol, indicating that the absorption was not slowed sufficiently to produce 'flip-flop' (absorption rate limited) kinetics. CYD complexation also reduced the F of salbu-

TABLE 1

Summary of the pharmacokinetic parameters (mean ± SE; n = 4) of salbutamol and its complex with HP-β-CYD after i.v. administration to rabbits

	Salbutamol (free)	Salbutamol (complexed)	HP-β-CYD (complexed)
$t_{1/2,\lambda_1}$ (min)	4.9 ±0.4	5.2 ± 0.7	18.2 ±2.3
$t_{1/2,z}$ (min)	101.6 ±7.3	139.7 ±10.5	75.2 ±6.2
CL (ml/min per kg)	25.0 ±3.7	25.4 ± 2.2	2.7 ±0.2
V_{ss} (l/kg)	2.50±0.10	4.37± 0.20	0.21±0.02

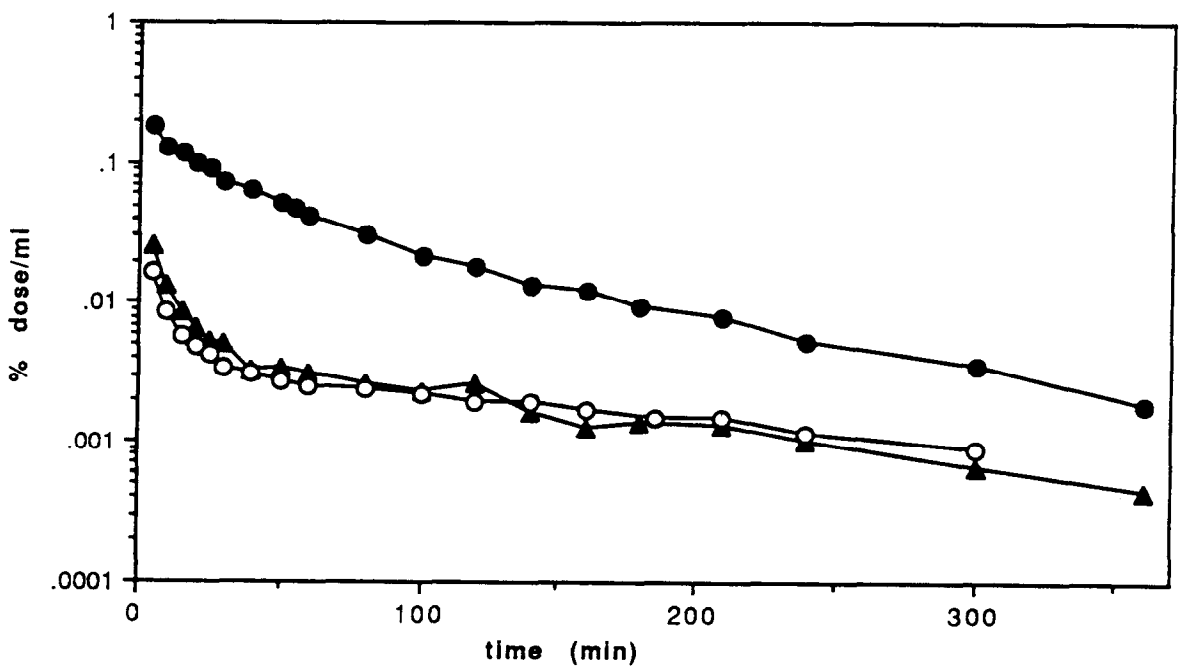

Fig. 1. Mean plasma concentrations of salbutamol (free) (▲), salbutamol (from complex) (○) and HP-β-CYD (from complex (●), following i.v. administration of salbutamol (1 mg/kg) or salbutamol complex (salbutamol, 1 mg/kg; HP-β-CYD, 6.38 mg/kg) to rabbits ($n = 4$). Plasma concentrations expressed as a percentage of administered dose.

TABLE 2

Summary of the pharmacokinetic parameters (mean ± SE; n = 4) of salbutamol and its complex with HP-β-CYD after i.t. administration to rabbits

	Salbutamol (free)	Salbutamol (complexed)	HP-β-CYD (complexed)
t_{max} (min)	13.6± 2.4	23.0± 1.2	107.0± 4.5
C_{max} (ng/ml)	1035.5±81.5	524.6±43.8	22770 ±2500
$t_{1/2,z}$ (min)	145.3±18.6	168.5±21.4	64.0± 2.9
$t_{1/2,a}$ (min)	10.6± 4.2	32.7± 6.1	26.2± 7.8
F (%)	109.2± 8.1	80.6± 8.0	67.8± 8.8

tamol to approx. 80% of that of the free drug. This value is much higher than that calculated after p.o. and rectal administration in rabbits (< 10%; Kurosawa et al., 1984) which was attributed to first-pass metabolism. In comparison, in humans the systemic availability of salbutamol after p.o. administration is 50% (Hutchings et al., 1987). The interspecies difference may well be due to different enzymatic activity. After nebulisation of salbutamol in humans, a low F was also noted (Vaisman et al., 1987). This was attributed to swallowing a large fraction of the aerosolised dose. The V_{ss} after i.v. administration in the rabbit was approx. 2.5 l/kg which is similar to that reported in humans (Price and Clissold, 1989) and is indicative of extensive extravascular distribution.

In this study, the i.t. plasma profile for HP-β-CYD was identical to that previously determined in the absence of salbutamol (Cabral Marques et al., 1991). No significant difference was found in any parameter ($t_{max} = 113 \pm 11$ and 107 ± 5 min; $t_{1/2,z} = 63 \pm 5$ and 64 ± 3 min; $F = 80 \pm 12$ and 68 ± 9%, respectively) for free HP-β-CYD and HP-β-CYD administered as salbutamol complex. The i.v. kinetics, however, showed a longer terminal half-life ($t_{1/2,z}$) for HP-β-CYD when complexed, i.e. 75.2 ± 6.2 min, compared to 44.2 ± 3.6 min for the free carrier (Cabral Marques et al.,

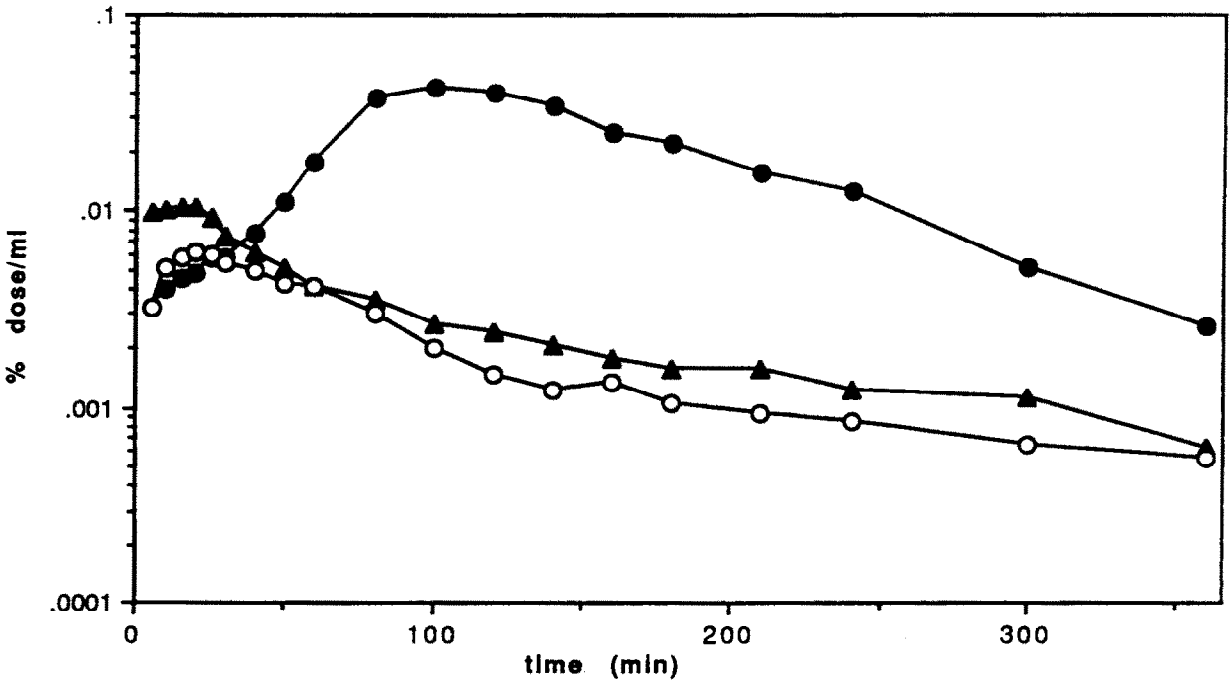

Fig. 2. Mean plasma concentrations of salbutamol (free) (▲), salbutamol (from complex) (○) and HP-β-CYD (from complex) (●), following i.t. administration of salbutamol (2 mg/kg) or salbutamol complex (salbutamol, 2 mg/kg; HP-β-CYD, 12.76 mg/kg) to rabbits ($n = 4$). Plasma concentrations expressed as a percentage of administered dose.

1991). The sampling period used for the i.v. studies with free HP-β-CYD (3 h) was shorter than that used for complexed HP-β-CYD (6 h). Hence, in the former studies, the distribution of free HP-β-CYD may not have been complete and thus the $t_{1/2,z}$ measured was not a true reflection of the true elimination half-life. Some other parameters were also significantly different (initial half-life $(t_{1/2,\lambda_1}) = 11 \pm 1$ and 18 ± 2 and CL = 3.6 ± 0.2 and 2.7 ± 0.2, respectively) for HP-β-CYD when free and complexed. The pharmacokinetics of HP-β-CYD showed a long lag time (34 min) before the start of absorption. Consequently, the t_{max} occurred much later than that of salbutamol which indicates that the complex dissociates within the lung, re-iterating its potential as a carrier for pulmonary drug delivery.

Conclusion

Although complexation did not retard the release of salbutamol sufficiently to justify its use as a sustained release formulation, this approach to achieving prolonged pulmonary drug release may be a useful strategy for drugs that exhibit stronger complexes with HP-β-CYD.

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