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Studies of cyclodextrin inclusion complexes. III. The pulmonary absorption of β-, DM-β- and HP-β-cyclodextrins in rabbits

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Summary

The fate of β-cyclodextrin (β-CYD) and some of its derivatives is reported following pulmonary administration in order to evaluate the feasibility of using β-CYDs for sustaining pulmonary drug action or for controlling systemic drug levels following pulmonary administration. Using a randomised cross-over design, the absorption and pharmacokinetics of β-, dimethyl(DM)-β- and 2-hydroxypropyl(HP)-β-CYDs have been investigated in five healthy New Zealand White male rabbits, after intravenous bolus dosing (i.v.) by means of a marginal ear vein and pulmonary administration via intratracheal instillation (i.t.) at the bifurcation of the trachea. No significant difference was found between the i.v. and i.t. terminal half-life ($t_{1/2,z}$) for β- or DM-β-CYDs, indicating the absence of pulmonary absorption rate-limited kinetics. The clearance of each CYD (3.4–3.9 ml/min per kg) was similar to the glomerular filtration rate. The steady-state volume of distribution (184–214 ml/kg) is fairly small, approximating that of extracellular fluid volume, indicating extravasation of the CYDs. Although the bioavailable fraction (66–80%) is similar for all CYDs, the HP-β-CYD is absorbed more slowly than the others (mean absorption time approx. 113 min compared to 26 and 21 min for β- and DM-β-CYDs, respectively). The times required to reach the maximum plasma level for β-, DM-β- and HP-β-CYD were 30, 22 and 113 min, respectively. From these studies it is concluded that of the three CYDs tested, HP-β-CYD has the best potential as a drug carrier for sustaining pulmonary release.

Introduction

Recently, cyclodextrin (CYD) derivatives have been shown to sustain the release rate of drugs in vitro (Uekama et al., 1990) and following subcutaneous (Uekama et al., 1989) and oral administration (Uekama et al., 1987; Hirayama et al., 1988l; Horiuchi et al., 1990). Such properties result from the formation of an inclusion complex of the drug within the torus of the CYD molecule. If the apparent stability constant of the complex is of sufficient magnitude, then slow dissociation (i.e. drug release) will occur within the biological milieu. In previous work (Cabral Marques et al., 1990a,b), we showed that salbutamol formed complexes with β-CYD and dimethyl(DM)-β-CYD which may therefore serve to control the release of the drug within the lung. No reports exist regarding the fate of CYDs following pul-

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monary delivery, therefore we have investigated the absorption and pharmacokinetics of β-, DM-β- and 2-hydroxypropyl(HP)-β-CYDs in five healthy New Zealand White (NZW) male rabbits after intravenous bolus (i.v.) and pulmonary administration via intratracheal instillation (i.t.).

Materials and Methods

Materials

β-CYD was obtained from Berk Ltd, Basingstoke, Hants, U.K. (Ensuiko Sugar Refining Co., Ltd). DM-β-CYD was purchased from Aldrich Chemical Co., Gillingham, Dorset, HP-β-CYD (degree of substitution = 7.0) was a generous gift from Lilly Research Centre Ltd, Surrey, U.K. [^{14}C]DM-β-CYD and [^{14}C]HP-β-CYD (degree of substitution = 5.1) were purchased from Izinta Isotope Trading Enterprise, Budapest, Hungary.

Methods

In a randomised cross-over study, five male NZW rabbits (2.7–4.2 kg) were given i.v. and i.t. doses (10 mg/kg body weight) of β-, DM-β- and HP-β-CYDs. i.v. and i.t. solutions contained DM-β- or HP-β-CYDs (36 mg/ml) in isotonic saline. β-CYD was given as a solution (18 mg/ml), intravenously and a suspension (due to its low solubility, i.e. 18.5 mg/ml) intratracheally at the bifurcation of the trachea, via a tubing-needle-syringe arrangement (Fig. 1). Each animal was allowed a minimum period of at least 1 week between each CYD administration in which to recover. The rabbits were housed separately and received food and water ad libitum.

The marginal ear vein was chosen for the collection of blood and administration of anaesthetic. In addition, the marginal vein on the contralateral ear was cannulated for i.v. administration of the CYDs. For ease of cannulation, the rabbits were sedated with an intramuscular injection of Hypnorm (Janssen Pharmaceuticals, Wantage (0.1–0.2 ml/kg). Hypnovel (Roche, Welwyn Garden City) (0.1–0.15 ml/kg) was the anaesthetic of choice for i.t. intubation. Anaesthesia was induced instantly and lasted for approx. 30 min. The animals had fully recovered 2 h after injection.

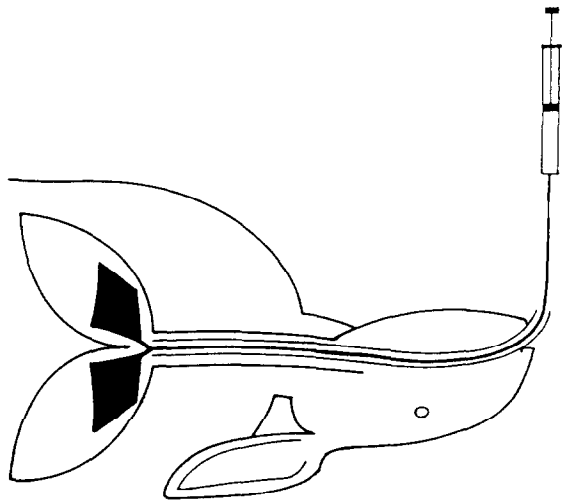

Fig. 1. Intratracheal delivery into rabbit lung.

To facilitate endotracheal intubation, the animal, once anaesthetised, was held in a supine position and the head and neck held horizontal. By grasping the tongue and guiding the tube along its symmetrical axis, the trachea could be entered. The success of intubation could be determined by the intermittent appearance of condensation within the tube as the animal was breathing or of fog on a mirror placed at the free end of the endotracheal tube. Once intubated, 0.28 ml/kg of a solution of DM- or HP-β-CYDs or a suspension of β-CYD were delivered into the lung, from a syringe through a 0.58 mm i.d. diameter tube which has been placed inside the endotracheal tube. After both tubes were withdrawn from the trachea, the animals was held in a reclining position to promote distribution of the dose throughout the lung.

Approx. 0.8 ml of blood from the marginal ear vein was collected into tubes containing 10 μl of heparinised saline (100 IU/ml) at the following times: predose and 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140 and 160 min for both routes of administration and at 210, 240 and 270 min for i.t. administration only.

The plasma was separated by centrifugation and stored at −20 °C before analysis of β-CYD

or used immediately (divided into two) for DM- and HP-β-CYDs. Plasma concentrations of CYDs were determined by the following methods: The HPLC method of Frijlink et al. (1987) was utilized with slight modification for β-CYD (method described below) and scintillation counting for the ^{14}C radiolabelled DM- and HP-β-CYDs.

Determination of β-CYD

CYDs are known to form inclusion complexes with many organic dye molecules (e.g. phenolphthalein). The decrease in colour intensity of the phenolphthalein has been found to be proportional to the β-CYD concentration. The method used was adopted from Frijlink et al. (1987). It consists of the determination of β-CYD concentration in biological samples by HPLC with negative colorimetric detection using post-column addition of an alkaline solution of phenolphthalein. The detection principle is based on the findings of Vikmon (1981).

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 30 s vortex mixing and centrifugation for 5 min at 3000 × *g* (9000 rpm), 185 μl of the upper layer was added to 35 μl of 1 M NaOH. After shaking for 30 s, 180 μl was injected onto the HPLC column (3.9 mm × 30 cm, 10 μm, μ-Bondapak Phenyl (Waters Associates), with pre-column filter) and assayed at 546 nm. The flow rates of the eluent (water) and the post-column agent (8 mM sodium carbonate and 0.06 mM phenolphthalein in 0.96% (v/v) ethanolic solution) were 2 ml min^{-1}.

Peak heights were used to calculate the β-CYD concentration based on calibration curves prepared from spiked plasma samples.

In order to check the purity of the [^{14}C]HP-β-CYD, TLC analyses were performed on silica gel plates (Poligram Sil N-HR for thin-layer chromatography, layer: 0.2 mm MN Silica Gel H-NR, batch: 12.82, Camlab, Cambridge) using a mixture of 1-propanol : ethyl acetate : water : 25% ammonia solution (6 : 2 : 5 : 3) as the solvent. 0.36 mg (10 μl) of HP-β-CYD (unlabelled compound) was applied to the silica gel plate and chromatographed once it reached room temperature, in a closed glass tank thoroughly saturated with the solvent vapour. The plate was then dried at 40–50 °C for 15 min and the chromatograms detected by spraying with concentrated sulphuric acid and heating at 100 °C (Koizumi et al., 1986). For [^{14}C]HP-β-CYD, the plate was dried and cut into 1 cm transverse strips which were placed in scintillation vials with 4 ml of scintillation cocktail and counted for 5 min.

An R_f of 0.81 was obtained for HP-β-CYD with no other spots suggesting the absence of impurities. Radioactivity was associated with only a single strip in the case of [^{14}C]HP-β-CYD, giving R_f values in the range, 0.77–0.85, in agreement with the data obtained for the unlabelled compound, suggesting again the absence of impurities.

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 programme, Minim (Purves, 1988). Clearance (CL), mean residence time (MRT), mean absorption time (MAT), bioavailable fraction (F) and steady-state volume of distribution (V_{ss}) were calculated from area measurements using standard procedures (Rowland and Tozer, 1989).

Pharmacokinetic parameters for the different CYDs were compared using two-way analysis of variance and Duncan's multiple range test (Duncan, 1955).

Results and Discussion

There are two possible methods of pulmonary drug administration, either as an intratracheal instillate or as an aerosol produced by a nebuliser or a metered dose device. The rate of solute absorption is reportedly higher from aerosols as they deliver more drug to the alveolar region whereas i.t. instillation delivers the drug over a smaller surface area and higher up in the respiratory tract where absorption is likely to be slower.

TABLE 1

Summary of the pharmacokinetic parameters (mean ± SE; n = 5) of β-, DM-β- and HP-β-CYDs after i.v. administration to rabbits

	β-CYD	DM-β-CYD	HP-β-CYD
$t_{1/2,\lambda_1}$ (min)	5.0 ± 0.3	25.4 ± 7.2	10.5 ± 1.3
$t_{1/2,z}$ (min)	41.5 ± 6.9	52.9 ± 8.2	44.2 ± 3.6
MRT (min)	57.2 ± 6.4	56.6 ± 5.1	52.2 ± 4.2
CL (ml/min per kg)	3.4 ± 0.3	3.9 ± 0.5	3.6 ± 0.2
V_{ss} (ml/kg)	188.4 ± 9.9	214.2 ± 11.7	184.6 ± 5.4

The absorption rates of 12 compounds with diverse physicochemical properties given by aerosol administration were found to be approximately twice that seen after intratracheal administration to rats (Brown and Schanker, 1983), and mice and rabbits (Schanker et al., 1986). However, aerosols exhibit significant differences in deposition due to particle size, tidal volume, frequency of breathing, lung volume and distribution of ventilation. The instillate method was therefore selected, in order to ensure that 100% of the dose was delivered directly to the lung, thus eliminating any deposition in the head region.

In the case of i.v. administration all of the CYDs behaved in an indistinguishable manner except for the initial half-life ($t_{1/2,\lambda_1}$) where DM-β-CYD was significantly different from the other two (Table 1). The plasma CYD profiles (Figs 2–4) were very similar for all rabbits and all CYDs.

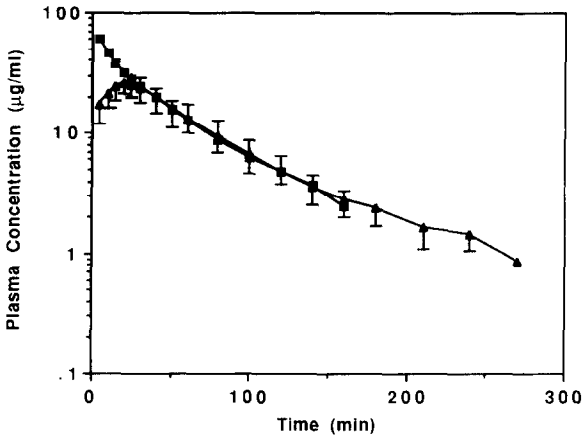

Fig. 3. Mean plasma concentration of DM-β-CYD following i.v. (■) and i.t. (▲) administration to rabbits. Each point is the mean of 5 rabbits with SE bars.

The clearance (CL) of each CYD is similar to glomerular filtration rate in the rabbit (Altman and Dittmer, 1972). The steady-state volume of distribution (V_{ss}) is fairly small, approximating that of extracellular fluid volume. This, in combination with the observed distribution phase after i.v. dosing, indicates extravasation of the CYDs. In a recent study in rats, the i.v. pharmacokinetics of β-CYD and HP-β-CYD were noted to be

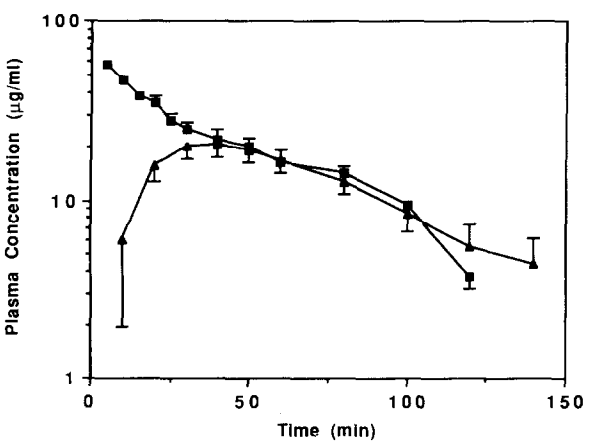

Fig. 2. Mean plasma concentration of β-CYD following i.v. (■) and i.t. (▲) administration to rabbits. Each point is the mean of 5 rabbits with SE bars.

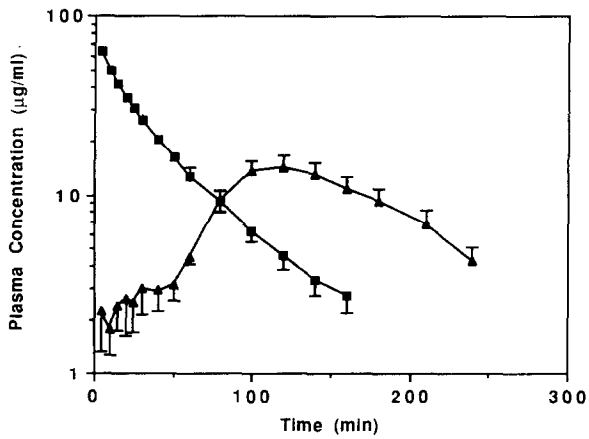

Fig. 4. Mean plasma concentration of HP-β-CYD following i.v. (■) and i.t. (▲) administration to rabbits. Each point is the mean of 5 rabbits with SE bars.

TABLE 2

Summary of the pharmacokinetic parameters (mean ± SE; n = 5) of β-, DM-β- and HP-β-CYDs after i.t. administration to rabbits

	β-CYD	DM-β-CYD	HP-β-CYD
t_{max} (min)	30.0 ± 4.5	22.4 ± 1.9	113.0 ± 10.6
C_{max} (μg/ml)	23.5 ± 1.9	26.8 ± 5.7	14.4 ± 2.4
$t_{1/2,z}$ (min)	44.2 ± 3.9	44.3 ± 6.8	63.0 ± 4.8
MAT (min)	26.1 ± 6.8	20.7 ± 4.0	113.0 ± 6.6
F (%)	65.9 ± 12.8	73.9 ± 13.2	79.8 ± 12.0

similar to each other and also to those of inulin (Frijlink et al., 1990).

Following i.t. instillation in the rabbit, the plasma β-CYDs' profiles (Figs 2–4) showed an increase in their levels to mean C_{max} values (maximum plasma levels) of 23.5 ± 1.9, 26.8 ± 5.7 and 14.4 ± 2.4 μg/ml for β-, DM- and HP-β-CYDs, respectively, which were not statistically different ($0.05 < p < 0.1$). The values for t_{max} (time to reach C_{max}) are given in Table 2 with mean values of 30.0 ± 4.5, 22.4 ± 2.0 and 113.0 ± 10.6 min for β-, DM- and HP-β-CYDs, respectively.

No significant difference was found between the various parameters calculated for the pulmonary absorption of β- and DM-β-CYD, i.e., a rapid peak plasma concentration following i.t. instillation was achieved in all the animals and at each sampling point there was little variation in concentration for the same CYD. The slight shift to the right of the β-CYD plasma concentration curve (reflected by the longer t_{max}) may be due to dissolution of the suspension. The HP-β-CYD showed different behaviour, i.e. an initial lag phase prior to attainment of C_{max} which resulted in a greatly extended t_{max} and a more prolonged post-peak decline.

Recently, DM-β-CYD has been shown to be an absorption enhancer for nasal delivery of 17β-oestradiol and progesterone (Hermens et al., 1990; Schipper et al., 1990). It was proposed that DM-β-CYD may exert direct effects on epithelial membranes, namely GI mucosa, rectal and percutaneous administration (Uekama and Otagiri, 1987). These observations may account for the rapid absorption peak for DM-β-CYD when given intratracheally.

There were no significant differences in bioavailable fraction (F) between the CYDs: β-, 66%; DM-β-, 74%; and HP-β-CYD, 80% (Table 2). These availabilities are considerably higher than observed for β-CYD after oral dosing (approx. 1%) to rats (Frijlink et al., 1990).

The terminal half-life ($t_{1/2,z}$) of each of the three CYDs was calculated from the terminal linear phase of the plasma concentration-time curves and is listed in Tables 1 and 2. A two-compartment disposition model was used for i.v. and a one-compartment model for i.t. analysis. There were no significant differences between the i.t. and i.v. routes for $t_{1/2,z}$ of β- and DM-β-CYD whereas for HP-β-CYD, $t_{1/2,z}$ was longer after i.t. dosing. This observation, however, needs to be interpreted with care, since the assay detection limit resulted in a shorter time interval being used for half-life determination after i.v. dosing. The absorption kinetics of HP-β-CYD were very different from those of the other CYDs and could not be described by a simple first- or zero-order process. The lag time observed may be associated with oligomer formation.

An MAT of nearly 2 h for HP-β-CYD makes it the best candidate for achieving 'sustained' release in the lung. It may also prove useful in controlling the thermodynamic activity of some drugs such as peptides known to self-associate, in order to optimise their absorption profile and bioavailability.

Conclusion

The i.v. kinetic parameters for all the CYDs are very similar. When given by the i.t. route the bioavailability (F) is the same for all the CYDs, however, the absorption kinetics of HP-β-CYD were very different as compared to the other CYDs and could not be described by a simple first- or zero-order process. It is absorbed more slowly than the other CYDs, as shown by its MAT.

From these results we may conclude that of the three CYDs tested, HP-β-CYD due to its lower absorption rate appears to have the best

potential as a carrier for sustaining pulmonary release.

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