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Report

The Pharmacokinetics of β-Cyclodextrin and Hydroxypropyl-β-cyclodextrin in the Rat

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Hydroxypropyl-β-cyclodextrin was analyzed by HPLC using postcolumn complexation with phenolphthalein and negative colorimetric detection, with a detection limit of 20 μg/ml. The pharmacokinetics of β-cyclodextrin and of hydroxypropyl-β-cyclodextrin were studied after intravenous administration to permanently cannulated rats. The pharmacokinetic behavior of both cyclodextrins was similar to that of inulin, showing rapid distribution over extracellular fluids. Elimination occurred through glomerular filtration. When a dose of 200 mg/kg β-cyclodextrin was administered the elimination rate was decreased, probably as a result of nephrotoxicity of β-cyclodextrin. Within 24 hr after administration most of the cyclodextrin dose was recovered unchanged in urine. After oral administration, only insignificant amounts of intact β-cyclodextrin were absorbed from the gastrointestinal tract.

KEY WORDS: β-cyclodextrin; hydroxypropyl-β-cyclodextrin; pharmacokinetics; absorption; intravenous administration; oral administration.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides, known for their ability to form inclusion complexes with many lipophilic drugs, thereby changing the physicopharmaceutical properties of these drugs. Complexation may increase aqueous solubility and bioavailability, improve stability, and affect the drug's effects (1,2). Increased drug solubility suggested the application of cyclodextrins and their derivatives in parenteral dosage forms (3). Studies of the effects of β-cyclodextrin on the pharmacokinetics of several barbiturates after intravenous and intraperitoneal administration to mice (4–6) showed that tissue distribution and drug effect were changed by complexation. On the other hand, Arimori and Uekama (7) reported that the pharmacokinetic behavior of prednisolone, administered intravenously and intramuscularly as a solution to rabbits, was not changed by the complexation with β- or γ-cyclodextrin. Similarly, Brewster *et al.* (8) found that the brain concentration and biological response of intravenously administered estradiol-dihydropyridine ester was not changed by hydroxypropyl-β-cyclodextrin complexation. β-Cyclodextrin was shown to decrease the local irritation caused by intramuscular injection of chlorpromazine (9,10). Further, a reduction of vitamin A toxicity was suggested by the successful treatment of a 2-year-old boy suffering from a life-threatening hypervitaminosis A with an infusion of 2-hydroxypropyl-β-cyclodextrin (11). Several years ago a soluble powder for injections of prostaglandin-E_1 stabilized by α-cyclodextrin has been introduced on the market in Japan.

Among the few studies on cyclodextrin pharmacokinetics Szabo *et al.* (12) found in a preliminary study that, after intravenous administration of dimethyl-β-cyclodextrin to rabbits, the plasma level decreased rapidly within 1–2 hr and, after intramuscular injection, the substance was completely excreted into urine within 24 hr. The pharmacokinetic behavior of ^{14}C-β-cyclodextrin administered orally to rats was described by Gerloczy *et al.* (13); but their analysis did not separate the parent cyclodextrin from metabolites, resulting from metabolism of cyclodextrins by microorganisms from the colon flora.

The previously reported HPLC assay for β-cyclodextrin (14) using negative colorimetric detection with postcolumn phenolphthalein complexation was modified for the analysis of hydroxypropyl-β-cyclodextrin. Further, the pharmacokinetic behavior of β-cyclodextrin and of hydroxypropyl-β-cyclodextrin after intravenous administration to rats and the absorption behavior of orally administered β-cyclodextrin are described.

MATERIALS AND METHODS

Materials

β-Cyclodextrin was kindly supplied by AVEBE, Veendam, The Netherlands. The 2-hydroxypropyl-β-cyclodextrin was a gift from Prof. Szejtli, Chinoin, Budapest, Hungary.

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The average molar degree of substitution was 2.7. Inulin was obtained from Merck, Darmstadt, F.R.G. The physiological saline used to prepare the injections was sterile and pyrogen free. Heparin was obtained from Leo Pharmaceutical Products, Weesp, The Netherlands. Lyophilized glucose-oxidase (250 U/mg) was obtained from Boehringer Mannheim, F.R.G. All other chemicals used were of analytical grade.

Instruments

The UV/Vis absorption measurements were performed on a Philips PU 8720 UV/VIS scanning spectrophotometer. The HPLC system consisted of two Waters 510 pumps (Waters Associates, Milford, MA), a U6K injector, and a Model 441 absorbance detector. A capillary tubing of 1.5 m (1.0-mm i.d., 1/16-in. o.d.) was used for mixing the column eluate with the postcolumn reagent.

β-Cyclodextrin Analysis

The analysis of β-cyclodextrin in plasma and urine was performed as described in a previous paper (14). To 100 μl plasma 150 μl of a trichloroacetic acid solution (6.7%) was added. After mixing and centrifugation at 1800*g*, 50 μl of 1 *M* sodium hydroxide solution was added to the clear supernatant. Of this solution, 170 μl was injected onto the HPLC column. The analytical column was a μBondapak Phenyl column (Waters Associates; mean particle diameter, 10 μm; 300 × 3.9-mm i.d.), used with a Chrompack R P guard column (75 mm × 2.1-mm i.d.). The column eluent was methanol:water (10:90), with a flow rate of 2.0 ml/min. The postcolumn reagent was 0.008 *M* sodium carbonate and 6×10^{-5} *M* phenolphthalein in water, also used at a flow rate of 2.0 ml/min. The capillary tubing was used for the mixing of postcolumn reagent and column eluate. The effluent was monitored at 546 nm.

Hydroxypropyl-β-cyclodextrin Analysis

The assay procedure was the same as for β-cyclodextrin, except for the chromatographic system. The analytical column was a μBondapak Phenyl column (Waters Associates; mean particle diameter, 10 μm; 150 × 3.9 mm i.d.). The column eluent was acetonitrile:water (5:95).

Inulin analysis

Plasma and urine samples containing inulin were analyzed according to the method of Heyrovsky (15) with some slight modifications. When necessary the samples were diluted with water. To 200 μl of sample, 200 μl of a freshly prepared glucose-oxidase solution in water (3.3 mg/ml) was added. The solution was mixed and incubated at 37°C for 1 hr. Next 200 μl of a trichloroacetic acid solution (20% in water) was added. After mixing and centrifugation (1800*g*), 200 μl of a indole-3-acetic acid solution in ethanol 96% (5 mg/ml) was added to 400 μl of the clear supernatant. After mixing and adding 4.0 ml hydrochloric acid (36%), the samples were incubated at 37°C for 3 hr. Subsequently, the absorption at 520 nm of the solution was measured. A calibration curve of plasma or urine samples, spiked with 0, 10, 25, 50, 100, and 250 μg/ml inulin, was measured simultaneously. The regression parameters from this line were used to calculate the inulin concentrations.

In Vivo Study Design

Intravenous Administration

Male Wistar rats (390–480 g) were used. The jugular vein of the rats was permanently cannulated according to the method described by Steffens (16). The rats were operated at least 1 week before the experiments. After attachment of the sampling tube the rats were placed in metabolic cages. The amounts of cyclodextrin or inulin to be administered were dissolved in 1.0 ml saline, except for the 200 mg/kg, for which 1.5 ml was used. The solutions were administered intravenously through the cannula. Blood samples of 250 μl were taken 1 hr before administration, the others at appropriate times after administration. The last three samples in the inulin experiment had a volume of 400 μl. After collection the blood samples were immediately placed in ice. Heparin was used as anticoagulant. Plasma samples were prepared by centrifugation (1800*g*). Urine samples were collected in a vessel placed in ice, over two periods, 0–24 and 24–48 hr after administration.

Oral Administration

In a pilot study two permanently cannulated male rats (400 and 410 g) were starved 16 hr prior to the experiment. They received drinking water ad libitum before and during the experiment. Both animals received 100 mg β-cyclodextrin dissolved in 5 ml water (37°C), through a stomach catheter. Blood and urine sampling was performed as described above. An additional study was performed with eight female Wistar rats (160–180 g). For every dose four of these rats were starved 16 hr previous to the experiment, and four had free access to food. All animals received drinking water ad libitum. Doses of 50, 100, and 150 mg β-cyclodextrin dissolved in 5 ml water (37°C) were administered through a polyethylene stomach catheter. The rats were placed in metabolic cages and urine was collected over the first 24 hr after administration.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic calculations were performed using the computer program Rugfit (17), an iterative least-squares regression analysis program, fitting the experimental data to equations with up to five exponential terms. The amounts absorbed after oral administration were compared using Student's *t* test. Differences were considered to be significant if $P < 0.05$.

RESULTS AND DISCUSSION

Analysis of Hydroxypropyl-β-cyclodextrin

The previously described assay of β-cyclodextrin using postcolumn complexation with phenolphthalein was applicable to hydroxypropyl-β-cyclodextrin, because it also decolorizes phenolphthalein upon complexation. However, hydroxypropyl-β-cyclodextrin is, unlike β-cyclodextrin, not a homogeneous substance but a mixture of β-cyclodextrin

derivatives, differently substituted with hydroxypropyl groups. Since these molecules have a range of retention times over approximately 16 min (Fig. 1), the detection limit was only 20 μg/ml. While the highest peak was used for quantification, the chromatographic elution pattern served to check for any changes in composition. The analysis was linear over the concentration range 20–500 μg/ml.

The use of a size-exclusion chromatographic column may circumvent the above-described problem, since with a Shim-Pack Diol-150 column hydroxypropyl-β-cyclodextrin is eluted as one peak (18).

Intravenous Administration

After intravenous administration of different β-cyclodextrin doses a rapid decrease in plasma concentration is observed to below the detection limit after 3 hr (Fig. 2). In a previous paper (14) a much slower elimination of β-cyclodextrin was reported (half-life, 114 vs 25 min) after a dose of 25 mg/kg, possibly as a result of different animal procedures. In the previous study the rats underwent surgery (cannulation of the carotid artery) immediately before the experiment and they were kept under general anesthesia during the experiment. The stress of anesthesia and surgery could affect the renal blood flow and, thereby, the elimination of β-cyclodextrin. It was therefore decided to use the permanently cannulated rat model for further experiments. In this model the rats are allowed to recover completely after placing the cannulas and no anesthesia is necessary during the experiments. Further, large rats were used since the frequent blood sampling in the beginning of the experiments will interfere less with normal renal physiology than in the case of relatively small rats.

The plasma levels found after administration of hydroxypropyl-β-cyclodextrin are given in Fig. 3. A dose of

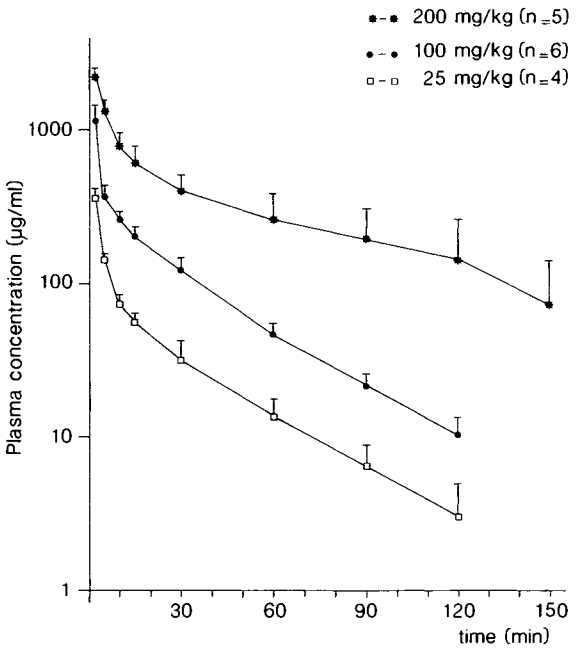

Fig. 2. Plasma disappearance curves after intravenous administration of β-cyclodextrin. Mean ± SD.

25 mg/kg was not administered for this substance, since plasma levels would rapidly fall below detection limits. The resultant pharmacokinetic parameters (Table I), indicate that linear kinetics are found for the 25 and 100 mg/kg β-cyclodextrin doses and for both hydroxypropyl-β-cyclodextrin doses. However, for the 200 mg/kg β-cyclodextrin dose the plasma clearance is decreased. Most of the β-cyclodextrin dose was recovered unchanged in the urine (Table I). The hydroxypropyl-β-cyclodextrin was also almost completely

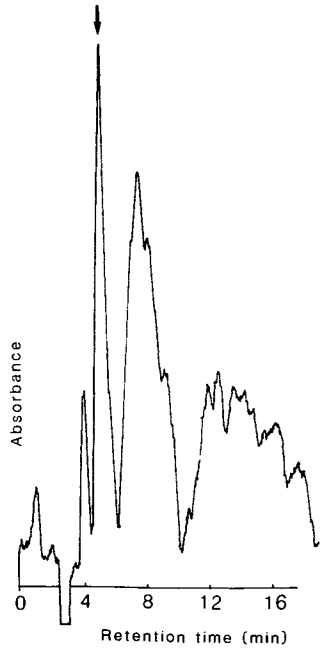

Fig. 1. Representative HPLC chromatogram of hydroxypropyl-β-cyclodextrin (250 μg/ml). The arrow indicates the peak measured.

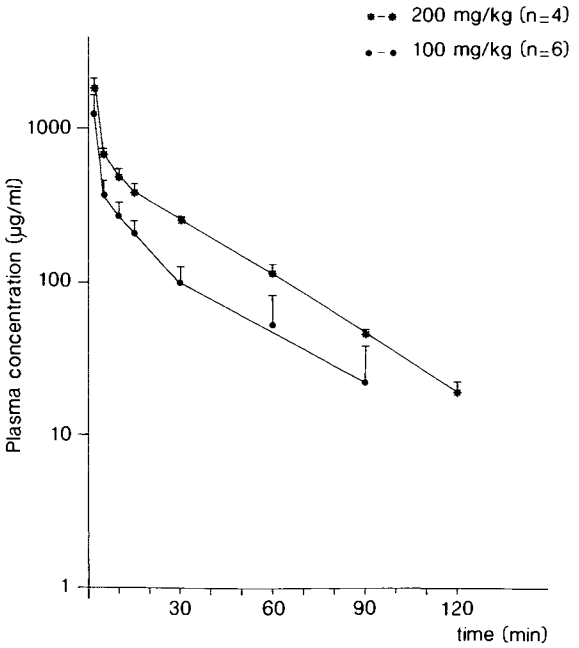

Fig. 3. Plasma disappearance curves after intravenous administration of hydroxypropyl-β-cyclodextrin. Mean ± SD.

Table I. Pharmacokinetic Parameters of β-Cyclodextrin (β-cd), Hydroxypropyl-β-cyclodextrin (hp-β-cd), and Inulin After Intravenous Administration

	β-cd			hp-β-cd		inulin
Dose (mg/kg)	25	100	200	100	200	100
$t_{1/2}\alpha$ (min)	1.5	1.5	2.9	1.4	1.0	1.5
$t_{1/2}\beta$ (min)	25.5	23.9	50.2	23.5	23.9	22.4
C_0 (μg/ml)	737	2150	3180	2520	4790	2670
C_1 (μg/ml)	666	1850	2520	2280	4160	2340
C_2 (μg/ml)	71	302	658	298	625	332
V_{ss} (ml/kg)	152	176	173	166	194	145
V_1 (ml/kg)	34	46	63	39	42	37
V_2 (ml/kg)	118	130	142	127	152	107
AUC (min · μg/ml)	4050	14800	58900	14600	27900	15700
CL (ml/min/kg)	6.2	6.2	3.4	6.9	7.2	6.4
Urine recovery (%)	96 ± 4	88 ± 7	95 ± 3	96 ± 10	98 ± 6	105 ± 3

recovered in urine and the elution pattern of the chromatogram from the urine sample was identical to that of the injected hydroxypropyl-β-cyclodextrin. These results suggest that no significant metabolism of intravenously administered cyclodextrins occurs.

Cyclodextrins are large hydrophylic molecules which are thought to be unable to pass across biological membranes (19,20), limiting the total volume of distribution to the extracellular fluid. Nearly all of the cyclodextrin administered was recovered unchanged in urine within 24 hr (Table I), while 24 hr after administration cyclodextrin was no longer detectable in urine, indicating that elimination occurs by glomerular filtration. To test these two hypotheses the pharmacokinetics of a 100 mg/kg intravenous dose of inulin were also determined. Inulin is, like cyclodextrins, a polysaccharide, composed of fructose units. The substance is distributed over the total extracellular volume and is eliminated solely by glomerular filtration (21). The plasma concentrations found after intravenous administration of inulin are presented in Fig. 4 and the pharmacokinetic parameters are given in Table I. As expected the pharmacokinetic behavior of cyclodextrins and inulin is similar, suggesting that the cyclodextrins are also distributed over the extracellular fluids and eliminated by glomerular filtration.

The 200 mg/kg dose of β-cyclodextrin leads to nonlinear elimination. Since glomerular filtration is expected not to be dose dependent, this high dose may have affected renal blood flow or cellular function of the kidney. Indeed severe signs of nephrotoxicity were found after parenteral administration of high doses of cyclodextrins (19,22,23). This nephrotoxicity could lead to a decrease in glomerular filtration rate, causing the observed decrease in elimination rate. This is in good agreement with the finding that after the 200 mg/kg dose the disappearance pattern of hydroxypropyl-β-cyclodextrin showed no deviation from linearity since this substance is much less nephrotoxic (2,24).

Oral Administration

The pilot experiment showed that plasma levels of β-cyclodextrin could be detected only between 10 and 60 min after administration and were near the detection limit (2–11 μg/ml). In fact the found plasma levels did not allow a reliable estimation of the bioavailability. Because most of the cyclodextrin administered intravenously was recovered in urine, the amount of β-cyclodextrin excreted into the urine during the first 24 hr after administration was taken as the amount absorbed from the gastrointestinal tract (Table II). The fractions absorbed are small and variable. A statistically significant difference between the results obtained with the starved and those with the fed rats could not be demonstrated. The results indicate an increase in gastrointestinal absorption up to the 100-mg dose, but no further increase is observed for the 150-mg dose. Gerloczy *et al.* (13) also found that probably a very small fraction of β-cyclodextrin was absorbed after oral administration. But they had

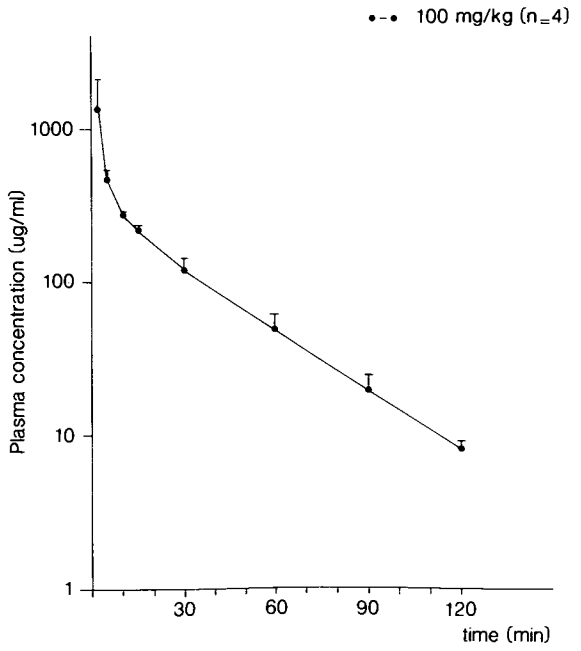

Fig. 4. Plasma disappearance curves after intravenous administration of inulin. Mean ± SD.

Table II. Amounts of β-Cyclodextrin Absorbed After Oral Administration: Mean ± SD (n = 4)

Dose (mg)	Amount absorbed (μg)	
	Starved	Nonstarved
50	460 ± 192 (0.92%)[a]	490 ± 285 (0.98%)
100	1090 ± 430 (1.09%)	1068 ± 372 (1.06%)
150	1096 ± 301 (0.73%)	996 ± 99 (0.64%)

[a] In parentheses the relative amount of the dose absorbed is presented.

difficulties in separating metabolites from β-cyclodextrin. In this study, this small absorption could be confirmed, since the analysis used is very selective (14).

The observed maximum in the gastrointestinal absorption of β-cyclodextrin could explain the very low toxicity of orally administered β-cyclodextrin (the oral LD_{50} for rats is more than 12.5 g/kg) (19,25). The mechanism through which absorption occurs is not completely understood. Szabo *et al.* (12) showed that the absorption of β-cyclodextrin was a passive transport and Irie *et al.* (26) suggested that the paracellular pathway might be the main route of absorption. Another mechanism could be fluid phase endocytosis of the mucosal cells in the gastrointestinal tract.

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