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 phenthraline 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 insulin, 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 pharmaceutical 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 E1 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 14C-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, Veen-dam, 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., \(\frac{1}{8}\)-in. o.d.) was used for mixing the column eluate with the postcolumn reagent.

\(\beta\)-Cyclodextrin Analysis

The analysis of \(\beta\)-cyclodextrin in plasma and urine was performed as described in a previous paper (14). To 100 \(\mu\)l plasma 150 \(\mu\)l of a trichloroacetic acid solution (6.7%) was added. After mixing and centrifugation at 1800g, 50 \(\mu\)l of 1 M sodium hydroxide solution was added to the clear supernatant. Of this solution, 170 \(\mu\)l was injected onto the HPLC column. The analytical column was a \(\mu\)Bondapak Phenyl column (Waters Associates; mean particle diameter, 10 \(\mu\)m; 300 \(\times\) 3.9-mm i.d.), used with a Chrompack R P guard column (75 mm \(\times\) 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 \(\times\) 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-\(\beta\)-cyclodextrin Analysis

The assay procedure was the same as for \(\beta\)-cyclodextrin, except for the chromatographic system. The analytical column was a \(\mu\)Bondapak Phenyl column (Waters Associates; mean particle diameter, 10 \(\mu\)m; 150 \(\times\) 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 \(\mu\)l of sample, 200 \(\mu\)l of a freshly prepared glucose-oxidase solution in water (3.3 mg/ml) was added. The solution was mixed and incubated at 37\(^\circ\)C for 1 hr. Next 200 \(\mu\)l of a trichloroacetic acid solution (20% in water) was added. After mixing and centrifugation (1800g), 200 \(\mu\)l of a indole-3-acetic acid solution in ethanol 96% (5 mg/ml) was added to 400 \(\mu\)l of the clear supernatant. After mixing and adding 4.0 ml hydrochloric acid (36%), the samples were incubated at 37\(^\circ\)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 \(\mu\)g/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 \(\mu\)l 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 \(\mu\)l. After collection the blood samples were immediately placed in ice. Heparin was used as anticoagulant. Plasma samples were prepared by centrifugation (1800g). 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 \(\beta\)-cyclodextrin dissolved in 5 ml water (37\(^\circ\)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 prior to the experiment, and four had free access to food. All animals received drinking water ad libitum. Doses of 50, 100, and 150 mg \(\beta\)-cyclodextrin dissolved in 5 ml water (37\(^\circ\)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 Rugit (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-\(\beta\)-cyclodextrin

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