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An intravenous toxicity study of 2-hydroxypropyl-β-cyclodextrin, a useful drug solubilizer, in rats and monkeys

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Summary

The subacute and subchronic intravenous (i.v.) toxicity of 2-hydroxypropyl-β-cyclodextrin (HPCD) was examined in Sprague-Dawley rats and cynomolgus monkeys. After either 14 or 90 days of alternate day dosing with either saline or 200 mg/kg HPCD, no toxicologically meaningful differences were observed in the following parameters: body weight, body weight gain, food consumption, hematology, clinical chemistry, organ weights, organ to brain weight ratio, organ to body weight ratio and macroscopic and microscopic histopathology. Subsequent to these studies, an acute high-dose study was performed in cynomolgus monkeys which indicated that a single i.v. dose of HPCD as high as 10 g/kg was not lethal.

Introduction

The inability to formulate poorly water soluble drugs into appropriate aqueous dosage forms has severely limited the application of many potentially useful agents. Several approaches have been proposed to circumvent this problem including the use of organic co-solvents, emulsions, liposomes and micelles (Davis and Illum, 1986). Unfortunately, all of the above-mentioned techniques have their associated problems. Organic co-solvents are irritating and often toxic and microparticulate formulations may direct drugs to undesirable loci such as the reticuloendothelial system (RES) or lung (Davis et al., 1987; Kirsh et al., 1987; O'Mullane et al., 1987). An attractive alternative to these methods is the use of cyclodextrins.

Cyclodextrins are cyclic amylose-derived oligomers which contain various numbers (α = six, β = seven, γ = eight) of α-1,4-linked glucose units. The number of these units determines the size of a cone-like cavity into which many compounds may include and form stable, water soluble complexes (Uekama, 1981; Szejtli, 1982; Uekama and Otagiri, 1987). Unfortunately, the cyclodextrin which is most useful for incorporating a number of drugs, i.e. β-cyclodextrin (β-CD), is poorly water soluble (1.8 g/100 ml at 25°C). This low aqueous solubil-

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ity of β-CD is associated with cytoplasmic crystals in rat renal tubules and severe nephrosis after parenteral administration (Frank et al., 1976). Several attempts have been made to improve the water solubility and therefore usefulness of β-CD. Alkylation or hydroxyalkylation of the cyclic oligomer disrupts hydrogen bonding which destabilizes the crystal lattice. In addition, these manipulations can transform the crystalline material into amorphous mixtures of isomeric cyclodextrins (Pitha and Pitha, 1984). The amorphous nature of modified β-CD as well as the alkylated moiety contribute to increasing the aqueous solubility of the preparations. Methylated cyclodextrins such as heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DMCD) are potent drug solubilizers but are also relatively lipophilic and possess surface activity causing them to be hemolytic and parenterally toxic (Pitha et al., 1988). The hydroxyalkyl derivative of β-CD, 2-hydroxypropyl-β-cyclodextrin (HPCD), lacks these unwanted physicochemical characteristics, is readily soluble in water (1 g/ml) and has been shown to be a useful drug solubilizer (Pitha, 1988; Yoshida et al., 1988). If, however, this material is to be exploited in i.v. formulations, its safety via this route must be demonstated. This communication examines the toxicity of HPCD in rats after repeated i.v. administration in both subacute and subchronic paradigms.

Materials and Methods

Chemistry

HPCD was prepared according to the method of Pitha (Pitha et al., 1986b). In this procedure, propylene oxide was added to a sodium hydroxide solution of β-CD. The crude material was purified by salt removal and extraction. The degree of substitution was determined by mass spectrometry using a Kratos MS 8ORFA double focusing instrument fitted with a fast atom gun. HPCD was analysed by fast atom bombardment while immobilized in a glycerol matrix. The average degree of substitution was calculated from the average mass which was obtained by dividing the product of the mass and intensities summed over the total number of isomers by the sum of the intensities.

The freeze-dried HPCD was then submitted for microcombustion analysis, residue on ignition and microchemical analyses including: heavy metals, arsenic, bromide and chloride (Schwartzkoph Microanalytical Laboratories, Woodside, NY). *Limulus* amebocyte lysate (LAL) tests were performed by Associates of Cape Cod, Woodshold, MS.

Propylene glycol was detected in cyclodextrin mixture by gas chromatographic analysis using a Chromsorb 101 (80–100 mesh, 146 cm × 2.4 mm) analytical column with a mobile phase of N_2 flowing at 50 cm^3 min (20 $lb/inch^2$). The column was maintained at 210 °C, the injector at 230 °C and the detector at 210 °C. The Gaw-Mac instrument was fitted with a flame ionization detector (H_2, 25 cm^3/min; O_2, 300 cm^3/min). Samples were prepared in water and 2 μl were injected. Solvent content was then determined using an external standard curve which was linear over the concentration range investigated. The retention time was 1.6 min of propylene glycol in this system.

Acetone was quantitated by HPLC in a system consisting of a Spectra-Physics (SP 8780) pump, autosampler (SP 8780), integrator (SP 4290) and an LDC/Milton Roy detector (SpectroMonitor D). Separation was achieved on a Spherisorb C-8, 5 μm (25 cm × 4.6 mm i.d.) analytical column (Alltech, Inc.). The mobile phase consisted of acetonitrile : ammonium acetate buffer (0.05 M, pH = 6) 58 : 42 containing 0.0012 M tetrabutylammonium perchlorate. The flow rate was 1.25 ml/min and the column was operated at 17.5 °C. Acetone was detected at 266 nm and the retention time for acetone in this system was 2.6 min. Samples were dissolved in water and quantitation performed based on an external standard curve. The limit of accurate detection in this system was about 2.2 μg/ml or ~ 0.001% acetone by weight.

Aqueous size exclusion chromatography (ASEC) was performed using an SSI 300 pump (Scientific Systems, Inc.). The column used was a polyglycerol methacrylate gel system (Shodex OH Pak KB803) operating at ambient temperature with an aqueous (100% H_2O) mobile phase and compounds were detected by changes in refractive index of the mobile phase (Spectra Physics SP

8430 RI detector). The retention time for HPCD in this system was 11.6 min, for β-CD, 13.0 min and for propylene glycol. 14.72 min. Unreacted β-CD was quantitated in the HPCD batches using HPLC. In the determinations, a Spherisorb-NH_2 25 cm × 4.6 mm i.d., 5 μm analytical column was used. The mobile phase consisted of aqueous acetonitrile (65 : 35 acetonitrile : H_2O), the flow rate was 1 ml/min and the column operated at ambient temperature. The pump and detector were the same as those used in the ASEC system. The retention time for β-CD in this system was 10.7 min. Standard curves of β-cyclodextrin were prepared in water and samples of 40 mg/ml of HPCD were submitted for analysis.

Animal studies–general

Weanling male and female Crl : CD®(SD)BR albino viral antibody free rats were obtained from Charles River Laboratories, Inc. (Portage, MI) and acclimated for at least 15 days prior to study initiation. During acclimation, animals were examined for overt signs of clinical abnormalities and if any were detected, animals were deleted from the study. All rats were singly housed in standard hanging wire cages in a climate controlled vivarium (temperature maximum range 69–75 °F; relative humidity maximum range, 30–70%; and 12 h light : 12 h dark cycle). Ground Rodent Chow (Purina Mills, Inc.) was provided ad libitum in glass containers which were designed to limit spillage and facilitate estimation of food consumption. Water was partially deionized and UV-sterilized and was provided ad libitum. Body weights at initiation of subacute dosing were 212.3 ± 9.2 g (mean ± SD; males) and 153.7 ± 5.5 g (females). Larger rats were used in the subchronic study after considering the feasiblity of reliable, repeated (46 times) tail vein injections. Body weights at initiation of subchronic dosing were 337.4 ± 8.9 (males) and 235.0 ± 10.4 (females)

Sixteen wild caught cynomolgus monkeys (Hazleton Research Products, Inc., Cumberland, VA) were used in the procedures and were acclimated for at least 11 weeks prior to study. All animals were conditioned and housed individually in stainless steel cages in an environmentally controlled vivarium (temperature range, 67–79 °F; relative humidity range, 30–70%; 12 h light : 12 h dark cycle). Animals were provided with Primate Chow (Purina) and tap water ad libitum. Body weights at initiation of the subchronic study were 3.22 ± 0.39 kg (mean ± SD) and 2.73 ± 0.33 kg for males and females, respectively.

Subacute study

Two groups of 10 Sprague-Dawley rats (five males and five females) received bolus injections of (via the tail vein) either sterile normal saline (1 ml/kg) or a 20% w/v solution of HPCD in sterile water (200 mg/kg per ml) every second day for 14 days. This dosing schedule was selected to complement pharmacokinetic studies which characterized drug-HPCD complexes (Brewster et al., 1988). During the period of compound administration, animals were monitored for overt toxicity and general robustness. Animals were weighed at the end of weeks 1 and 2 and food consumption was monitored during the same period. After the eighth dose (cumulative dose 1.6 g/kg) rats were fasted overnight, anesthetized with ketamine, and blood samples were collected from the retro-orbital plexus; they were then killed and necropsied for macroscopic abnormalities. Selected organs were then removed and weighed. Selected tissues were subsequently fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned and stained for microscopic examination by a veterinary pathologist. Specific parameters examined in this and other studies included: (1) body weight and body weight change, (2) food consumption, (3) hematology including total erythrocyte count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), total leukocyte count (WBC), relative and differential leukocyte count including neutrophils (N-SEG), lymphocytes (L), monocytes (M), eosinophils (E), basophils (B), immature neutrophils (BAND-N) and nucleated red blood cell count (NRBC), (4) clinical chemistry including glucose (GLU), blood urea nitrogen (BUN), alanineaminotransferase (ALT), total protein (TPRO), albumin (ALB), globulin (GLOB), albumin/ globulin ratio (A/G), alkaline phos-

phatase (ALKPHOS), cholesterol (CHOL), triglycerides (TRIG), total bilirubin (T BILI), calcium (Ca), inorganic phosphate (PO_4), aspartate aminotransferase (AST), creatinine (CREAT), sodium (Na), potassium (K) and chloride (Cl), (5) organ weight, organ to brain weight and organ to body weight for brain, heart, liver, kidney, adrenal glands, salivary glands, spleen, thymus, thyroid, testes, prostate, epididymides, ovaries, uterus, and pituitary, (6) macroscopic examination of all orifices; the carcass, cranial and nasal cavities; thoracic, abdominal and pelvic cavities and viscera, the external surface for lesions, (7) microscopic appearance of the following tissues: adrenal glands, aorta, bone, epididymides, esophagus, eyes, heart, intestine, kidneys, liver, lung, lymph nodes, mammary gland, ovaries, pancreas, parathyroids, sciatic nerve, pituitary gland, prostate, salivary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, thyroid gland, tongue, urinary bladder, uterus, vagina and any gross lesions noted in macroscopic examination. Examination of brain included the following: cerebellum/pons, cerebral cortex, hypothalamus, thalamus, arcuate nucleus, median eminence and caudate nucleus.

Subchronic studies

Based on the results from the subacute study, a similar study was undertaken to examine the same parameters identified above after 13 weeks of dosing. Then rats of each sex were treatred via tail vein injection with normal saline (1 ml/kg) or HPCD (200 mg/kg per ml) every second day for 91 days (cumulative dose 9.2 g/kg). This dosing schedule was maintained to complement evaluation of a drug complexed in HPCD. General robustness, body weight and food consumption were monitored at weekly intervals. Other parameters were examined at scheduled animal sacrifice (24 h after the 46th dose).

In a complementary study, 8 cynomolgus monkeys (4 males, 4 females) received bolus injections (saphenous vein) of either sterile normal saline (1 ml/kg) or HPCD (200 mg/kg per ml) every second day for 91 days. Animals were monitored twice daily for cageside obvious indication of toxic effects. Body weight and food consumption was recorded weekly. Animals were fasted overnight and blood samples were collected from the femoral vein before and after 4, 8 and 13 weeks of treatment. Overnight urine samples were also collected at these times. Animals were anesthetized with pentobarbital, exsanguinated and necropsied 24 h after the last dose. The same parameters identified in the subacute study were examined.

Acute high-dose study

A high dose i.v. study was completed in cynomolgus monkeys (4 total, 2 males and 2 females). In this study, animals received a dose of 2 g/kg HPCD in sterile water on day 0 and a dose of 10 g/kg HPCD on day 3. The HPCD was prepared as a 50% (w/v) solution with sterile water and infused via the saphenous vein over a 1-h time period in restrained animals. Animals were monitored for mortality and morbidity through day 7.

Data evaluation

The difference between means for the saline and HPCD treated animals for each sex was evaluated for each of the following data: body weight, food consumption, clinical chemistry and hematology, urine pH and specific gravity; organ weight, organ-to-body weight percentage and organ-to-brain weight ratios.

Levene's test (Levene, 1980) was used to evaluate homogeneity of variance. If heterogeneity of variance at $P < 0.05$ was found, $\log_{10}$, square, reciprocal, angular and rank transformations were used to stabilize variance. Standard one-way analysis of variance was done on the homogeneous or ranked data. Resulting F values exceeding the 5.0% two-tailed probability level are considered statistically significant. Data are shown as arithmetic mean $\pm$SD.

Results

Chemistry

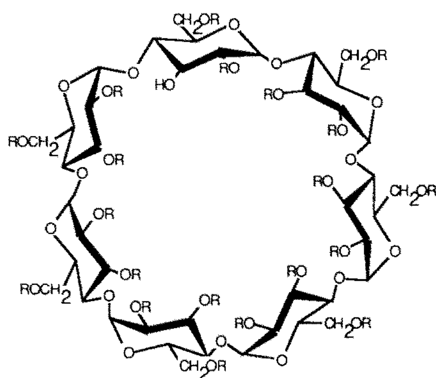
The HPCD mixture used in the toxicity evaluation was prepared by reacting propylene oxide with β-CD which was solubilized in aqueous base.

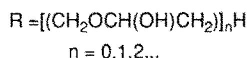
The reaction mixture was neutralized with hydrochloric acid, the resulting salt removed by ion exchange chromatography and the crude product obtained by lyophilization. The powder was then suspended in acetone to remove traces of propylene glycol and polypropylene glycols which formed during the progress of the reaction. The filtered product was then taken up in water, freeze-dried, redissolved in water and refreeze-dried. The material obtained was rigorously characterized by various analytical techniques. Trace quantities of propylene glycol were determined by gas chromatography, unreacted β-cyclodextrin and acetone were quantitated by reversed phase HPLC using refractive index and ultraviolet detection, respectively and polypropylene glycols were detected using aqueous size exclusion chromatography (ASEC). The HPCD mixture was also characterized in terms of its heavy metal, arsenic and halogen content, its residue on ignition, by microcombustion analysis and by its specific rotation. Results of these determinations are given in Table 1.

TABLE 1

Selected information on batch characteristics of HPCD

Structure:





Degree of substitution:		7.0
Residue on ignition:		0.02%
Residual acetone (%):		< 0.01%
Residual propylene glycol (%):		< 0.15%
Unreacted β-cyclodextrin:		< 0.1%
Arsenic (ppm):		< 0.1
Heavy metals (as lead) (ppm):		< 10
Bromide (ppm):		< 5
Chloride (ppm):		< 5
Microcombustion analysis:	C, %:	46.80
	H, %:	7.65
Specific rotation [α]:		130° (589 nm, RT)

The HPCD system is a very complex mixture of literally billions of possible positional and optical isomers similar, in this sense, to hydroxypropyl cellulose and other pharmaceutical starches. Given this, specific information about the content and batch uniformity of the mixture is extremely important. Parameters used to assay HPCD included fast atom bombardment mass spectrometry (FAB-MS) and ASEC. Fig. 1 shows the FAB-MS for the material used in the toxicity evaluation. The isomeric mixture is characterized by a mean degree of substitution of approximately seven around which is a symmetrical distribution of isomers. This pattern is characteristic of a particular batch of HPCD and is useful in confirming batch integrity. Fig. 2 shows the ASEC trace for HPCD. The peak assymetry and retention time produced by a sample are related to the molecular weights of the components and the isomeric distribution and, again, are characteristic for a particular batch. Finally, the HPCD was not pyrogenic as measured by the standard *Limulus* amebocyte lysate (LAL) test for endotoxins. Thus, even though HPCD is a complex mixture, it can be chemically well characterized.

Subacute studies, rats

There were no treatment related abnormalities observed and no deaths occurred during the antemortum phase of the study. No significant difference in body weight or body weight gains (Table 2) were detected between saline and HPCD treated animals; although body weight gain tended to increase faster in male HPCD vs control rats. Similarly, there was no difference in food consumption between the groups (Table 3). Table 4 indicates that there were no significant alterations in hematological parameters between treatment groups or between treatment groups. In addition, there were no differences in clinical chemistry values (Table 5). Organ weight, organ to brain and organ to body weight ratios were not different

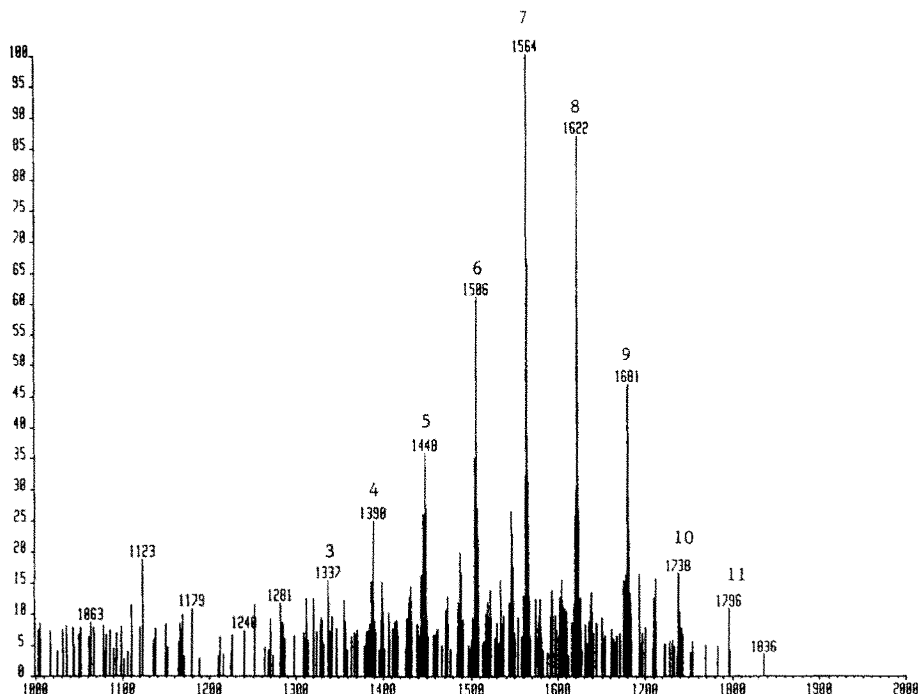


Fig. 1. Fast atom bombardment mass spectrum (FAB-MS) of 2-hydroxypropyl-β-cyclodextrin. The numbers above each mass represent the degree of substitution. The average degree of substitution which is calculated by summing the products of the individual mass and intensities and dividing by the sum of the intensities is 7.014 in this example. The spectrum was obtained by glycerol immobilization of the sample.

between the saline and HPCD treated groups. At necropsy, no macroscopic differences were related to treatment and the injection site was not remarkable. Finally, microscopic examination of the tissues mentioned in the methods section revealed no treatment related effects.

Subchronic studies – rats

There were no deaths or notable cageside observations prior to scheduled animal killing. Body weight, cumulative body weight gain (Table 6) and food intake were not different between saline and HPCD treated groups. The apparent, but not statistically significant increased body weight gain indicated from results of the 2 week study was not seen in 13 weeks of treatment (Tables 2 and 6). Organ weights were not different as shown on Table 7. Statistical evaluation of organ to body weight and organ to brain weight ratios identified only 4 of 70 comparisons as significant. These were all in one group and related. They included a decrease in brain to body weight in females, increased left and right kidney to brain weight in females and increased spleen to brain ratio in females. The toxicological significance of these identified differences is uncertain as these calculated ratios, which differed only 8 to 14% between saline and HPCD groups, may reflect biological variation in the female brain weight. Only one statistically significant difference was found in both sexes for the clinical pathology parameters

TABLE 2

Body weight gain (g) in rats treated i.v. every second day for 14 days with either saline or 200 mg/kg HPCD

Time	Male		Female	
	Saline	HPCD	Saline	HPCD
Week 1	36.8 ± 5.3 [a]	45.7 ± 4.7	16.8 ± 6.6	16.0 ± 5.0
Week 2	36.6 ± 5.0	41.7 ± 5.2	19.9 ± 4.38	18.0 ± 4.2

[a] Mean ± SD (5 animals/group).

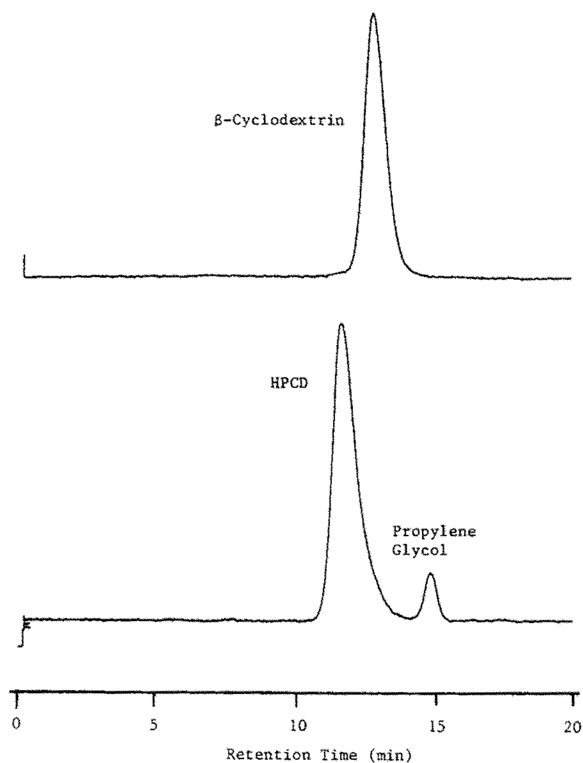


Fig. 2. Aqueous size exclusion chromatography of cyclodextrins. The upper trace is a chromatogram of β-cyclodextrin while the lower trace is a sample of HPCD (average degree of substitution = 7) spiked with a small amount of propylene glycol.

TABLE 3

Weekly food consumption (g) in rats treated i.v. every second day for 14 days with either saline or 200 mg/kg HPCD

Time	Male		Female	
	Saline	HPCD	Saline	HPCD
Week 1	152.3 ± 8.1 [a]	144.8 ± 7.8	97.8 ± 12.5	107.9 ± 10.6
Week 2	151.5 ± 10.8	151.1 ± 5.2	106.4 ± 9.5	112.1 ± 12.5

[a] Mean ± SD (5 animals/group).

examined (Tables 8 and 9). A slight increase in serum creatinine was found in HPCD treated rats. Other identified differences found only in one sex were: decrease in MCV in males, increased WBC and lymphocytes in females and decreased urine pH (6.8 ± 0.4 saline vs 6.2 ± 0.3 HPCD) in females. None of these changes were associated with concurrent changes in related clinical pathology variables, macroscopic or microscopic morphology. The site of injections was unremarkable at necropsy.

Monkeys

There was no difference in the incidence of antemortum observations between treatment groups. Similarly body weight and food consump-

TABLE 4

Hematology results in male and female rats given either saline or 200 mg/kg HPCD i.v. every second day for 14 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
RBC ($\times 10^6$/mm^3)	8.02 ± 0.19 [a]	7.55 ± 0.17	7.67 ± 0.41	7.32 ± 1.00
HGB (g/dl)	15.6 ± 0.41	14.6 ± 0.51	14.8 ± 0.80	13.6 ± 3.06
HCT (%)	50.9 ± 0.7	48.6 ± 1.7	49.2 ± 2.6	47.2 ± 5.6
MCV (fl)	63 ± 1.10	64 ± 2.20	64 ± 1.5	65 ± 2.7
MCH (pg)	19.5 ± 0.1	19.3 ± 0.6	19.3 ± 0.5	18.4 ± 2.4
MCHC (%)	30.6 ± 0.5	30.0 ± 0.4	30.0 ± 0.3	28.6 ± 3.8
PLT ($\times 10^3$/mm^3)	949 ± 56	1020 ± 79	1105 ± 115	924 ± 395
NRBC (/100 WBC)	0 ± 0.4	0 ± 0.0	0 ± 0.0	0 ± 0.0
WBC ($\times 10^3$/mm^3)	8.7 ± 2.2	7.9 ± 1.2	5.1 ± 0.8	7.4 ± 2.6
N-SEG ($\times 10^3$/mm^3)	0.7 ± 0.3	0.6 ± 0.4	0.8 ± 0.3	0.5 ± 0.2
L ($\times 10^3$/mm^3)	7.8 ± 2.1	7.1 ± 1.1	4.2 ± 0.7	6.8 ± 2.4
M ($\times 10^3$/mm^3)	0.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
E ($\times 10^3$/mm^3)	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
B ($\times 10^3$/mm^3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Abbreviations are defined in Materials and Methods.
[a] Mean value ± SD; $n = 5$/group.

TABLE 5

Serum chemistry values for male and female rats given either saline or 200 mg/kg HPCD every second day for 14 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
GLU (mg/dl)	107 ± 17 [a]	94 ± 7	103 ± 12	100 ± 14
TPRO (G/dl)	6.3 ± 0.3	6.3 ± 0.2	6.5 ± 0.1	6.5 ± 0.4
ALB (G/dl)	3.0 ± 0.1	3.0 ± 0.1	3.2 ± 0.1	3.1 ± 0.1
GLOB (G/dl)	3.3 ± 0.3	3.3 ± 0.2	3.3 ± 0.1	3.4 ± 0.3
A/G (ratio)	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
T-BILI (mg/dl)	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
CHOL (mg/dl)	36 ± 16	33 ± 7	48 ± 12	52 ± 19
TRIG (mg/dl)	56 ± 34	57 ± 17	41 ± 11	35 ± 12
AST (IU/l)	109 ± 32.5	124 ± 32.5	102 ± 24.3	102 ± 162
ALT (IU/l)	22 ± 4.1	24 ± 4.1	19 ± 2.7	21 ± 2.3
ALKPHOS (IU/l)	127 ± 15	137 ± 26	100 ± 18	109 ± 16
BUN (mg/dl)	11.2 ± 1.1	11.2 ± 0.9	11.5 ± 0.4	13.2 ± 2.5
CREAT (mg/dl)	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1
Ca (mg/dl)	10.0 ± 0.4	9.8 ± 0.3	9.9 ± 0.3	10.1 ± 0.8
PO_4 (mg/dl)	9.4 ± 0.7	9.7 ± 0.3	8.3 ± 0.9	8.3 ± 0.8
Na (mmol/l)	142 ± 1.2	142 ± 1.5	142 ± 0.8	141 ± 1.1
K (mmol/l)	4.4 ± 0.3	4.7 ± 0.2	4.5 ± 0.4	4.5 ± 0.2
Cl (mmol/l)	105 ± 4	106 ± 2	108 ± 2	107 ± 3

Abbreviations are defined in Materials and Methods.
[a] Mean value ±SD; $n = 5$/group.

tion were not different and remained stable over the treatment period. Results of hematology and serum chemistry parameters evaluated from blood samples obtained after 13 weeks of dosing are shown on Tables 10 and 11, respectively. Significant changes were increased MCV and MCH and decreased Na in male monkeys and increased Cl in females. The toxicological significance of these differences is less clear as male monkeys assigned to the HPCD treatment group had significantly increased MCV and lower K prior to treatment while females assigned to HPCD treatment had significantly increased Na and Cl before dosing. Further, clinical pathology parameters monitored after 5 and 9 weeks of dosing did not indicate consistent significant difference between saline and HPCD treated monkeys for any of the variables.

TABLE 6

Cumulative body weight gain (g) in rats treated i.v. every second day for 91 days with either saline or 200 mg/kg HPCD

Treatment week	Male		Female	
	Saline	HPCD	Saline	HPCD
1	32.7 ± 6.0 [a]	33.9 ± 7.4	9.7 ± 9.8	14.4 ± 15.5
4	112.7 ± 16.4	115.9 ± 14.2	27.4 ± 11.0	32.8 ± 13.2
8	183.7 ± 30.2	193.7 ± 25.4	50.7 ± 15.8	57.6 ± 12.9
13	214.3 ± 29.9	224.4 ± 32.0	65.3 ± 11.6	77.7 ± 19.3

[a] Mean ±SD; $n = 10$/group.

Significant changes in organ weight, organ to body weight and organ to brain weight ratios were found in only 4 of the 110 monitored parameters. Statistically significant increases were found in uterine weight (saline, 5.7 ± 1.4 g vs HPCD, 8.8 ± 1.9 g), uterine to body weight and uterine to brain weight ratios as well as the right (but not left) salivary gland to body weight ratio. It is unlikely that these differences are toxicologically significant as uterine weights are known to vary considerably in wild-caught cynomolgus monkeys and normal biological variation in this relatively small ($n = 4$) group size may account for the differences. Morphological observations at necropsy and histological examination were comparable. All observa-

TABLE 7

Organ weights for male and female rats given either saline or 200 mg / kg HPCD i.v. every second day for 91 days

Organ	Males		Females	
	Saline	HPCD	Saline	HPCD
Brain (g)	2.13 ± 0.07 [a]	2.11 ± 0.11	2.00 ± 0.07	1.91 ± 0.07
Heart (g)	1.50 ± 0.16	1.55 ± 0.09	0.97 ± 0.14	1.00 ± 0.06
Liver (g)	13.5 ± 1.9	14.1 ± 1.4	7.3 ± 1.0	7.6 ± 0.8
Rat kidney (g)	1.66 ± 0.18	1.74 ± 0.14	0.93 ± 0.08	1.01 ± 0.09
Rat adrenal (mg)	27.6 ± 3.9	27.3 ± 4.4	30.1 ± 4.6	31.0 ± 4.3
Spleen (mg)	784 ± 135	822 ± 86	416 ± 75	473 ± 54
Thymus (mg)	345 ± 124	315 ± 113	264 ± 75	240 ± 89
Rat thyroid (mg)	13.4 ± 2.0	13.9 ± 3.4	10.7 ± 4.0	10.6 ± 2.0
Rat salivary Gland (mg)	433 ± 51	449 ± 50	277 ± 27	280 ± 23
Rat testes (g)	1.70 ± 0.12	1.77 ± 0.22	–	–
Prostate (g)	1.23 ± 0.31	1.11 ± 0.27	–	–
Rat epididymes (mg)	760 ± 52	754 ± 65	–	–
Rat ovary (mg)	–	–	62 ± 7	57 ± 10
Uterus (mg)	–	–	794 ± 296	711 ± 110
Pituitary (mg)	11.5 ± 2.4	10.2 ± 3.0	16.8 ± 6.3	17.9 ± 4.3
Body wt. (g)	517.6 ± 36.4	529.4 ± 30.4	275.4 ± 14.3	290.5 ± 22.9

[a] Mean ± SD; $n = 10$ animals/group.

tions were common in wild-caught monkeys. No injection site irritation was evident macroscopically.

Acute high-dose study

There was no mortality in this study and, with the exception of some hematuria which occurred

TABLE 8

Hematology results in male and female rats given either saline or 200 mg / kg HPCD i.v. every second day for 91 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
RBC ($\times 10^6$/mm^3)	9.16 ± 0.20 [a]	9.49 ± 0.70	8.53 ± 0.70	8.20 ± 0.59
HGB (g/dl)	14.6 ± 0.59	14.8 ± 1.10	14.2 ± 1.0	13.6 ± 0.9
HCT (%)	49.6 ± 1.7	49.6 ± 3.3	48.4 ± 3.0	46.9 ± 2.9
MCV (fl)	54 ± 2	52 ± 1 [b]	57 ± 1	57 ± 2
MCH (pg)	16.0 ± 0.6	15.6 ± 0.6	16.7 ± 0.5	16.7 ± 0.5
MCHC (%)	29.5 ± 0.6	29.8 ± 0.7	29.4 ± 0.7	29.1 ± 0.9
PLT ($\times 10^3$/mm^3)	1037 ± 96	998 ± 108	891 ± 95	908 ± 227
NRBC (/100 WBC)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
WBC ($\times 10^3$/mm^3)	10.3 ± 3.1	9.8 ± 2.4	4.3 ± 1.3	5.7 ± 0.8 [b]
N-SEG ($\times 10^3$/mm^3)	1.6 ± 0.6	1.2 ± 0.5	0.7 ± 0.2	0.9 ± 0.5
L ($\times 10^3$/mm^3)	8.4 ± 3.1	8.2 ± 2.2	3.5 ± 1.2	4.7 ± 0.7 [b]
M ($\times 10^3$/mm^3)	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
E ($\times 10^3$/mm^3)	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
B ($\times 10^3$/mm^3)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.0 ± 0.0

Abbreviations are defined in Materials and Methods.

[a] Mean value ± SD; $n = 10$ animals/group.

[b] Significantly different from saline-treated group $P < 0.05$.

TABLE 9

Serum chemistry values for male and female rats given either saline or 200 mg / kg HPCD every second day for 91 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
GLU (mg/dl)	112 ± 15 [a]	119 ± 13	108 ± 11	105 ± 9
TPRO (G/dl)	7.0 ± 0.4	7.4 ± 0.4	8.2 ± 0.7	8.1 ± 0.7
ALB (G/dl)	3.7 ± 0.5	3.6 ± 0.3	4.1 ± 0.6	3.8 ± 0.5
GLOB (G/dl)	3.3 ± 0.3	3.6 ± 0.4	4.0 ± 0.7	4.3 ± 0.8
A/G (ratio)	1.2 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	0.9 ± 0.3
T-BILI (mg/dl)	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
CHOL (mg/dl)	40 ± 9	35 ± 6	52 ± 13	54 ± 12
TRIG (mg/dl)	59 ± 27	59 ± 24	66 ± 35	77 ± 47
AST (IU/l)	123 ± 26	105 ± 19	118 ± 36	99 ± 29
ALT (IU/l)	35 ± 10	31 ± 5	40 ± 19	33 ± 7
ALKPHOS (IU/l)	78 ± 13	8.5 ± 21	42 ± 12	36 ± 12
BUN (mg/dl)	15 ± 2	15 ± 2	16 ± 2	15 ± 2
CREAT (mg/dl)	0.9 ± 0.1	1.0 ± 0.1 [b]	0.9 ± 0.0	1.0 ± 0.1 [b]
Ca (mg/dl)	10.5 ± 0.6	10.8 ± 0.4	10.9 ± 0.5	10.8 ± 0.7
PO_4 (mg/dl)	5.7 ± 0.8	6.2 ± 1.1	4.8 ± 0.9	5.4 ± 0.9
Na (mmol/l)	140 ± 40	140 ± 2	141 ± 2	141 ± 2
K (mmol/l)	4.7 ± 0.2	4.5 ± 0.2	4.2 ± 0.2	4.1 ± 0.3
Cl (mmol/l)	102 ± 3	101 ± 2	102 ± 2	101 ± 2

Abbreviations are defined in Materials and Methods.
[a] Mean value ± SD; $n = 10$ animals/group.
[b] Significantly different from saline-treated group $P < 0.05$.

TABLE 10

Hematology results in male and female monkeys given either saline or 200 mg / kg HPCD i.v. every second day for 91 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
RBC ($\times 10^6$/mm^3	6.95 ± 0.49 [a]	6.45 ± 1.00	7.00 ± 0.25	6.51 ± 0.50
HGB (g/dl)	12.3 ± 0.9	12.7 ± 1.6	12.2 ± 0.7	11.8 ± 0.8
HCT (%)	42.5 ± 3.8	44.4 ± 5.8	41.3 ± 2.3	41.2 ± 2.4
MCV (fl)	61 ± 2	69 ± 2 [b]	59 ± 3	63 ± 4
MCH (pg)	17.7 ± 3	19.8 ± 1.1 [b]	17.5 ± 0.8	18.2 ± 1.5
MCHC (%)	29.0 ± 0.6	28.6 ± 0.8	29.7 ± 1.1	28.7 ± 0.5
PLT ($\times 10^3$ mm^3)	498 ± 81	424 ± 93	412 ± 44	506 ± 105
NRBC (/100 WBC)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
WBC ($\times 10^3$/mm^3)	14.7 ± 1.8	12.7 ± 4.5	8.9 ± 16	8.2 ± 1.3
N-SEG ($\times 10$ mm^3)	4.7 ± 0.7	4.6 ± 2.8	2.9 ± 1.6	2.4 ± 1.1
L ($\times 10^3$/mm^3)	9.2 ± 1.7	7.4 ± 1.4	5.2 ± 0.6	5.3 ± 0.9
M ($\times 10^3$/mm^3)	0.6 ± 0.7	0.3 ± 0.3	0.2 ± 0.1	0.2 ± 0.1
E ($\times 10^3$/mm^3)	0.2 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	0.2 ± 0.2
B ($\times 10^3$/mm^3)	0.1 ± 0.1	0.0 ± 0.1	0 ± 0.0	0 ± 0.0

Abbreviations are defined in Materials and Methods.
[a] Mean value ± SD; $n = 4$ animals/group.
[b] Significantly different from saline-treated group $P < 0.05$.

TABLE 11

Serum chemistry values in male and female monkeys given either saline or 200 mg / kg HPCD i.v. every second day for 91 days

Parameters	Males		Females	
	Saline	HPCD	Saline	HPCD
GLU (mg/dl)	94 ± 8 [a]	83 ±13	86 ± 13	89 ±13
TPRO (g/dl)	9.1 ± 0.2	8.8 ± 0.6	9.2 ± 0.3	9.0 ± 0.7
ALB (g/dl)	4.6 ± 0.4	4.8 ± 0.3	4.3 ± 0.1	4.3 ± 0.5
GLOB (g/dl)	4.4 ± 0.2	4.1 ± 0.3	4.9 ± 0.4	4.7 ± 0.3
A/G (ratio)	1.0 ± 0.2	1.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
T-BILI (mg/dl)	0.3 ± 0.0	0.3 ± 0.1	134 ± 26	132 ±13
CHOL (mg/dl)	144 ± 24	126 ±22	35 ± 9	34 ± 3
TRIG (mg/dl)	40 ± 15	34 ± 4	35 ± 9	34 ± 3
AST (IU/l)	36 ± 4	32 ± 9	30 ± 2	33 ± 3
ALT (IU/l)	47 ± 19	33 ±17	51 ± 14	38 ±25
ALK PHOS (IU/l)	797 ±420	590 ±77	308 ±182	197 ±48
BUN (mg/dl)	21 ± 4	18 ± 3	21 ± 3	22 ± 3
CREAT (mg/dl)	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.1
Ca^{2+} (mg/dl)	11.2 ± 0.8	10.4 ± 0.3	10.0 ± 0.3	10.5 ± 0.3
PO_4^{3-} (mg/dl)	6.0 ± 1.1	5.6 ± 0.6	4.4 ± 0.6	4.6 ± 0.8
Na (mmol/l)	158 ± 1	153 ± 3 [b]	151 ± 2	154 ± 2
K (mmol/l)	5.1 ± 0.5	4.6 ± 0.1	4.2 ± 0.9	4.9 ± 0.1
Cl (mmol/l)	110 ± 2	108 ± 2	107 ± 2	112 ± 2 [b]

Abbreviations are defined in Materials and Methods.

[a] Mean value ±SD; $n = 4$ animals/group

[b] Significantly different from saline-treated group $P < 0.05$

in two animals at the 10 g/kg dose, there were no HPCD-related demonstrable toxic manifestations.

Discussion

The use of cyclodextrins to improve the solubility and other pharmaceutical properties of drugs is expanding. These starch derivatives, especially the water soluble cyclodextrins, can be of great benefit in parenteral formulations assuming that these materials can be given safely by this route. The parent β-CD produces a number of untoward effects in rats when given s.c. or i.p. (Frank et al., 1976; Perrin et al., 1978; Hiasa et al., 1981). These effects, which are not manifested after oral administration, include an increased blood urea nitrogen (BUN) (Perrin et al., 1978), a decrease in the rate of body weight gain, a decrease in the weight of the liver and a decrease in the liver to body weight ratio (Hiasa et al., 1981). Most striking is an increase in the weight of the kidney which results in dramatic increases (100%) in the kidney to body weight ratio. Other effects include a decrease in the activity of various serum enzymes associated with renal function such as succinic dehydrogenase, alkaline phosphatase, glucose-6-phosphatase and β-gluconoridase (Hiasa et al., 1981). Histologically parenteral β-CD administration produces a series of changes in the vacuolar appearance of the kidney proximal tubules. These changes include cytoplasmic vacuolation, cellular distruction and the formation of giant lysosomes (Frank et al., 1976). The lysosomes are embedded with microcrystalline material, presumably β-CD. These histological alterations are associated with mitochondrial degeneration and nephrosis. The events responsible for these toxic reactions appear to involve the tubular reabsorption of the cyclodextrin and concentration in vacuoles. The low aqueous solubility may cause precipitation and the resulting microcrystalline structures may rupture lysosomes and lead to nephrosis. These processes impart to β-CD a relatively low LD_{50}. Reported LD_{50} values range from 300 mg/kg to approximately 800 mg/kg (Frank et al., 1976; Szejtli,

1982; Muller and Braun, 1985). The fact that the low water solubility of β-CD is associated with its toxicity can be appreciated by considering the i.v. toxicity of γ-cyclodextrin (γ-CD). This material has an aqueous solubility of approximately 23 g/100 ml (12-fold that of β-CD) and is relatively non-toxic after i.v. administration (LD_{50} i.v. > 2400 mg/kg) (Matsuda et al., 1983).

Attempts to increase the aqueous solubility of β-CD via alkylation have been successful. DMCD and the corresponding trimethylated derivative (TMCD) can be dissolved to an extent of 57 and 31% w/v, respectively, in water (Yoshida et al., 1988). In addition, the methylated derivatives of β-CD form more stable complexes with many drugs than those obtained with β-CD. Unfortunately, these lipophilic compounds also lower the surface tension of water and as a result lyse red blood cells at relatively low concentrations (Yoshida et al., 1988). This property causes these materials to be fairly toxic (LD_{50} i.v. for DMCD 200 mg/kg) after parenteral administration (Muller and Braun, 1985). In addition, these materials are irritating to mucous membranes when applied topically. The amorphous HPCD derivative is readily water soluble (> 100% w/v), hydrophilic, easily and reproducible prepared and ammenable to extensive chemical characterization. HPCD has been applied to numerous biomedical tasks and published reports indicate that it is not acutely toxic after oral, i.p., i.v., i.m., topical or intracerebral (i.c.) administration (Pitha et al., 1986a; Carpenter et al., 1987; Anderson et al., 1988; Brewster et al., 1988; Pitha et al., 1988). The LD_{50} for this material has not been measured but based on work presented here as well as elsewhere, the toxicity of HPCD is approaching that of glucose.

Subacute or subchronic administration (14 or 90 days) of HPCD to rats and monkeys did not result in any consistent statistically significant alteration in any of the morphological or clinical pathology parameters examined. Occasional changes were limited to one sex and/or differed in direction of change between species (i.e. MCV). The measured BUNs were not elevated after HPCD treatment, there was no effect of HPCD on body weight gain or on liver to body weight ratio. In addition, the kidney was not impaired by repeated HPCD administration as illustrated by the unaltered kidney to body weight ratio, absolute kidney weight and BUN levels. There were no noticeable histological pecularities such as vacuolization or necrosis associated with the kidney or for that matter, with any tissue. Also, massive doses of HPCD (10 g/kg) were not acutely lethal to monkeys. Further, other studies have shown that HPCD is not mutagenic (Brewster, unpublished results). Additional studies with HPCD are warranted to identify the toxicological properties of this potential pharmaceutical excipient. Studies should also examine the toxicology of HPCD-drug inclusion complexes. Parameters such as hemolytic properties may vary with the included or incorporated drug. Our studies indicate that HPCD in aqueous solutions of 10–40% (w/v) are not hemolytic in vivo (unpublished observation) with 23% solution approximately isotonic. The apparent lack of HPCD toxicity after acute or subchronic i.v. administration observed in the present studies supports the use of HPCD as a solubilizing excipient in i.v. and other parenteral formulations.

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