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The use of 2-hydroxypropyl-β-cyclodextrin as a vehicle for intravenous administration of dexamethasone in dogs

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Summary

Dexamethasone (Dex) was administered to 6 mongrel dogs in a dose of 5 mg/kg as the sodium salt of its phosphate ester or as an inclusion complex in 2-hydroxypropyl-β-cyclodextrin (HPCD). Blood and urine were collected and analyzed for dexamethasone by HPLC. The area under the plasma concentration time curve (AUC) of dexamethasone during the first hour was significantly greater after the administration of the Dex-HPCD complex compared to the phosphate ester prodrug (1.63 ± 0.13 vs 1.04 ± 0.10 μg h ml^{-1}). In addition, the renal clearance (CL_{ren}) was significantly higher after administration of the HPCD form (0.91 ± 0.20 vs 0.52 ± 0.20 ml min^{-1} kg^{-1}). There was no difference between treatments for other pharmacokinetic parameters of dexamethasone. Hence, intravenous injection of dexamethasone in HPCD allows direct administration of this poorly soluble steroid without any prodrug conversion. The higher plasma levels during the first hour after administration may be advantageous for the treament of emergency situations.

Introduction

Dexamethasone is a synthetically derived glucocorticoid which is widely used for the treatment of asthmatic, allergic and rheumatic diseases. Intravenous administration of dexamethasone is therapeutically useful in emergency medicine for treating shock reactions, heart attacks or edemas of the brain and the lungs. Unfortunately, glucocorticoid dosage forms suitable for intravenous administration are limited because of the very low solubility of these drugs in water. Therefore, the commercially available products are salts of either the phosphate or succinate esters of the steroids. These prodrugs have to be cleaved by esterases to release the active drug (Rohdewald et al., 1987; Möllmann et al., 1988). This bioactivation occurs rapidly in man leading to maximum plasma levels of dexamethasone within 10 min after intravenous administration (Rohdewald et al., 1987). While these products are generally well tolerated, i.v. administration of the phosphate esters produces a syndrome described by patients as intense anxiety and feeling like 'one is covered with ants'. As these untoward effects are not associated with hemisuccinate prodrugs, dephosphorylation may be involved in the genesis of this reaction.

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Food, cosmetic and pharmaceutical industries have taken advantage of cyclodextrins (CD) to form inclusion complexes with compounds to improve aqueous solubility of lipophilic moieties, stabilize labile compounds and mask unpleasant taste (Szejtli, 1982, Lach and Cohen, 1963; Uekama, 1981). However, the unmodified α, β and γ cyclodextrins are not useful for parenteral drug formulations due to their limited solubility and significant renal toxicity (Frank et al., 1976; Hiasa et al., 1981; Müller and Brauns, 1985). Amorphous mixtures of modified CD including 2-hydroxypropyl-β-cyclodextrin isomers (HPCD) retain the ability to form inclusion complexes but do not have limitations associated with crystaline CD such as renal toxicity (Brewster et al., 1990). Further, aqueous solubility of HPCD is far greater than parent β-CD which results in vastly improved aqueous solubility of numerous compounds (Uekama and Otagiri, 1987). The solubility of dexamethasone in water could be improved from 0.08 mg/ml to 65 mg/ml by preparing a complex containing 63 mg dexamethasone/g HPCD.

It was the objective of the present study to investigate the use of HPCD as a vehicle for intravenous injection of dexamethasone and to compare the resulting pharmacokinetics to dexamethasone phosphate administration in dogs.

Materials and Methods

Drugs and chemicals

The sodium salt of the phosphate ester of dexamethasone was purchased from Diosynth (Oss, The Netherlands) and the dexamethasone HPCD inclusion complex was provided by Pharmatec Inc. (Alachua, FL). The dexamethasone HPCD-complex was made following procedures previously described (Brewster et al., 1988; Pitha and Pitha, 1985; Pitha et al., 1986). Briefly, a 43.5% w/v HPCD solution was prepared by dissolving one part cyclodextrin into two parts deionized, 18.3 Ω water (Barnstead Nanopure II Ultrapure water system). The HPCD used was characterized by fast atom bombardment mass spectrometry to have an average molecular degree of substitution of 7.0. The isomeric mixture ranged from 3–11 in 2-hydroxypropyl substitution. An excess of dexamethasone was suspended in the HPCD solution and was equilibrated for 24 h. The system was then filtered through 0.45 μm polyvinylidine difluoride membranes and the filtrate frozen in liquid nitrogen. The solid was lyophilized (Labconoco Freeze Drier model 18) and then powdered by passing the lyophilized solid through a 60 mesh (250 μm) sieve. The degree of drug incorporation was found to be 62.7 mg of dexamethasone per gram of complex as determined by HPLC. The stability constant for a 1 : 1 complex of dexamethasone and HPCD was approximately 2000 M^{-1}.

All chemicals and solvents used for HPLC analysis were of analytical grade and purchased from Fisher (Atlanta, GA).

Experimental design

Six mongrel dogs (weight 18–25 kg) were used. Animals were purchased from and housed in the Department of Animal Resources, University of Florida. All studies were approved by the Instutional Animal Care and Use Committee prior to initiation. Each dog received dexamethasone (5 mg/kg) as the phosphate ester and as the HPCD inclusion complex in a cross-over design with a wash-out period of at least 1 week separating the treatments.

Prior to the experiment one cephalic and the contralateral jugular vein were cannulated and an urinary catheter was fitted for quantitative collection of urine. The dog was maintained in a sling restraint (Alice King Chattem Medical Arts, Los Angeles, C.A.) during the 8 h sampling period. An isotonic sodium chloride solution (2 ml/kg/h) was infused to provide continous urine production. Dexamethasone sodium phosphate was dissolved in normal saline for injection to give a solution containing 25 mg/ml of steroid. The dexamethasone was administered as 25 mg/ml in a 40% (w/v) HPCD vehicle. Dexamethasone (5 mg/kg) was administered as a bolus injection into the cephalic vein (0.2 ml/kg). Blood was collected at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after drug administration and centrifuged to separate the plasma. Urine

was obtained at time 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h and the volume was measured. Aliquots of plasma and urine were stored until analysis at −20 °C.

High-performance liquid chromatography

The concentrations of dexamethasone in plasma and urine were determined using a modification of an HPLC method previously described (Derendorf et al., 1986). Briefly, the HPLC equipment consisted of a Milton Roy Constametric high pressure pump, a Milton Roy UV-detector and a Hewlett Packard 3392A integrator. The chromatographic separation was achieved using a Spherisorb ODSII column (150 × 4.6 mm i.d., particle size 5 μm). The mobile phase consisted of acetonitrile/water 27 : 73 with 1 ml of 44 N phosphoric acid added to each liter of eluent. The flow rate was 1 ml/min. The injection volume was 20 μl. Under these conditions α-methylprednisolone (internal standard) and dexamethasone were eluted after 6.0 and 7.0 min, respectively and were monitored at 254 nm. For the quantitation of dexamethasone, calibration samples were prepared by spiking blank plasma or urine with different amounts of dexamethasone ranging from 0.1–5.0 μg/ml for plasma and from 2.5–250 μg/ml for urine. The assay allowed quantitation of dexamethasone of less than 50 ng/ml. The coefficient of variation did not exceed 8.2%.

Sample preparation

To 0.5 ml of plasma or urine, 0.05 ml of a solution of α-methylprednisolone in methanol (100 μg/ml) was added followed by 1 ml of a saturated solution of ammonium sulfate in water. The mixture was shaken with 3 ml of ethyl acetate for 15 min and centrifuged. The upper organic layer was removed and evaporated under a nitrogen stream at 40 °C in a waterbath. After reconstitution in 200 μl acetonitrile-water (1 : 1) 20 μl were injected into the HPLC system.

Pharmacokinetic calculations

The plasma data were fitted to a two-compartment body model using non-linear regression (RSTRIP, 1989). The area under the plasma concentration time curve (AUC), the terminal elimination half-time ($t_{1/2\beta}$) and the mean residence time (MRT) were derived directly from the regression program. Total body clearance (CL_{tot}) was calculated as D/AUC, where D is the dose. The volume of the central compartment V_c was calculated as D/Cp_0 after administration of the inclusion complex and as $k_a D/[a(k_a - \alpha) + b(k_a - \beta)]$ after the phosphate, where Cp_0 is the calculated plasma concentration at time 0, k_a is the formation rate constant for the conversion of dexamethasone phosphate to dexamethasone, and α and β are hybrid constants as determined by nonlinear regression. The volume of distribution at steady-state V_{dss} was calculated as $CL_{tot}\mathrm{MRT}$ after the HPCD complex and as $CL_{tot}(\mathrm{MRT} - 1/k_a)$ for the phosphate. The volume of distribution at pseudo steady state V_{darea} was determined as CL_{tot}/β. The calculation of the pharmacokinetic parameters after administration of the phosphate assumes complete conversion of the prodrug.

The renal clearance, CL_{ren}, was determined as the slope of a plot of plasma AUC versus cumulative renal elimination.

Statistics

The calculated pharmacokinetic parameters were tested for significant differences using the paired Student's t-test. P values ≤ 0.05 were considered to be of statistical significance.

Results

The mean plasma concentration time curves of both treatments are presented in Fig. 1. The pharmacokinetic parameters derived from the plasma data are shown in Table 1. The difference in plasma levels during the first hour after administration results in higher AUC values after the HPCD preparation (1.63 ± 0.13 vs 1.04 ± 0.10 μg h ml^{-1}). All other parameters calculated from plasma data are not statistically different.

The cumulative elimination of dexamethasone into urine is presented in Fig. 2. The faster renal elimination after the HPCD preparation is especially evident during the first 5 h with a renal clearance (CL_{ren}) of 0.91 ± 0.20 ml min^{-1} kg^{-1}

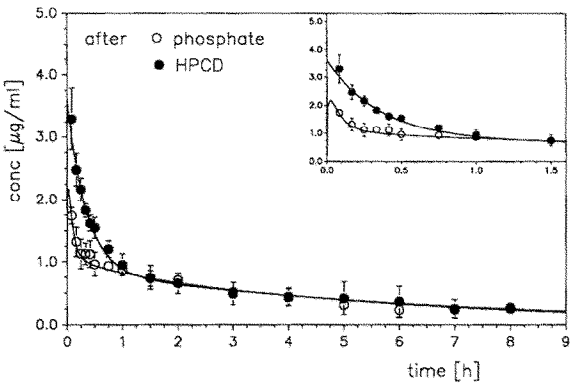

Fig. 1. Plasma concentration time curves of dexamethasone after intravenous administration of the sodium salt of its phosphate ester and an inclusion complex in 2-hydroxypropyl-β-cyclodextrin (HPCD), respectively. Data points represent means of 6 dogs. Vertical bars indicate standard deviation.

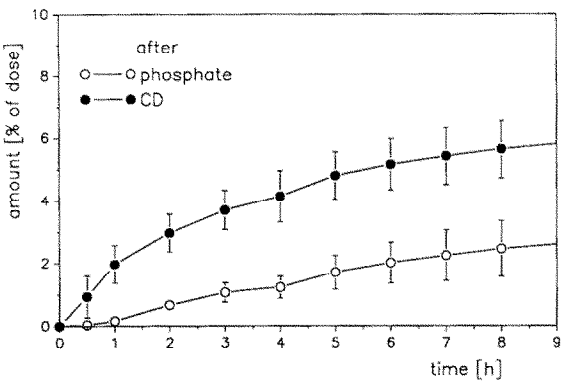

Fig. 2. Cumulative urinary elimination of unchanged dexamethasone. Data points represent means of 6 dogs. Vertical bars indicate standard deviation.

after HPCD administration and 0.52 ± 0.20 ml min^{-1} kg^{-1} after the phosphate ester (Fig. 3).

Discussion

The pharmacokinetics of dexamethasone in dogs were investigated after the intravenous administration of the steroid as an HPCD inclusion complex as well as the sodium salt of the phosphate ester. The plasma levels of dexamethasone after the administration of the inclusion complex were significantly higher compared to the concentrations achieved after the ester. The reason for this phenomenon can be explained by the nearly instantaneous availability of dexamethasone from

TABLE 1

Pharmacokinetic parameters of dexamethasone

Dog	AUC ($\mu g\ h\ ml^{-1}$)	AUC (0–1 h) ($\mu g\ h\ ml^{-1}$)	$t_{1/2\beta}$ (h)	MRT (h)	CL_{tot} ($ml\ min^{-1}\ kg^{-1}$)	CL_{ren}	V_c (l/kg)	V_{dss} (l/kg)	V_{darea} (l/kg)
After dexamethasone sodium phosphate									
1	5.70	1.20	5.38	6.56	14.62	0.77	2.71	5.76	6.80
2	6.34	1.00	4.29	6.05	13.14	0.33	1.26	4.73	4.89
3	6.18	0.98	3.91	5.47	13.48	0.37	0.93	4.42	4.57
4	4.45	1.14	3.85	5.38	18.73	n.a. [a]	1.90	6.04	6.24
5	4.95	0.89	3.89	5.53	16.84	0.70	1.58	5.55	5.67
6	5.26	1.00	3.81	5.36	15.84	0.43	1.18	5.08	5.23
mean	5.48	1.04 [b]	4.19	5.73	15.44	0.52 [b]	1.59	5.26	5.57
STD	0.67	0.10	0.55	0.44	1.95	0.20	0.59	0.77	
After dexamethasone HPCD inclusion complex									
1	7.90	1.59	4.71	6.22	10.55	0.98	1.47	3.94	4.30
2	5.33	1.72	3.67	4.13	15.63	1.23	1.11	3.87	5.10
3	9.58	1.86	4.60	6.09	8.70	0.65	1.16	3.18	3.46
4	4.48	1.56	5.30	4.63	18.60	n.a. [a]	1.93	5.17	8.50
5	6.75	1.52	4.99	6.32	12.35	0.95	1.35	4.68	5.33
6	5.95	1.51	4.43	5.40	14.01	0.75	1.62	4.54	5.37
mean	6.67	1.63	4.62	5.47	13.31	0.91	1.44	4.23	5.34
STD	1.69	0.13	0.51	0.83	3.26	0.20	0.28	0.64	1.56

[a] No data available; [b] statistically significant difference ($P < 0.05$).

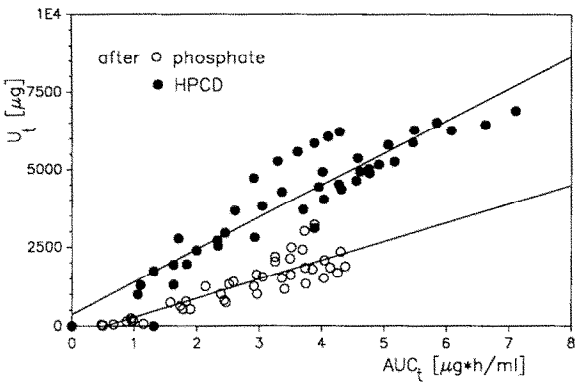

Fig. 3. Plot of the areas under the plasma concentration time curves from time 0 to time t versus the amount of dexamethasone eliminated unchanged in urine until time t. The slope of the linear regression lines represents the mean renal clearance ($n = 5$) of unchanged dexamethasone after the two dosage forms.

the cyclodextrin in the blood. In contrast, data from the ester prodrug-treated dogs suggest that the cleavage of the steroid ester requires time and thus delays the release of the parent compound.

In this respect the HPCD preparation seems to be superior to the common dexamethasone phosphate ester, since there is no prodrug conversion needed. In addition, the absence of dephosphorylation in high dose dexamethasone therapy may mitigate the acute side effects observed after phosphate administration. The urinary data show that after administration of HPCD the renal clearance of dexamethasone is increased. Usually, dexamethasone is tubularly reabsorbed and an increase in renal clearance would suggest a decreased reabsorption. This increase in renal clearance may be related to formation of a dexamethasone-2-hydroxypropyl-β-cyclodextrin complex in the tubular fluid. The cyclodextrin derivative is a small starch derivative which should be rapidly cleared by the kidney. Approximately two grams of cyclodextrin were administered to each dog with the dose of dexamethasone as inclusion complex. While this dose should be sufficiently diluted in the blood stream to result in rapid complex dissociation, upon concentration in the nephron, high levels of cyclodextrin may be produced. These levels may be sufficient to effect a subsequent recomplexation and decrease tubular reabsorption of dexamethosone. This would lead to an increase in renal clearance of the drug. These effects should be manifested early in the time course of the experiment, as excretion of the cyclodextrin should decline rapidly with time. In fact, the data show that the enhanced renal elimination is observed in the first five hours after drug administration.

High dose therapy with 2-hydroxypropyl-β-cyclodextrin has been used to accelerate the renal elimination of fat soluble vitamins, especially vitamin A (Pitha, 1982; Pitha and Szente, 1983; Carpenter et al., 1987) and cholesterol. While the amount of material used in this study was lower than those used to accelerate the elimination of hyperphysiological levels of endogenous substances, a circumstance where locally high concentrations of the cyclodextrin are in intimate contact with locally high concentrations of the administered drug may lead to in vivo recomplexation and accelerated elimination. Again these effects are transient and do not significantly alter the half-life or the mean residence time of dexamethasone, since the total fraction of the drug eliminated into the urine within 8 h is only 5.5%. Also, these effects do not appear to have toxicological significance, as the drug should be cleared more slowly, not more rapidly, if the kidney were impaired.

It would be useful to investigate the pharmacokinetics of HPCD itself. Recently, preliminary data became available describing plasma levels of HPCD in rats (Szathmary, 1989). However, due to the limited number of data points and the short observation period no conclusion about the kinetic behaviour of HPCD could be drawn.

In summary, formulation of lipophilic drugs as inclusion complexes in the amorphous modified cyclodextrin 2-hydropropyl-β-cyclodextrin may be an alternative to the prodrug approach for parenteral administration of these compounds.

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