Oral Pharmacokinetics of Carbamazepine in Dogs from Commercial Tablets and a Cyclodextrin Complex

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Abstract □ The extent of absorption of carbamazepine from a 2-hydroxypropyl-β-cyclodextrin/carbamazepine complex was significantly greater and the rate of absorption was faster when compared with an immediate-release carbamazepine tablet in the dog. Six dogs were dosed orally in a two-way crossover study in which the tablet was compared with an equivalent dose of the complex in solution. The area under the curve of concentration versus time for the complex was 5.6 times greater than the tablet, whereas the mean time to reach maximum concentration for the tablet was 1.4 hours versus 0.5 hours for the complex. The complex, therefore, had a greater rate and extent of absorption. A rapidly acting and better absorbed carbamazepine product has the potential to decrease the daily dose of carbamazepine, increase its utility as emergency treatment of epileptic seizures, and provide an acceptable alternative dosage form in patients who are unable to swallow tablets.

Carbamazepine (CBZ) is the drug of choice for the prophylactic management of partial seizures with complex symptomatology, generalized tonic-clonic seizures, and mixed seizures patterns.[1,2] The drug has become the best selling anticonvulsant in the world. Commercial success can be directly attributed to its favorable therapeutic profile. However, some clinically significant disadvantages of the antiepileptic drug relate to the available formulations. A loading dose cannot be administered orally (po) because of the slow absorption profile of commercially available tablets. Importantly, no parenteral formulations are available for patients unable to take tablets. Additionally, intravenous (iv) loading doses cannot currently be given.[3] CBZ is available in the United States as an immediate-release tablet with a time to reach maximum concentration (t_{max}) in humans ranging from 4 to 32 h.[4,5] This slow and highly variable absorption rate is even found in normal healthy volunteers and typical of both the innovator and generic tablet forms of the drug.[6] Very limited water solubility and poor wettability properties of the drug contribute to slow and variable absorption of the immediate-release form of commercially available tablets.[7] These physical drug characteristics have also impeded development of both an iv formulation and a po solution for CBZ. Both types of formulations could provide important therapeutic options for the epileptic surgical, pediatric, or trauma patient who is unable to swallow or adequately maintain relatively slow-release tablets.

Cyclodextrins are hydrophilic, cyclic, non-reducing oligosaccharides, composed of 6–8 glucopyranose units.[8] One of their uses in the pharmaceutical industry is to increase the solubility of poorly water soluble compounds.[8–10] Modified cyclodextrins provide additional advantages. For example, 2-hydroxypropyl-β-cyclodextrin (HPβCD) was demonstrated to be useful for the solubilization and stabilization of various drugs.[11] The amorphous mixture of drug and HPβCD is highly water soluble, unlike β-cyclodextrin, but retains the high complexing properties of the parent β-cyclodextrin.[12,13] When used orally, HPβCD and other cyclodextrins are not absorbed into the systemic circulation.[12] The purpose of this study was to evaluate the pharmacokinetics of a solution of a HPβCD/CBZ complex[14] in comparison to a commercially available standard CBZ tablet in dogs.

Experimental Section

Materials—CBZ for the complex and analytical standard was obtained from Sigma Chemical Company, St. Louis, MO. For the analytical procedure, carbamazepine-10,11-epoxide standard was purchased from Alltech Associated, State College, PA. The internal standard, tolybarb (5-ethyl-5-*p*-tolylbarbituric acid), was purchased from Aldrich Chemical Company, Inc., Milwaukee, MN. Methanol, acetonitrile, and methylene chloride were HPLC quality and were purchased from American Burdick and Jackson, Muskegan, MI. All other reagents were of reagent grade quality. Buffers were prepared with deionized water that had been passed through a MilliQ purification system. All buffers used in the HPLC mobile phase were filtered through a 0.45-μm filter (Millipore Corp., Bedford, MA).

Complex Preparation—The HPβCD and the complex were prepared as previously described.[14] The HPβCD solution was made with deionized water (Barnstad Nanopure II Ultrapure Water System). An excess of CBZ was sonicated in the HPβCD solution for 24 h at room temperature. The resulting suspension was centrifuged and filtered through 0.45-μm polyvinylidine difluoride membranes (Millex, HV4, Millipore). The filtrate was frozen in liquid nitrogen and lyophilized (Labconco Model 18 Freeze-Drier). The solid was then milled and passed through a 60-mesh sieve. The degree of drug incorporation in the HPβCD/CBZ complex was determined by HPLC to be 61.0 mg CBZ per gram of HPβCD/CBZ complex.

In Vivo Study Design—This was a two-way, randomized, crossover study in six fasted beagle dogs (three males and three females) in which the treatments were as follows: (a) CBZ tablets (Tegretol, 200-mg tablets, Geigy Pharmaceuticals) and (b) HPβCD/CBZ complex. The dose for both treatments consisted of 200 mg of CBZ. Forty-five milliliters of deionized water was administered by gavage to each dog after dosing with one 200-mg Tegretol tablet. The 3.28 g of HPβCD/CBZ complex was dissolved in 25 mL of deionized water (at room temperature). This solution was administered to the fasting dogs by gavage within 10 min after its dissolution. The complex was completely dissolved and formed a clear solution. The bottle that contained the complex was washed immediately with two consecutive 10-mL quantities of deionized water and administered to the dogs by gavage. The dogs thus received the same fluid volume for the two treatments.

Serial blood samples were drawn via a central venous catheter at 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, and 24.0 h after the dose. Blood samples were placed into tubes containing K_2EDTA to prevent clotting and centrifuged immediately to obtain the plasma. Plasma samples were collected and stored at −20 °C until analyzed.

Analytical Method—Plasma samples were analyzed for CBZ by an HPLC method validated for canine plasma. The CBZ and internal standard (tolybarb) were extracted from plasma with solid-phase extraction columns. Extraction columns (ClinElut, size CE 1001,

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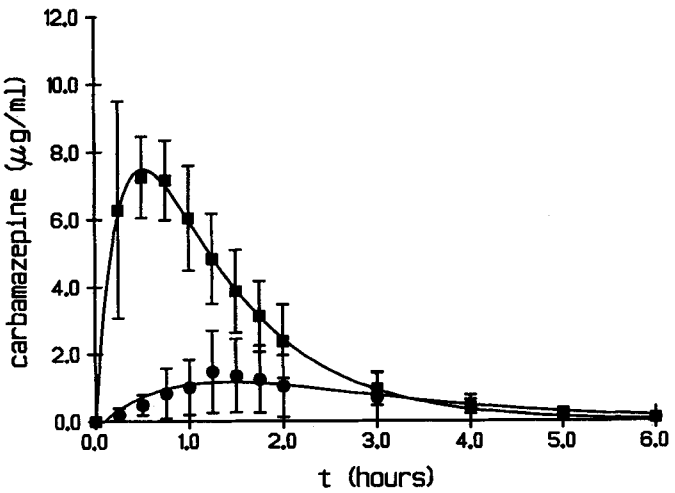

Figure 1—The mean plasma CBZ levels following the single-dose administration of Tegretol 200-mg tablets (-●-) and the HPβCD complex (200 mg carbamazepine) (-■-) to six beagle dogs.

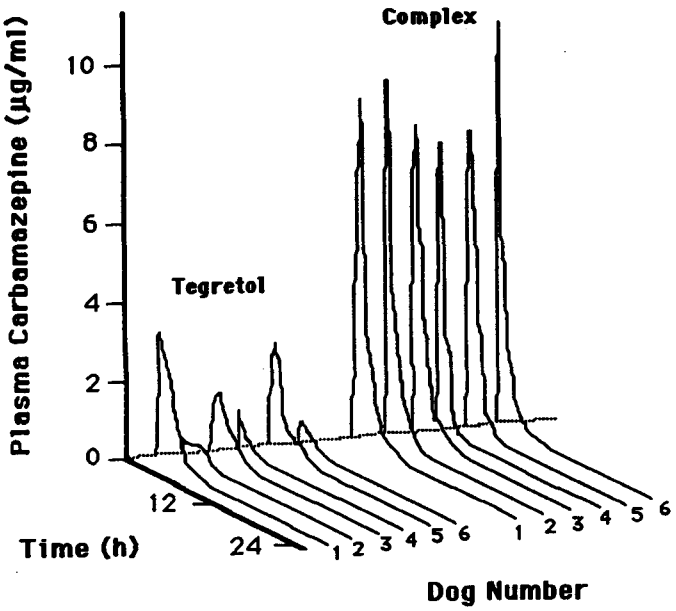

Figure 2—The individual plasma CBZ levels following the single-dose administration of Tegretol tablets (200 mg) and the HPβCD complex (200 mg of CBZ) to six beagle dogs.

Analytichem International, Harbor City, CA) were placed over 16 × 85-mm disposable glass centrifuge tubes. The columns were preconditioned with 100 μL of a 1 M potassium phosphate buffer (pH 6.8). One hundred microliters of the internal standard (5 μg/mL) were added to the top of the column followed by 200 μL of plasma. The column was allowed to equilibrate for 5 min. After equilibration, 10 mL of methylene chloride was added, and the effluent was collected in glass tubes. A total of ~9.0 mL of the methylene chloride was recovered from each extraction. The effluent was evaporated from the tube at 50 °C under a stream of nitrogen. The residue was reconstituted with 50 μL of methanol and placed in a microinjection vial for injection into the HPLC. The injection volume was 10 μL.

The method used to detect and quantify the plasma CBZ was a modification of a published method.[15] The liquid chromatograph used was a Waters M6000 isocratic pump system equipped with an automatic injector (712 WISP, Waters Chromatography, Milford, MA), an RCM-100 radial compression module with a 8 mm × 10 cm Nova-Pak 4 micron ODS cartridge (PN86342, Waters Chromatography, Milford, MA), and a variable wavelength UV detector (model 490, Waters Chromatography, Milford, MA). The mobile phase consisted of methanol:acetonitrile:25 mM potassium phosphate buffer (pH 7.0; 10:30:60) and was premixed and deaerated by sonication and vacuum. The flow rate was 2.0 mL/min. Detection of CBZ and internal standard was by UV absorption at 210 nm. Retention times of tolybarb and CBZ were 4.1 and 5.9 min, respectively. Carbamazepine-10,11-epoxide was separated from CBZ (retention time, 3.3 min) but was not quantified. The absorbance was recorded with a recording integrator (model 3392, Hewlett Packard, Avondale, PA), and quantification was based on peak height measurements.

Standard curves were developed with canine plasma for the range of CBZ of 0.33 to 5.0 μg/mL. The method was linear between this range of concentrations (r^2 = 0.9996). The precision data were obtained from replicate determinations at 2.5 μg/mL. Within-run CVs were <6.0% and between-run CVs were <4.2%.

Pharmacokinetic Analysis—The CBZ concentration–time data were evaluated for the extent and rate of CBZ absorption. Area under the curve to the last time point (AUC_{24}) was calculated with the log-linear trapezoidal rule to the last time point. The ratio of the AUC_{24} for the complex divided by the AUC_{24} for Tegretol was used to evaluate the extent of absorption. The maximum plasma concentration (C_{max}) and the ratio of the C_{max} values were also used as measure of the extent of absorption. The t_{max} and its ratio were used to evaluate the rate of absorption. The data were analyzed statistically with the paired t test.

Results and Discussion

CBZ was significantly better absorbed from the HPβCD/CBZ complex than from the CBZ tablet (Figures 1 and 2 and Table I). The mean AUC ratio (complex/tablet) was 5.6 (range: 1.8–11.6). In addition, the CV for the complex AUC was considerably less (22%) than that for the tablet AUC (78%). The AUC for the HPβCD/CBZ complex was significantly greater ($p < 0.01$) than that for the tablet. The more than fivefold mean increase in the AUC was unexpected and surprising. This increased extent of absorption and increased reproducibility of the AUC values from the HPβCD/CBZ complex may be due to the increased water solubility of the complex.

The terminal rate constant of CBZ was 1.00 ± 0.39 h^{-1} for the HPβCD/CBZ units complex. Because of slow and probably

Table I—Mean and Individual Pharmacokinetic Data

Dog	Tegretol (T)			HPβCD/CBZ Complex (C)			C/T Ratio		
	AUC, μg · h/mL	C_{max}, μg/mL	t_{max}, h	AUC, μg · h/mL	C_{max}, μg/mL	t_{max}, h	AUC, μg · h/mL	C_{max}, μg/mL	t_{max}, h
1	7.97	3.25	1.25	14.82	8.59	0.75	1.97	2.64	0.60
2	1.82	0.54	3.00	15.31	8.94	0.50	7.43	16.71	0.17
3	4.26	1.60	1.50	14.18	7.77	0.75	3.45	4.84	0.50
4	0.98	0.92	0.50	7.68	7.23	0.25	7.47	7.86	0.50
5	5.84	2.62	1.00	11.71	7.40	0.50	1.79	2.82	0.50
6	1.15	0.59	1.00	13.26	10.12	0.25	11.56	17.04	0.25
Mean	3.67	1.59	1.38	12.83	8.34	0.50	5.61	8.65	0.42
SD	2.85	1.13	0.56	2.82	1.10	0.22	3.86	6.64	0.17
CV%	78	71	62	22	13	44	69	77	44
Median	3.04	1.26	1.13	13.72	8.18	0.50	5.44	6.35	0.50

incomplete release of CBZ from the tablet, low plasma CBZ levels were obtained. The slow and prolonged absorption precluded a precise calculation of a terminal rate constant from the tablet data because absorption and elimination rate constants were of the same order of magnitude.

This increased solubility also permitted the CBZ to be absorbed significantly faster than the CBZ in the tablet as is illustrated in the significantly shorter t_{max} values, 0.50 and 1.38 h, for complex and tablet, respectively. The accelerated rate and greater extent of absorption combined to produce a large increase ($p < 0.01$) in the C_{max} values from the HPβCD/CBZ complex. The mean C_{max} increased from 1.59 μg/mL (CV = 71%) for Tegretol to 8.34 μg/mL (CV = 13%) for the HPβCD/CBZ complex. The reduced variability of absorption from the complex is reflected in the lower CV seen for the C_{max} data.

It would appear from these data that solubilization of CBZ from tablets is the rate-limiting step for the absorption of the drug in the dog. Placing CBZ in solution by complexing it with HPβCD permits the rate-limiting step to shift to the process of absorption through the gut wall. Removing the variability of unreliable dissolution results in a greater total and a faster rate of absorption of CBZ from the HPβCD/CBZ complex. Improved bioavailability for a CBZ product has the potential to decrease the daily dose of CBZ needed for anticonvulsant therapy. Whereas CBZ is generally reported to have a relatively high oral bioavailability from tablets,[1,3,5] some studies have reported increased seizure risk due to inadequate bioavailability of both generic and innovator tablet products.[16,17] Rapid absorption from a solution of a CBZ complex could also result in increased use of CBZ for the emergency treatment of status epilepticus and provide an acceptable alternative dosage form to patients who are unable to swallow tablets.

Pediatric patients suffering from refractory seizures showed therapeutic plasma levels of CBZ and seizures were controlled within 1 h of drug administered as a suspension through nasogastric tubes.[18] A solution is expected to result in more rapid plasma therapeutic levels compared with po suspensions. Recent studies in rats confirmed that the t_{max} was shortened and the C_{max} was increased after dosing with CBZ complex compared with a po suspension.[19]

In addition to providing a more truly immediate-release po formulation, HPβCD may be useful as a parenteral formulation. Previous studies showed that the solution was well tolerated as an iv bolus in rats and provided the expected seizure protection in murine models of epilepsy.[14] Parenteral dosing could be important for trauma and surgical patients and offer the clinician the potential to closely regulate therapeutic plasma concentrations of CBZ during concurrent treatment with drugs known to alter the pharmacokinetic profile of antiepileptic drugs. Thus, plasma CBZ levels could be regulated by rate of iv administration and not be subject to variable rates of disintegration, dissolution, and absorption. Whereas the results from this canine study require verification in humans, the cyclodextrin complex represents the first successful improvement in the bioavailability of CBZ.

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