The Preparation and Evaluation of a Tablet Dosage Form of Cyclosporine in Dogs

Hisham Y. Abdallah[1,2] and Michael Mayersohn[1,3]

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Cyclosporine (CsA) is commercially available for oral administration as a solution in olive oil with alcohol and an emulsifier. To improve its variable absorption and low patient acceptability, several oral formulations were prepared and tested *in vitro* and *in vivo* in dogs. A tablet formulation prepared by direct compression was then selected for comparison with the commercial oil solution placed into soft gelatin capsules. The study involved a randomized crossover design in six dogs. In order to determine absolute bioavailability and to compensate for any time-dependent changes in clearance, an intravenous tracer dose of ^{3}H-CsA was administered along with each oral test product on each of two occasions. Absolute bioavailability (mean ± SD) was 46.0 ± 11.1 and 45.4 ± 9.9% for the capsules and tablets, respectively. C_{max}, t_{max}, and mean absorption time were not significantly different between the two products. No differences were observed in the pharmacokinetics of the intravenously administered CsA in the two experiments, which were separated by 8–13 days. We conclude that the proposed tablet formulation for CsA is equivalent in dogs to the commercial dosage form placed into soft gelatin capsules.

KEY WORDS: cyclosporine; dosage form; bioavailability; pharmacokinetics; dogs; tracer.

INTRODUCTION

CsA is commercially available for oral administration as a solution in olive oil with alcohol and an emulsifier. The preparation is administered by dispersing the required dose in a suitable vehicle such as milk or juice. Absorption following oral administration is slow, incomplete, and highly variable (1). Objectionable taste and difficulty in measuring the required dose by visually impaired patients are other shortcomings of the current formulation.

In the present study we report the results of experiments designed to develop an improved oral dosage form for CsA. We investigated several approaches including *in situ* micronization and a directly compressible tablet formulation. The dog was chosen as the animal model. The information generated from the present study will allow better understanding of the pharmacokinetics of CsA in the dog, an animal which is extensively used in experimental organ transplantation.

In order to avoid the potential problem of time-dependent changes in CsA kinetics suggested in some literature reports (2–5), we simultaneously administered unlabeled CsA in the oral formulations and an intravenous tracer dose of ^{3}H-CsA.

MATERIALS AND METHODS

HPLC

Both CsA and the internal standard, dihydrocyclosporin C (CsC), were provided by Sandoz Corporation, East Hanover, NJ. For the standard curve, blood was spiked with CsA to produce final concentrations of 62.5, 125, 250, 500, 1000, and 2000 ng/ml. Tritiated CsA (mebmt-β-^{3}H-CsA, 14.1 mCi/mg; nominal concentration, 1 mCi/ml solution in 95% ethanol) was purchased from Amersham Corporation (Arlington Heights, IL). Purity of ^{3}H-CsA, as determined by HPLC, was 85%.

CsA was extracted from whole blood by a modification of the method of Carruthers *et al.* (6). Two milliliters of blood was mixed with 100 μl of CsC solution (10 μg/ml) and 4 ml of 0.09 *N* HCl and shaken with 10 ml of diethyl ether (Baker Resi-Analyzed reagent, J. T. Baker, Phillipsburg, NJ) for 15 min. After centrifugation at 1500*g* for 10 min, the ether extract was transferred to another tube containing 4 ml of 0.09 *N* NaOH, shaken for 10 min, and centrifuged for 10 min. The ether extract was then dried with about 1 g of exsiccated sodium sulfate and evaporated to dryness under nitrogen at 30°C. The residue was reconstituted in 400 μl of the mobile phase and 400 μl of hexane and centrifuged for 3 min. One hundred microliters of the mobile phase layer was injected onto an Altex Ultrasphere-CN column (4.6 × 250 mm, 5 μm) maintained at 55°C, using a mobile phase of 45% acetonitrile and 2% tetrahydrofuran in water pumped at 1.5 ml/min and UV detection at 214 nm. Peak height ratios of drug to internal standard were calculated for each sample and the concentration of CsA was determined by referring to a standard curve generated on the same day. Tritiated CsA was obtained by automated fraction collection (Frac 100, Pharmacia, Piscataway, NJ) of the HPLC fraction corresponding to unchanged CsA, followed by scintillation counting. The coefficients of variation for within-day measurements were 12.4, 5.9, and 2.9% for CsA concentrations of 80, 150, and 800 ng/ml, respectively. The corresponding day-to-day values were 8.5, 10.1, and 5.1%. The analytical recovery of both unlabeled and ^{3}H-CsA was 80%.

Preparation of CsA Tablets

CsA tablets were prepared by direct compression. Each tablet contained 100 mg CsA, 250 mg mannitol, 200 mg microcrystalline cellulose (Avicel PH 102, FMC Corp., Rockland, ME), 20 mg stearic acid, and 10 mg sodium dodecyl sulfate (SDS).

Pharmacokinetic Study of Cyclosporine in Dogs

Six healthy adult female greyhound dogs, weighing 24–31 kg (mean, 27.6 ± 2.1 kg), were used for the study. The animals underwent physical examination and received the required vaccinations. Animals were fasted overnight and for 4 hr after dosing; free access to water was permitted at all

[1] Department of Pharmaceutical Sciences, College of Pharmacy, The University of Arizona, Tucson, Arizona 85721.
[2] Present address: Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Salk Hall, Pittsburgh, Pennsylvania 15261.
[3] To whom correspondence should be addressed.

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times. On two separate occasions, and in a randomized crossover fashion, each dog received a total oral dose of 400 mg CsA in the form of 4 experimental tablets or as the commercial solution filled into 4 soft gelatin capsules, followed by 150 ml of whole milk. Along with the oral dose each dog received an intravenous bolus injection of 202.4 μCi of ^{3}H-CsA in 5 ml saline via an antecubital vein. A washout period of at least 1 week was allowed between the two experimental treatments.

Blood samples, 5–7 ml, were obtained from a jugular vein through an indwelling, 8-in 14-G Teflon catheter and placed into EDTA Vacutainers. Samples were obtained before and at 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 15, 24, 30, and 48 hr after dosing. Samples were stored frozen until assayed.

Data Analysis

Concentration–time data obtained as a result of both oral and intravenous administration of CsA were analyzed by model-independent methods using standard pharmacokinetic calculations (7).

The initial volume of distribution, V_1, was determined according to Khor *et al.* (8) as follows: the rate of change of concentration during each sampling interval after intravenous administration was plotted against the midpoint of the corresponding time interval. The area under the resulting rate curve, AURC, was determined by the trapezoidal rule, with extrapolation to infinity by dividing the last point by the terminal disposition rate constant obtained from the slope of the intravenous log concentration–time plot. V_1 was calculated from

$$V_1 = \text{Dose}_{iv}/\text{AURC}$$

The significance of the difference in the various pharmacokinetic parameters between the two treatments was determined by a Wilcoxon signed rank test for the equality of two medians with paired observations (9).

RESULTS

Cyclosporine Assay in Whole Blood

Representative chromatograms of blank blood and blood from one of the dogs following CsA administration are presented in Fig. 1. The retention times for CsC and CsA were 6.5 and 7.5 min, respectively.

Pharmacokinetics of Intravenous and Oral Cyclosporine

The concentration–time profiles of ^{3}H-CsA in the six dogs after intravenous administration in both experiments are shown in Fig. 2. The pharmacokinetic parameters derived from these profiles are presented in Table I. There were no statistically significant differences in any of the disposition parameters when comparing the results of experiments 1 and 2.

Figure 3 illustrates CsA blood concentration–time profiles in the six dogs following administration of the commercial CsA oral solution filled into soft gelatin capsules and the experimental tablets. Pharmacokinetic parameters derived from the oral data are presented in Table II. In one of the dogs (37-B) the capsule gave much higher CsA concentrations than did the tablets, with values of bioavailability, F, of 59.3 and 29.8%, respectively. Shortly after this dog had received the tablets, the venous catheter inserted into its jugular vein was accidentally detached from the needle. For the rest of the experimental day, the dog looked malaise and remained inactive. Dog 37-C, on the other hand, exhibited higher CsA bioavailability from the tablets (45.9%) than from the capsules (30.6%). Dog 398 was the only animal to show two peaks in the CsA blood concentration–time profile (Fig. 3). The first peak occurred at 1.5 hr, followed by a decline and a plateau that continued for 6 hr, and then another peak was observed. Shortly after this dog was given the tablets, it was injured by a sharp piece of metal protruding from the

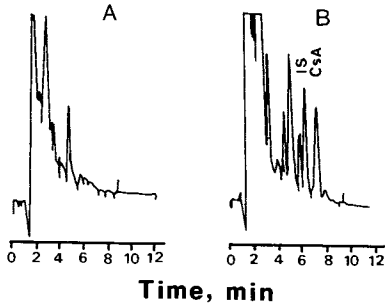

Fig. 1. Representative chromatograms obtained from the HPLC assay of blank blood (A) and blood from dog 37-C, 13.3 hr following oral dosing with 400 mg CsA (B). Estimated CsA concentration, 595 ng/ml.

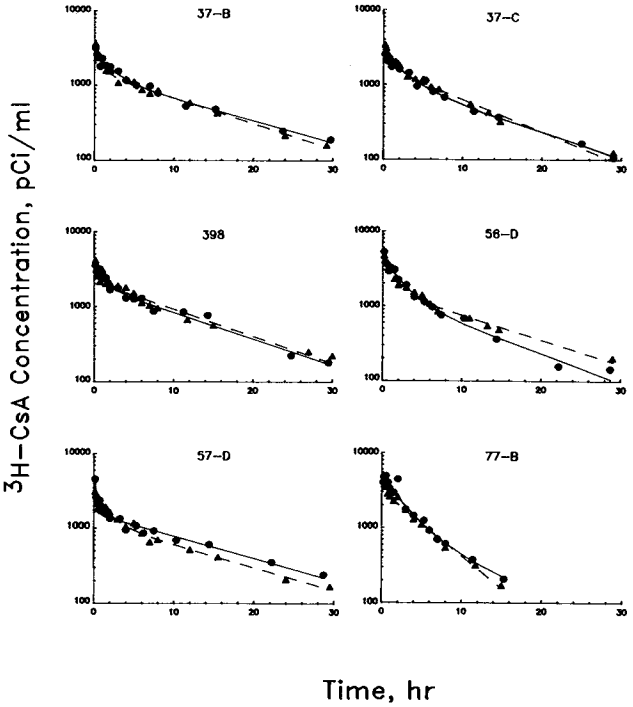

Fig. 2. Blood concentrations of ^{3}H-CsA in six dogs following intravenous doses of 202.4 μCi in two experiments (1, ●; 2, ▲). The solid and dashed lines represent best fits of the data obtained by biexponential equations with a weighting factor of $1/Y^2$ for experiments 1 and 2, respectively.

Table I. Pharmacokinetic Parameters for Unchanged ^{3}H-CsA in Dogs Obtained by Area Analysis Following an Intravenous Dose of 202.4μCi

Dog No.	Expt No.	AUC (pCi · hr/ml)	TBC (L/hr/kg)	β (hr^{-1})	$t_{1/2}$ (hr)	MRT (hr)	Volume (L/kg)		
							V_{area}	V_{ss}	V_c
37-B	1	22,870	0.316	0.0641	10.81	13.45	4.93	4.25	1.93
	2	21,052	0.343	0.0719	9.64	12.13	4.71	4.16	1.26
37-C	1	18,145	0.406	0.0813	8.53	10.24	4.99	4.16	2.52
	2	20,963	0.351	0.0851	8.15	12.15	4.13	4.27	1.18
398	1	27,672	0.240	0.0821	8.44	11.84	2.92	2.84	1.35
	2	27,885	0.238	0.0654	10.60	13.11	3.64	3.12	1.25
56-D	1	22,908	0.368	0.0866	8.00	9.01	4.25	3.32	1.22
	2	26,520	0.318	0.0655	10.58	12.00	4.86	3.82	1.34
57-D	1	25,063	0.283	0.0617	11.23	14.96	4.59	4.23	1.00
	2	20,673	0.340	0.0686	10.10	12.72	4.96	4.34	1.82
77-B	1	21,106	0.355	0.1254	5.53	5.52	2.83	1.96	1.47
	2	17,413	0.430	0.1822	3.80	4.09	2.36	1.76	1.15
Mean	1	22,961	0.328	0.0835	8.30[a]	9.75[a]	4.09	3.46	1.58
SD		3,263	0.061	0.0229	2.41	4.42	0.97	0.93	0.55
Median		22,889	0.336	0.0817	8.49	11.04	4.42	3.74	1.41
Mean	2	22,418	0.337	0.0898	7.72[a]	9.27[a]	4.11	3.58	1.33
SD		3,970	0.062	0.0459	4.74	7.83	0.99	1.00	0.25
Median		21,008	0.342	0.0703	9.87	12.14	4.42	3.99	1.26
		NS[b]	NS		NS	NS	NS	NS	NS

[a] Harmonic mean and pseudo-standard deviation, calculated according to Lam *et al.* (*J. Pharm. Sci.* 74:229–231, 1985).
[b] Experiment 1 vs 2, Wilcoxon signed rank test for equality of two medians of paired observations at the 0.05 significance level. NS, not significant.

cage fence. After receiving topical treatment, the dog remained inactive for most of the rest of the day.

The average maximum CsA blood concentration, C_{max}, was 1685 ± 524 ng/ml for the capsules and 1493 ± 363 ng/ml for the tablets. Time to reach the peak, t_{max}, was 2.63 ± 1.29 hr (range, 1.5–5.0 hr) and 3.32 ± 2.56 hr (range, 1.5–6.0 hr) for the capsules and the tablets, respectively. Mean absorption time, MAT, was 2.76 ± 2.67 and 2.96 ± 2.33 hr for the capsules and the tablets, respectively. Mean CsA absolute bioavailability from the capsules was 46.0 ± 11.1%. The corresponding value for the tablets was 45.4 ± 9.9%. None of the parameters calculated from the oral data differed significantly between the tablet and the capsules (Table II).

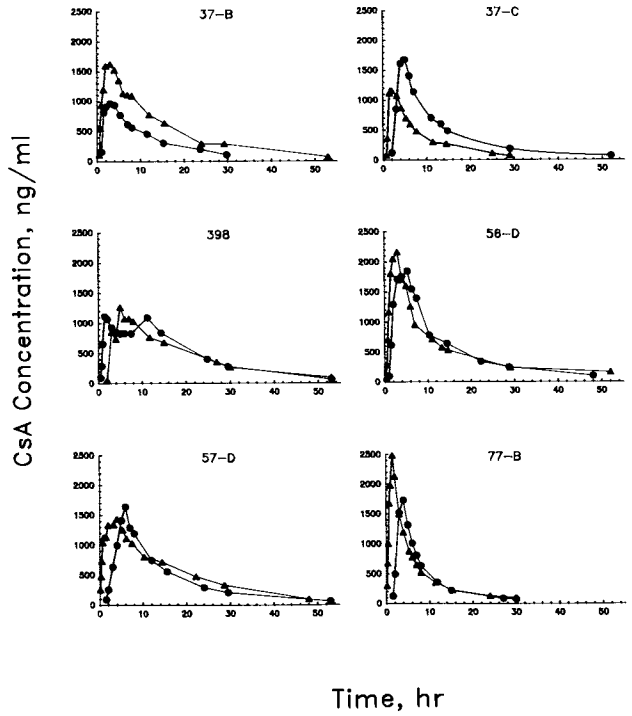

Fig. 3. CsA blood concentrations in six dogs following oral doses of 400 mg in the form of the commercial oil solution filled into soft gelatin capsules (▲) and formula III tablets (●).

DISCUSSION

Preliminary Experiments

Several approaches were attempted to improve the performance of the CsA oral formulation. The first approach used a water-miscible liquid carrier in which CsA is highly soluble, triacetin (glyceryl triacetate, 590 mg/ml; S. H. Yalkowsky, unpublished observations). Triacetin is partially water-soluble (1:15) and easily digested by esterases. CsA solution (250 mg/ml) was prepared in triacetin with 2% polysorbate 80 and filled into soft gelatin capsules, each containing 1 ml of the solution. When tested for *in vitro* dissolution, the capsules showed no detectable CsA release for up to 22 hr. In an *in vivo* study, only one of three dogs showed measurable CsA blood concentrations after taking 500 mg CsA in this formulation.

We subsequently prepared solid dispersions with polyethylene glycol 6000 (PEG). CsA/PEG melts containing 15 and 20% of the former were prepared, filled into hard gelatin capsules, and tested *in vitro*. Placebo capsules disintegrated rapidly; however, the inclusion of CsA in the melt made the

Table II. Pharmacokinetic Parameters of Cyclosporine in Dogs Following an Oral Dose of 400 mg as Soft Gelatin Capsules or Tablets

Dog No.	Dosage form	AUC (ng · hr/ml)	C_{max} (ng/ml)	t_{max} (hr)	MAT (hr)	F
37-B	Capsules	24,679	1621	3.0	3.22	0.593
	Tablets	13,455	965	3.0	1.44	0.298
37-C	Capsules	10,987	1151	2.0	1.13	0.306
	Tablets	19,021	1674	5.0	1.73	0.459
398	Capsules	24,234	1264	5.0	6.87	0.440
	Tablets	25,385	1110	1.5	5.39	0.464
56-D	Capsules	26,415	2161	3.0	8.20	0.504
	Tablets	25,856	1846	5.3	6.36	0.571
57-D	Capsules	27,448	1435	4.0	2.42	0.554
	Tablets	22,062	1638	6.0	4.37	0.540
77-B	Capsules	15,192	2480	1.5	3.32	0.364
	Tablets	13,524	1726	4.0	5.48	0.393
Mean	Capsules	21,493	1685	2.63[a]	2.76[a]	0.460
SD		6,744	524	1.29	2.67	0.111
Median		24,457	1520	3.00	3.27	0.472
Mean	Tablets	19,884	1493	3.32[a]	2.96[a]	0.454
SD		5,535	363	2.56	2.33	0.099
Median		20,542	1656	4.50	4.88	0.462
Significance[b]		NS	NS	NS	NS	NS

[a] Harmonic mean and pseudo-standard deviation, calculated according to Lam *et al.* (*J. Pharm. Sci.* 74:229–231, 1985).
[b] Capsules vs tablets, Wilcoxon signed rank test for equality of two medians of paired observations at the 0.05 significance level. NS, not significant.

mixture too hydrophobic to disintegrate in a reasonable time. This effect of CsA on the wettability of PEG took place only when the mixture was prepared by heating. When the two ingredients were triturated, the mixture was readily wetted and the capsules disintegrated in 15 min. It appears that the more intimately CsA was mixed with PEG, the more the former imparted its hydrophobic nature on the mixture.

Tablets of CsA prepared by direct compression with varying amounts of mannitol, Avicel, PEG, and SDS were tested. The removal of PEG from the tablet formulation and its replacement with mannitol resulted in marked improvement in the properties of the tablets. Tablets with no mannitol or with 100 mg mannitol and 150 mg PEG failed the USP XX disintegration test, while those containing 250 mg mannitol and no PEG exhibited excellent disintegration and dissolution properties. The tablets with a high mannitol content were also harder and glossier than those which contained less mannitol.

Tablets as well as hard gelatin capsules containing CsA/PEG trituration were tested in two dogs and compared to the commercial solution filled into soft gelatin capsules. The tablets produced CsA blood concentrations higher than those obtained following the soft gelatin capsules in both dogs. The hard gelatin capsules gave rise to CsA concentrations lower than or comparable to those obtained following soft gelatin capsules. The mean AUCs for the soft capsules, hard capsules, and tablets in the two dogs were 21,381, 14,655, and 21,020 ng · hr/ml, respectively. This observation, with the added advantage of ease of manufacture of tablets compared to capsules, made us choose tablets as the final formulation to be tested in more animals in comparison to the commercial dosage form.

Administering the commercial dosage form to dogs in the same way as it is taken by patients proved problematic. It was not possible to force the dog to drink the olive oil solution dispersed in milk because of the objectionable taste of the product, while administration via gastric tube might influence absorption. The two choices left were to administer the undiluted solution by a syringe placed at the back of the dog's tongue or to fill the solution into soft gelatin capsules. We wanted to avoid the stress involved in the former procedure because of handling and the objectionable taste. When tested *in vitro* the capsule shells disintegrated in less than 5 min. Furthermore, literature reports have shown almost identical AUCs when comparing Sandoz's soft gelatin capsules to those obtained following the oral solution (10–12). It seemed reasonable, therefore, to fill the solution into capsules. The administration of the capsules or tablets with water was difficult; the dogs had to be forced to drink water by a syringe, which made them resist and attempt to vomit. Milk did not seem to influence CsA absorption in the preliminary studies, and therefore, the dogs were allowed to drink 150 ml of milk after swallowing the capsules or tablets.

Pharmacokinetics of Intravenous and Oral Cyclosporine

The dog was chosen as the animal model because of a similar gastrointestinal anatomy and physiology to man (13). Greyhounds were more readily available to us than any other breed of dogs, and female dogs were chosen because of their smaller size, permitting the use of smaller doses of ^{3}H-CsA in order to obtain measurable blood concentrations of radioactivity.

Our experimental design permits the unequivocal determination of whether differences in AUC after oral administration are due to differences in absorption, distribution, or elimination. This design also allows the examination of the assumption of time invariance of CsA kinetics in dogs during

short-term experiments involving occasional dosing. The biotransformation pathways of the tracer compound used are expected to be identical to those of the unlabeled drug since the tritium label is located at a position where metabolism is known not to occur, namely, the β carbon of amino acid 1 (1).

None of the pharmacokinetic parameters determined showed a statistically significant difference between experiment 1 and experiment 2 (Table I). Generally, the capsules gave rise to a more rapid onset of absorption and earlier peak blood concentrations than did the tablets (Fig. 3); the differences in t_{max} and MAT, however, were not statistically significant (Table II). There was no significant difference in bioavailability between the tablets and the capsules (Table II). The problems which occurred on the experimental days with dogs 37-B and 398 not only provide a possible explanation for the difference in behavior of these two dogs compared to other dogs, but also suggest another factor potentially influencing CsA pharmacokinetics, namely, activity and posture. We are not aware of any report that addresses the differences in CsA kinetics between supine and ambulatory subjects.

The only other study that examined absolute bioavailability of the commercial oil solution in dogs (14) yielded a mean percentage absorbed of 20%, with a range of 10 to 31%. We obtained higher values for the commercial preparation filled into soft gelatin capsules, 46.0 ± 11.1% (range, 30.6 to 59.3%).

A nonparametric statistical test was used to compare the pharmacokinetic parameters obtained in experiments 1 and 2, namely, the Wilcoxon signed rank test for the equality of two medians of paired observations. Nonparametric tests assume no particular shape of the distribution of the observed values. The assumptions made are that the distribution is continuous and symmetric and that the error terms in individual values are mutually independent. Because of the small sample size in the present study and the scarcity of information in the literature about the pharmacokinetic parameters of CsA in dogs, we could not substantiate the assumption of a particular distribution in our data. The means and standard deviations presented for the parameters determined are strictly descriptive of the sample and no claim is made that these values estimate a larger population of individuals.

In conclusion, we have prepared a tablet formulation that is more palatable to the patient than the current olive oil solution. Provided that bioequivalence can be shown in human subjects, our preparation may prove superior to Sandoz's improvement over the existing dosage form, namely, the soft gelatin capsules. Directly compressible tablets are easier to manufacture than capsules, and they do not suffer from the aftertaste of oil that may be experienced in some patients after swallowing the capsules.

The results of the present study indicate that during the time between the two experiments, 8–13 days, there was no change in the pharmacokinetics of CsA in the dogs. Therefore, experiments in normal dogs involving separate, occasional oral and intravenous administration over short periods of time should be considered acceptable. For studies with longer durations, involving more frequent CsA administration, or performed in subject populations where time invariance has not been verified, simultaneous oral and intravenous administration is recommended.

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