Pharmacokinetics, Mass Balance, and Induction Potential of a Novel GABA Uptake Inhibitor, CI-966 HCl, in Laboratory Animals

Louis L. Radulovic,[1,2] Howard N. Bockbrader,[1] and Tsun Chang[1]

Received April 6, 1992; accepted March 16, 1993

CI-966 exhibits anticonvulsant properties in various animal models. The drug acts by inhibiting synaptic uptake of γ-aminobutyric acid (GABA). Oral absorption of CI-966 in dogs given 1.39 mg/kg is rapid with a t_{max} of 0.7 hr. In rats given 5 mg/kg oral, a mean t_{max} of 4.0 hr was observed. Following iv administration of the same respective doses, elimination $t_{1/2}$ in dogs and rats averaged 1.2 and 4.5 hr. Absolute oral bioavailability of CI-966 was 100% in both species. Following oral dosing of [^{14}C]CI-966 HCl to dogs, fecal, and urinary excretion accounted for 89% and 2.3% of the ^{14}C dose, respectively. In bile-duct cannulated rats, biliary excretion is the major elimination pathway of radioactivity (75%). Urinary and fecal excretion accounted for 4.1 and 12%, respectively. CI-966 does not induce or inhibit mouse hepatic mixed function oxidases, as determined by hexobarbital sleeping time.

KEY WORDS: GABA uptake inhibitor; anticonvulsant; pharmacokinetics; dose proportionality; mass balance; enzyme induction/inhibition.

INTRODUCTION

CI-966 HCl, 1-[2-[bis[4-(trifluoromethyl)phenyl]-methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid monohydrochloride (Fig. 1), is a potential anticonvulsant which differs structurally and mechanistically from currently marketed anticonvulsants (1). CI-966 acts by blocking the synaptic reuptake of GABA, while having minimal effect on release of GABA.

GABA receptors mediate inhibitory neurotransmission in various regions in the brain and spinal cord and regulate several physiological processes (2,3). Malfunctions in the central GABA system are presumed to contribute to the development of epilepsy, dyskinesia, and other central nervous system diseases (4–6). Hence, drugs which affect GABA may be of therapeutic value. Currently marketed anticonvulsants, such as phenytoin, carbamazepine, and valproic acid, act on voltage-sensitive sodium channels and do not affect GABA uptake at therapeutic concentrations.

Based on pharmacological studies performed in laboratory animals (7), CI-966 should be effective against simple partial seizures, complex partial seizures which are often refractory to available medications, and generalized tonic-clonic seizures. CI-966 was ineffective in a rat model for absence seizures.

During development, studies were conducted to determine the pharmacokinetics, dose proportionality, mass balance, and induction potential of CI-966 HCl in laboratory animals. Results of these studies are reported here.

MATERIALS

CI-966 HCl and ^{14}C-labeled drug were synthesized by the Chemistry and Radiochemistry Departments at Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI (8). [^{14}C]CI-966 HCl had a specific activity of 22.0 μCi/mg and a radiochemical purity of at least 99%. PD 126561 (internal standard; IS), 1-[2-[bis[4-(trifluoromethoxy)-phenyl]-methoxy]ethyl]-3-piperidine carboxylic acid, monohydrochloride, was synthesized by the Chemistry Department at Parke-Davis Pharmaceutical Research Division, and was used as the internal standard in the rat HPLC assay. Bond Elut CN end-capped cartridges were obtained from Analytichem International (Harbor City, CA). HPLC-grade water and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). HPLC-grade dimethyl sulfoxide (DMSO) and dimethylacetamide (DMA) were acquired from Burdick and Jackson (Muskegon, MI). Ready Gel was obtained from Beckman Instruments (Fullerton, CA). Combusto-cones, Carbo-sorb II, and Permafluor V were purchased from Packard Instruments (Downers Grove, IL). All other chemicals used were ACS grade or better.

METHODS

CI-966 was administered as the hydrochloride salt. Doses are expressed as free-base equivalents.

Single-Dose Pharmacokinetic Study

Two groups of six fasted, male Wistar rats were administered single 5 mg/kg oral or iv CI-966 doses in 15% DMA in 5% dextrose. Serial blood samples (1 mL) were drawn up to 32 hr postdose (ten samples per rat). Plasma was separated and analyzed for CI-966 using a HPLC-UV procedure.

In a nonblind, randomized, four-way crossover study, four fasted female dogs received single 0.46, 1.39, and 2.78 mg/kg oral doses of CI-966 HCl and a single 1.39 mg/kg iv dose. All doses were dissolved in 15% DMA in 5% dextrose. Serial blood samples (3 mL) were taken up to 8 hr postdose (nine samples per dog). Plasma was separated and stored frozen until analyzed for CI-966 by HPLC. CI-966 is stable in dog plasma at −20°C for at least 5 months. Analysis of variance and Tukey's Studentized range test were used to determine significance of mean CI-966 pharmacokinetic parameter differences between dogs and/or treatments (9).

^{14}C Mass Balance Studies

Six male Wistar rats were anesthetized with ether and a silastic cannula (0.025-in. ID × 0.047-in. OD) was surgically implanted into the common bile duct. Following overnight

[1] Pharmacokinetics/Drug Metabolism Department, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105.

[2] To whom correspondence should be addressed at Pharmacokinetics/Drug Metabolism, Parke-Davis Pharmaceutical Research Division, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

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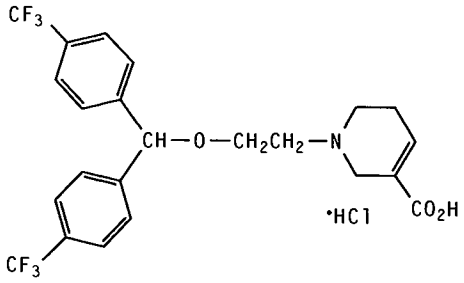

Fig. 1. Structure of CI-966 HCl.

recovery, each fasted rat received a single 14.5 mg/kg (142.5 μCi/kg) oral dose of [^{14}C]CI-966 HCl (hot plus cold drug) based on a mean weight of 351 g. Urine, bile, and feces were collected up to 120 hr postdose. Only three of the six animals completed the study. One rat (Rat 4) was found dead at 48 hr postdose. Rats 5 and 6 were sacrificed following the 32- to 48- and 72- to 96-hr collection intervals, respectively, due to their moribund state. Morbidity of Rat 6 was most likely due to obstruction of the bile duct cannula and not drug related. As for Rats 4 and 5, cause of death was uncertain. Results from Rats 4, 5, and 6 were excluded from data analyses.

Two fasted beagle dogs per gender received a single 2.77 mg/kg (0.5 μCi/kg) oral dose of [^{14}C]CI-966 HCl (hot plus cold drug). Dogs received from 5.3 to 5.6 μCi [^{14}C]CI-966. Urine and feces samples were collected up to 120 hr postdose.

Urine and feces from the rat and dog studies were analyzed for total radioactivity by liquid scintillation counting (LSC). Each sample was counted for 10 min in a Packard Tri-Carb liquid scintillation counter Model 4530 using external standardization for quench correction. Limit of quantitation was three times the mean background count of predose samples. Fecal samples were combusted in a Packard Tri-Carb sample oxidizer Model 306 prior to LSC. The resulting $^{14}CO_2$ was trapped with Carbo-sorb II and counted in Permafluor V.

Induction/Inhibition Study

A hexobarbital sleeping time study was conducted in mice to determine the effect of CI-966 on hepatic cytochrome P-450 monooxygenases. Thirty male CD-1 mice (≈25 g) were randomly assigned to three treatment groups: (1) vehicle, 10% DMA in 5% dextrose; (2) CI-966 HCl solution, 10 mg/kg QD; and (3) phenobarbital solution, 80 mg/kg QD. Vehicle and CI-966 HCl dissolved in vehicle were administered by intraperitoneal (ip) injection for 10 consecutive days from Day 1 through Day 10. Phenobarbital in saline was given ip for 5 days, Days 6 through 10. Following a 2-day washout period, all three treatment groups received a single 75 mg/kg ip dose of hexobarbital in saline. Once sedated, the mice were placed on their sides and the time necessary for the animals to right themselves was recorded. Studentized *t* test and Duncan's multiple range test were used to determine significance of mean hexobarbital sleeping time differences between treatments (9).

Analysis of Plasma CI-966 Concentrations

Sensitive reversed-phase HPLC-UV procedures were developed for quantitation of CI-966 in rat and dog plasma. The assays were capable of detecting from 0.05 to 8.0 μg/mL CI-966. Fifty microliters of calibration standard stock solution, 50 μL of IS (16 μg/mL PD 126561), and 1 mL of 25 m*M* ammonium phosphate buffer, pH 9 (Buffer A), were added to 400 μL of rat plasma. Samples were applied to 1-mL Bond Elut CN cartridges (end-capped), which were previously conditioned with 3 × 1 mL acetonitrile and 3 × 1 mL Buffer A. Cartridges were washed with 2 × 1 mL Buffer A and 2 × 0.3 mL of 20% acetonitrile in Buffer A. CI-966 and IS were eluted with 300 μL of 50% acetonitrile in Buffer A.

The above procedure was modified slightly for isolation of CI-966 from dog plasma since an endogenous peak cochromatographed with IS. One hundred microliters of calibration standard stock solution and 1 mL of buffer A were added to 400 μL of dog plasma. Samples were applied to the CN cartridges, which were conditioned as described previously. Cartridges were washed with 2 × 1 mL of Buffer A and 0.5 mL of 20% acetonitrile in Buffer A. CI-966 was eluted with 300 μL of 50% acetonitrile in Buffer A.

Fifteen microliters of 1 *N* HCl was added to both rat and dog isolates to adjust pH prior to HPLC analysis. Isolates were resolved on a Supelcosil LC-18-DB HPLC column (5 μm, 4.6 ID × 250 mm; Supelco Inc., Bellefonte, PA). Mobile phase consisted of 25 m*M* ammonium phosphate buffer monobasic, pH 3:acetonitrile (39:61). Flow was maintained at 1 mL/min. Absorbance was monitored at 230 nm. Rat plasma CI-966 concentrations were quantitated by regressing peak-height ratios as a function of CI-966 concentrations. Quantitation of CI-966 in dog plasma was performed by absolute peak height. A weighting factor of 1/concentration was used in the linear regression analyses.

Pharmacokinetic Analysis

CI-966 pharmacokinetic parameters were derived using model independent methods. Maximum plasma concentration (C_{max}) and its corresponding time (t_{max}) were obtained from observed data. Elimination rate constant (λz) was estimated as the absolute value of the slope of a least-squares regression of natural logarithm plasma concentration–time profile during the terminal phase. Apparent elimination half-life ($t_{1/2}$) was calculated 0.693/λz. Harmonic mean elimination $t_{1/2}$ was calculated using the Jackknife method (10). Area under the plasma concentration–time curve (AUC) was estimated using the trapezoidal rule. AUC(0-*t*ldc) was calculated from time 0 to *t*ldc, the time of last detectable concentration (ldc). AUC(0-∞) was determined by summing AUC(0-*t*ldc) and ldc/λz. Plasma clearance (CL_p) was calculated as Dose (mg/kg)/AUC(0-∞)$_{iv}$. Steady-state volume of distribution (Vd_{ss}) was calculated as (Dose · AUMC/[AUC(0-∞)^2], where dose is expressed as mg/kg and AUMC is the area under the curve of the product of time *t* and drug concentration from time 0 to infinity. Absolute bioavailability (*%F*) was determined as [AUC(0-∞)$_{po}$/AUC(0-∞)$_{iv}$] · 100%.

RESULTS AND DISCUSSION

Pharmacokinetics and Oral Bioavailability

Mean plasma CI-966 concentration–time profiles in rats and dogs are shown in Figs. 2 and 3, respectively. Mean

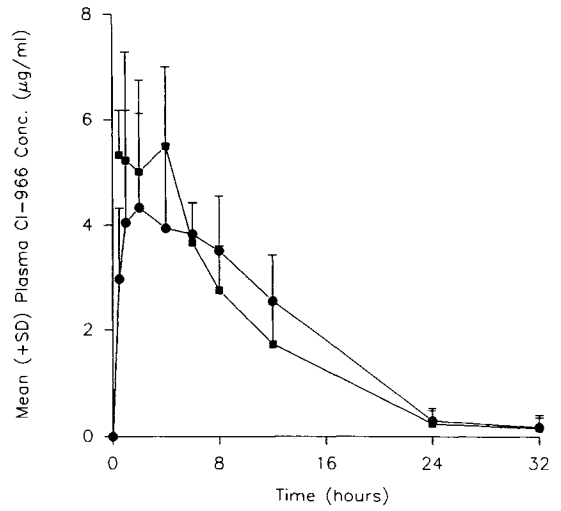

Fig. 2. Mean concentration–time profiles following oral (circles) and iv (squares) administration of 5 mg/kg CI-966 HCl to rats.

pharmacokinetic parameters in rats and dogs are provided in Table I. The large variability (%RSD) in t_{max} is attributed to two rats in which maximum plasma CI-966 concentrations were not observed until 8 hr postdose. The reason for this anomaly is unknown. Yet pharmacokinetic parameters derived for the single 5 mg/kg oral and iv doses administered to rats were comparable. Differences in mean AUC(0-∞) values were less than 10%. Mean λz values also differed by approximately 10%. Harmonic mean elimination $t_{1/2}$ averaged 4.5 hr for both routes of administration. Mean absolute bioavailability of CI-966 HCl in Wistar rats was 108%, indicating that drug was completely absorbed when administered orally as a solution. ANOVA results for λz and AUC(0-∞) values derived for the single 1.39 mg/kg oral and iv doses administered to dogs revealed no statistically significant difference among treatment means. Harmonic mean elimination $t_{1/2}$ values were similar and averaged 1.2 hr. CI-966 HCl given as an oral solution was completely absorbed by dogs.

Noteworthy differences in the pharmacokinetics of CI-966 in rats and dogs were the delay in absorption of CI-966 in rats as reflected in t_{max} (4 hr in rats as compared to 0.8 hr in dogs) and a 4.8-fold increase in CL_p in dogs (7.61 mL/min/kg) compared to rats (1.59 mL/min/kg). Vd_{ss} values in rats (0.72 L/kg) and dogs (0.86 L/kg) were comparable.

Results from the four-way crossover study to determine dose proportionality in beagle dogs are reported in Table II. Mean t_{max} values ranged from 0.5 to 0.8 hr, suggesting rapid absorption. No significant differences were observed among treatment means for t_{max}. CI-966 exhibited linear pharma-

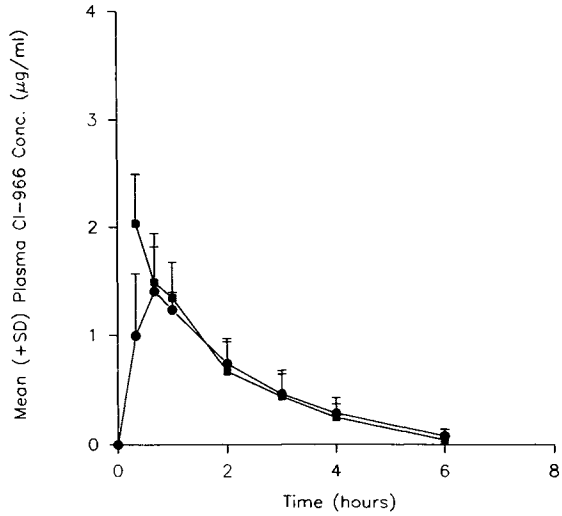

Fig. 3. Mean concentration–time profiles following oral (circles) and iv (squares) administration of 1.39 mg/kg CI-966 HCl to dogs.

Table I. Mean (%RSD[a]) Pharmacokinetic Parameters Obtained Following Single Oral and iv Doses of CI-966 HCl to Male Wistar Rats (N = 6) and Female Beagle Dogs (N = 4)

	Wistar rats (5 mg/kg)		Beagle dogs (1.39 mg/kg)	
	Oral	iv	Oral	iv
C_{max} (μg/mL)	5.20 (29.2)	—	1.45 (26.1)	—
t_{max} (hr)	4.0 (82.2)	—	0.8 (44.2)	—
λz (hr^{-1})	0.146 (34.4)	0.164 (36.1)	0.55 (8.55)	0.61 (11.7)
$t_{1/2}$ (hr)	4.7 (34.7)	4.2 (36.6)	1.3 (10.8)	1.1 (13.3)
AUC (0–∞) (μg · hr/mL)	62.1 (22.8)	57.5 (30.6)	3.36 (31.1)	3.38 (35.9)
F (%)	108	—	102 (13.8)	—
CL_p (mL/min/kg)	—	1.59 (35.6)	—	7.61 (37.5)
Vd_{ss} (L/kg)	—	0.72 (25.2)	—	0.86 (29.3)

[a] Percentage relative standard deviation.

Table II. Mean (%RSD[a]) CI-966 Pharmacokinetic Parameters Obtained Following Oral Administration of 0.46, 1.39, and 2.78 mg/kg CI-966 HCl to Female Beagle Dogs (N = 4)

Parameter	Dose (mg/kg) 0.46	1.39	2.78	ANOVA result
C_{max} (μg/mL)	0.305 (27.6)	1.45 (26.1)	2.61 (30.5)	NP[b]
Normalized C_{max}[c] (μg/mL)	0.663 (27.6)	1.04 (26.1)	0.940 (30.5)	NS[d]
t_{max} (hr)	0.5 (46.2)	0.8 (44.2)	0.7 (117)	NS
λz (hr^{-1})	0.558 (40.3)	0.550 (8.55)	0.579 (12.1)	NS
$t_{1/2}$ (hr)	1.3 (45.9)	1.3 (10.8)	1.2 (12.0)	NP
AUC(0–∞) (μg · hr/mL)	0.686 (41.0)	3.36 (31.1)	6.08 (23.9)	NP
Normalized AUC(0–∞)[c] (μg · hr/mL)	1.49 (41.0)	2.42 (31.1)	2.19 (23.9)	P = 0.007

[a] Percentage relative standard deviation.
[b] Statistical comparison not performed.
[c] Normalized to a dose of 1 mg/kg.
[d] Not statistically significant ($P > 0.05$).

cokinetics. Harmonic mean elimination $t_{1/2}$ values ranged from 1.2 to 1.3 hr. Mean λz values were not significantly different and averaged 0.6 hr^{-1}. Mean C_{max} values increased with increasing dose. When C_{max} was normalized for dose, mean value of the 0.46 mg/kg oral dose was approximately 60% of the values obtained for the 1.39 and 2.78 mg/kg oral doses; however, the difference in treatment means was not statistically significant. Mean AUC(0-∞) values also increased with increasing dose. The normalized mean AUC(0-∞) value for the 0.46 mg/kg oral dose was approximately 60% of the values obtained for the two higher doses and was significantly different. These results indicated that mean AUC(0-∞) values for the 1.39 and 2.78 mg/kg dose group were proportional to dose but less than proportional with the 0.46 mg/kg dose group. Similar trends in normalized mean AUC(0-∞) and C_{max} values for the 0.46 mg/kg dose suggest reduced bioavailability.

[^{14}C]CI-966 Mass Balance

In bile duct-cannulated rats, mean (%RSD) cumulative urinary excretion of radioactivity accounted for only 4.09% (61.6). Mean cumulative biliary excretion accounted for 74.6% (4.7), with peak excretion occurring in the 8- to 24-hr collection. An additional 12.0% (26.6) of the radioactivity was recovered in the feces. The presence of radioactivity in the feces of bile duct-cannulated rats suggests that CI-966 and/or its metabolites are also secreted in the gastrointestinal tract, since oral bioavailability of CI-966 is 100% in rats. Mean total excretion of radioactivity was 90.7% (1.2).

In dogs, mean (%RSD) cumulative urinary excretion of radioactivity was 2.29% (39.0). Approximately 89% (10.2) of the ^{14}C dose was eliminated in the feces within 72 hr postdose. As in rats, CI-966 or its metabolites probably undergo biliary excretion or are secreted in the gastrointestinal tract since oral bioavailability in dogs is 100%. Mean total ^{14}C excretion was 91.0% (10.3).

Induction/Inhibition Study

A hexobarbital sleeping time study was conducted in mice to determine the effect of CI-966 on hepatic cytochrome P-450 activity. Duration of hexobarbital sleeping time has been shown to be correlated with hepatic cytochrome P-450 activities. Enzyme inducers, such as phenobarbital, enhance the metabolism of hexobarbital and reduce sleeping time (11). Mean hexobarbital sleeping times for vehicle (13.2 ± 5.6 min) and CI-966 (12.7 ± 2.6 min) treatment groups were not significantly different, but mean hexobarbital sleeping time of the phenobarbital (8.4 ± 2.2 min) treatment group was significantly different ($P = 0.019$) from that of the vehicle control. Since repeated administration of 10 mg/kg/day CI-966 for 10 days failed to produce any effect on hexobarbital sleeping time as compared to vehicle control, CI-966 is not considered an inducer or inhibitor of hepatic cytochrome P-450 in mice.

CONCLUSIONS

Since published pharmacokinetic data are lacking on other GABA uptake inhibitors such as Tiagabine (NO-328, Novo-Nordisk; A-70569, Abbott), SKF-89976-A, and SKF-100844-A (both Smith Kline & French compounds), comparison of CI-966's disposition in laboratory animals is impossible. Moreover, CI-966 differs mechanistically from marketed anticonvulsants. However, as with carbamazepine, phenytoin, and valproic acid (12), CI-966 is highly bound to plasma proteins.

CI-966 exhibits good bioavailability and readily passes the blood–brain barrier (7). At anticipated therapeutic doses (1 to 2 mg/kg orally), CI-966 pharmacokinetics are linear.

ACKNOWLEDGMENTS

The authors would like to acknowledge Mr. Robert Boncyzk and Mr. James Burleigh for their technical assistance, without which the studies could not have been performed.

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