Pharmacokinetics and Anticonvulsant Effect of a New Hypnotic, CL 284,846, in Rats[1]

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Purpose. CL 284,846 (CL846) is an investigational non-benzodiazepine agent with hypnotic, anxiolytic, myorelaxant and anticonvulsant properties. This study assessed the pharmacokinetics and anticonvulsant action of CL846 in female Sprague-Dawley rats.

Methods. CL846 pharmacokinetics were examined after either an iv bolus dose (2.5 mg/kg) or a 6-hr infusion (0.4 mg/kg/hr). CL846 pharmacodynamics were evaluated with a pentylenetetrazol (PTZ) infusion 5 min after a CL846 in bolus dose (0 to 10 mg/kg). CL846 and the derived metabolite CL 284,859 (CL859) concentrations in serum and brain tissue were determined by HPLC with fluorescence detection.

Results. Both the steady-state volume of distribution (1636 ± 162 and 1804 ± 293 ml/kg, after bolus and infusion administration, respectively) and systemic clearance (19.1 ± 7.1 and 22.2 ± 4.3 ml/min/kg for bolus and infusion administration, respectively) were high. No differences in pharmacokinetic parameters were noted between the two modes of administration. The relationship between anticonvulsant effect and brain/serum concentrations was well described by an E_{max} model. CL846 was as effective as triazolam in antagonizing PTZ-induced seizures.

Conclusions. Under theconditions of the present study, CL846 pharmacokinetics were linear and stationary. Further evaluation of the anticonvulsant properties of CL846 is warranted, including the potential development of tolerance, which is well known for benzodiazepines.

KEY WORDS: anticonvulsant; CL 284,846; CL 284,859; pentylenetetrazol; pharmacokinetics; pharmacodynamics.

INTRODUCTION

Insomnia is a prevalent disorder that affects 10 to 35% of the population (1,2). When pharmacologic treatment is indicated, benzodiazepines have proven to be superior to other agents. The use of benzodiazepines is limited, however, by undesirable effects such as synergistic depression of the central nervous system (CNS) when used concomitantly with other psychotropic drugs, impairment of psychomotor performance, and development of tolerance upon repeated administration (3). These adverse effects have lead to the search for other drugs with the selectivity and safety of benzodiazepines. CL 284,846 (CL846), a pyrazolopyrimidine (Figure 1), is a novel non-benzodiazepine agent that may offer several advantages over benzodiazepines.

CL846 evidences pharmacologic effects (hypnotic, anxiolytic, myorelaxant, and anticonvulsant) that are typical of benzodiazepines and probably are mediated by binding to the benzodiazepine-GABA receptor complex, which increases the frequency of chloride channel opening with a subsequent inhibition of neuronal activity (4). CL846 displaces flunitrazepam from neuronal binding sites *in vitro* (IC_{50} 205 nM) and increases the binding of *t*-butylbicyclophosphorothionate, a compound known to bind to a site affiliated with the benzodiazepine-GABA receptor complex (American Cyanamid Co., unpublished results). The decrease in motor activity and impairment of grip strength caused by CL846 *in vivo* is attenuated by RO 15-1788, a specific antagonist of the benzodiazepine receptor. CL846 is orally active in a variety of animal tests, with an ED_{50} ranging from 2.6 to 15 nM, similar to the ED_{50} values for triazolam (1.1–13 nM) and flurazepam (8–64 nM). CL846 shows a smaller degree of tolerance than triazolam, as expressed by the increased ED_{50} for impaired motor activity and grip strength during multiple administration, and is associated with a smaller potentiation of CNS depression when administered concurrently with ethanol (American Cyanamid Co., unpublished results).

The desethyl metabolite of CL846 (CL 284,859 [CL859]) (Figure 1), the primary metabolite formed in the rat, is believed to be pharmacologically active but non-sedating; the ED_{50} of CL859 is approximately 50-fold higher than that of CL846 in tests of inclined screen grip strength and motor activity. The metabolite is much less potent than CL846 in displacing flunitrazepam from the benzodiazepine-GABA receptor (IC_{50} = 11 μM).

Although numerous pharmacologic actions of CL846 have been investigated in experimental animals, characterization of the concentration-effect relationship has received little attention. Determination of this relationship is necessary to assess the potential therapeutic or toxic concentration range; when combined with pharmacokinetic data, this relationship allows prediction of the magnitude and duration of pharmacologic effect under differing routes or modes of administration.

Antagonism of PTZ-induced convulsions has been used extensively in the screening of benzodiazepine-type compounds and various investigational antiepileptics (5). Retrospective examination has shown that this model correlates well with clinical efficacy against certain types of seizures (6). The PTZ model was used previously in this laboratory to evaluate the pharmacodynamics of triazolam, a benzodiazepine hypnotic that shares many pharmacologic and pharmacokinetic properties with CL846 (7). The present studies were undertaken to assess the pharmacokinetics of CL846 after an iv bolus dose or a 6-hr infusion in rats, and to characterize the concentration-anticonvulsant effect relationship of CL846 after iv bolus administration.

MATERIALS AND METHODS

Chemicals

CL846, CL859 and CL 218,872 were provided gener-

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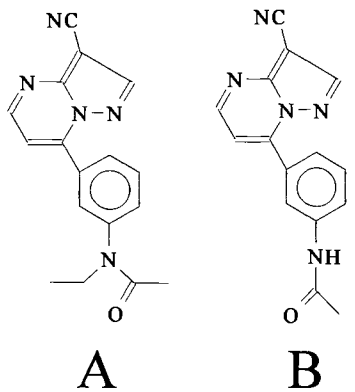

Fig. 1. Chemical structures of CL846 (A) and CL859 (B).

ously by American Cyanamid Company (Pearl River, New York); PTZ was purchased from Sigma Chemical Co. (St. Louis, Missouri). All other reagents and solvents were obtained from commercial sources and were used without additional purification. For the pharmacokinetic studies, CL846 was dissolved in an aqueous solution of 15% N,N-dimethylacetamide; for the pharmacodynamic study, CL846 was dissolved in an aqueous solution of 35% polyethyleneglycol (PEG) 300.

Animals

Female Sprague-Dawley rats (Hilltop Laboratory Animals, Scottdale, Pennsylvania), weighing 225-270 g, were housed in individual stainless steel cages in temperature-controlled rooms with a 12-hr light per day cycle. The animals were allowed free access to food and water, and were acclimated for at least 2 weeks prior to experimentation.

Pharmacokinetic Studies

On the day prior to the experiment, rats were anesthetized with ether and a silicone rubber cannula was inserted into the right external jugular vein; for iv infusion studies, both the right jugular and right femoral veins were cannulated. On the day of the experiment, CL846 was injected through the jugular cannula as a bolus (2.5 mg/kg, n = 4 rats) or was infused (0.4 mg/kg/hr for 6 hr) into the femoral vein (n = 5 rats). Blood samples (100–200 μl) were obtained through the jugular vein cannula prior to drug administration and at timed intervals throughout the experiment (10–14 samples per animal). Blood samples were allowed to clot, centrifuged, and the serum was harvested and stored at −20°C pending HPLC analysis.

In a separate experiment, the disposition of CL846 in brain tissue and serum was evaluated after an iv bolus dose (0.8 mg/kg) to 18 rats. Three rats were sacrificed by decapitation at each time point (2.5, 5, 30, 60, 120 and 240 min after drug administration); trunk blood and brain tissue were collected after sacrifice and stored at −20°C pending analysis.

Pharmacodynamic Study

Seizures were induced prior to (baseline) and at various times after CL846 treatment by infusion of PTZ (7.5 mg/min) into the jugular vein until occurrence of clonic convulsions. Seizure sensitivity was expressed as the dose of PTZ required to produce clonic convulsions. One day after determination of the baseline PTZ dose, 33 rats were randomized to receive one of the following CL846 doses (0 [PEG vehicle], 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 mg/kg), administered as a bolus. In preliminary studies, the PEG 300 vehicle (750 μl) previously was found not to affect PTZ-induced seizures in the rat. Five min after CL846 administration (the time of maximal effect as determined in a preliminary experiment), PTZ was infused until seizures occurred. Immediately post-seizure, the animal was sacrificed by decapitation for collection of trunk blood and brain tissue. PTZ infusions were administered at the same time each day, and the observer was blinded to the dose of CL846.

Octanol-Water Partition Coefficient

The liposolubility of CL846 and CL859 was assessed by determining the octanol-water partition coefficient according to standard techniques. Two weighed samples of CL846 and CL859 each were dissolved in 1 ml octanol, and 5 ml of distilled water or potassium phosphate buffer (pH 7.4) was added. After shaking (60 min) and centrifuging (10 min), drug concentrations in aliquots of both phases were determined by HPLC in duplicate. The partition coefficient was calculated as the log of the ratio of drug concentrations in the organic and the aqueous phase.

Assay

A specific and sensitive HPLC method with fluorescence detection (8) was used with slight modifications. Samples of serum (100 μl) or brain tissue homogenate (0.2 g brain tissue homogenized in 200 μl of saturated sodium chloride in water) were added to tubes containing CL 218,872 as an internal standard, and proteins were precipitated with 20 μl ice-cold acetonitrile. The supernatant was filtered, evaporated to dryness, and reconstituted in 100 μl of mobile phase prior to analysis. Samples with concentrations exceeding the upper limit of the standard curve were diluted with water (serum) or saturated sodium chloride (brain tissue) prior to preparation. Calibration curves for both analytes were linear through 250 ng/ml serum or 250 ng/g brain tissue (correlation coefficients >0.99). The lower limit of quantitation was 5 ng/ml in 100 μl serum and 2.5 ng/g in 0.2 g brain tissue. In serum, the interday coefficients of variation were 10.8% (CL846) and 8.5% (CL859) at 10 ng/ml, and 1% for both analytes at 250 ng/ml. In brain tissue, the interday coefficients of variation were 12.4% (CL846) and 9.3% (CL859) at 10 ng/g, and 5.2% (CL846) and 4.1% (CL859) at 250 ng/g. Mean recovery of both analytes exceeded 80% regardless of the matrix.

Pharmacokinetic Data Analysis

CL846 concentration-time profiles were analyzed by non-compartmental methods according to standard techniques to estimate systemic clearance (Cl), steady-state volume of distribution (V_{SS}), terminal elimination rate constant (λ_Z), and mean residence time (MRT) (9). Compartmental pharmacokinetic analysis also was performed. CL846 and CL859 concentration-time profiles from individual rats were

fit simultaneously with the following differential equations (and associated initial conditions), assuming a one-compartment model with linear disposition for both parent and metabolite:

$$\textit{CL846 (bolus)}: \frac{dC_{846}}{dt} = -(k_f + k_{10[CL846]}) \cdot C_{846};$$

$$C^0_{846} = \frac{X_0}{V_{846}}$$

$$\textit{CL846 (infusion)}: \frac{dC_{846}}{dt} = \frac{k_0}{V_{846}} - (k_f + k_{10[CL846]}) \cdot C_{846};$$

$$C^0_{846} = 0$$

$$\textit{CL859}: \frac{dC_{859}}{dt} = k_f \cdot C_{846} - k_{10[CL859]} \cdot C_{859};\ C^0_{859} = 0$$

where X_0 is the bolus dose of CL846 administered, k_0 is the zero-order infusion rate for CL846, V_{846} is the volume of distribution for CL846, k_f is the apparent first-order rate constant for formation of CL859, $k_{10[CL846]}$ is the apparent first-order elimination rate constant for CL846 (representing all other routes of clearance), and $k_{10[CL859]}$ is the first-order elimination rate constant for CL859. Since mass balance was not performed, the volume of distribution for the metabolite was indeterminate. Parameter estimates were obtained by nonlinear least-squares regression with PCNONLIN (SCI Software, Lexington, KY).

Pharmacodynamic Data Analysis

Anticonvulsant effect (E) was defined as the ratio of Day 2 to Day 1 (baseline) PTZ threshold convulsive doses. Serum and brain tissue concentration data were fit simultaneously with the following equation:

$$E = 1 + \frac{E_{max} \cdot C_{846}}{EC_{50} + C_{846}}$$

where E is the observed PTZ threshold dose ratio at a specific concentration (C_{846}) in serum or brain tissue, 1 is the baseline effect, E_{max} is the estimated maximum effect (assumed to be a unique value regardless of the matrix under consideration), and EC_{50} is the drug concentration (serum or brain tissue) associated with 50% of the maximum effect. Assessment of the goodness of fit of the model to the observed data was based on the Akaike's Information Criteria (AIC), residual plots, coefficient of determination and standard error of the parameter estimates.

Statistical Analysis

All pharmacokinetic parameters are presented as the mean and standard deviation. Statistical comparisons were made between the 2 groups using the Student's *t* test for parallel groups. Pharmacodynamic parameters are presented as the estimate and standard error returned by the nonlinear regression analysis program.

RESULTS

Pharmacokinetic Studies

Mean serum concentration-time profiles for CL846 and CL859, after either bolus or infusion administration of CL846, are presented in Figure 2. Non-compartmental pharmacokinetic parameters for CL846 are presented in Table I. Systemic clearance was high and relatively variable, ranging between 11 and 28 ml/min/kg. Steady-state volume of distribution was large (1404 to 2266 ml/kg) which, coupled with the high systemic clearance, yielded a terminal half-life of approximately 60 min. CL859 serum concentrations declined slightly more slowly than those of the parent compound, although the difference was not marked, indicating that the elimination of this metabolite was possibly formation-rate limited. Model-dependent pharmacokinetic parameters are presented in Table II. Compartmental analysis revealed that a 1-compartment model for both parent drug and metabolite was adequate to describe the data, and the derived parameters were in agreement with those obtained by non-compartmental methods. None of the parameters differed statistically between bolus dose and infusion administration protocols.

Mean concentration-time profiles for CL846 and CL859 in serum and brain tissue after an iv bolus dose of CL846 are displayed in Figure 3. CL846 entered the brain very rapidly, with maximum concentrations observed at 2.5 min (*i.e.*, the earliest sample collected). The decline in brain tissue concentrations paralleled that in serum, consistent with rapid

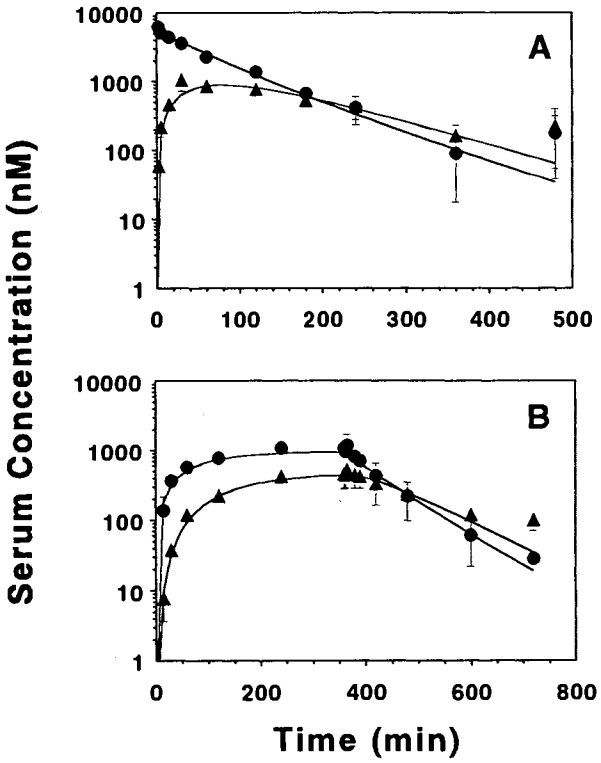

Fig. 2. Serum CL846 (●) and CL859 (▲) concentration-time profiles after a 2.5-mg/kg iv bolus dose (A) or a 0.4-mg/kg/hr iv infusion (B) of CL846. Bars represent mean ± SE; solid lines indicate the average of the individual fitted profiles.

Table I. Non-Compartmental Pharmacokinetic Parameters (mean ± SD) After iv Administration of CL846

Parameter	Bolus Dose (2.5 mg/kg, n = 4)	Infusion (0.4 mg/kg/hr [6 hr], n = 5)
Cl (ml/min/kg)	19.1 ± 7.1	22.2 ± 4.3
V_{ss} (ml/kg)	1636 ± 162	1804 ± 293
λ_z (min^{-1})	0.0102 ± 0.0037	0.0129 ± 0.0035
MRT (min)	95.7 ± 41.3	83.3 ± 16.5
$\frac{CL859\ AUC_{0\to\infty}}{CL846\ AUC_{0\to\infty}}$	0.51 ± 0.09	0.52 ± 0.07

distribution across the blood-brain interface. The brain tissue:serum concentration ratio for CL846 was 0.52 ± 0.15 and did not vary with time post-administration. CL859 concentrations in brain tissue were substantially lower than those in serum; the brain tissue:serum concentration ratio for the metabolite was 0.13 ± 0.081 and also did not vary with time post-administration of the parent drug.

Pharmacodynamic Study

Anticonvulsant effect could be related to the concentration of CL846 in either serum or brain tissue (Figure 4). Based on AIC considerations, the relationship was best described by a simple E_{max} model as compared to its sigmoidal counterpart (data not shown). The maximum PTZ threshold dose ratio as estimated by nonlinear least-squares regression was 12.9 ± 1.6; the EC_{50} values were 3300 ± 579 ng/ml and 1856 ± 321 ng/g for serum and brain tissue, respectively. Figure 5 displays the relationship between serum and brain tissue CL846 concentrations when results from the pharmacokinetic and pharmacodynamic experiments were combined. Brain tissue concentrations increased linearly with serum concentrations; the equation describing this relationship ($y = 0.447 \cdot x - 2.3$) was consistent with a brain:serum partition coefficient of approximately 0.5.

Octanol-Water Partition Coefficient

The log of CL846 partition coefficient between octanol and water or phosphate buffer (pH 7.4) was 1.05 ± 0.001 and 1.10 ± 0.1, respectively. Liposolubility of the metabolite was similar to that of the parent; the log partition coefficients for CL859 were 1.20 ± 0.20 and 1.08 ± 0.1 in water and phosphate buffer, respectively.

DISCUSSION

The present study is the first report describing CL846 pharmacokinetics in the rat. Characterization of CL846 disposition is necessary for the prediction of CL846 concentration-time profiles under different administration regimens, as well as to ascertain the potential linearity and stationarity of drug disposition. The lack of differences in pharmacokinetic parameters obtained after bolus and infusion administration in the present study suggest that linear and stationary disposition of this agent probably prevail within the pharmacologically relevant concentration range.

CL846 disposition in male Sprague-Dawley rats was evaluated in an unpublished study conducted at American Cyanamid Company after iv bolus administration of a 5-mg/kg dose. The mean clearance was 12.3 ± 3.7 ml/min/kg, V_{SS} was 1071 ± 97 ml/kg, the mean residence time was 98 ± 42 min, and the metabolite:parent AUC ratio was 0.633 ± 0.342. The clearance and steady-state volume of distribution were approximately 30% lower than in the present experiments, raising the question of whether the disposition of this agent differs between genders as noted for many drugs (10–12). Although a direct comparison between the two studies cannot be performed, as the rats were obtained from different suppliers and the studies were conducted at different sites and times, the potential gender difference in CL846 disposition warrants investigation in a prospective study.

CL846 elimination in rats was rapid with a terminal half-life of approximately 1 hr, which is similar to that observed in humans. The clearance and steady-state volume of distribution cannot be compared between species, as the drug was administered orally in a clinical study and the systemic bioavailability of CL846 in humans is unknown (8). In the rat, disposition of the major metabolite of CL846 could be described with a simple 1-compartment model, and the AUC ratio for the metabolite as compared to the parent drug was approximately 0.5. Metabolism of CL846 in humans appears to differ considerably from that in rats; metabolite to parent AUC ratios in healthy male volunteers were less than 2% (8). The consequences of this difference in metabolism may be minor, as CL859 is thought not to contribute to the sedative activity of the parent. *In vivo* and *in vitro* studies (in human liver slices and S9 fractions) of CL846 have shown that the primary route of metabolism is through oxidation, leading to the formation of 5-oxo-CL846 and 5-oxo-CL859; these metabolites were observed in urine of healthy volunteers as the respective glucuronide conjugates. Tautomerization of the oxo metabolites leads to the formation of the respective hydroxy-CL846 and CL859 (13,14). The 5-oxo-CL846 and

Table II. Model-Dependent Pharmacokinetic Parameters (mean ± SD) After iv Administration of CL846

Parameter	Bolus Dose (2.5 mg/kg, n = 4)	Infusion (0.4 mg/kg/hr [6 hr], n = 5)
Cl (ml/min/kg)	19.8 ± 7.4	25.9 ± 7.9
V (ml/kg)	1456 ± 97	1966 ± 706
k_f (min^{-1})	0.00593 ± 0.00224	0.00760 ± 0.00179
$k_{10[CL846]}$ (min^{-1})	0.00757 ± 0.00370	0.00639 ± 0.00480
$k_{10[CL859]}$ (min^{-1})	0.0129 ± 0.0039	0.0160 ± 0.0049

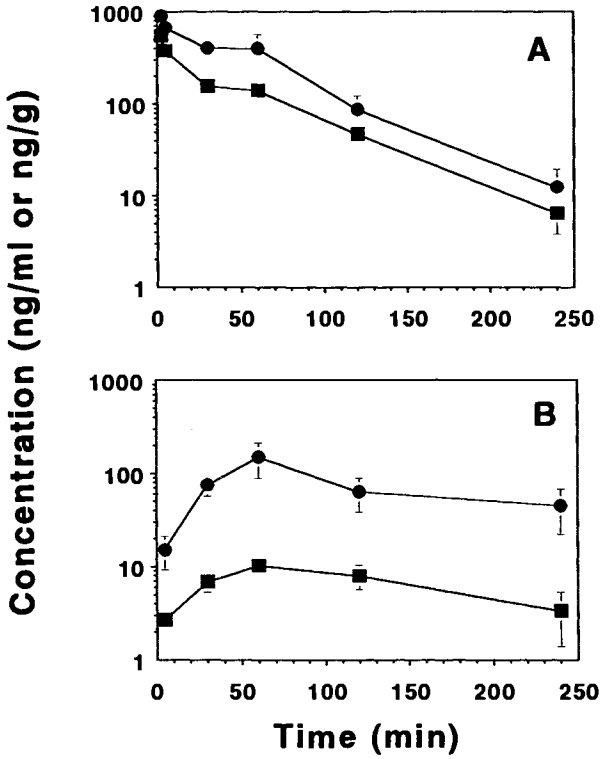

Fig. 3. Serum (●) and brain tissue (■) concentration-time profiles for CL846 (A) and derived CL859 (B) after a 0.8-mg/kg iv bolus dose of CL846. The data are presented as the mean ± S.E. for 3 rats per time point.

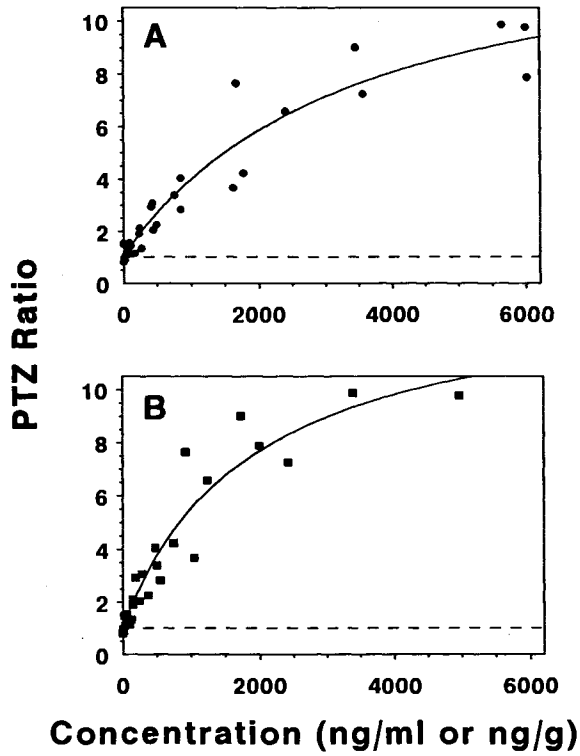

Fig. 4. Relationship between anticonvulsant effect and CL846 serum concentrations in serum (A) or brain tissue (B) 5 min after an iv bolus dose. Symbols represent observations in individual rats; the solid lines represent the best fit of the simple E_{max} model to the observed data.

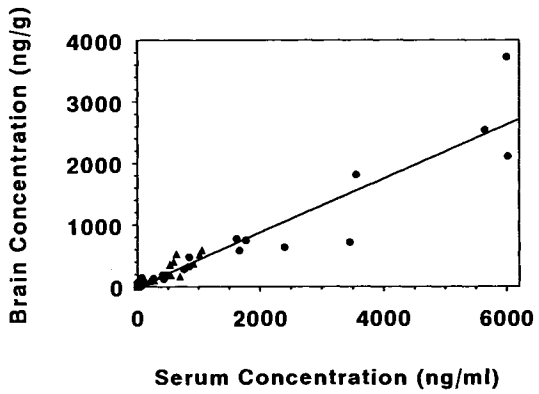

Fig. 5. Relationship between serum and brain tissue CL846 concentrations during the pharmacokinetic (▲) and pharmacodynamic (●) studies.

5-oxo-desethyl metabolites did not displace flunitrazepam from the benzodiazepine receptor and did not show activity in a drug discrimination test (American Cyanamid Co., unpublished results).

It has been suggested that benzodiazepine disposition in brain tissue would be more closely related to concentrations at the site of action, and consequently would show a better correlation with pharmacologic effect, than systemic concentrations (15). An attempt was made to describe simultaneously CL846 concentration-time profiles in serum and in brain. However, reliable estimates of intercompartmental transfer rate constants could not be obtained due to the fact that the CL846 concentration ratio in the two matrices was both concentration- (Figure 5) and time-independent (*i.e.*, the concentration-time profile in brain tissue paralleled that in serum; Figure 3). Similar observations have been made in this laboratory with triazolam (unpublished results). Thus, pharmacokinetic-pharmacodynamic modeling could be performed with equal efficacy based on concentrations in either matrix.

CL846 is less lipophilic than triazolam (log of partition coefficients were approximately 1.0 and 2.2 for CL846 and triazolam, respectively). This finding is consistent with differences between the two compounds in both the brain:serum ratio (0.5 for CL846 and 2.5 for triazolam) and the steady-state volume of distribution (1.6 L/kg for CL846 and 3.9 L/kg for triazolam). In the case of the metabolite, however, the brain to serum ratio was 4-fold smaller than that of the parent drug despite a similar liposolubility. This discrepancy between liposolubility and brain:serum partitioning cannot be attributed only to differences in protein binding, [fraction bound of 0.70 for CL846 and less than 0.81 for CL859], and may be due to the possibility that brain tissue concentrations of CL846 and CL859 are controlled significantly by binding to the receptor. The influence on systemic disposition of drug binding to the effector site has been proposed for angiotensin-converting enzyme inhibitors, for which disposition was modeled with a saturable protein compartment, presumably representing the angiotensin-converting enzyme (16).

As shown previously with other benzodiazepines (17), as well as anticonvulsants such as valproic acid (18) and phenobarbital (19), a relationship was observed between

drug concentrations and inhibition of PTZ-induced seizures that could be described with an E_{max} model. The simple relationship between drug concentration and effect can be explained by the rapid distribution of CL846 into the biophase and the direct mechanism of anticonvulsant action, *i.e.*, binding of the drug to the benzodiazepine-GABA receptor complex. The ratio of EC_{50} estimates based on brain versus serum concentrations was 0.56, which was in agreement with the brain:serum ratio (0.52 ± 0.15) determined experimentally and confirmed the lack of dissociation between serum and brain concentrations. The maximum inhibition of PTZ-induced seizures was similar to the E_{max} of triazolam (12.9 versus 11.5, respectively) and both drugs were much more potent than clobazam, oxazepam, flunitrazepam, phenobarbital and valproic acid, for which E_{max} ranged between 2 and 4 (17–19). In a series of studies conducted at American Cyanamid, after oral administration of both drugs the CL846 ED_{50} estimates for antagonism of convulsions induced by PTZ, biccuculine, picrotoxin and strychnine were 2.6, 14.4, 6.6, 9.8 and 63.5 nmole/kg, respectively; for triazolam, the ED_{50} estimates were 4.4, 1.5, 1.8, 1.8 and 4.4 nmole/kg (7). Although the methods used to measure anticonvulsant effect were different, the ED_{50} of CL846 in the present study (~16 nmole/kg) is consistent with the values reported previously. In contrast, triazolam ED_{50} was substantially smaller after iv administration (~0.3 nmole/kg). The difference in apparent efficacy between studies may be due to the low bioavailability of triazolam after oral administration (20). CL846 possesses substantial anticonvulsant properties comparable to benzodiazepines, and may be useful in the acute or chronic treatment of epilepsy. Although benzodiazepines are potent inhibitors of convulsions in a wide spectrum of epileptic disorders, the chronic use of these agents is limited by the development of tolerance that occurs in 30 to 60% of the patients (21). Additional studies are underway to evaluate the anticonvulsant effects of CL846 during continuous administration in comparison to triazolam.

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