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

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Received March 14, 1995; accepted June 1, 1995

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 pentyleneetrazol (PETZ) 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 (163 ± 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 E50 model. CL846 was as effective as triazolam in antagonizing PETZ-induced seizures.

Conclusions. Under the conditions 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; pentyleneetrazol; 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 sympathetic 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 (IC50 205 nM) and increases the binding of 2-butyrbicyclophosphorothionate, 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 ED50 ranging from 2.6 to 15 nM, similar to the ED50 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 ED50 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 ED50 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 (IC50 = 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 PETZ-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 PETZ 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-
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 (Vss), terminal elimination rate constant (k2), and mean residence time (MRT) (9). Compartmental pharmacokinetic analysis also was performed. CL846 and CL859 concentration-time profiles from individual rats were...