Pharmacokinetics and Cerebrospinal Fluid Penetration of CI-994 (N-Acetyldinaline) in the Nonhuman Primate

Luca Riva, Susan M. Blaney, Robert Dauser, Jed G. Nuchtern, John Durfee, Leticia McGuffey, and Stacey L. Berg

Pediatric Clinic, San Gerardo Hospital, Monza, Italy 20050 [L. R.], and Texas Children’s Cancer Center and Texas Children’s Hospital, Baylor College of Medicine, Houston, Texas 77030 [S. M. B., R. D., J. G. N., J. D., L. M., S. L. B.]

ABSTRACT
CI-994 is a substituted benzamide derivative that has demonstrated significant antitumor activity in vitro and in vivo against a broad spectrum of murine and human tumor models. Its mechanism of action is still unknown but seems to be novel compared with existing anticancer drugs. We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of CI-994 in nonhuman primates. Three animals (total 4 doses) received an 80 mg/m² dose of CI-994 administered over 20 min, and one animal received a dose of 100 mg/m². Serial plasma and fourth ventricular CSF samples were obtained from 0 to 4320 min after administration of the 80-mg/m² dose, and only plasma samples were obtained after the 100-mg/m² dose. CI-994 was measured using a previously validated reverse-phase high-performance liquid chromatography assay. Elimination of CI-994 from plasma was triexponential (4 of 5 cases) or biexponential (1 of 5 cases), with a terminal half life (t½) of 7.4 ± 2.5 h, volume of distribution of 15.5 ± 1.8 L/m², and clearance of 40 ± 6 ml/min/m². The area under the concentration-time curve (AUC) for the 80-mg/m² dose was 125 ± 17 μg/hr. CI-994 was first detected in CSF at the completion of the i.v. infusion. Peak concentrations of CI-994 in CSF were 3.4 ± 0.3 μM. Elimination from CSF was monoeponential (2 of 4 cases) or biexponential (2 of 4 cases) with a terminal t½ in CSF of 12.9 ± 2.5 h and AUC of 55 ± 18 μg/hr. The AUC_{CSF}/AUC_{plasma} ratio was 43 ± 10%. This study demonstrates that there is excellent CSF penetration of CI-994 after i.v. administration. Additional studies are needed to evaluate the potential role of CI-994 in the treatment of central nervous system neoplasms.

INTRODUCTION
CI-994 [4-(acetylamino)-N-(2'-aminophenyl)benzamide], an acetylated dinaline derivative, demonstrates significant antitumor activity in vitro and in vivo against a broad spectrum of murine and human tumor models, including leukemia, pancreatic, colon, and mammary carcinomas, osteogenic sarcoma, and prostate carcinomas (1–4). Although both dinaline and CI-994 possess similar antitumor activity (5), CI-994 was developed rather than the parent compound to avoid potentially significant interpatient variability that could result from differences in acetylator phenotype (6). The mechanism of action of CI-994 is unclear but may be related to the loss of a Mtr 16,000 nuclear protein whose function is currently unknown (7). In rats and dogs, the dose-limiting toxicity of CI-994 after prolonged oral administration is myelosuppression (6). In a Phase I study of daily oral administration of CI-994 in adults with solid tumors, the dose-limiting toxicities were neutropenia and thrombocytopenia, and the maximum tolerated dose was 8 mg/m²/day for 8 weeks. To date, minor responses have been noted (8).

Although dinaline was initially developed as an anticonvulsant (9), little is known about the central nervous system penetration of either dinaline or CI-994. In addition, detailed pharmacokinetic studies have not been published. In this article, we report the pharmacokinetics and CSF penetration of CI-994 in the nonhuman primate.

MATERIALS AND METHODS
Animals. Four adult male Rhesus monkeys (Macaca mulatta) weighing 8.1–11.5 kg were used for this study. The animals were fed High Protein Monkey Diet No. 5045 by Lab Diet (St. Louis, MO) and were group-housed in accordance with the Guide for the Care and Use of Laboratory Animals (10). Blood samples were drawn from a catheter placed in either the internal jugular or the saphenous vein. CSF samples were drawn from a s.c. Ommaya reservoir attached to an indwelling Pudenz catheter, with the tip located in the fourth ventricle. As previously described (11), this model permits drug infusion and repetitive blood and CSF sampling in unanesthetized animals.

Drugs. CI-994 (PD 123654; MW 269.3), dinaline (PD 104208; MW 227.26) and the internal standard (PD 123651; MW 241.3) were supplied in powder form by Parke-Davis (Ann Arbor, MI). The dose of CI-994 was dissolved to a final concentration of 3 mg/ml in a solution of 5% Dextrose and 0.9% sodium chloride/dimethylacetamide/ethanol/PEG 200 (4:3:2:1) and filtered through a 0.22 μm filter prior to administration.

Animal Experiments. CI-994 was administered as a 20 min i.v. infusion. Three animals received a total of four doses of...
80 mg/m²; one animal received a dose of 100 mg/m². Blood samples were collected before the infusion; at the end of the infusion; and 5, 10, 15, and 30 min and 1, 2, 4, 6, 8, and 24 h after the end of infusion in all of the experiments. In three experiments, blood samples were also obtained at 48 and 72 h after the end of infusion. Plasma was immediately separated by centrifugation at 1500 rpm and frozen at −30°C until analysis.

Ventricular CSF samples were obtained in three animals (total of four doses). CSF samples were collected before the infusion; at the end of the infusion; and 15 and 30 min and 1, 2, 4, 6, 8, and 24 h after the end of infusion in all of the experiments; in three experiments, samples were also collected at 48 and 72 h after the end of infusion. CSF samples were frozen at −30°C until analysis.

**HPLC Assay.** CI-994 and dinaline concentrations were measured using a modification of a previously described reverse-phase HPLC method (12). Plasma samples underwent solid-phase extraction using 3 ml C₁₈ Bond Elut columns (Varian, Harbor City, CA), which had previously been rinsed with 3 ml of methanol and 3 ml of distilled H₂O. Five hundred µl of the sample were loaded, the column was washed with 3 ml of distilled H₂O, and the drug was eluted with 2 ml of acetonitrile. Eluates were evaporated to dryness under nitrogen at 37°C. Samples were then reconstituted in 500 µl of mobile phase and filtered through a 0.45 µm filter (Ultrafree-MC; Millipore Corporation, Bedford, MA) before injection. The recovery of CI-994, dinaline, and the internal standard from plasma, after solid-phase extraction, were 99%, 98%, and 95% respectively. CSF samples were directly injected on the HPLC system without solid-phase extraction.

The HPLC system consisted of a Nova-Pack C₁₈, 4 µm 3.9 × 150 mm column (Millipore Corporation, Waters Chromatography, Milford, MA) and a Nova-Pack C₁₈ guard column (Millipore Corporation, Waters Chromatography, Milford, MA) with a mobile phase of 0.1 M ammonium acetate (pH 5.8)/methanol/acetonitrile (80/20/10) at a flow rate of 1.0 ml/min. Peaks were monitored on a Waters Model 490E programmable multiwavelength detector at 275 nm, or on a Waters Model 996 photodiode array detector (Millipore Corporation, Waters Chromatography). The retention times were 5 min for dinaline, 10 min for CI-994, and 14 min for the internal standard. A separate standard curve was made in plasma or PBS (for CSF samples) each day. Standard curves were linear from 0.02 µM to 50 µM of CI-994.

**Pharmacokinetic Analysis.** Postinfusion concentration-time data were fitted to both biexponential (n = 2) and triexponential (n = 3) equations for plasma and monoeponential (n = 1) and biexponential (n = 2) equations for CSF with MLAB (13), using the formula:

\[ C(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i t} \]

where C is the drug concentration at time t, Aᵢ is the intercept, and λᵢ is the rate constant. Akaike’s information criterion was used to determine the best fit equation (14). The t₁/₂ for each phase of elimination was calculated by dividing 0.693 by the rate constant (λᵢ) for that phase. Other pharmacokinetic parameters were calculated using model-independent methods. The steady-state volume of distribution (Vdᵢₙ) was calculated from the area under the moment curve (15).

Subsequently, the five-compartment model shown in Fig. 1 was fitted simultaneously to the concentration-time data from the four experiments in which both plasma and CSF drug concentrations were measured. The results of this analysis were used to simulate CI-944 concentrations in plasma and CSF concentrations during 56 days of daily, single-dose oral administration of an 8-mg/m² dose of CI-994. On the basis of preclinical data (16), bioavailability of 100% and rapid (10 min) absorption were assumed. The CSF volume was fixed at 10 ml, the approximate CSF volume of the rhesus monkey. Simulations were performed using ADAPT II software (17).

**RESULTS**

i.v. administration of CI-994 was well tolerated. Fig. 2 shows a representative graph of plasma and CSF CI-994 concentrations after an 80-mg/m² i.v. dose. Tables 1 and 2 show the pharmacokinetic parameters for CI-994 in plasma and CSF. The
plasma area under the concentration-time curve (AUC, mean ± SD) after an 80 mg/m² dose was 125 ± 17 μM·h, the terminal \( t_{1/2} \) of CI-994 was 7.4 ± 2.5 h, the clearance was 40 ± 6 ml/min/m², and the volume of distribution (Vd) was 15.5 ± 1.8 liters/m². The peak plasma concentration of CI-994 was 23.3 ± 4.8 μM. CI-994 was detected in CSF by the end of the infusion. The maximum CSF concentration occurred 1–4 h after the end of the infusion, with a mean CSF peak of 3.4 ± 0.3 μM. Elimination of the drug from CSF was protracted, with CI-994 still detectable at 72 h after the end of infusion in 3 of 3 animals, and quantifiable (concentration ≥ 0.02 μM) in 2 of 3 animals. The \( t_{1/2} \) of CI-994 in CSF was 12.9 ± 2.5 h and the AUC was 55 ± 18 μM·h. The AUC_{CSF/AUC_{plasma}} ratio was 43 ± 10%.

Small quantities of dinaline (<0.1 μM) were identified in plasma samples for a short time (≤6 h) after CI-994 administration. In addition, two possible metabolites of CI-994 with retention times of approximately 3 and 5.9 min were detected in plasma. However, the calculated concentrations of these metabolites in CI-994 equivalents were negligible, and they were not analyzed further.

The pharmacokinetic behavior of CI-994 in plasma and CSF through 48 h after drug administration could be well described by a model incorporating three plasma and two CSF compartments (Fig. 1). Fig. 3 shows the measured plasma and CSF CI-994 concentrations and the concentrations predicted by this model. The pharmacokinetic parameters obtained from this model are listed in Table 3. The model-dependent clearance of CI-994 calculated from these parameters (\( k_{10}V_I \)) is 45 ml/min/m² (where \( k_{10} \) is the rate constant for elimination from the central plasma compartment and \( V_I \) is the volume of distribution of the central compartment), in good agreement with the model-independent clearance of 40 ± 6 ml/min/m².

**DISCUSSION**

After short i.v. infusion in nonhuman primates, CI-994 is cleared from the plasma relatively rapidly, with a \( t_{1/2} \) of 7.4 ± 2.5 h. In contrast, the \( t_{1/2} \) of CI-994 in CSF is nearly twice as long, 12.9 ± 2.5 h. Because of this difference in half-lives, the plasma and CSF concentration-time curves cross at approximately 24 h, with CSF concentrations exceeding those in plasma after that time (Fig. 2). Although the peak CSF concentration, 3.4 ± 0.3 μM, is only 15% of the peak plasma concentration (23.3 ± 4.8 μM), the prolonged CSF exposure results in a high AUC_{CSF/AUC_{plasma}} ratio of 43 ± 10%. This excellent CSF penetration exceeds that of most commonly used anticancer agents (18).

The pharmacokinetic behavior of CI-994 in plasma and CSF after i.v. drug administration can also be described by a model incorporating three plasma and two CSF compartments. Because CI-994 is administered in low daily doses in the clinical setting (8), we used the parameters from the five-compartment model to simulate the plasma and CSF concentrations of CI-994.
when the drug is administered at a dose of 8 mg/m²/day for 8 consecutive weeks. The predicted mean concentration of CI-994 is approximately 0.5 μM in plasma and 0.2 μM in CSF with this dose and schedule. Because CI-994 concentrations as low as approximately 0.3 μM inhibit tumor growth in vitro (9), prolonged low-dose administration of this drug may approach active concentrations in both plasma and CSF.

The poor outcome of treatment of many central nervous system tumors, especially of leptomeningeal metastases, may in part result from the paucity of anticancer agents that achieve adequate antitumor concentrations in the CSF. Our data show that CI-994 penetrates into CSF very well after relatively high-dose i.v. administration and suggest that daily low-dose administration may also produce significant CSF as well as plasma drug concentrations. Further evaluation of the activity of CI-994 in central nervous system tumors, including recurrent central nervous system leukemia, is warranted.

REFERENCES
Pharmacokinetics and Cerebrospinal Fluid Penetration of CI-994 (N-Acetyldinaline) in the Nonhuman Primate

Luca Riva, Susan M. Blaney, Robert Dauser, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/6/3/994

Cited articles  This article cites 13 articles, 1 of which you can access for free at: http://clincancerres.aacrjournals.org/content/6/3/994.full.html#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.