Cancer Chemother Pharmacol (2004) 53: 527–532
DOI 10.1007/s00280-004-0765-6

ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetic study of BNP1350 in nonhuman primates

Received: 10 October 2003 / Accepted: 11 November 2003 / Published online: 2 March 2004

Abstract *Purpose*: BNP1350 (7-[(2-trimethylsilyl) ethyl]-20(S)-camptothecin, karenitecin), a highly lipophilic camptothecin, a high percentage of which is maintained in the active lactone form under physiologic conditions, has recently entered clinical trials in adults and children. BNP1350 has shown significant preclinical antitumor activity against a wide variety of adult and pediatric tumor cell lines. This study was undertaken to define the pharmacokinetics of BNP1350 in both plasma and cerebrospinal fluid (CSF) in a nonhuman primate model. *Methods*: Four nonhuman primates with indwelling Ommaya reservoirs received BNP1350, 0.1 mg/kg i.v, administered as a 60-min infusion. Frequent plasma and CSF samples were obtained for quantitation of BNP1350 concentrations using reverse-phase high-pressure liquid chromatography (HPLC). *Results*: Disappearance of the lactone form from the plasma was biexponential with a mean distribution half-life of 57.5 min (CV ±33%) and an elimination half-life of 457 min (CV ±24%). The volume of distribution for the central compartment was 1.36 l/kg (CV ±27%) and clearance from the central compartment was 10.6 ml/kg per minute (CV ±28%). The peripheral compartment volume of distribution was 1.96 l/kg (CV ±8.4%). Peak CSF lactone concentration, which occurred at 12 to 25 min after the end of the infusion, was 0.33 n *M* (CV ±71%). *Conclusions*: The ratio of the CSF AUC to the plasma AUC was less than 5% (range 0.4% to 3.0%), similar to other highly protein-bound topoisomerase inhibitors such as 9-aminocamptothecin and SN-38 (the active metabolite of irinotecan).

Keywords BNP1350 · Karenitecin · Topoisomerase I inhibitor · CSF penetration · Pharmacokinetics

This work was supported in part by NICHD U01 HD37242.

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Introduction

BNP1350 (Fig. 1), a novel semisynthetic highly lipophilic camptothecin derivative, was specifically designed to have high oral bioavailability, insensitivity to MDR/MPR/LRP tumor-mediated drug resistance, absence of glucuronidation, and increased lactone stability at physiologic pH as compared to other camptothecins [1]. BNP1350 exerts its cytotoxic effect via inhibition of topoisomerase I, an intranuclear enzyme that relaxes supercoiled DNA by creating single-strand DNA breaks that are subsequently religated by this enzyme [2]. Topoisomerase I inhibitors stabilize the covalent complex between topoisomerase I and DNA, resulting in enzyme-linked DNA breaks that cannot be religated in the presence of the drug [3, 4]. There is also experimental evidence that the lactone form of camptothecins binds directly and noncovalently to double-stranded DNA and single-stranded DNA structures in the absence of topoisomerase I, and that this binding may play an additional role in their anticancer activity [5].

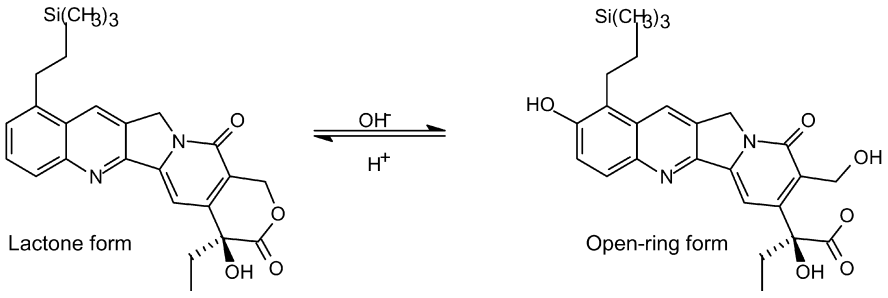

Fig. 1 Lactone and open ring forms of BNP1350

BNP1350 has potent activity in vitro against adult tumor cell lines including lung, prostate, breast, colon, ovarian, melanoma, and head and neck tumors [6, 7], as well as against pediatric malignancies including medulloblastoma, neuroblastoma, and rhabdomyosarcoma [8]. Substantial in vivo antitumor activity has also been observed following treatment with BNP1350 (both orally and intraperitoneally) in mice with subcutaneously implanted xenografts derived from a variety of tumors including prostate, breast, non-small-cell lung and colon cancer, melanoma, and central nervous system [9, 10, 11].

The CSF penetration of BNP1350, a lipophilic topoisomerase I inhibitor, has not previously been characterized in nonhuman primates. Studies of the CSF penetration of water-soluble camptothecin analogs have shown that the degree of penetration of the lactone form of topotecan is approximately 30%, whereas penetration of the lactone forms of 9-aminocamptothecin and SN-38, the active metabolite of irinotecan, are both less than 5% [12, 13]. In this study, CSF and plasma pharmacokinetics of BNP1350 were examined in a nonhuman primate model that has previously been shown to be predictive of CSF penetration by camptothecins in humans [14, 15].

Materials and methods

Drug

BNP1350 in its clinical formulation was provided by BioNumerik Pharmaceuticals (San Antonio, Tx.) in single-dose vials containing 0.5 mg BNP1350 in 5 ml of the cosolvent vehicle comprising N-methylpyrrolidone, PEG300, Tween 80, ethanol, and citric acid. The appropriate dose of drug was further diluted at a ratio of 1:4 with 5% dextrose and administered over 60 min through either a peripheral or central venous catheter.

Animals

Four adult rhesus monkeys (*Macaca mulatta*), ranging in weight from 10.3 to 11.8 kg, were used in these experiments. The animals were fed High Protein Monkey Diet #5045 from Lab Diet (St. Louis, Mo.) and group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [16]. Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein opposite the site of drug administration. In three of the animals, CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a subcutaneously implanted Ommaya reservoir [17].

Experiments

The plasma pharmacokinetics of BNP1350 were studied in four animals and the CSF pharmacokinetics in three animals following i.v. administration of BNP1350 at a dose of 0.10 mg/kg over 60 min. Serial blood samples were obtained mid-infusion, at the end of the infusion, and 5, 15 and 30 min and 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after the end of the infusion. Plasma samples were separated immediately by centrifugation at 12,000 *g* for 2 min in a rapid acceleration/deceleration centrifuge. CSF samples (approximately 300 µl) were obtained from a fourth ventricular Ommaya reservoir in three animals 45 min into the infusion, at the end of the infusion, and 10, 30, 40, 60 and 90 min and 2, 4, 6, 8 and 10 h after completion of the infusion. The reservoir was pumped four times before and after each sample collection to ensure adequate mixing with the ventricular CSF.

Sample analysis

Samples from plasma and CSF were analyzed for BNP1350 using a reverse-phase HPLC method as described below. The method used is a modification of a previously described HPLC method for measuring BNP1350 [18]. The lactone and total drug concentrations of BNP1350 were measured in plasma. Because of the limited sample volume, only the active lactone form of BNP1350 was measured in the CSF.

The lactone form of BNP1350 was extracted from plasma samples using solid-phase extraction (SPE) carried out on Bond Elut C8 cartridges, 1 ml/100 mg (lot no. 060601), produced by Varian Associates (Harbor City, Calif.). The SPE was done at ambient temperature after the cartridges had been conditioned with 1 ml acetonitrile (ACN) and rinsed with 1 ml water. Plasma was immediately applied and pressure was adjusted to provide a flow rate of 0.5 ml/min. The SPE cartridges were then washed with 1 ml water. Additional washing was performed using 1 ml 10% ACN in water. BNP1350 was eluted with 1 ml 100% ACN. The eluent was evaporated at 37°C under a nitrogen flow at 10 psi. Dry sample was reconstituted in a 0.5 ml matrix solvent. An aliquot of 100 µl from each sample was then injected for analysis.

To extract the total BNP1350 (lactone and carboxylate forms) from plasma, the same method as described above was used except that 1 ml of plasma sample was acidified with 20 µl 10% phosphoric acid. Acidified samples were vortexed and incubated for at least 30 min at ambient temperature. The concentration of the open ring form was calculated by subtracting the concentration of the lactone from the concentration of total drug. CSF samples were analyzed for BNP1350 lactone by immediate direct injection of a 100-µl aliquot of CSF onto the HPLC column.

HPLC analysis of BNP1350 was carried out using a Waters (Milford, Mass.) HPLC system composed of a 600E multisolvent delivery system, a 717 Plus autosampler, and a 474 scanning fluorescence detector. The system was controlled and data were acquired and processed by the Waters software Millennium chromatography manager, v 3.15.01. The fluorescence detector was set at an excitation wavelength of 365 nm and emission was monitored at 420 nm. Separation was achieved using a Waters reverse-phase column (Nova-Pak C18 150×3.9 mm), as well as a guard column

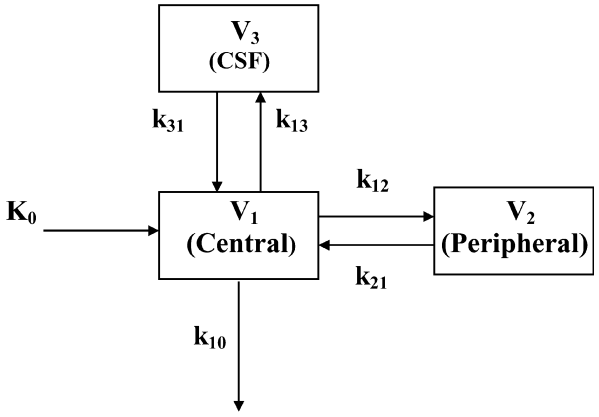

Fig. 2 The pharmacokinetic model for describing the plasma and CSF disposition of BNP1350 consists of central (*box* V_1) and peripheral compartments (*box* V_2) with first-order irreversible elimination from the central compartment and a third compartment (*box* V_3) for CSF. K_0 represents the drug infusion. The rate constant k_{10} represents the elimination of BNP1350 from the central compartment, k_{12} represents movement of BNP1350 from the central to the peripheral compartment, and k_{21} represents the reverse. The rate constant k_{13} represents movement of BNP1350 from the central compartment to the CSF, and k_{31} represents the reverse. V_1 is the volume of distribution of the central compartment, V_2 the volume of distribution of the peripheral compartment, and V_3 the volume of the CSF compartment (fixed at 10 ml for this model)

(Nova-Pak C18 20×3.9 mm). Retention material particles were 4 μm with a 60 Å pore size.

The lactone form of BNP1350 was eluted under isocratic conditions using a mobile phase consisting of a 50:50 (v/v) mixture of triethylamine and ACN with a retention time of 9.0 min and a total run time of 15 min. The flow rate of 1 ml/min produced a back pressure of 103 bar. Standard curves were linear ($r^2 = 0.998$) over the range 0.3 to 20 n *M*. The lower limit of quantitation was 0.31 n *M*. Interassay variability was 5.0% at 1 n *M* and 5.7% at 20 n *M*. Intraassay variability was 6.1% at 2 n *M*. The method was validated for plasma and reference solvent (10% DMSO + 1% BNP-PF4 in 0.2% phosphoric acid).

Pharmacokinetic analysis

Noncompartmental methods were used to calculate the area under the concentration versus time curve (AUC), total body clearance (Cl_{TB}), mean residence time (MRT), and steady-state volume of distribution (Vd_{ss}) for the lactone and total (lactone plus carboxylate) forms of BNP1350. The AUC was derived by the linear trapezoidal method and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. Cl_{TB} was determined by dividing the dose by the AUC. The MRT was calculated by dividing AUMC (the area under the first moment versus time curve) by the AUC. The model-independent (non-compartmental) Vd_{ss} was calculated using the area under the moment curve, correcting for infusion time [19]. The fraction of drug penetrating into the CSF was derived from the ratio of the AUCs in CSF and plasma.

A model consisting of central and peripheral compartments with first-order irreversible elimination from the central compartment and a third compartment for CSF (Fig. 2) was fitted to the plasma and CSF concentration versus time data from the individual BNP1350 i.v. infusion experiments using MLAB (Civilized Software, Bethesda, Md.) [20]. Pharmacokinetic modeling was done using only the lactone concentration-time data since the conversion to the open ring form was negligible (less than 10%). The model equations were as follows:

$$\frac{dX_1}{dt} = Infusion - k_{10}X_1 - k_{12}X_2 - k_{13}X_3$$
$$\frac{dX_1}{dt} = k_{12}X_1 - k_{21}X_2$$
$$\frac{dX_3}{dt} = k_{13}X_1 - k_{31}X_3$$
$$C_1 = \frac{X_1}{V_1}$$
$$C_3 = \frac{X_3}{V_3}$$

In the above equations X represents the amount of lactone in a compartment, k with a subscript represents the rate constant for transfer from a compartment, V represents the volume of a compartment, and C represents lactone concentration. The CSF volume of distribution was fixed at 10 ml, the approximate volume of CSF in the rhesus monkey. The rate constants and volume of the central compartment were estimated for each animal using MLAB. Best-fit parameters were determined by minimizing the residual squared error weighted by the inverse concentration squared. The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were calculated from the model rate constants. The volume of the peripheral compartment was estimated from the equation $V_2 = k_{12}/k_{21} \cdot V_1$. The model-dependent CSF clearance was calculated from the product of k_{31} (the first-order BNP1350 rate constant for elimination from the CSF) and V_3 (the CSF volume).

Results

Model-independent plasma pharmacokinetic parameters for the BNP1350 lactone and total drug following an i.v. dose of 0.1 mg/kg are provided in Table 1. The clearances and volumes of distribution shown were calculated by noncompartmental (model-independent) methods. As shown in Table 1, the parameters for the lactone and total drug were equivalent.

Figure 3 shows a representative concentration versus time curve of predicted vs actual BNP1350 concentrations in plasma and CSF. The distribution and elimination of BNP1350 lactone were well-described by a model consisting of central and peripheral compartments with first-order irreversible elimination from the central compartment and a third compartment for CSF. Model-dependent plasma pharmacokinetic parameters for the lactone are shown in Table 2. Since the lactone form represents more than 90% of the total measurable drug, the parameters for the lactone are equivalent to those that would be obtained if total drug were modeled instead.

As shown in Table 2, the plasma disappearance of the drug was best described by a two-compartment model with a mean distribution half-life of 57.5 min (CV ±33%) and an elimination half-life of 457 min (CV ±24%). The central compartment volume of distribution of the lactone was 1.36 l/kg (CV ±27%) and the clearance from the central compartment was 10.6 ml/kg per minute (CV ±28%). The peripheral compartment volume of distribution was 1.96 l/kg (CV ±8.4%).

Table 3 summarizes the CSF model-independent and model-dependent pharmacokinetic parameters. Peak CSF concentrations appeared 12 to 25 min after the end of the infusion and were 0.33 n *M* (CV ±71%), less than 1% of the peak plasma concentration. The ratio of the CSF AUC to the plasma AUC was less than 5% in all three monkeys from which CSF samples were available (range 0.4% to 3.0%). Clearance from the CSF was

Table 1 Model-independent plasma pharmacokinetic parameters for both total drug and lactone in each animal

Animal	Total drug					Lactone form				
	C_{max} (n *M*)	AUC (μ*M*·min)	Cl_{TB} (ml/min/kg)	MRT (min)	V_{dss} (l/kg)	C_{max} (n *M*)	AUC (μ*M*·min)	Cl_{TB} (ml/min/kg)	MRT (min)	V_{dss} (l/kg)
J128	124	30.0	7.4	519	3.6	133	31.6	7.1	394	2.6
J0	198	22.4	10.0	265	2.3	158	23.0	9.7	309	2.7
J124	107	16.6	13.4	235	2.8	105	16.2	13.7	238	2.9
L984	53.3	10.8	20.7	220	3.9	44.2	10.4	21.5	237	4.5
Mean	121	19.9	12.9	310	3.2	110	20.3	13.0	295	3.1
SD	59.8	8.2	5.8	141	0.7	49.0	9.1	6.3	74	0.9
CV (%)	50	41	45	45	24	44	45	48	25	28

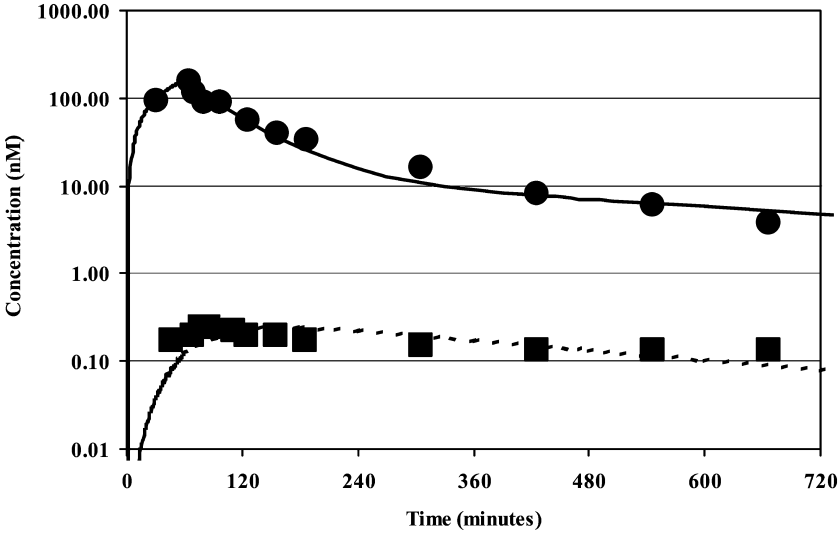

Fig. 3 Plasma (●) and CSF (■) concentrations of BNP1350 are shown for a representative experiment with i.v. bolus administration of BNP1350 at a dose of 0.1 mg/kg over 60 min

Table 2 Model-dependent pharmacokinetic parameters for the lactone form of BNP1350 in plasma

Animal	k_{10} (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{13} (min^{-1})	k_{31} (min^{-1})	V_1 (l/kg)	V_2 (l/kg)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	$Cl_{central}$ (ml/kg/min)
J128	0.0046	0.0027	0.0023	3.94×10^{-8}	0.0107	1.63	1.91	83.0	545	7.4
J0	0.0115	0.0059	0.0025	2.37×10^{-8}	0.0035	0.89	2.08	37.7	436	10.2
J124	0.0086	0.0036	0.0035	1.42×10^{-8}	0.0080	1.70	1.75	51.2	309	14.7
L984	0.0081	0.0032	0.0019	NA	NA	1.24	2.11	58.0	539	10.1
Mean	0.0082	0.0039	0.0026	2.58×10^{-8}	0.0074	1.36	1.96	57.5	457	10.6
SD	0.0028	0.0014	0.0007	1.27×10^{-8}	0.0036	0.38	0.17	19.0	111	3.0
CV (%)	35	37	27	49	49	28	8.4	33	24	28

Table 3 CSF pharmacokinetic parameters for the lactone form of BNP1350

Animal	Model-independent parameters				Model-dependent parameters		
	C_{max} (n *M*)	AUC (μ*M*·min)	MRT (min)	AUC_{CSF}/AUC_{plasma} (%)	k_{31} (min^{-1})	$t_{1/2}$ (min)	Cl (ml/h)
J128	0.60	0.95	1,580	3.01	0.0107	64.8	6.41
J0	0.25	0.32	1,680	1.41	0.0035	200.0	2.08
J124	0.15	0.07	532	0.41	0.0080	87.0	4.78
L984	NA	NA	NA	NA	NA	NA	NA
Mean	0.33	0.45	1,261	1.61	0.0074	117.3	4.42
SD	0.24	0.46	630	1.31	0.0036	72.5	2.19
CV (%)	71	102	50	82	49	62	49

4.42 ml/h (CV ±49%). MRT for the lactone form of the drug in the CSF was 1260 min (21 h) (CV ±50%).

Discussion

The plasma and CSF pharmacokinetics of the novel camptothecin BNP1350 were characterized in a nonhuman primate model that has previously been shown to be predictive of CSF drug penetration in humans [13, 14]. The disappearance of BNP1350 from plasma was described well by a two-compartment model with a rapid distribution half-life of 57.5 min (CV ±33%) and a slower elimination half-life of 457 min (CV ±24%).

The lipophilic nature of BNP1350 was demonstrated by the large volume of distribution and long plasma MRT. The estimated central and peripheral volumes of distribution (1.36 l/kg, CV ±28%, and 1.96, CV ±8.4%) greatly exceeded the total body water of nonhuman primates (approximately 0.7 l/kg), while the plasma MRT of both the total and the lactone forms of BNP1350 were approximately 5 h (310 min, CV ±45%, for the total drug and 295 min, CV ±25%, for the lactone form).

In this study the lactone stability of BNP1350 was also documented. More than 90% of total measured drug was present in the lactone form in plasma. These results demonstrate a tremendous potential pharmacologic advantage with regard to lactone stability of BNP1350 compared to other camptothecins. In contrast, after topotecan administration to nonhuman primates, more than 60% of total drug in plasma is in the open ring form by 30 min after infusion, and the ratio of mean AUCs of the lactone form to the total drug is 0.36 in plasma and 0.48 in CSF.

CSF penetration of BNP1350, estimated from the ratio of the plasma AUC to the CSF AUC, was less than 5% (range 0.4% to 3.0%). This is similar to the values observed for 9-aminocamptothecin and SN-38 (the active metabolite of irinotecan), but less than previously reported for topotecan (CSF:plasma ratio 30%). The CSF clearance of BNP1350 was 4.42 ml/h (CV ±49%), which is consistent with bulk flow as the primary mechanism of BNP1350 elimination from the CSF. From a clinical perspective, the relatively low CSF penetration of BNP1350 may be somewhat offset by its long CSF MRT of 1260 min (CV ±50%). It is well known that the duration of exposure to BNP1350 and other camptothecins is directly proportional to cytotoxicity that is substantial relative to the incremental changes in cytotoxicity resulting from increasing drug concentrations. In addition, the coefficient of variation of the BNP1350 plasma pharmacokinetic parameters were all 50% or less. This may be clinically important since interpatient variability in drug levels is reported to be substantial for other camptothecins that are in use in the clinic. A more consistent plasma level of drug is highly desirable, particularly for cytotoxic agents.

Camptothecins are a relatively new class of anticancer agents that have come into widespread clinical use over the last 10 years. One of the disadvantages to the commercially available topoisomerase inhibitors is that the less-active, open-ring carboxylate form predominates at physiologic pH. As demonstrated in this preclinical study, the active lactone form of BNP1350 is the predominant form of the drug. Phase I and II clinical trials of this agent are currently in progress.

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