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ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetics of rebeccamycin (NSC 655649) in nonhuman primates

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Abstract *Purpose*: The rebeccamycins, indolocarbazole topoisomerase I poisons originally discovered in actinomycetes, have shown activity in vitro against a range of adult and pediatric tumors. The derivative NSC 655649 (diethylaminoethyl analog of rebeccamycin, or DEAE rebeccamycin) is currently undergoing early-phase human studies and has shown some signs of antitumor activity. We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of NSC 655649 after systemic administration in a nonhuman primate model that is predictive of anticancer drug behavior in humans. *Design*: A dose of 400 mg/m^2 was infused over 1 h to three rhesus monkeys. Serial blood and CSF samples were collected. Rebeccamycin concentrations were measured by high-pressure liquid chromatography. Pharmacokinetic analysis was performed using compartmental and noncompartmental methods. *Results*: A two-compartment or three-compartment model described rebeccamycin pharmacokinetics in plasma adequately. In two animals, the three-compartment model provided a better fit, and in one animal, the two-compartment model was better. The terminal half-life was 730 ± 410 min, the AUC was 3130 ± 425 μ*M* min, and the clearance was 190 ± 25 ml/min/m^2. Rebeccamycin was below the limit of quantitation in all CSF samples. The animals had some nausea and agitation during and shortly after the infusion that responded to treatment with prochlorperazine or diazepam. Otherwise, rebeccamycin was well tolerated with minimal toxicity. *Conclusion*: Rebeccamycin penetrates poorly into the CSF following an intravenous infusion. Therefore, systemically administered rebeccamycin is unlikely to be an important agent for the treatment of leptomeningeal tumors. Because the drug is associated with local irritation at injection sites, it is not an ideal candidate for development as an intrathecal agent. However, the role of rebeccamycin in the treatment of parenchymal brain tumors should be determined in clinical trials.

Keywords Plasma · Cerebrospinal fluid · Pharmacokinetics · Rebeccamycin

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Introduction

The indolocarbazole anticancer agents include both the staurosporine derivatives, whose mechanism of action appears to be protein kinase C inhibition, and the rebeccamycin derivatives. The rebeccamycins are indolocarbazoles originally discovered in actinomycetes [1, 2]. Although the mechanism of action of the rebeccamycins is not entirely clear, it appears that these agents are topoisomerase I poisons, and that different derivatives may interact at different sites on the topoisomerase I molecule [3–7]. The rebeccamycins have shown activity in vitro against a range of adult [1] and pediatric [8] tumors. The derivative NSC 655649 is currently undergoing early-phase human studies and has shown some signs of antitumor activity [9–11].

We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of NSC 655649 (diethylaminoethyl analog of rebeccamycin, or DEAE rebeccamycin) after systemic administration in a nonhuman primate model

that is highly predictive of anticancer drug behavior in humans [12].

Materials and methods

Drug

Rebeccamycin was supplied by the Division of Cancer Treatment and Diagnosis, NCI (Bethesda, Md.). The drug was diluted in normal saline to a final concentration of 2–2.4 mg/ml and infused via a central venous catheter.

Monkeys

Three adult male rhesus monkeys (*Macaca mulatta*) weighing 10.4–12.3 kg were used in these experiments. The animals were fed Open Formula Extruded Non-Human Primate Diet twice daily and group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [13]. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir [12]. The reservoir was pumped three times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF. Drug was administered through a surgically implanted central venous catheter. Blood samples were drawn through a catheter placed in the contralateral femoral or saphenous vein.

Experiments

A dose of 400 mg/m^2 was infused over 1 h to each of three rhesus monkeys. Blood samples and ventricular CSF were collected immediately prior to the dose, at 30 min during the infusion, at the end of the infusion, and at 15 and 30 min, and 1, 2, 4, 6, 8 and 24 h after the infusion in all animals. Additional samples were obtained at 10 h in one animal and 48 h in one animal. Ventricular CSF samples were collected at similar time points. Plasma was separated immediately by centrifugation at 12,000 *g* for 10 min in a rapid acceleration/deceleration centrifuge. Plasma and CSF were frozen immediately after collection. Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks after rebeccamycin infusion. Animals were also observed on a daily basis for a minimum of 3 weeks after infusion for any evidence of clinical toxicity.

Sample analysis

Rebeccamycin concentrations in the monkey plasma and CSF samples were analyzed using a modification of a previously described high-pressure liquid chromatography (HPLC) assay [10] (Fig. 1). Briefly, 2 ml of plasma was mixed with 1% phosphoric acid and applied to an Oacis solid-phase extraction cartridge (Waters, Milford, Mass.) that had been rinsed with 1 ml methanol and 1 ml water. The cartridge was then washed with 1 ml water followed by 1 ml 5% methanol and the sample eluted with 3 ml ethanol. The eluate was dried under nitrogen at 37°C. CSF samples were injected directly, without extraction. The HPLC system consisted of a Multisolvent Delivery System 600E, an Autosampler 717 plus, and a PDA 996 (Waters). Samples were reconstituted in 1 ml mobile phase prior to injection. Separation was achieved on Luna C18(2), 3 μm, 4.6×150 mm column (Phenomenex, Torrance, Calif.) with isocratic elution consisting of 65% 20 m *M* monobasic potassium phosphate acidified with phosphoric acid (pH 2.5) and 35% acetonitrile (v/v) at a flow rate of 1.0 ml/min at room temperature. The injection volume was 100 μl (plasma) or 200 μl (CSF) and detection was at 318 nm. The limit of quantitation was 0.040 μ*M* in plasma and 0.015 μ*M* in CSF. Internal standard was not used. Recovery from plasma was 82 ± 7%; the intraday coefficient of variation was 4% at 0.07 μ*M*, 6% at 1.5 μ*M*, and 2% at 75 μ*M*. Interday coefficients of variation at those concentrations were 7, 4, and 2%. The standard curve was linear from 0.037 to 75 μ*M* in plasma.

Pharmacokinetic analysis

Plasma concentration–time data were modeled in ADAPT II [14]. Both two-compartment and three-compartment models were fitted to the data. Akaike's Information Criterion [15] was used to determine the best fit. Pharmacokinetic parameters (clearance, volume of distribution at steady state, and half-lives) were derived from the estimates of model parameters using standard techniques [16]. The areas under the concen-

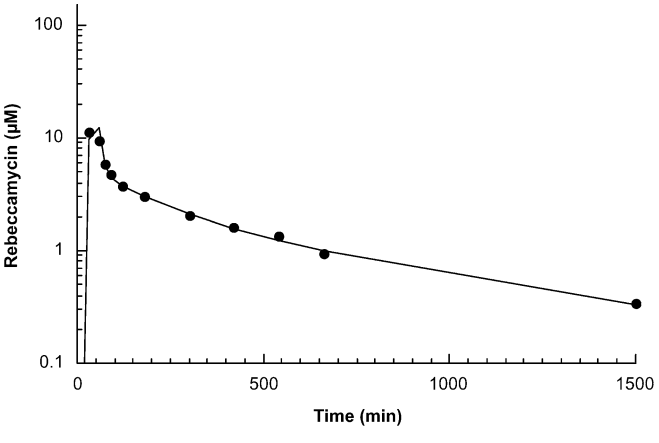

Fig. 1 Measured (*solid symbols*) vs model-predicted (*line*) rebeccamycin concentrations in a nonhuman primate (animal 1) after a 1-h infusion of 400 mg/m^2

Table 1 Pharmacokinetic parameters for rebeccamycin after a 1-h infusion of 400 mg/m^2 in the nonhuman primate

Animal	v_d(l/m^2)	Cl_{TB} (ml/min/m^2)	$t_{1/2alpha}$ (min)	$t_{1/2\ terminal}$ (min)	AUC (μM min)
1	10.4	215	6.3	570[a]	2650
2	9.4	175	6.1	1200[a]	3470
3	10.1	175	7.8	430[b]	3270
Mean	9.9	190	6.7	730	3130
SD	0.5	25	0.9	410	425

[a]Three-compartment model provides best fit.
[b]Two-compartment model provides best fit.

tration–time curve (AUC) were determined by the linear trapezoidal method and extrapolated to infinity using the terminal rate constant [16].

Results

The pharmacokinetic parameters of rebeccamycin in plasma after a 1-h intravenous infusion in the nonhuman primate are presented in Table 1. In two animals, the three-compartment model provided a better fit, and in one animal, the two-compartment model was better. The terminal half-life was 730 ± 410 min, the AUC was 3130 ± 425 μM min, and the clearance was 190 ± 25 ml/min/m^2. Rebeccamycin was below the limit of quantitation in all CSF samples.

The animals had some agitation during and shortly after the infusion that responded to treatment with prochlorperazine or diazepam. Otherwise rebeccamycin was well tolerated with minimal clinical or laboratory evidence of toxicity.

Discussion

The promising clinical activity of the camptothecin derivatives topotecan and irinotecan has created considerable interest in the development of new topoisomerase I inhibitors. Rebeccamycin appears to inhibit topoisomerase I through a mechanism different from that of the camptothecins [3–7]. Phase I studies of rebeccamycin have been conducted in adults and children [9–11] and phase II studies are ongoing. The pharmacokinetic parameters described here are in good agreement with those found in the human studies. In adults and children, the reported rebeccamycin clearance ranges from approximately 5 to 8 l/h/m^{2}and the drug exhibits a variable but prolonged terminal half-life, ranging from 34 to 154 h [9–11, 17]. These results compare with a clearance of approximately 11 l/h/m^2 and a terminal half-life of approximately 12 h in our study. It is likely that some of the variability, especially in half-life, resulted from the different doses and schedules employed in the different studies. For the animal studies, we chose a dose lower than the human maximum tolerated dose in order to avoid toxicity; using a higher dose might have resulted in identifying a more prolonged terminal half-life if drug concentrations in plasma remained above the limit of quantitation for the assay at late time points.

We showed that rebeccamycin penetrated poorly into the CSF following an intravenous infusion, indicating that rebeccamycin does not cross the intact blood–brain barrier. In addition, because the drug has been associated with local irritation at injection sites in the phase I studies, it is not an ideal candidate for development as an intrathecal agent. Therefore, systemically administered rebeccamycin is unlikely to be an important agent for the treatment of leptomeningeal tumors. However, penetration into the CSF may not be an ideal surrogate marker for penetration into brain tumors, since many brain tumors have a disrupted blood–brain barrier as evidenced by their gadolinium uptake [18]. Thus the role of rebeccamycin in the treatment of parenchymal brain tumors will need to be determined based on additional preclinical and clinical information about its antitumor activity.

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