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Plasma and Cerebrospinal Fluid Pharmacokinetics of Thalidomide and Lenalidomide in Nonhuman Primates

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Abstract

Purpose—Thalidomide, originally developed as a sedative, was subsequently identified to have antiangiogenic properties. Lenalidomide is an antiangiogenic and immunomodulatory agent that has been utilized in the treatment of patients with brain tumors. We studied the pharmacokinetics and cerebrospinal fluid (CSF) penetration of thalidomide and lenalidomide in a nonhuman primate model.

Methods—A dose of 50 mg of thalidomide or 20 mg of lenalidomide were administered once orally to each of three rhesus monkeys. Plasma and CSF samples were obtained at specified intervals and the thalidomide or lenalidomide concentrations were determined by high-performance liquid chromatography with tandem mass spectrometry. Pharmacokinetic parameters were estimated using noncompartmental methods. CSF penetration was calculated as area under the concentration-time curve (AUC) CSF/AUC plasma.

Results—For thalidomide, the median apparent clearance (Cl/F) was 2.9 mL/min/kg, the median plasma AUC was 80 $\mu\text{M}\cdot\text{hr}$, and the median terminal half-life ($t_{1/2}$) was 13.3 hours. For lenalidomide, the median Cl/F was 8.7 mL/min/kg, the median AUC was 9 $\mu\text{M}\cdot\text{hr}$, and the median $t_{1/2}$ was 5.6 hours. Thalidomide was detected in the CSF of all animals, with a median penetration of 42%. Lenalidomide was detected in the CSF of 2 of 3 animals, with a CSF penetration of 11% in each.

Conclusion—Thalidomide and lenalidomide penetrate into the CSF after oral administration of clinically relevant doses. Plasma exposure to lenalidomide was similar in our model to that observed in studies involving children who have brain tumors. These results support further development of lenalidomide for the treatment of central nervous system malignancies.

Keywords

thalidomide; lenalidomide; pharmacokinetics; CSF penetration; nonhuman primates

INTRODUCTION

Central nervous system (CNS) tumors are the most common solid tumor in children, comprising 25% of all childhood cancers [1]. Overall survival for children with brain tumors is approximately 75% [2]; however, children with high-grade gliomas and diffuse intrinsic pontine gliomas fare poorly [3, 4]. Similarly, children whose cancer metastasizes to the CNS often have poor outcomes. Therefore, new drugs are needed for malignant childhood brain tumors.

Initially withdrawn from the market due to teratogenic effects of the drug, thalidomide was approved in 1998 by the US Food and Drug Administration (FDA) for the management of erythema nodosum leprosum [5]. Further investigation of thalidomide characterized its antiangiogenic and immunomodulatory effects [6]. Lenalidomide (CC-5013, Revlimid® brand drug; Celgene Corporation, Summit, NJ) is a compound with enhanced immunomodulatory potency and decreased sedative and neurotoxic properties [7]. It is approved by the FDA for the treatment of adults with transfusion-dependent del(5q) myelodysplastic syndrome and, in combination with dexamethasone, for the treatment of multiple myeloma [8].

The mechanism of action directly responsible for lenalidomide's anti-tumor activity is not known. It inhibits the secretion of pro-inflammatory cytokines [9]; increases the secretion of anti-inflammatory cytokines [9]; induces T-cell proliferation [10] and interleukin-2 and interferon- γ production [11]; suppresses endothelial response to angiogenic molecules [12]; and promotes G1 cell cycle arrest and apoptosis of malignant cells [13]. Since tumor growth is believed to be partly dependent on the ability of a tumor to neovascularize, angiogenesis inhibitors may play a role in the prevention of tumor growth or decrease the risk for tumor metastases. Lenalidomide has been well-tolerated in adults and children with recurrent CNS tumors [14–16]. The CNS penetration of thalidomide and lenalidomide remains unknown, and this information is important to determine potential tumor targets for this class of drugs, including their use in leptomeningeal disease. Thus we studied the cerebrospinal (CSF) penetration of thalidomide and lenalidomide in a nonhuman primate model that has been highly predictive of anticancer drug distribution in humans [17].

MATERIALS AND METHODS

Drug

Thalidomide and lenalidomide were supplied by Celgene Corporation (Summit, NJ) as a powder. Thalidomide 50 mg was diluted in 6.5 mL 1% carboxymethylcellulose and lenalidomide 20 mg was diluted in 2.5 mL 1% carboxymethylcellulose before oral administration.

Animals

Four adult male rhesus monkeys (*Macaca mulatta*) weighing 10 to 16.8 kg were used in these experiments, which were approved by the Institutional Animal Care and Use Committee. The animals were fed Purina Lab Diet 5045 High Protein Monkey Diet twice daily and housed in accordance with Guide for the Care and Use of Laboratory Animals [18]. Drug was given orally. Blood samples were drawn through a catheter placed in the saphenous vein. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir [17].

Experiments

Thalidomide 50 mg (3.2–3.6 mg/kg) or lenalidomide 20 mg (1.2–2 mg/kg) were administered once orally to three animals. Blood and ventricular CSF were collected immediately before the dose and at 30, 60, 90 minutes, and 2, 3, 4, 6, 8, 10, and 24 hours after the drug was administered. Plasma was separated immediately by centrifugation at 1,500 rpm for ten minutes. Plasma and CSF were frozen immediately after collection and diluted 1:1 with Sorensen's citrate buffer (pH 1.5; prepared by combining 70 mL 0.1M citric acid, 5 mL 0.1M sodium citrate dihydrate, and 425 mL water, and adjusting the pH with concentrated HCl) and stored at -60°C until analysis. 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 three weeks after drug administration. Animals were also observed on a daily basis for a minimum of three weeks after drug administration for any evidence of clinical toxicity.

Sample analysis

The calibration standard and quality control samples were prepared in artificial CSF (aCSF) (Online Resource 1) or rhesus monkey plasma (Bioreclamation, Hicksville, NY) mixed (1:1, v/v) with Sorensen's citrate buffer. $^{13}\text{C}_5$ -lenalidomide (synthesized by Celgene Corporation) and thalidomide- d_4 (Toronto Research Chemicals, North York, ON, Canada) in methanol were used as internal standards. The text has been update to include the following: The analytical dynamic ranges for lenalidomide and thalidomide are 3.82–1928 μM and 18.82–3872 μM , respectively. Three levels of quality controls were included in each run (Online Resource 2).

For CSF analysis, 80 μL of each thawed sample was mixed with 20 μL of internal standard working solution. Then samples were vortexed and centrifuged at 2,000 rpm for three minutes before being injected into the high performance liquid chromatography (HPLC)-tandem mass spectrometry system for analysis. For plasma analysis, 100 μL of each thawed sample was mixed with 300 μL of internal standard working solution to precipitate proteins. Then samples were vortexed and centrifuged at 3,500 rpm for 15 minutes. The supernatants (350 μL per sample) were transferred and mixed with 40 μL of methanol containing 5% formic acid, then evaporated to dryness under N_2 at 35°C and reconstituted into 100 μL of water/acetonitrile/formic acid (80:20:0.4), for lenalidomide or water/methanol/formic acid (50:50:0.1), for thalidomide. For each sample, 10 μL of sample was injected onto a HPLC system consisting of Shimadzu LC-20ADXR UFLC pumps with Shimadzu SIL-20ACXR autosampler (Shimadzu, Shanghai, China) using a Phenomenex Luna C18 (2) column (50×2.0 mm, 5 μm) (Torrance, CA). The mobile phase consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) at a flow rate of 0.4 mL/minute. A gradient elution was performed at 5–30% mobile phase B from 0 to 1 minute, 30–95% B from 1 to 2 minutes, 95% B from 2 to 2.5 minutes, and 5% B from 2.6 to 4 minutes. Compounds were detected using an API4000 QTrap mass spectrometer (Ontario, Canada).

Pharmacokinetic analysis

Noncompartmental methods were employed to derive the pharmacokinetic parameters. Terminal half-lives ($t_{1/2}$) were estimated using first order kinetics. For both plasma and CSF, the areas under the concentration-time curve (AUC) were determined by the linear trapezoidal method to the last measured concentration (AUC_{last}) and extrapolated to infinity (AUC_{inf}) using the terminal rate constant [19]. CSF penetration was calculated as $\text{AUC}_{\text{inf}}^{\text{CSF}}/\text{AUC}_{\text{inf}}^{\text{plasma}}$.

RESULTS

Pharmacokinetic parameters after oral administration of thalidomide and lenalidomide are presented in Tables 1 and 2. For thalidomide, the median apparent clearance (Cl/F) was 2.9 mL/min/kg (range, 2.6–3.6), median plasma AUC_{inf} was 80 $\mu\text{M}\cdot\text{hr}$ (range, 80–88), and the median plasma $t_{1/2}$ was 13.3 hours (range, 8.1–13.7). For lenalidomide, the median Cl/F was 8.7 mL/min/kg (range, 6.6–18.7), the median plasma AUC_{inf} was 9 $\mu\text{M}\cdot\text{hr}$ (range, 7–13), and the median plasma $t_{1/2}$ was 5.6 hours (range, 4.4–8.2). For thalidomide, the median CSF AUC_{inf} was 33 $\mu\text{M}\cdot\text{hr}$ (range, 32–40), and the median CSF penetration was 42% (range, 36–50). For lenalidomide, drug was detected in the CSF of 2 of the 3 animals. The CSF AUC_{inf} was 1.0 and 1.4 $\mu\text{M}\cdot\text{hr}$, and in both animals, the CSF penetration was 11%. Mean plasma and CSF concentrations for thalidomide (Figure 1) and lenalidomide (Figure 2) are shown. The animals tolerated the drugs without significant clinical or laboratory toxicity.

DISCUSSION

Thalidomide and lenalidomide have both been used successfully in the treatment of malignancies, especially myelodysplastic diseases. The CNS penetration of these agents is of interest because thalidomide has prominent CNS toxicity, i.e., sedation [20], while lenalidomide has shown some activity in patients with brain tumors [21]. While both of these agents have been studied in children [16, 21, 22], their CSF penetration has not been described in detail. In this study, we demonstrated that there was notable CNS penetration of thalidomide (42%) and lenalidomide (11%) following oral dosing in the nonhuman primate. This degree of penetration compares well to that of standard chemotherapy drugs commonly used for systemic treatment of leptomeningeal disease, such as cytarabine, methotrexate, and corticosteroids, whose CNS penetration ranges from 3–20% [23].

In our study, the 50 mg dose of thalidomide given to the nonhuman primates is comparable to a dose of approximately 65–100 mg/m² in humans. The median Cl/F of thalidomide (59 mL/min/m²) was very similar to that seen in children (55 mL/min/m²) [22] and adults (85 mL/min/m²) [24]. The $t_{1/2}$ was also similar, with a mean of approximately 13 hours in the primates, 6 hours in children [22], and 5–6 hours in adults [24, 25, 26, 27].

The 20 mg dose of lenalidomide used in our study compares to approximately 25–40 mg/m² in humans. The Cl/F of lenalidomide (174 mL/min/m²) is higher than the Cl/F of thalidomide (59 mL/min/m²), and comparable to that of lenalidomide in adults (116 mL/min/m²) [28] and children (136–234 mL/min/m²) [16, 21]. Also, the $t_{1/2}$ of 5.6 hours for lenalidomide in non-human primates is slightly longer than that of approximately 2.5 hours in children [16, 21] and 4 hours in adults [28].

In our study, thalidomide exhibited higher CSF penetration (42%) than lenalidomide (11%). In the phase I clinical trial of thalidomide, 20% of children who received thalidomide experienced somnolence [22]. In contrast, somnolence was not reported in either phase I study of lenalidomide in children [16, 21] nor in the phase I study of lenalidomide in adults with CNS tumors [15]. It is not known whether the increased CSF exposure of thalidomide compared with lenalidomide is related to the difference in side effect profile observed between the two drugs.

Clinical responses to lenalidomide have been observed in children with CNS tumors. In the study by Warren and colleagues, out of 47 patients evaluable for response, there were two partial responses and 23 patients with stable disease > 6 months [21]. Although we observed moderate CSF penetration of lenalidomide in 2 of the 3 animals, lenalidomide was below the limit of quantitation in the third. It is likely that CNS penetration is better in tumors that have a disrupted blood-brain barrier compared with the intact blood-brain barrier in our

model. Taken together, our data support the further evaluation of lenalidomide in patients with CNS tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Mean plasma (■) and CSF (○) concentrations of thalidomide after oral administration of a 50 mg dose

Fig. 2.
Mean plasma (■) and CSF (○) concentrations of lenalidomide after oral administration of a 20 mg dose

Pharmacokinetic parameters for thalidomide after oral administration in nonhuman primates

Table 1

Animal	Plasma AUC _{inf} (μM•hr)	CSF AUC _{inf} (μM•hr)	Cl/F (mL/min/kg)	Cl/F (mL/min/m ²) ^a	t _{1/2} (hr)	Peak Plasma Concentration (μM)	Peak CSF Concentration (μM)	AUC _{inf} CSF/ AUC _{inf} plasma (%)
1	80	40	2.6	52	8.1	5.5	2.8	50
2	80	33	2.9	59	13.3	3.7	2.0	42
3	88	32	3.6	73	13.7	5.0	1.8	36
Median	80	33	2.9	59	13.3	5.0	2.0	42

AUC_{inf}: area under the curve extrapolated to infinity; CSF, cerebrospinal fluid; Cl/F, apparent clearance; t_{1/2}, half-life;

^a approximate

Table 2
Pharmacokinetic parameters for lenalidomide after oral administration in nonhuman primates

Animal	Plasma AUC _{inf} (μM•hr)	CSF AUC _{inf} (μM•hr)	Cl/F (mL/min/kg)	Cl/F (mL/min/m ²) ^a	t _{1/2} (hr)	Peak Plasma Concentration (μM)	Peak CSF Concentration (μM)	AUC _{inf} CSF/ AUC _{inf} plasma (%)
1	9	1.0	8.7	174	5.6	1.4	0.11	11
3	13	1.4	6.6	132	4.4	2.7	0.18	11
4	7	BQL	18.7	374	8.2	0.7	NA	NA
Median	9	1.2	8.7	174	5.6	1.4	0.14	11

AUC_{inf}: area under the curve extrapolated to infinity; CSF, cerebrospinal fluid; Cl/F, apparent clearance; t_{1/2}, half-life; BQL, below quantifiable limit of detection; NA, not able to perform analysis;
^a approximate