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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of intravenously administered ABT-751 in non-human primates

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Abstract
Purpose ABT-751 is an orally bioavailable sulfonamide that binds to the colchicine binding site on β-tubulin and inhibits microtubule polymerization. The plasma and cerebrospinal fluid (CSF) pharmacokinetics of ABT-751, after a short intravenous infusion, were evaluated in a non-human primate (*Macaca mulatta*) model that is highly predictive of the CSF penetration of drugs in humans.
Materials and methods Plasma and CSF samples were collected over 24 h after 7.5 mg/kg (150 mg/m^2) ABT-751 infused over 0.25–0.70 h, and ABT-751 concentrations in plasma and CSF were quantified using a validated HPLC-MS/MS assay. Pharmacokinetic parameters in plasma and CSF were derived using non-compartmental methods.
Results and conclusion Plasma disappearance was bi-exponential with a terminal half-life of 13 h. The mean ± SD clearance was 100 ± 18 ml/min m^2, the mean ± SD volume of distribution at steady state was 1.3 ± 0.5 l/kg, and the mean ± SD mean residence time was 4.6 ± 1.8 h. The mean ± SD peak ABT-751 concentration in CSF was 0.26 ± 0.08 μM, and the mean ± SD CSF half-life of 1.3 ± 0.3 h. CSF penetration was limited (mean ± SD AUC_{CSF}:AUC_{plasma}, 1.1 ± 0.3%) relative to total (protein-bound + free) plasma drug concentrations, but the CSF concentrations approximated the estimated free drug concentrations in plasma.

Keywords ABT-751 · Cerebrospinal fluid · Non-human primate · Pharmacokinetics · Tubulin · Microtubule

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Introduction

ABT-751 is an orally bio-available sulfonamide that binds to the colchicine binding site on β-tubulin and inhibits microtubule polymerization [4]. The drug has broad anti-tumor activity in preclinical models [9, 14] and has undergone phases I and II trials in adults, and phase I testing in children [1–3, 8, 10]. The clinical experience with ABT-751 in central nervous system tumors is limited, but on the 7-day dosing schedule, one patient with an anaplastic astrocytoma experienced a 21% reduction in tumor size and stable disease for 39 weeks [5].

The cerebrospinal fluid (CSF) penetration of the standard tubulin-binding agents, such as the vinca alkaloids and taxanes, is low [6, 7]. These agents are natural products and are large molecules (molecular weight >800). They are also substrates for P-glyprotein (P-gp), which is highly expressed in brain capillary endothelial cells and the choroid plexus epithelium [13], which are the anatomic sites of the blood:brain barrier and blood:CSF barrier, respectively. P-gp may play a role in excluding these agents from the central nervous system. ABT-751 is a smaller molecule (molecular weight, 371 amu) and is not a P-gp substrate [9, 14], suggesting that ABT-751 may have more substantial penetration into the central nervous system.

We investigated the CSF penetration of ABT-751 after an intravenous dose in a non-human primate model that has been predictive of the central nervous system pharmacology of anticancer drugs in humans [11]. The plasma pharmacokinetics after intravenous dosing is also presented.

Materials and methods

Drug

ABT-751 (chemical name, *N*-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide), was provided by Abbott Laboratories and administered to animals intravenously over 15–42 min at a dose of 7.5 mg/kg (150 mg/m^2). ABT-751 was dissolved in polyethylene glycol and normal saline or D_5W (50% V:V in the first two animals, 35% V:V in the third and fourth animals). The drug concentration in the infusate was 3 mg/ml in the first animal and 1.5 mg/ml in the subsequent animals. The drug solution was sterilized by passing it through a 0.22 μm Millipore filter and then infused into the animals through a central venous catheter.

Animals

Four adult Rhesus monkeys (*Macaca mulatta*) ranging in weight from 11.8 to 15.3 kg were used for this study. The experimental protocol was reviewed and approved by the National Cancer Institute's Animal Care and Use Committee. Animals are fed NIH 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 [12]. Blood samples were drawn through a temporary saphenous vein catheter, placed contralateral to the site of drug administration. CSF was drawn from a chronically indwelling fourth ventricular Pudenz catheter that is attached to a subcutaneously implanted Ommaya reservoir [11]. The reservoir was pumped prior to and after each sample.

Experiments

Blood samples (3 ml) were collected in tri-potassium EDTA tubes prior to ABT-751 infusion, and at 5–10 min into the infusion, at the end of the infusion, and then approximately 0.5, 1, 2, 4, 6, 8, 10, and 24 h after the end of infusion. Plasma was separated by centrifuge at 0–5°C within 1 h after collection. CSF samples were drawn prior to the infusion, then approximately 0.5, 1, 2, 4, 6, 8, 10, and 24 h after the end of infusion. All plasma and CSF samples were frozen at −70°C until assayed. The first animal that received the drug did not have an Ommaya reservoir. The purpose of this first experiment was ensure the safety and tolerability of the dose and route of administration of ABT-751 in the animal model prior to administering the agent to animals with Ommaya reservoirs. Plasma samples only were obtained from this first animal, and plasma and CSF samples were obtained from the subsequent three animals.

Sample analysis

Concentrations of ABT-751 were quantified in plasma and CSF with a validated HPLC-MS/MS assay. The drug was separated from the plasma using liquid-liquid extraction with a mixture of ethyl acetate and hexane. Plasma (0.2 ml) was combined and briefly vortexed with 50 μl of internal standard (A-318730; prepared in acetonitrile:0.1% TFA [10:90, by volume]) in a 96-well plate. The samples were extracted by vortexing with 1 ml ethyl acetate:hexane (1:1 by volume), the plate was centrifuged at 2,000 rpm for 2 min; the upper organic layer was automatically transferred to a clean 96-well plate (Tomtec Quadra Dispenser) and evaporated to dryness with a gentle stream of dry nitrogen at low heat (~37°C). The samples were reconstituted by vortexing with 0.5 ml mobile phase. CSF (0.15 ml) was combined with 50 μl internal standard and vortexed prior to hPLCMS/ MS analysis.

Spiked standards were prepared in both blank monkey plasma and phosphate buffered saline (CSF substitute). Spiked standards were analyzed simultaneously with the samples. ABT-751 and the internal standard were separated from each other and co-extracted contaminants on a 100 x 3 mm Keystone Aquasil 5 μm C18 column with an acetonitrile:0.1% trifluoroacetic acid mobile phase (40:60, by volume) at a flow rate of 0.7 ml/min. Analysis was performed on a Sciex API3000™ Biomolecular Mass Analyzer with a turboionspray interface using MRM detection at *m/z* 372.1 → 201.2 for ABT-751 and *m/z* 368.3 → 181.3 for the internal standard. Peak areas were determined using Sciex MacQuan™ software.

The plasma standard curve was linear (correlation coefficient > 0.999) over the concentration range 0.15–14.67 μM, with mean accuracy values from 96.7 to 109.3%. Samples with concentrations above the linear range of the standard curve were diluted prior to analysis. The limit of quantification was estimated to be ~0.016 μM from a 0.2 ml plasma sample. The CSF ABT-751 standard curve was linear (correlation coefficient > 0.993) over the concentration range

0.030–2.93 μM, with mean accuracy values from 92.3 to 103.6%. The limit of quantification was estimated to be ~0.016 μM from a 0.15 ml CSF sample. All plasma and CSF samples from the four experiments were assayed in a single batch. The intra-day coefficients of variation for the assay were <6%.

Pharmacokinetic analysis

The area under the concentration versus time curve (AUC) in plasma and CSF was derived using the linear trapezoid method and extrapolated to infinity ($AUC_{0-\infty}$) by adding C_{last}/β, where C_{last} is the ABT-751 concentration at the last measurable time point and β is the elimination rate constant, which was estimated using linear regression of the terminal portion of the log-transformed concentration time curve. The percentage of drug penetrating into the CSF was derived from the ratio of the $AUC_{0-\infty}$ in CSF to the $AUC_{0-\infty}$ in plasma. The half-life was determined by dividing 0.693 by the elimination rate constant, and the clearance was derived from dose/$AUC_{0-\infty}$. The volume of distribution at steady state (Vd_{ss}) and the mean residence time (MRT) were calculated from the AUC and the area under the moment curve.

Results

After a short intravenous infusion of ABT-751, the peak plasma concentration (at the end of the infusion) averaged 44 ± 3 μM. Disappearance of the drug from plasma was bi-exponential (Fig. 1) with a mean terminal half-life of 13 h. Pharmacokinetic parameters derived from the plasma drug ABT-751 concentrations for the four individual animals are listed in Table 1. The mean ± SD clearance was 100 ± 18 ml/min m^2, the mean ± SD Vd_{ss} was 1.3 ± 0.5 l/kg and the mean ± SD MRT was 4.6 ± 1.8 h.

ABT-751 was measurable in CSF at the first time point (0.25–0.65 h after the end of the short infusion) and the peak concentrations were at this first time point in all three animals. The mean ± SD C_{max} in CSF was 0.26 ± 0.08 μM, which was well below the peak plasma concentration. ABT-751 disappeared from CSF rapidly (Fig. 1) with a mean ± SD half-life of 1.3 ± 0.3 h. The more prolonged terminal elimination phase observed in the plasma drug concentration was not observed in CSF, but may have been below the level of detection of the assay. Pharmacokinetic parameters derived from the CSF ABT-751 concentrations are listed in Table 2. The mean ± SD AUC_{CSF}:AUC_{plasma} was 1.12 ± 0.34%.

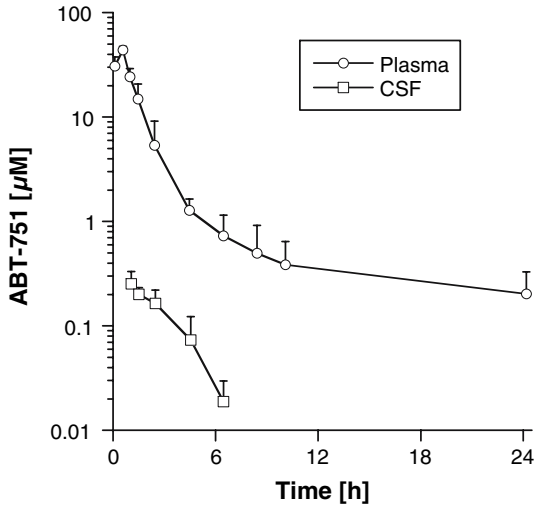

Fig. 1 Mean and standard deviation (*error bars*) plasma (*circle*) and CSF (*squares*) ABT-751 concentration–time profiles from the three animals that had CSF sampling via the Ommaya reservoir

All animals tolerated the dose of 7.5 mg/kg. The first two animals were transiently pale and somewhat lethargic after the infusion of ABT-751 over 0.25 and 0.33 h. These symptoms were not observed in the third and fourth animals that received 0.7 h infusions. The first and third animal showed signs of nausea around the time of the infusion. No other toxicities were observed during or after the infusion and all animals were asymptomatic 24 h after the dose.

Table 1 Plasma pharmacokinetic parameters for ABT-751 after a short intravenous infusion of 7.5 mg/kg (150 mg/m^2) in four non-human primates

Animal	AUC_{0-last} (μM h)	$AUC_{0-\infty}$ (μM h)	EOI (μM)	Half-life (h)	Clearance (ml/min m^2)	Vd_{ss} (l/kg)	MRT (h)
R829	59.8	62.1	44.9	11.5	125	1.40	3.73
B9078	63.9	67.3	39.9	18.2	99.5	1.28	4.27
15398	67.3	75.5	44.2	15.4	89.2	1.93	7.23
G4	77.4	79.1	47.7	6.66	84.7	0.774	3.05

AUC_{0-last} the area under the concentration–time curve to the last measured time point (24 h); *$AUC_{0-\infty}$* the AUC extrapolated to infinity; *EOI* the plasma concentration at the end of the infusion; *Vd_{ss}* the volume of distribution at steady state; and *MRT* the mean residence time

Table 2 CSF pharmacokinetic parameters for ABT-751 after a short intravenous infusion of 7.5 mg/kg (150 mg/m^2) in three non-human primates

Animal	$AUC_{0\text{-last}}$ (μM h)	$AUC_{0\text{-}\infty}$ (μM h)	C_{max} (μM)	Half-life (h)	AUC_{CSF}:AUC_{plasma} (%)
B9078	0.96	1.00	0.34	1.2	1.5
15398	0.76	0.81	0.25	1.7	1.1
G4	0.59	0.64	0.19	1.1	0.81

$AUC_{0\text{-last}}$ the area under the concentration-time curve to the last measured time point; $AUC_{0\text{-}\infty}$ the AUC extrapolated to infinity; C_{max} the peak CSF concentration; AUC_{CSF}:AUC_{plasma} % exposure to ABT-751 in CSF relative to the plasma drug exposure in the same animal

Discussion

We used a non-human primate model that is predictive of the CSF pharmacology of anticancer drugs in humans to study the plasma disposition and CSF penetration of ABT-751 after a short intravenous infusion. The drug exposure (AUC) in CSF was limited (1.1%) relative to the exposure to ABT-751 in plasma.

The prolonged terminal elimination phase of ABT-751 in plasma was not detected in the CSF, presumably because the CSF concentrations had dropped below the level of detection of the assay before reaching the terminal phase. This could result in an underestimation of the AUC_{CSF}. In order to assess the impact of missing a terminal CSF elimination phase that is parallel to that seen in plasma, the AUC in CSF can be extrapolated to infinity using the terminal rate constant in plasma rather than the terminal rate constant in CSF. This recalculation results in a 43% higher AUC_{CSF}, but the mean AUC_{CSF}:ACU_{plasma} using this recalculated AUC_{CSF} is 1.6%, which is not substantially higher than the 1.1% derived by using the CSF terminal slope to extrapolate the AUC_{CSF} to infinity.

CSF protein levels are substantially lower than protein levels in plasma, and drugs that are highly protein-bound in plasma, like ABT-751, which is 99.6–99.7% protein-bound in monkey plasma in vitro (ABT-751 Investigational Drug Brochure), will generally have very limited CSF penetration when total (protein-bound + free) CSF drug concentrations are compared to total plasma drug concentrations. Drug effect is usually dependent on free drug concentration at the effect site. With this highly (>99%) protein-bound agent, CSF concentrations approximate free drug concentrations in plasma, suggesting that the non protein-bound drug can readily cross the blood–CSF barrier, albeit at very low concentrations.

Peripheral neuropathies are a prominent toxicity of most tubulin-binding agents, including ABT-751, but the incidence of central nervous system toxicity from the tubulin-binding agents is low. The limited central nervous system drug penetration of the tubulin-binding agents may account for these differential peripheral and central nervous system toxic effects and may enhance the therapeutic indices of these drugs.

In conclusion, after an intravenous infusion of ABT-751 to non-human primates, the penetration of drug into the CSF was limited, which is consistent with the extensive plasma protein binding of the drug. CSF ABT-751 concentrations approximated free (non protein-bound) drug concentrations in plasma estimated from the previously measured extent of protein binding in monkey plasma in vitro.

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References

1. Benson AB, Kindler HL, Jodrell D, Hagey A, Coates AI, Meek KA, Cernohous P, Gordon GB (2005) Phase 2 study of ABT-751 in patients with refractory metastatic Colorectal Carcinoma (CRC). Proc Am Soc Clin Oncol 23:255s
2. Cho SY, Adamson PC, Hagey A, Widemann BC, Maris JM, Fox E, Medina DM, Cai Y, Gordon G, Balis FM (2004) Phase I trial and pharmacokinetic study of ABT-751, an orally bioavailable tubulin binding agent, in pediatric patients with refractory solid tumors. Proc Am Soc Clin Oncol 22:147
3. Fox E, Adamson PC, Hagey A, Widemann BC, Maris JM, Cohn SL, Cai Y, Medina DM, Meek KA, Balis FM (2005) Phase I trial of oral ABT-751 in pediatric patients: preliminary evidence of activity in neuroblastoma (NBL). Proc Am Soc Clin Oncol 23:806s
4. Galmarini CM (2005) ABT-751 (Abbott). Curr Opin Investig Drugs 6:623–630
5. Hande KR, Hagey A, Berlin JD, Cai Y, Meek K, Kobayashi H, Lockhart AC, Medina DM, Sosman JA, Gordon GB, Rothenberg ML (2006) The pharmacokinetics and safety of ABT-751, a novel, orally bioavailable sulfonamide antimitotic agent: results of a phase 1 study. Clin Cancer Res 12:2834–2840
6. Herrington JD, Di Nunno L, Rinehart JJ (1998) Lack of CNS penetration of docetaxel in a patient with leptomeningeal carcinomatosis. Ann Pharmacother 32:611–612
7. Kellie SJ, Barbaric D, Koopmans P, Earl J, Carr DJ, de Graaf SS (2002) Cerebrospinal fluid concentrations of vincristine after bolus intravenous dosing. Cancer 94:1815–1820
8. Kobayashi H, Hande KR, Berlin JD, Roth BJ, Sosman JA, Lockhart AC, Hagey A, Meek K, Coates A, Rothenberg ML

(2004) Phase I results of ABT-751, a novel microtubulin inhibitor, administered daily × 7 every 3 weeks. Proc Am Soc Clin Oncol 22:147
9. Koyanagi N, Nagasu T, Fujita F, Watanabe T, Tsukahara K, Funahashi Y, Fujita M, Taguchi T, Yoshino H, Kitoh K (1994) In vivo tumor growth inhibition produced by a novel sulfonamide, E7010, against rodent and human tumors. Cancer Res 54:1702–1706
10. Mauer AM, Szeto L, Belt RJ, Schwartzberg LS, Hagey A, Coates AI, Meek KA, Cernohous P, Gordon GB (2005) Preliminary results of a Phase 2 study of ABT-751 in patients (pts) with taxane-refractory non-small cell lung carcinoma. Proc Am Soc Clin Oncol 23:654s
11. McCully CL, Balis FM, Bacher J, Phillips J, Poplack DG (1990) A rhesus monkey model for continuous infusion of drugs into cerebrospinal fluid. Lab Anim Sci 40:520–525
12. National Research Council (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington
13. Warren KE, Patel MC, McCully CM, Montuenga LM, Balis FM (2000) Effect of P glycoprotein modulation with cyclosporin A on cerebrospinal fluid penetration of doxorubicin in non-human primates. Cancer Chemother Pharmacol 45:207–212
14. Yoshimatsu K, Yamaguchi A, Yoshino H, Koyanagi N, Kitoh K (1997) Mechanism of action of E7010, an orally active sulfonamide antitumor agent: inhibition of mitosis by binding to the colchicine site of tubulin. Cancer Res 57:3208–3213