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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of tasidotin (ILX-651) and its metabolites in non-human primates

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Abstract
Purpose To evaluate the plasma and cerebrospinal fluid (CSF) pharmacokinetics and CSF penetration of tasidotin and metabolites in a nonhuman primate model.
Methods Tasidotin 0.75 mg/kg was administered intravenously. The plasma and CSF concentrations of tasidotin and its metabolites were determined. Pharmacokinetic parameters were estimated using model-independent and model-dependent methods.
Results The mean (±SD) CSF:plasma AUC ratio for tasidotin was 1.1 ± 0.4. For tasidotin, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate the plasma AUCs (mean ± SD) were 30 ± 10, 54 ± 19 and 12 ± 2 μM min, and apparent plasma half-lives were 27 ± 4, 229 ± 73 and 100 ± 29 min. The plasma clearance of tasidotin was 44 ± 14 ml/min/kg. The CSF AUC and half-life of tasidotin was 28 ± 10 μM min and 96 ± 40 min. The model-dependent plasma clearance was 35 ml/min/kg for tasidotin and 2 ml/min/kg for tasidotin-C-carboxylate.
Conclusions Tasidotin penetrates into the CSF well and further evaluation of its activity in the treatment of central nervous system malignancies should be considered.

Keywords Tasidotin · ILX651 · Pharmacokinetics · CSF · Non-human primate

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Introduction

Inhibitors of tubulin are widely utilized and highly effective anticancer therapies. However, current therapies are limited by systemic toxicity and the development of resistance [1]. The treatment of primary or metastatic central nervous system (CNS) malignancies with tubulin inhibitors is further complicated by poor penetration of these agents into the CNS [2, 3]. New agents that inhibit microtubules by novel mechanisms may provide efficacy in resistant tumors, and agents with improved side effect profiles may allow increased dose intensity. Understanding the CNS penetration and pharmacokinetics of these new agents provides important pre-clinical data when considering their use in the treatment of CNS malignancies.

Tasidotin HCl (ILX651, N,N-dimethyl-Lvalyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-tert-butylamide hydrochloride, molecular weight 643.31) is a crystalline, water soluble, third generation dolastatin-15 analogue. The dolastatins bind to tubulin in the vinca domain and inhibit microtubule assembly and depolymerization, leading to inhibition of the cell cycle in mitosis as well as to apoptosis [4–6]. Tasidotin appears to antagonize microtubule assembly

and to induce a prolonged lag phase in microtubule assembly [7], a novel mechanism of microtubule inhibition differing from that of the microtubule stabilizers (taxanes and epothilones) and the tubulin inhibitors (vinca alkaloids and dolastatin). Its metabolite tasidotin-C-carboxylate has recently been described as a 10–30 times more potent inhibitor of microtubule dynamics than the parent tasidotin. However, it inhibits cell proliferation only weakly (IC_{50} ~4 mM) in vitro [8]. Thus its contribution to tumor inhibition after tasidotin administration is uncertain.

Tasidotin is 85–100% unbound in mouse, rat, dog and human plasma, and tasidotin-C-carboxylate is 100% unbound in all species. Tasidotin is extensively metabolized and excreted by biliary (major) and urinary (minor) routes. In vitro free tasidotin enters cells by an unidentified transporter. It is then metabolized within the cell to tasidotin-C-carboxylate, which is further metabolized to yield free proline and noncytotoxic desprolyl-tasidotin-C-carboxylate (unpublished data). In humans, tasidotin shows rapid elimination with a half-life of less than an hour. In contrast, the tasidotin-C-carboxylate concentration peaks around 5 h after administration and has a half-life of about 10 h [9–12].

In pre-clinical studies, tasidotin has activity against a variety of tumor types including melanoma, non-small cell lung cancer, renal, prostate, breast, colon, CNS, ovarian, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma and leukemia [13–15]. In phase 1 studies in adults, tasidotin was generally well tolerated with a dose limiting toxicity of neutropenia. Other grade 3 toxicities described in these studies included diarrhea, vomiting, ileus, elevated transaminases, and pyrexia [9, 10, 12]. Phase 2 studies have been completed in adult patients with melanoma, non-small cell lung cancer and prostate cancer [11].

Most microtubule inhibitors have poor CNS penetration [2, 16]. In order to assess the CNS penetration of tasidotin, we studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate after intravenous (IV) administration in a nonhuman primate model that has been highly predictive of anticancer drug distribution in humans [17].

Materials and methods

Drug

Tasidotin was supplied by Genzyme Oncology, San Antonio, TX in 30 mg vials containing 10 mg/ml of Tasidotin. The dose of the drug was diluted in 0.9% sodium chloride to a final total volume of 50 mL.

Animals

The study was approved by the Institutional Animal Care and Use Committee. Six healthy adult male rhesus monkeys (Macaca mulatta) weighing 11.4–15.9 kg were used in these experiments. The monkeys were provided with water ad libitum, were fed a commercial diet (Lab Diet® 5045) twice daily and supplemented with fresh fruits and vegetables. The colony is group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [18]. Animals were observed on a daily basis for a minimum of 4 weeks post-infusion for any evidence of clinical toxicity. Clinical laboratory studies including complete blood counts, electrolytes, and liver and renal function tests were obtained at baseline and weekly for at least 4 weeks following the infusion of tasidotin.

Experiments

Tasidotin 0.75 mg/kg was administered IV over 30 min through a surgically implanted central venous catheter or through a catheter placed in the femoral or saphenous vein. Blood samples were drawn through a catheter placed in the contralateral femoral or saphenous vein. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir. Blood samples (2.5 ml in EDTA) were obtained prior to the start of the infusion, 15 min into the infusion, at the end of the infusion and at 5, 10, 15, 30, 45, 60, 90 min, and 2, 4, 6, 8, 10, and 24 h after completion of the infusion. Plasma was immediately separated by centrifugation for 10 min at 4,400 rpm and stored at −80°C until analysis. CSF samples (0.25 ml) were obtained prior to the start of the infusion, 15 min into the infusion and at the end of the infusion, then at 30 and 90 min, and 2, 4, 6, 10, and 24 h following the end of the infusion. In the first animal, only blood samples were collected and the 8 and 10 h time points were not obtained. The sampling schedule was extended in the later animals to more clearly characterize the tasidotin metabolites. Two animals had incomplete sampling due to technical difficulties. Samples were analyzed by MicroConstants, Inc (San Diego, CA) for the concentrations of tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate using a previously described liquid chromatography tandem mass spectrometry assay with a linear range from 1 to 500 ng/ml [9, 19].

Pharmacokinetic analysis

Pharmacokinetic parameters for tasidotin and its 2 metabolites (tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate) in plasma and CSF were estimated using noncompartmental methods. Concentrations below the lowest

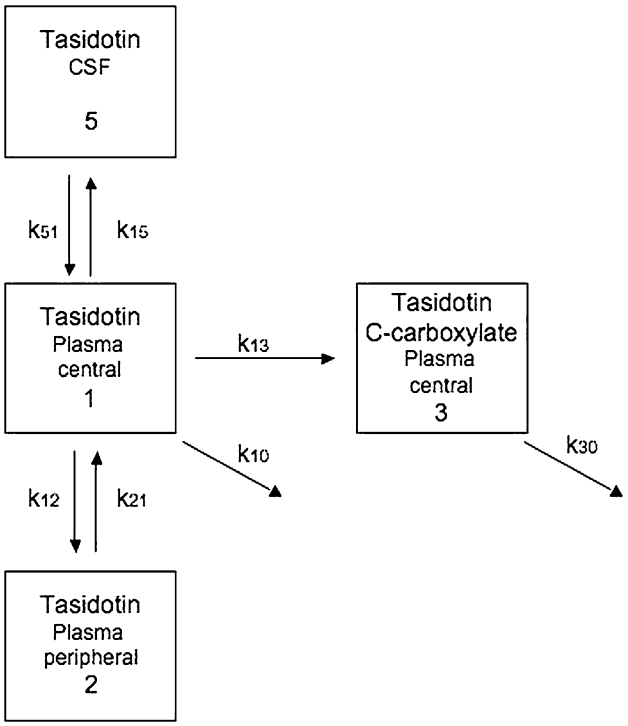

Fig. 1 Compartmental model for tasidotin in plasma and CSF and tasidotin-C-carboxylate metabolite in plasma where *k with a subscript* represents the rate constant for transfer between the indicated compartments or out of the body

limit of quantification (<1.00 ng/ml or 1.6 nM) were set equal to 0 prior to detection of the drug or metabolite and to missing following clearance of the drug or metabolite. The terminal half-life was estimated by linear regression over the last three concentration-time data points for all analytes in the plasma and tasidotin in the CSF, and over the last two data points for desprolyl-tasidotin-C-carboxylate in the CSF. The area under the concentration-time curve (AUC) was calculated using the trapezoidal method and extrapolated to infinity by adding the quotient of the final plasma or CSF concentration divided by the terminal rate constant. Clearance was calculated from the equation clearance = total dose administered/AUC. The steady state volume of distribution (Vd_{ss}) was calculated from the area under the moment curve and corrected for infusion time. The CSF penetration for tasidotin and desprolyl-tasidotin-C-carboxylate was calculated as the ratio of the CSF AUC to the plasma AUC. Tasidotin-C-carboxylate was either not detected or only briefly detected in the CSF so its CSF AUC could not be calculated.

As a secondary analysis, a model was developed that could be fit to the plasma and CSF drug concentrations for tasidotin and tasidotin-C-carboxylate in all six animals simultaneously using ADAPT II software [20].This model (Fig. 1) incorporates two plasma compartments for tasidotin and a single plasma compartment for tasidotin-C-carboxylate, as well as a single CSF compartment for tasidotin. The CSF volume was fixed at 1.5 ml/kg, which is the approximate volume of the CSF in the rhesus monkey. Clearance was determined by multiplying the elimination constant(s) by the volume of the compartment.

Results

The animals tolerated the infusion well without significant clinical or laboratory toxicity. The pharmacokinetic parameters of tasidotin, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate in the plasma, as determined using noncompartmental methods, are shown in Table 1. Pharmacokinetic parameters for tasidotin and desprolyl-tasidotin-C-carboxylate in the CSF are shown in Table 2. The concentrations in plasma and CSF of tasidotin, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate following administraion of 0.75 mg/kg of tasidotin are shown for a representative animal in Fig. 2. The mean (± SD) CSF:plasma AUC ratio for tasidotin was 1.1 ± 0.4 and for

Table 1 Model independent pharmacokinetic parameters of tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate in plasma following a 0.75 mg/kg IV infusion

Subject	Tasidotin				Tasidotin-C-carboxylate		Desprolyl-tasidotin-C-carboxylate	
	Cl (ml/min/kg)	Vd_{ss} (L/kg)	AUC_{inf} (μM min)	$t_{½}$ (min)	AUC_{inf} (μM min)	$t_{½}$ (min)	AUC_{inf} (μM min)	$t_{½}$ (min)
S1	25	0.9	47	34	94	250	14	77
S2	39	1.0	30	23	57	221	8	93
S3	34	1.1	35	30	52	237	14	142
S4	55	2.3	21	27	36	175	12	148
S5	69	1.3	17	23	43	365	15	73
S6	43	1.1	28	25	41	128	11	107
Mean	44	1.3	30	27	54	229	12	100
SD	14	0.5	10	4	19	73	2	29

Table 2 Model independent pharmacokinetic parameters of tasidotin and desprolyl-tasidotin-C-carboxylate in CSF and CSF/Plasma AUC ratios

	Tasidotin		Desprolyl-tasidotin-C-carboxylate		CSF/Plasma AUC	
	AUC_{inf} (μM min)	$t_{1/2}$ (min)	AUC_{inf} (μM min)	$t_{1/2}$ (min)	Tasidotin	Desprolyl-tasidotin-C-carboxylate
S3	36	58	7.3	89	1.0	0.5
S4	38	151	8.4	208	1.8	0.7
S5	12	116	a	a	0.7	a
S6	28	59	6.4	152	1.0	0.6
Mean	28	96	7.4	150	1.1	0.6
SD	10	40	0.8	49	0.4	0.1

a Insufficient time points collected to characterize adequately

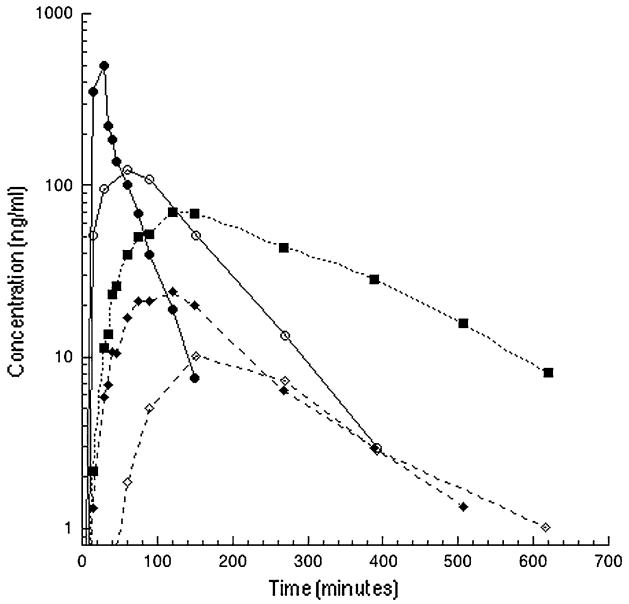

Fig. 2 Concentration versus time curves of tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate in plasma and CSF following administration of tasidotin 0.75 mg/kg IV over 30 min in a representative non-human primate. Tasidotin (*solid line*) in plasma (*filled circles*) and CSF (*open circles*), tasidotin-C-carboxylate (*dotted line*) in plasma (*filled squares*), and desprolyl-tasidotin-C-carboxylate (*dashed line*) in plasma (*filled diamonds*) and CSF (*open diamonds*)

desprolyl-tasidotin-C-carboxylate was 0.6 ± 0.1 For tasidotin, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate the plasma AUCs (mean ± SD) were 30 ± 10, 54 ± 19 and 12 ± 2 μM min, respectively. The apparent half-lives in plasma for tasidotin, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate were 27 ± 4, 229 ± 73 and 100 ± 29 min. The clearance of tasidotin from plasma was 44 ± 14 ml/min/kg. The CSF AUC and half-life were 28 ± 10 μM min and 96 ± 40 min for tasidotin and 7.4 ± 0.8 μM min and 150 ± 49 min for desprolyl-tasidotin-C-carboxylate.

The model dependent parameters for tasidotin and tasidotin-C-carboxylate are shown in Table 3. The model dependent clearance of tasidotin from plasma was 35 ml/min/kg and of tasidotin-C-carboxylate was 2 ml/min/kg.

Table 3 Model dependent parameters for tasidotin and tasidotin-C-carboxylate

Parameter	Estimate
k_{10} (min^{-1})	0.14
k_{12} (min^{-1})	0.37
k_{13} (min^{-1})	0.0096
k_{15} (min^{-1})	0.000062
k_{21} (min^{-1})	0.094
k_{30} (min^{-1})	0.0020
k_{51} (min^{-1})	0.0072
V_1 (ml/kg)	228
V_2 (ml/kg)	786
V_5 (ml/kg) (fixed)	1.5

Discussion

The elimination of tasidotin from plasma in the nonhuman primates was similar to that previously described in humans with a mean half-life for tasidotin of 27 min and clearance of 44 ml/min/kg in the animals compared with 18–45 min and 15–38 L/h/m^2 (approximately 7–17 ml/min/kg) in the adults. Similarly, in the nonhuman primates we report a tasidotin-C-carboxylate mean half-life of 229 min compared with 396–516 min in humans. Desprolyl-tasidotin-C-carboxylate was not described in the phase 1 studies [9, 10, 12].

Tasidotin has demonstrated potent and broad anti-tumor efficacy in vitro with IC_{50}s ranging from 2 to 300 nM (1.2–182 ng/mL) [21]. Tasidotin inhibits mitoses and proliferation at concentrations around 70 nM with its primary effects on microtubule dynamics [8]. In the nonhuman primate, peak CSF tasidotin concentrations ranged from 53 to 272 nM and exceeded 70 nM for 2.5–3.5 h in three of the four animals. The dose studied in the nonhuman primate, 0.75 mg/kg, is approximately equivalent to 15 mg/m^2. Since the MTD of tasidotin in humans is approximately 30 mg/m^2 administered on a daily or every other day schedule for three to five doses, it is likely that CSF concentrations in humans approach cytotoxic levels for a more prolonged period. Using the parameters derived from the pharmacokinetic model described above (Table 3), we

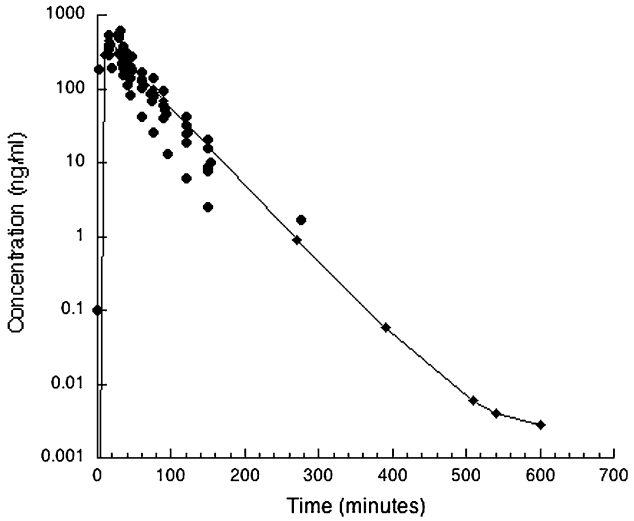

Fig. 3 Model predicted (*line with diamond*) versus measured (*circles*) concentrations of tasidotin in plasma over time following administration of 0.75 mg/kg IV over 30 min in the nonhuman primate

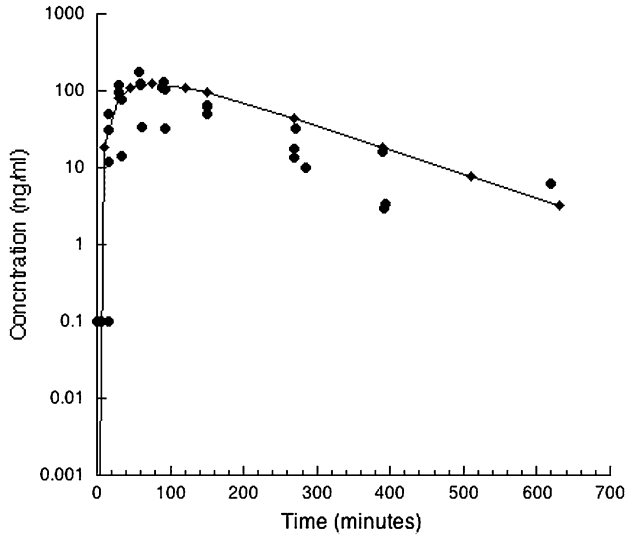

Fig. 4 Model predicted (*line with diamond*) versus measured (*circles*) concentrations of tasidotin in CSF over time following administration of 0.75 mg/kg IV over 30 min in the nonhuman primate

simulated the plasma (Fig. 3) and CSF (Fig. 4) concentrations of tasidotin after a 30 mg/m^2 dose in humans given as a 30-min infusion, which is similar to the adult MTD. Predicted CSF concentrations of tasidotin rapidly exceeded 70 nM and remained >70 nM for ~4 h following each infusion. Desprolyl-tasidotin-C-carboxylate was also detected in significant amounts in the CSF. This metabolite, however, is believed to be inactive and therefore its prolonged CSF exposure seems unlikely to be clinically important.

In contrast to tasidotin and desprolyl-tasidotin-C-carboxylate, tasidotin-C-carboxylate was not detected in significant amounts in the CSF. This may be due either to poor penetration into the CSF or to rapid metabolism to desprolyl-tasidotin-C-carboxylate by an unidentified prolinase.

Both tasidotin and desprolyl-tasidotin-C-carboxylate exhibit prolonged apparent half-lives in the CSF and high CSF:plasma AUC ratios, approximately 100% for tasidotin and 60% for desprolyl-tasidotin-C-carboxylate. The peak concentrations in the CSF were about 30% of the plasma C_{max} for both the parent compound and desprolyl-tasidotin-C-carboxylate (Fig. 2). This high penetration may be related to their relative lack of protein binding. Other microtubule inhibitors exhibit limited CNS penetration. Vincristine was not detected in CSF after bolus IV dosing [2]. Several studies of the taxanes have described poor blood brain barrier penetration due to extrusion by P-glycoprotein, although paclitaxel has been shown to penetrate to some extent into brain tumor tissue despite limited penetration into the CSF or normal brain [22–24]. Thus, although microtubule inhibitors often demonstrate preclinical activity against CNS malignancies, their clinical utility may be limited by low CNS penetration. In contrast, the high CNS exposure of tasidotin is unusual for microtubule inhibitors and suggests that further evaluation of this agent in the treatment of CNS malignancies is warranted.

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