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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of the histone deacetylase inhibitor, belinostat (PXD101), in non-human primates

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Abstract
Purpose Histone deacetylases (HDAC) are involved in the regulation of gene transcription. Aberrant HDAC activity has been associated with tumorigenesis, and, therefore, HDACs are potential targets for the treatment of cancers, including tumors of the central nervous system (CNS). Belinostat is a novel, potent, pan-HDAC inhibitor with antiproliferative activity on a wide variety of tumor cell lines. We studied the cerebrospinal fluid (CSF) penetration of intravenous (IV) belinostat in a non-human primate model as a surrogate for blood:brain barrier penetration.
Design Five adult rhesus monkeys received increasing doses of belinostat (10–60 mg/kg) as a 30-min IV infusion. Serial blood and CSF samples were collected over 48 h. Plasma and CSF concentrations of belinostat were quantified with an LC/MS/MS assay. Pharmacokinetic parameters were calculated using non-compartmental methods, and CSF penetration is expressed as the ratio of the area under the concentration-time curve (AUC) in CSF to the AUC in plasma.
Results Belinostat was cleared rapidly from plasma with a half-life of 1.0 h, a mean residence time of 0.47 h, and a clearance of 425 ml/min/m^2. CSF penetration of belinostat was limited. CSF drug exposure was <1% of plasma drug exposure and <10% of free (non-protein bound) plasma drug exposure.
Conclusion IV belinostat is rapidly cleared from plasma and has limited penetration into the CSF.

Keywords Cerebrospinal fluid · Pharmacokinetics · Histone deacetylase · PXD101 · Belinostat

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Introduction

Histones are chromatin proteins packaged with DNA in the nucleosome. Modification of these histone proteins plays a fundamental role in regulating gene expression [1]. Acetylation and deacetylation of histones is controlled by the activities of histone acetyltransferases (HAT) and histone deacetylases (HDAC), respectively. Deacetylation of histones is associated with condensation of chromatin and repression of gene transcription, and aberrant HDAC activity may be associated with tumor formation [2, 3]. Inhibition of HDAC has been shown to induce expression of genes associated with cell cycle arrest and tumor suppression [4, 5]. HDAC inhibitors are being investigated as antitumor agents for a variety of cancers. There is interest in evaluating HDAC inhibitors in patients with CNS tumors because of reports of in vitro activity in glioma cell lines [6, 7].

Belinostat is a low-molecular weight HDAC inhibitor with activity in vitro against human tumor cell lines, and in vivo against human tumor xenografts [8]. Histone deacetylase enzyme activity in cell lysates is inhibited by belinostat at IC_{50} values of 9–100 nM [8]. Belinostat is currently being evaluated in a number of Phase Ib/II combination and Phase II monotherapy clinical trials for solid tumors and hematologic malignancies.

CNS tumors are difficult to treat, in part, due to the presence of the blood: brain barrier (BBB), which is comprised of brain capillary endothelial cells that restrict entry of anticancer agents into the CNS. Determining the penetration of drugs into the CSF is used as a surrogate for BBB penetration and is useful for identifying agents that may prove useful for treating CNS tumors. A non-human primate model has been used to quantify CSF drug penetration after systemic administration and has been highly predictive of CNS pharmacology in humans [9]. We studied the CSF penetration of the HDAC inhibitor, belinostat, in this animal model.

Materials and methods

Drug preparation and administration

Belinostat (mw 318) was supplied by the investigational drug branch of the Cancer Therapy Evaluation Program (CTEP), NCI (Bethesda, MD) as a powder, and reconstituted with L-arginine 100 mg/ml to prepare an intravenous formulation (final belinostat concentration, 50 mg/ml) just prior to infusion. Belinostat was administered via a central catheter as a 30-min infusion at escalating doses of 10–60 mg/kg (human equivalent 200–1,200 mg/m^2).

Non-human primate model

Five adult male rhesus monkeys (*Macaca mulatta*) weighing 11.2–13.7 kg were used for the study. The animals were fed NIH open formula extruded non-human primate diet and group housed indoors in accordance with the *Guide for the Care and Use of Laboratory Animals* [10]. Blood for pharmacokinetic sampling was obtained via a temporary saphenous venous catheter. For CSF sampling, the initial animal (dosed at 10 mg/kg) had a temporary lumbar catheter. The remaining four animals had a pudenz catheter permanently implanted in the 4th ventricle and attached to a subcutaneous Ommaya reservoir, which allows for repeated CSF sampling in unanesthetized animals [9].

Pharmacokinetic sampling

Blood was collected in heparinized tubes prior to the dose of belinostat, at the completion of the infusion, and at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 10, 24 and 48 h following the completion of the infusion. Plasma was immediately separated by centrifugation and plasma samples were stored at –70°C in polypropylene tubes. CSF (0.3 ml aliquots) was collected prior to the dose of belinostat and at 0.5, 1, 2, 4, 6, 8, 10, 24 and 48 h after the dose and frozen immediately at –70°C in polypropylene tubes.

Belinostat assay

The concentrations of belinostat in plasma and CSF were measured using a gradient LC/MS/MS procedure. Samples were analyzed with an internal standard, Bufexamac (B-0760, Sigma Aldrich). Plasma and CSF extraction was carried out using the Waters Sirocco^{TM} protein precipitation plates with 96-well collection plates. Twenty microliters of the clear filtrate was injected into the chromatographic system. Chromatography was performed using a Waters Alliance 2795 separation module (Waters, Milford) equipped with a Waters Sunfire 50 × 2.1 mm 1D, 5 μm column operated at 40°C. Mobile phase A was 0.05% formic acid and mobile phase B was neat acetonitrile of HPLC gradient grade. The following gradient was used: 0–4.0 min linear gradient from 10 to 90% B, 4.1–7.0 equilibration of the column with 10% B. Flow rate was 0.3 ml/min. Detection was performed by a Waters Premier Quattro in ESI positive mode. The applied MRM transition was 319.0 to 92.8 m/z with cone voltage 25 V and collition energy 20 V. Recovery of the assay was 100%. The lower limit of quantification (LOQ, estimated as a signal-to-noise ratio of 10:1) was ~3 ng/ml in CSF and ~8 ng/ml in plasma. The precision was better than 20% at 10 ng/ml and ranged from 9.1 to 12.1% at 100 and 1000 ng/ml in both matrices. The accuracy at 100 ng/ml was 98.7 and 98% in plasma and CSF, respectively. The method was linear in the range from LOQ to 50,000 ng/ml, with $r = 0.9938$ and 0.9922 for plasma and CSF, respectively.

Pharmacokinetic analysis

Plasma and CSF concentration-time data were analyzed using non-compartmental methods. The area under the concentration-time curves (AUC) for plasma and CSF belinostat were derived using the linear trapezoidal method and extrapolated to infinity ($AUC_{0\text{-}\infty}$). Clearance is defined as the dose/plasma $AUC_{0\text{-}\infty}$. The volume of distribution at steady state (Vd_{ss}) and mean residence time (MRT) were derived from the area under the moment curve. The CSF penetration is defined as $AUC^{CSF}_{0-\infty} \Big/ AUC^{plasma}_{0-\infty}$. CSF penetration was also evaluated relative to free (non-protein bound) plasma AUC based on 93% protein binding of belinostat in humans (Investigators' Brochure) using a modified equation:

$$\frac{AUC_{0-\infty}^{CSF}}{AUC_{0-\infty}^{plasma} \bullet 0.07}$$

Results

Plasma and CSF pharmacokinetics

After a short IV infusion belinostat was rapidly cleared from the plasma in non-human primates (Fig. 1). The end of infusion belinostat plasma concentration ranged from 16,000 to 47,900 ng/ml, but plasma concentrations fell to below 10 ng/ml by 10 h after the start of the infusion. The mean plasma half-life was 1.0 h, the mean MRT was 0.47 h, and the mean clearance was 425 ml/min/m^2 (Table 1). The clearance was also variable with a range of 257 to 677 ml/min/m^2, but the clearance did not appear to be dose-dependent over the dosage range of 10 to 60 mg/kg. This variability in clearance resulted in a poor correlation between dose and $AUC_{0-\infty}$ (Fig. 2).

Penetration of belinostat into the CSF was limited (Fig. 1; Table 2). The mean $AUC_{0-\infty}^{CSF} / AUC_{0-\infty}^{plasma}$ was 0.67% in the four animals receiving doses of 25 mg/kg and above. Lumbar CSF belinostat concentrations were too low (too few measurable time points) in the animal that received 10 mg/kg to calculate an $AUC_{0-\infty}$ in CSF. The peak belinostat CSF concentration in this animal was 22 ng/ml and the end of infusion plasma concentration was

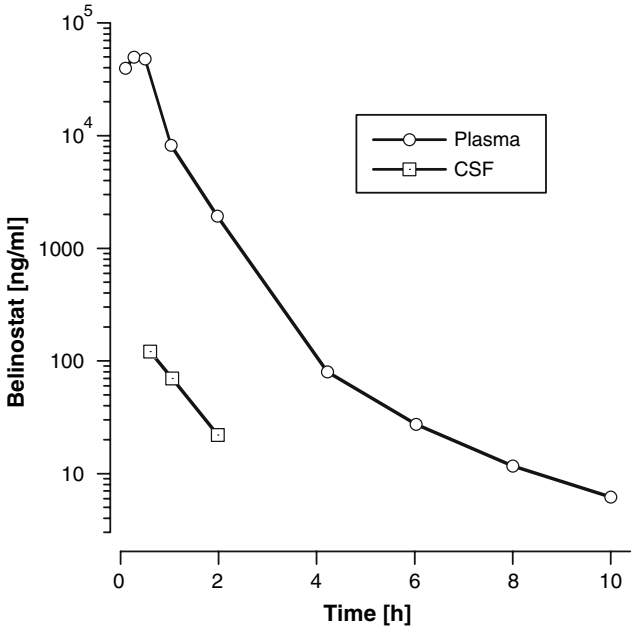

Fig. 1 Belinostat plasma and CSF concentrations over time in the non-human primate that received the 35 mg/kg dose infused IV over 30 min

Table 1 Belinostat plasma pharmacokinetic parameters

Dose (mg/kg)	$AUC_{0-\infty}^{plasma}$ (ng h/ml)	$T_{1/2}$ (h)	Clearance (ml/min/m^2)	Vd_{ss} (L/kg)	MRT (h)
10	13000	0.47	258	0.20	0.26
25	19200	1.23	435	0.31	0.24
35	43000	1.58	274	0.38	0.46
50	34700	0.89	481	1.11	0.77
60	27100	0.89	677	1.26	0.62

16,000 ng/ml. Peak CSF belinostat concentrations in the remaining four animals that received 25–60 mg/kg ranged from 77 to 328 ng/ml.

Belinostat is 93% protein-bound in plasma (Investigator Brochure), and CSF is essentially protein free. Therefore we also calculated the CSF penetration of belinostat relative to free (non-protein bound) plasma $AUC_{0-\infty}$. Even taking into account protein binding in plasma, the CSF penetration of belinostat was limited. The mean $AUC_{0-\infty}^{CSF} / AUC_{0-\infty}^{free\ plasma}$ was 9.6%.

Toxicity

No significant clinical or laboratory toxicities were observed in these five animals after a single 30 min IV infusion of belinostat at a dose of up to 60 mg/kg. The two animals receiving the highest doses (50, 60 mg/kg) developed post-infusion nausea. The animal at the 50 mg/kg dose level developed a dermatitis and superficial pyoderma that was most likely related to the antibacterial scrub for CSF sampling.

Discussion

Belinostat has limited penetration into the CSF after intravenous administration relative to total plasma drug exposure (<1%) and free (non-protein bound) plasma drug exposure (<10%). However, peak CSF belinostat concentrations achieved after relatively high doses (35 to 60 mg/kg in the non-human primate, equivalent to 700 to 1,200 mg/m^2 in humans) resulted in peak CSF concentrations of 77 to 327 ng/ml (0.24 to 1.0 μM), which are above the concentrations required to inhibit HDAC (EC_{50}s for rhHDACs range from 0.003 to 0.248 μM [11]).

The plasma pharmacokinetics in the non-human primate model is similar to that reported in humans [12]. At doses of 150 to 1,200 mg/m^2 administered as a 30 min IV infusion, the half-life in humans was 0.5 to 1.0 h and plasma concentrations were undetectable by 8 to 10 h after the dose. However, histone H4 hyperacetylation in peripheral

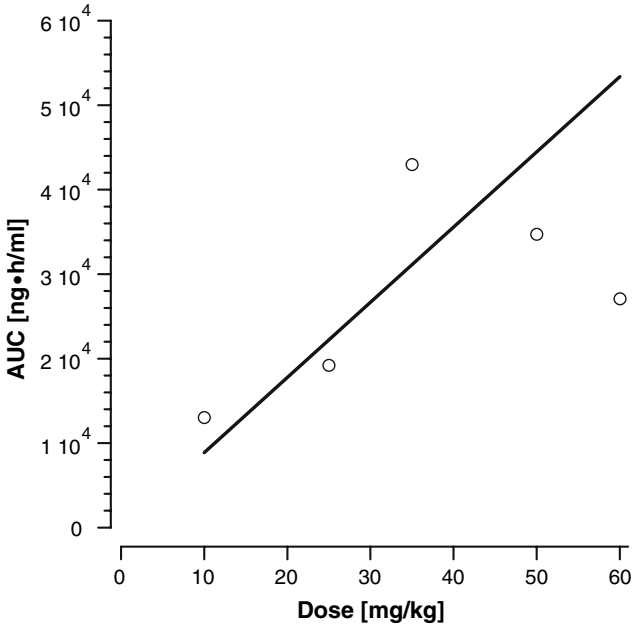

Fig. 2 Relationship between belinostat dose and the area under the plasma concentration-time curve. The line of proportionality is shown

Table 2 Belinostat CSF pharmacokinetic parameters

Dose (mg/kg)	$AUC^{CSF}_{0-\infty}$ (ng h/ml)	$T_{1/2}$ [h]	$\frac{AUC^{CSF}_{0-\infty}}{AUC^{plasma}_{0-\infty}}(\%)$	$\frac{AUC^{CSF}_{0-\infty}}{AUC^{freeplasma}_{0-\infty}}(\%)$
10	–[a]	–	–	–
25	132	0.31	0.68	9.8
35	140	0.56	0.33	4.7
50	150	0.75	0.43	6.3
60	339	0.42	1.25	17.9

[a] Unable to calculate due to too few time points with measurable belinostat concentrations

blood mononuclear cells was sustained for up to 24 h, suggesting that the pharmacodynamic effect of the drug was long-lasting despite the rapid drug clearance.

Prior studies of CSF penetration of the hydroxamic acid class of HDAC inhibitors are limited. Hockly et al. did show that suberoylanilide hydroxamic acid (SAHA) complexed with HOP-β-CD could cross the BBB of mice and increase histone acetylation, but only at the higher dose level of 200 mg/kg and not at the lower dose level of 100 mg/kg [13]. Our study is the first published study of CSF penetration for the hydroxamic class of HDAC inhibitors in a nonhuman primate. We show that although potentially effective concentrations of belinostat are attained in the CSF, penetration is limited and effective concentrations are maintained for relatively short periods of time. However, HDAC inhibitors have diverse mechanisms of action, including induction of proliferation arrest, differentiation and apoptosis of cancer cells as well as antiangiogenesis, [14, 15] and the exact target is unclear. Unlike cell-cycle specific cytotoxic agents, HDAC inhibitors may not necessarily need to be present in effective concentrations for long periods to exert their effects, as demonstrated in clinical trials of SAHA in adults which showed that histones isolated from PBMCs had consistent accumulation of acetylated histones post-therapy [16].

The role of HDAC inhibitors, particularly belinostat, in the treatment of CNS tumors remains unclear, but HDAC inhibition is an attractive target, given the preclinical activity demonstrated against glioma cell lines, and radiosensitizing capabilities demonstrated in vitro [17, 18]. Future studies may include intratumoral delivery of these agents via mechanisms such as convection-enhanced delivery in an effort to bypass the blood: brain barrier. In any case, the likely benefit of belinostat will be the synergistic activity demonstrated when combined with other antiglioma modalities including chemotherapy and radiation.

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