Published in final edited form as:

Cancer Chemother Pharmacol. 2020 April ; 85(4): 827–830. doi:10.1007/s00280-019-04021-y.

Characterizing the pharmacokinetics of panobinostat in a non-human primate model for the treatment of diffuse intrinsic pontine glioma

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Abstract

Purpose—Diffuse intrinsic pontine glioma (DIPG) is one of the deadliest forms of childhood cancers. To date, no effective treatment options have been developed. Recent drug screening studies identified the HDAC inhibitor panobinostat as an active agent against DIPG cells lines and animal models. To guide in the clinical development of panobinostat, we evaluated the CNS pharmacokinetics of panobinostat using CSF as a surrogate to CNS tissue penetration in a pre-clinical nonhuman primate (NHP) model after oral administration.

Methods—Panobinostat was administered orally to NHP ($n = 3$) at doses 1.0, 1.8, 2.4, and 3.0 mg/kg (human equivalent dose: 20, 36, 48, 60 mg/m², respectively). The subjects served as their own controls where possible. Serial, paired CSF and plasma samples were collected for 0–48 h. Panobinostat was quantified via a validated uHPLC-MS/MS method. Pharmacokinetic (PK) parameters were calculated using non-compartmental methods.

Results—CSF penetration of panobinostat after systemic delivery was low, with levels detectable in only two subjects.

Conclusion—The CSF penetration of panobinostat was low following oral administration in this pre-clinical NHP model predictive of human PK.

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Author contributions LR, CM, CP, and KW: experimental design; LR, CM, AO, and RC: experimental implementation; LR, CM, AO, and CP: data analysis; LR, CM, AO, RC, CP, WF, and KW: manuscript writing, editing, and approval.

Conflict of interest The authors declare no conflicts of interest.

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Keywords

Panobinostat; Non-human primate (NHP); Pharmacokinetics; Diffuse intrinsic pontine glioma (DIPG)

Introduction

Diffuse intrinsic pontine glioma (DIPG) is one of the deadliest forms of childhood cancer, with a median survival time of less than one year from diagnosis [1, 2]. Following the recent demonstration of safe biopsies and the development of patient-derived cell cultures and orthotopic xenograft models [3, 4], the tumor biology of DIPG is rapidly being unraveled. Recent genomic analyses of DIPG tumor tissue revealed that the majority of DIPGs exhibit histone variants H3.3 (*H3F3A*) and H3.1 (*HIST1H3B* or *HIST1H3C*), which result in a Lys27M (K27M) mutation in more than 60% of all cases [5–7].

High-throughput drug screening identified histone deacetylase (HDAC) inhibitors, particularly panobinostat [Molecular Weight (MW) = 349.43 g/mol], as active agents against DIPG cell lines [8]. Panobinostat is marginally soluble in water, and its solubility is pH dependent, with highest solubility around pH 3.0. The plasma protein binding is around 90%, and logP (a measure of lipophilicity) is 2.643. Panobinostat is a substrate for both ABCB1 [(p-glycoprotein (p-gp)] and ABCG2 [breast cancer resistance protein (BCRP)] [9]. Panobinostat demonstrated growth inhibition in 12 out of 16 DIPG cell lines with an IC₅₀ of 100 nM and significantly decreased tumor growth in an orthotopic xenograft mouse model with both systemic delivery and administration via CED [9]. Panobinostat is the active ingredient of Farydak® and is currently approved for the treatment of multiple myeloma. Panobinostat shows promise as a treatment for DIPG and is currently being evaluated in a pediatric clinical trial ([NCT02717455](#)).

A major obstacle in the treatment of CNS tumors is achieving target exposure (i.e., adequate concentration over time) at the tumor site. The blood–brain barrier (BBB), which is composed of neurovascular units, endothelial efflux transporters and tight junctions, makes it difficult for many therapeutic agents to reach the brain parenchyma [10]. Because of panobinostat's significant pre-clinical activity in DIPG, we evaluated the CNS penetration of the agent after systemic delivery in a pre-clinical nonhuman primate (NHP) model predictive of pharmacokinetics in humans, using CSF as a surrogate to CNS tissue penetration, to guide the clinical development of the agent [11, 12].

Materials and methods

Ethical approval

All NHP studies were approved by the National Cancer Institute Animal Care and Use Committee. The NHPs were cared for in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, Eighth Edition [13].

Subjects

Adult male rhesus macaque (*Macaca mulatta*) monkeys ($n = 3$) were used, serving as their own controls, if possible, and receiving panobinostat at each dose level in serial studies following a washout period of two weeks or greater. Each animal had a previously implanted indwelling, sub-cutaneous intravenous jugular and femoral ports for blood sampling and an indwelling, CSF ventricular reservoir in the lateral ($n = 1$) or fourth ventricle ($n = 2$) for sampling. The macaques had been recovered successfully from all surgeries, without complication, for a period of 6 months or greater and had previously participated in PK studies following a minimum washout period of two weeks or greater. All macaques were determined eligible for study, as defined by being within physiological normal limits, via veterinary physical examination and clinical chemistries with complete blood counts. The macaques were monitored post-administration for clinical toxicity following each dose via daily observation and clinical chemistries with complete blood counts for a period of two weeks.

Agents

Panobinostat (Selleck Chemicals, Houston, TX) was supplied as a powder and stored at -20°C for use in assay validation and sample quantification. For NHP studies, panobinostat was supplied by Novartis Pharma AG.

Study design and procedure

Panobinostat was orally administered as a powder in a small food treat at the doses 1.0, 1.8, 2.4, and 3.0 mg/kg (human equivalent dose (HED) = 20, 36, 48, and 60 mg/m²; $n = 3$). Otherwise, food was withheld for a minimum of 12 h prior to and four hours post-administration. Paired serial plasma and CSF samples were collected at 0, 30 min., 1, 2, 3, 4, 6, 8, 24, and 48 h. Samples were spun and frozen at -80°C until analysis.

Sample analysis

Panobinostat was extracted from plasma via liquid–liquid extraction with methyl *tert*-butyl ether and from CSF via a Waters[®] Ostro[™] phospholipid removal plate. In vivo panobinostat concentrations for standard curves, quality controls, and study samples were quantified using a validated ultra-high performance liquid chromatography-tandem mass spectrometry (uHPLC-MS/MS) method (lower limit of detection (LLOD) in plasma and CSF = 0.14 and 0.72 nM, respectively; lower limit of quantification (LLOQ) in plasma and CSF = 0.29 and 1.43 nM, respectively.)

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using non-compartmental methods with Phoenix[®] WinNonlin 6.4 (Certara, Cary, NC). The maximum concentration (C_{max}) and time to C_{max} (T_{max}) were recorded as observed values. The areas under the plasma and CSF concentration vs. time curves were calculated using the linear up/log down trapezoidal rule to time infinity (AUC_{inf}). The elimination rate corresponding to the terminal elimination phase was calculated as the slope of the best-fit line through the terminal phase (K_{el}). The half-life ($t_{1/2}$) was calculated as $\ln 2 / K_{\text{el}}$ for the terminal phase. Microsoft Excel 2016 for

Mac, version 15.28, was used to calculate K_{el} , $t_{1/2}$, and the mean and standard deviation for each parameter.

Statistical analysis

Data presented are the mean $\pm$ SD from three independent experiments unless indicated otherwise. All statistical tests were two-sided. For comparisons between groups, a Student *t*-test was used.

Results

Pharmacokinetics of NHP CSF penetration study

Overall, CSF penetration of panobinostat in NHP following oral dosing was low. In CSF, detectable levels of panobinostat were present in only two subjects—Subject #3 (1.0 mg/kg) and Subject #1 (1.8 mg/kg and 3.0 mg/kg). However, panobinostat was only quantifiable in one sample for Subject #1 (1.8 mg/kg) at 1 h; the detectable signals for Subject #3 (1.0 mg/kg) at 30 min, 1 h, and 2 h and Subject #1 (3.0 mg/kg) at 2, 3, and 4 h were under the LLOQ. The plasma concentration vs. time profile (Fig. 1) followed a triphasic pattern of elimination as seen in a previous clinical pharmacokinetic study [14]. The plasma C_{max} ranged from 5.08 to 149.6 nM, with T_{max} of 1.0 h. Mean dose-normalized AUC_{inf} was 7.65 ($\pm$ 2.64) h*nM/mg (Table 1).

Discussion

Our studies indicate limited CNS penetration of panobinostat following systemic delivery, which suggests that more targeted methods of administration may be necessary for the effective treatment of DIPG.

Our increasing understanding of genetics and epigenetics has significantly contributed to the identification of histone gene mutations [5–7] and its potential for therapeutic targeting. HDAC inhibitors are potent histone modifiers that increase histone acetylation by inhibiting histone deacetylation, leading to an open chromatin structure and gene activation [15]. Panobinostat, a non-selective FDA-approved HDAC inhibitor, has been used for treatment of various cancers [16], and recent studies have indicated its potent antitumor activity against DIPG in vitro and effects on DIPG growth in vivo [9, 17]. Responses in the murine model may be attributed to species differences in the BBB and potential BBB breakdown in these tumor-bearing models [17]. Moreover, mice are evolutionarily a distinctly lower species to humans, having dissimilar brain organization and BBB physiology and metabolism and, therefore, differing pharmacokinetics than humans [18]. Efficacy studies in tumor-bearing mice and PK studies in NHP are complementary and are necessary to develop a complete profile of an agent's clinical potential.

Although the physico-chemical properties of panobinostat (small MW of 349.43 g/mol and logP of 2.643) suggest that the compound has the potential to cross the BBB, it is also a substrate for efflux transporters ABCB1 (P-gp) and ABCG2 (BCRP), which may serve as the major barriers to its brain penetration.

Our current understanding of the BBB and CNS penetration of agents is incomplete. There are several examples in the clinic, e.g., vincristine, carboplatin, and dabrafenib, where agents have limited CSF (and CNS) penetration, yet patients with CNS tumors respond to treatment with these agents. This pre-clinical NHP model uses CSF as a surrogate for CNS tissue penetration, addressing CSF penetration of an agent in the absence of tumor. As microdialysis is the gold standard for PK assessment in tissue, several studies have demonstrated a predictive correlation between CSF and microdialysis drug concentrations [19]. While murine tumor-bearing models address the potential efficacy of an agent, NHP tumor-bearing models are not possible due to a competent and intact immune system, and therefore, the two translational models should be utilized in tandem with in vitro studies to gain a complete understanding of an agent's biological profile. While many may argue that the intact BBB of an NHP does not mirror the integrity of the barrier seen in tumor tissue, it has been shown that all GBMs contain regions of intact BBB [20]. As a pharmacodynamic (PD) effect following panobinostat administration has been observed in patients, the assumption cannot be made that a direct correlation exists between pharmacokinetics (i.e., drug exposure) and PD effect, and correlative PK and PD studies in tumor-bearing animals and patients would help address this issue.

In conclusion, panobinostat is a potent HDAC inhibitor with activity in in vitro and in vivo pre-clinical murine models against DIPG, with low CSF penetration in a non-tumor bearing NHP pre-clinical model. The correlation between observed patient PD and pre-clinical PK is unclear. Optimizing clinical trial design for DIPG utilizing panobinostat will require the continued use of both PK and PD pre-clinical animal models.

Funding

This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research. We would also like to acknowledge the following organizations and programs for supporting our studies: DIPG ALL-In Initiative (the Pediatric Brain Tumor Foundation, A Kids' Brain Tumor Cure, McKenna Claire Foundation, Musella Foundation, Prayers from Maria Foundation).

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Fig. 1.
Average panobinostat concentration vs. time profiles in plasma after oral doses of 1.0, 1.8, 2.4, and 3.0 mg/kg

Average pharmacokinetic parameters of panobinostat in nonhuman primate plasma after doses of 1.0, 1.8, 2.4, and 3.0 mg/kg

Table 1

Dose (mg/kg)	Amt. given (mg)	Half-life (h ⁻¹)	T _{max} (h)	C _{max} /dose (nM/mg)	Plasma AUC _{0-∞} /dose (h [*] ·nM/mg)	Cl (L/h)
1.0 (n = 3)	12.0	32.2	0.900	0.918	10.5	322.3
1.8 (n = 3)	21.5	6.76	1.17	2.20	9.30	1544
2.4 (n = 3)	27.9	13.5	1.17	0.861	5.88	821.5
3.0 (n = 3)	35.5	13.4	1.00	1.12	4.97	800.3
Average	24.2	16.5	1.06	1.27	7.66	872.0
Std. dev	9.96	11.0	0.133	0.627	2.66	503.8
%CV	41.1	66.5	12.6	49.2	34.7	57.77

*There were no quantifiable levels of panobinostat in the CSF