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Cerebrospinal fluid penetration of the colony-stimulating factor-1 receptor (CSF-1R) inhibitor, pexidartinib

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Abstract

Purpose—Pexidartinib (PLX3397) is a colony-stimulating factor-1 receptor (CSF-1R) inhibitor under clinical evaluation for potential CNS tumor treatment. This study aims to evaluate plasma pharmacokinetic parameters and estimate CNS penetrance of pexidartinib in a non-human primate (NHP) cerebrospinal fluid (CSF) reservoir model.

Methods—Five male rhesus macaques, each with a previously implanted subcutaneous CSF ventricular reservoir and central venous lines, were used. NHPs received a single dose of 40 mg/kg pexidartinib (human equivalent dose of 800 mg/m²), administered orally as 200 mg tablets. Serial paired samples of blood and CSF were collected at 0–8, 24, 48, and 72 h. Pex-idartinib concentrations were assayed by Integrated Analytical Solutions, Inc. (Berkeley, CA, USA) using HPLC/MS/MS. Pharmacokinetic (PK) analysis was performed using noncompartmental methods.

Results—Samples from four NHPs were evaluable. Average ($\pm$ SD) plasma PK parameters were as follows: $C_{\max}$ = 16.50 ($\pm$ 6.67) μ g/mL; $T_{\max}$ = 5.00 ($\pm$ 2.58) h; AUC_{last} = 250.25 ($\pm$ 103.76) h* μ g/mL; CL = 0.18 ($\pm$ 0.10) L/h/kg. In CSF, pexidarti-n ib was either quantifiable (n = 2), with $C_{\max}$ values of 16.1 and 10.1 ng/mL achieved 2–4 h after plasma $T_{\max}$, or undetected at all time points (n = 2, LLOQ_{CSF} = 5 ng/mL).

Conclusion—Pexidartinib was well-tolerated in NHPs, with no Grade 3 or Grade 4 toxicities. The CSF penetration of pex-idartinib after single-dose oral administration to NHPs was limited.

Keywords

Central nervous system; Pharmacokinetics; Brain tumor; Macrophage; Tyrosine kinase inhibitor; Glioma

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Conflict of interest The authors declare they have no conflict of interest.

Introduction

Drug delivery to the central nervous system (CNS) must overcome protective barriers between CNS tissue and vasculature. These barriers utilize cellular tight junctions and efflux transporters to prevent toxic substances from accessing CNS tissue unless they can passively diffuse or bind to specific transport proteins. Many anticancer drugs lack the physicochemical properties necessary to cross these barriers. Despite their efficacy against tumors in systemically accessible regions of the body, chemotherapeutic agents demonstrate minimal efficacy against gliomas in part due to insufficient CNS exposure. Even if a drug is able to penetrate certain regions of a glioma, its permeability can be restricted at other regions due to barrier heterogeneity and variable tumor disruption of the barrier [1, 2].

Alternatives to pharmacotherapy are also limited for high-grade CNS tumors. Gliomas are often located near vital brain structures, and glioma cells diffusely infiltrate surrounding tissue, making total surgical resection unlikely [3]. Radiotherapy may have an effect on malignant gliomas in the short-term, but it only minimally extends patient survival [4]. Therefore, there is a critical need for CNS-penetrant anticancer drugs to be identified and pursued.

Pexidartinib (PLX3397), an inhibitor of macrophage colony-stimulating factor receptor (CSF-1R), is FDA approved for adults with tenosynovial giant cell tumor and under investigation for CNS tumors [5]. Within the tumor microenvironment, macrophages can serve both pro-inflammatory and immuno-suppressive roles, the latter of which supports tumor growth by promoting angiogenesis and limiting the adaptive immune response against tumor cells [6]. CSF-1 is a growth factor that regulates macrophage proliferation and differentiation; it appears to contribute to a positive feedback loop between tumor cells, which secrete CSF-1, and macrophages, which respond with tumor-promoting activity [7]. Depleting endogenous CSF-1 in mice causes a macrophage deficiency that is associated with a significant reduction in tumor stroma and vasculature; introducing exogenous CSF-1 has the opposite effect [8]. Pexidartinib mimics CSF-1 depletion by blocking the activity of CSF-1 at its receptor tyrosine kinase, potentially limiting the support that tumors receive from macrophages in their microenvironment [9].

Pexidartinib is one of the most broadly tested CSF-1R inhibitors, but its potential for treating CNS malignancies is not well understood. Several preclinical mouse models demonstrated that pexidartinib can influence macrophage depletion and tumor growth and invasion in nervous tissue [10–12]. Clinical trials of pexidartinib have now been approved in newly diagnosed and advanced solid tumors and leukemia. While patients in a phase I trial for tenosynovial giant-cell tumor (non-CNS) showed a 52% response rate and at least 40% tumor reduction with pexidartinib, patients in a phase II trial for glioblastoma showed a more modest response, with 8.6% progression-free survival at 6 months [5, 9]. However, the glioblastoma patients exhibited high tumor penetrance of pexidartinib at 70% of plasma concentrations, along with pharmacodynamic effects, warranting further research into the use of pexidartinib for other CNS malignancies [5].

Assessing the CNS penetrance and full pharmacokinetic profile of pexidartinib is an important preliminary step to optimizing clinical trial design for CNS tumors. In this study, we assessed plasma and cerebrospinal fluid (CSF) pharmacokinetics of pexidartinib following systemic administration in a non-human primate (NHP) model that is predictive of pharmacokinetics in humans [13, 14].

Materials and methods

Animals

Five adult male rhesus macaques (*Macaca mulatta*), weighing 8.5–13.9 kg, were utilized. The National Cancer Institute (NCI) Animal Care and Use Committee (ACUC) approved this study. All macaques were cared for in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals, Eighth Edition [15] and socially housed, where possible.

Study clearance and monitoring

Physiological and neurological assessments consisting of veterinary physical examination, blood chemistries, and complete blood count were done for each subject prior to study. All subjects were within physiological normal limits and eligible for study. Following pexidartinib administration, the subjects were observed daily for clinical complications. Clinical chemistries and complete blood counts were collected twice weekly for 2 weeks.

Study design and procedure

Each NHP had previously implanted CSF reservoirs and ventricular catheter systems. The ventricular catheter, located in the lateral ($n = 3$) or fourth ($n = 2$) ventricle, was attached to a subcutaneous CSF reservoir as shown in Fig. 1 [13]. Additionally, the NHPs were previously instrumented with subcutaneous jugular and femoral venous IV port systems. The NHPs fasted 12 h prior to and 4 h post drug administration. Ketamine (10 mg/kg, Zetamine, VetOne Boise, ID) sedation was utilized to access and aseptically prepare the CSF reservoir and ports for sampling as well as prepare the subject for restraint. Pre-study plasma and CSF samples were collected. The NHPs were restrained, via the pole and collar system, for drug administration and sample collection. Environmental enrichment, including food treats, videos, and human interaction, was provided during the restraint period.

Pexidartinib, supplied as 200 mg tablets by Plexxikon Inc. (Berkeley, CA, USA) under agreement with the NCI, was administered orally to un-sedated but restrained macaques, via piller at a target dose of 40 mg/kg (human equivalent dose of 800 mg/m²). Serial paired blood and CSF samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h. Blood, collected via the femoral IV port at a volume of 3.0 mL, was placed in sodium heparin tubes and centrifuged at 3000 rpm for 5 min. The resulting plasma was frozen at -80°C . Ventricular CSF, collected via the CSF reservoir at a volume of 300 μL , was frozen at -80°C .

Pharmacokinetic analysis

Plasma and CSF pexidartinib concentrations were assayed by Integrated Analytical Solutions, Inc. (Berkeley, CA, USA) utilizing a validated high-performance liquid chromatography/tandem mass spectrometry (HPLC–MS/MS) method. The lower limit of quantitation (LLOQ) was 25 and 5 ng/mL for plasma and CSF, respectively. Pharmacokinetic (PK) parameters for pexidartinib in plasma and CSF were determined via non-compartmental analysis using Phoenix WinNonlin® v8.0 (Certara Pharsight, Cary, NC). The values for the maximum concentration ($C_{\max}$) and time to $C_{\max}$ ($T_{\max}$) were observed and recorded. The area under the plasma concentration vs. time curve (AUC_{last}) was determined by utilizing the linear up/log down trapezoidal rule to the last time point. Dose-normalized $C_{\max}$ and AUC_{last} were calculated by dividing by mg/kg dose for comparison across dose levels and species. The slope of the best fit line drawn through a minimum of three terminal concentration points determined the elimination rate (K_{el}). The natural log of 2 ($\ln 2$) divided by K_{el} was utilized to calculate the half-life ($T_{1/2}$). AUC extrapolated to time infinity (AUC_{INF}) was calculated by adding AUC_{last} to ($C_{\text{last}}/K_{\text{el}}$). Dose/ AUC_{INF} was utilized to calculate apparent oral clearance (CL/F). Apparent oral volume of distribution in the terminal phase (V_z/F) was calculated as CL/F divided by K_{el} .

Results

Four of the five NHPs were evaluable for PK analysis; one NHP was not evaluable due to a technical difficulty with the CSF reservoir unrelated to the study. Each NHP received 40 mg/kg pexidartinib (equivalent to a human dose of 800 mg/m²) rounded up to the next whole tablet size of 200 mg.

Following oral administration, on average, plasma $C_{\max}$ of 16.50 ± 6.67 µg/mL occurred at $T_{\max}$ of 5 h, and plasma AUC_{last} was 250.25 ± 103.76 h*µg/mL (Table 1). The elimination occurred in a single phase, with a mean half-life of 15.1 h.

Pexidartinib was quantifiable in the CSF of two of the four NHPs: CSF pexidartinib concentrations ranged from 5.49–16.1 ng/mL from 6–48 h in NHP 1 and from 5.36–10.1 ng/mL from 2–6 h in NHP 2 (Table 1). These CSF concentrations were 0.04–0.51% of the observed plasma concentrations at the same time point. Pharmacokinetic parameters could not be calculated for CSF due to the lack of quantifiable samples.

Pexidartinib was well-tolerated in all five subjects, with no Grade 3 or Grade 4 toxicities or adverse events observed. NHP 1 experienced a Grade 2 increase in creatinine, and NHPs 2 and 3 experienced Grade 1 increases in ALT, all of which resolved to normal values without clinical intervention.

Discussion

In this study, we assessed the penetrance of pexidartinib into the CSF of NHPs following oral administration to optimize the clinical trial design for pexidartinib in patients with CNS tumors. While the plasma exposure of pexidartinib was substantial and prolonged, it resulted in limited and variable CSF penetrance in the four NHPs.

In prior preclinical studies, pexidartinib plasma exposure has varied by species, with mice exhibiting greater exposure, but rats and dogs reporting less exposure, than the NHPs studied here [9]. This pharmacokinetic variability likely results from variations in study design, such as choice of pexidartinib formulation and prandial status, and biological differences between species.

A prior NHP study of pexidartinib also reported differing pharmacokinetics, with a four-fold greater dose of 160 mg/kg pexidartinib yielding nearly the same plasma AUC_{INF} as this study ($237 \text{ h} \cdot \mu\text{g/mL}$) [9]. Species and methodological differences may again contribute to this discrepancy in oral absorption of pexidartinib. However, the data also suggest a saturation of absorption in NHPs at high dose levels, possibly due to limited pexidartinib solubility in gastrointestinal fluid. Further research is needed to understand and address the limitations of these factors on pexidartinib oral dosing and absorption.

Unlike other preclinical studies, this NHP study shares consistent results with human studies of pexidartinib plasma pharmacokinetics. In a dose-escalation clinical trial ranging from 200–1200 mg (3.3–16.7 mg/kg) pexidartinib, dose-normalized plasma C_{max} and plasma AUC_{0-24} , averaged across all dose levels, remained within 20% of the values measured in this NHP study [9]. In phase I and phase II trials, 600 and 1000 mg doses yielded dose-normalized C_{max} values that ranged from 2–86% greater than the average dose-normalized C_{max} in this study [5, 16]. These results confirm that human plasma pharmacokinetics were adequately reproduced in the NHP preclinical model.

CNS penetrance was assessed by measuring pexidartinib levels in NHP CSF, which was used as a surrogate of CNS tissue penetration. CSF exposure was less than 1% of plasma concentrations in two NHPs, and not quantifiable in the other two NHPs. These results contrast existing data, outlined below, on the CNS penetrance of pexidartinib, and in doing so, they emphasize new considerations needed for the treatment of CNS tumors.

In 2016, Butowski et al. reported that pexidartinib achieved 70% tumor penetrance in glioblastoma (GBM) patients when dosed to steady state (QDx7), but it showed no significant efficacy [5]. Future studies could explore whether steady-state treatment can significantly improve CNS exposure (via increased plasma concentrations that thereby increase CSF concentrations). However, the lack of observed efficacy in Butowski et al.'s study suggests that the CNS distribution of pexidartinib was insufficient, with a lack of exposure at the tumor microenvironment. Butowski et al. analyzed surgically-resected tissue primarily from contrast-enhancing areas, which, by definition, indicates a disrupted blood–brain barrier (BBB). While GBMs and other CNS tumors are known to compromise BBB integrity, they are notably heterogeneous and often contain unresectable regions that maintain their BBB [1, 2]. In the GBM patients, it is possible that pexidartinib penetrated mainly the disrupted BBB around the tumor, allowing tumor cells within the intact BBB to promote recurrence. By using a non-tumor bearing model, our NHP study provides evidence that pexidartinib has limited ability to cross the intact BBB.

Recently, Yan et al. studied pexidartinib CNS penetrance in non-tumor bearing mice, finding 12% tissue penetrance in response to a 100 mg/kg oral dose [17]. As a lipophilic compound,

pexidartinib could preferentially partition into brain tissue from CSF. However, Yan et al. administered pexidartinib in a 5% DMSO vehicle, potentially enhancing the solubility of pexidartinib beyond the level of the tablet formulation used for this study and for patients. Anatomical and physiological differences between the blood–brain barrier and blood-CSF barrier could have also contributed to differences in pexidartinib penetrance [1, 18]. Additionally, empirically-reported differences between murine and primate BBB transport pathways may allow more efficient removal of pexidartinib from a primate brain compared to a murine brain [19, 20]. Human BBB protein expression is known to more closely match that of NHPs, which suggests similarly limited CNS penetrance in humans [19].

This CSF penetrance study provides novel and important data regarding pexidartinib CNS exposure over time, but we need to improve our ability to interpret this preclinical data for the context of patients. Further research needs to elucidate the roles that murine-primate differences and brain-CSF differences play in the CNS penetrance of pexidartinib. Additionally, improved sampling strategies, such as micro-dialysis to provide in situ drug exposure, may improve our understanding of pexidartinib disposition in the CNS. This information is critical for the accurate correlation of pharmacokinetic parameters with pharmacodynamic effects.

In conclusion, pexidartinib has limited CSF penetrance in NHPs following oral administration of a single dose. This may be attributable to species-specific plasma exposure and CNS physiology along with the non-tumor bearing nature of the model. Optimizing clinical trial design requires the use of both pharmacokinetic and pharmacodynamic preclinical animal models. The scope of this model helps define the complex clinical scenario presented by high-grade recurrent glioblastoma patients and the requirements for efficacious pexidartinib treatment.

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Fig. 1.
Locations of CSF ventricular reservoirs in the NHPs studied

Table 1

Pexidartinib plasma pharmacokinetics in each NHP

Subject	Weight kg	Dose mg/kg	T _{max} h	C _{max} µg/mL	T _{1/2} h	Vz/F L/kg	CL L/h/kg	AUC _{last} h*µg/mL
Plasma								
1	13.9	43.2	6	23.4	9.93	1.88	0.131	313
2	9.6	41.7	2	9.70	20.39	9.32	0.317	122
3	10.3	38.8	4	12.0	15.65	3.91	0.173	213
4	11.1	36.0	8	20.9	14.57	2.08	0.099	353
Average	11.23	39.93	5.00	16.50	15.13	4.30	0.18	250.25
Std. Dev	1.89	3.19	2.58	6.67	4.29	3.47	0.10	103.76
CSF								
1	13.9	43.2	8	0.0161				
2	9.6	41.7	6	0.0101				
Average	11.23	39.93	7.01	0.0131				
Std. Dev	1.89	3.19	1.43	0.0042				