Brain Distribution and Bioavailability of Elacridar after Different Routes of Administration in the Mouse

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ABSTRACT:

The objective of this study was to determine the bioavailability and disposition of elacridar (GF120918; *N*-(4-(2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl)phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide) in plasma and brain after various routes of administration in the mouse. Elacridar is a potent inhibitor of P-glycoprotein and breast cancer resistance protein and has been used to examine the influence of these efflux transporters on drug distribution to brain. Friend leukemia virus strain B mice were administered 100 mg/kg elacridar either orally or intraperitoneally. The absolute bioavailability of elacridar after oral or intraperitoneal dosing was determined with respect to an intravenous dose of 2.5 mg/kg. At these doses, the absolute bioavailability was 0.22 for oral administration and 0.01 for intraperitoneal administration. The terminal half-life of elacridar was approximately 4 h after intraperi-

toneal and intravenous administration and nearly 20 h after oral dosing. The brain-to-plasma partition coefficient ($K_{p, \text{brain}}$) of elacridar increased as plasma exposure increased, suggesting saturation of the efflux transporters at the blood-brain barrier. The $K_{p, \text{brain}}$ after intravenous, intraperitoneal, and oral dosing was 0.82, 0.43, and 4.31, respectively. The low aqueous solubility and high lipophilicity of elacridar result in poor oral absorption, most likely dissolution-rate-limited. These results illustrate the importance of the route of administration and the resultant plasma exposure in achieving effective plasma and brain concentrations of elacridar and can be used as a guide for future studies involving elacridar administration and in developing formulation strategies to overcome the poor absorption.

Introduction

Elacridar (GF120918; *N*-(4-(2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl)phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide) is a potent inhibitor of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) (Witherspoon et al., 1996; Allen et al., 1999). It is a third-generation inhibitor (Tan et al., 2000) and was initially described as a multidrug resistance-reversal agent (Hyafil et al., 1993), where it restored the sensitivity of multidrug-resistant tumors to doxorubicin.

Elacridar has been used extensively in vitro and in vivo as a P-gp and BCRP inhibitor. When coadministered with P-gp substrates such as topotecan and paclitaxel, elacridar improved their oral absorption by inhibiting intestinal P-gp, thereby preventing efflux of substrate drugs into the intestinal lumen (Kruijtz et al., 2002; Bardelmeijer et al., 2004). The role of P-gp and BCRP in limiting the distribution of substrate drugs across the blood-brain barrier (BBB) has been examined using elacridar as a dual inhibitor of both P-gp and BCRP.

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Coadministration of elacridar improved the brain penetration of several substrate molecules, such as morphine and amprenavir (Letrent et al., 1998; Edwards et al., 2002). Elacridar also significantly increased brain distribution of several tyrosine kinase inhibitors (TKIs) including imatinib, dasatinib, gefitinib, sorafenib, and sunitinib (Bihorel et al., 2007; Chen et al., 2009; Lagas et al., 2009, 2010; Agarwal et al., 2010, 2011b; Tang et al., 2012a). It was shown that brain penetration of the TKI sunitinib and its active metabolite is limited by the P-gp and BCRP at the BBB. Oral administration of elacridar improved the brain penetration of sunitinib in wild-type mice by 12-fold such that it was equal to that seen in *Mdr1alb*($-/-$)*Bcrp1*($-/-$) mice (Tang et al., 2012a,b). Elacridar has also been administered to glioma xenograft-bearing mice to enhance the brain penetration of paclitaxel (Hubensack et al., 2008).

The common objective of many of the above studies was to use P-gp and Bcrp inhibition by elacridar as a strategy to enhance distribution of substrate drugs into the brain. One such therapeutic area where this could be particularly useful is in the treatment of devastating brain tumors such as glioma. Many molecularly targeted TKIs that are currently being evaluated in glioma do not effectively cross an intact BBB because of P-gp- and Bcrp-mediated efflux (Breedveld et al., 2005; Bihorel et al., 2007; Chen et al., 2009; Lagas et al., 2009). Coadministration of elacridar with TKIs that do not effectively cross the BBB because of P-gp- and BCRP-mediated efflux could lead to improved efficacy of these drugs in glioma as a result of their

ABBREVIATIONS: GF120918, *N*-(4-(2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl)phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; BBB, blood-brain barrier; TKI, tyrosine kinase inhibitor; FVB, Friend leukemia virus strain B; AUC, area under the concentration-time curve; $AUC_{0-\infty}$, AUC from time zero to infinity.

enhanced delivery across the BBB. Because these TKIs are intended for chronic administration, for elacridar to effectively improve delivery, it must also be administered chronically. To accomplish this efficiently, we must have a better understanding of the factors that could affect the systemic bioavailability and brain distribution of elacridar.

Chronic administration of elacridar has several difficulties, mainly arising because of its unfavorable physicochemical properties. Elacridar is practically insoluble in water and is poorly soluble in most other aqueous solvents, and it is extremely lipophilic ($\log P = 5.67$) (Padowski and Pollack, 2010). This makes it difficult to formulate elacridar as an injectable. These poor physicochemical properties also mean that its oral absorption will most likely be dissolution-rate-limited (Ward and Azzarano, 2004). This will also contribute to observed variability in plasma and tissue concentrations in preclinical studies. Intersubject variability in exposure after oral dosing has been observed in clinical trials (Planting et al., 2005). Brain penetration of elacridar in mice has been shown to be dose-dependent and influenced by the presence of P-gp and BCRP at the BBB. This has been elegantly demonstrated using radiolabeled elacridar positron emission tomographic imaging (Kawamura et al., 2011a,b). All of these factors contribute to highly variable adsorption and disposition of elacridar in both preclinical models and clinical applications.

Although elacridar has been used to alter the brain penetration of a wide variety of drugs, the factors influencing the brain distribution of elacridar itself have not been carefully elucidated. The objective of this study was to describe the pharmacokinetics of elacridar in plasma and brain after different routes of administration and to estimate the systemic bioavailability of elacridar. The results from the current study will be helpful in determining the dose and route of administration of elacridar in future studies that may involve chronic administration, particularly in preclinical studies using the mouse model.

Materials and Methods

Materials. Elacridar, mol. wt. 563.64, was purchased from Toronto Research Chemicals (North York, ON, Canada). Hydroxypropylmethylcellulose (Methocel E5LV) was obtained from Dow Chemical Company (Midland, MI). All other chemicals used were reagent-grade or high-performance liquid chromatography-grade from Sigma-Aldrich (St. Louis, MO).

Animals. In vivo studies were conducted in Friend leukemia virus strain B (FVB) wild-type mice (Taconic Farms, Germantown, NY). All animals were 8 to 10 weeks old at the time of the experiment. All mice were maintained under a 12-h light/dark cycle, had unlimited access to food and water, and were maintained under a temperature-controlled environment. All studies were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Intravenous Administration of Elacridar. The elacridar intravenous dosing solution was prepared on the day of the experiment by dissolving elacridar in a vehicle containing dimethyl sulfoxide, propylene glycol, and saline, 2:2:1 (v/v/v), at a concentration of 1.25 mg/ml. FVB wild-type mice were given intravenous doses of 2.5 mg/kg (2 μ l volume/g b.wt.) in the tail vein. Blood and brain were collected at 0.5, 1, 2, 4, and 8 h after the dose ($n = 4$ at each time point). Animals were euthanized by use of a carbon dioxide chamber. Blood was collected by cardiac puncture, and plasma was obtained by centrifugation at 7500 rpm for 10 min at 4°C. The whole brain was quickly removed from skull and rinsed with ice-cold saline. Brains were immediately flash-frozen with liquid nitrogen. The specimens were stored at -80°C until analysis by liquid chromatography-tandem mass spectrometry.

Intraperitoneal and Oral Administration of Elacridar. Elacridar for intraperitoneal and oral dosing was prepared on the day of the experiment by preparing a stable suspension of elacridar, using 0.5% hydroxypropylmethylcellulose and 1% Tween 80 to obtain a 10-mg/ml formulation. Mice received an intraperitoneal dose of 100 mg/kg by injection into the peritoneal cavity. For oral administration, mice received a dose of 100 mg/kg by oral gavage. Blood and brain were sampled at 15 min, 0.5, 1, 2, 4, and 8 h after intraperitoneal

dosing and at 0.5, 1, 2, 4, 8, 17, and 24 h after oral dosing. Plasma and brain samples were collected and processed in the manner described in the previous section.

Analysis of Elacridar by Liquid Chromatography-Tandem Mass Spectrometry. The concentrations of elacridar in mouse plasma and brain were determined by high-performance liquid chromatography coupled with mass spectrometry. Frozen brain samples were thawed and homogenized with 3 volumes of 5% bovine serum albumin using a tissue homogenizer (Thermo Fisher Scientific, Waltham, MA). Fifty microliters of plasma and 100 μ l of brain homogenate were spiked with 20 ng of internal standard tyrphostin (AG 1478) and 100 μ l of a buffer, pH 11 (0.1 M sodium hydroxide and 0.04 M sodium bicarbonate). Samples were extracted by vigorously vortexing with 1 ml of ethyl acetate for 5 min and then centrifugation at 7500 rpm for 15 min at 4°C. Six-hundred microliters of organic layer was transferred to microcentrifuge tubes and dried by a gentle stream of nitrogen. Samples were reconstituted in 100 μ l of mobile phase and were transferred to autosampler vials. A 5- μ l volume was injected using a temperature-controlled autosampler maintained at 10°C. Chromatographic analysis was performed using an Agilent Technologies (Santa Clara, CA) Eclipse XDB-C18 RRHT threaded column (4.6 mm i.d. $\times$ 12.5 mm, 5 μ). The mobile phase was composed of acetonitrile: 20 mM ammonium formate (with 0.1% formic acid) (42:58 v/v) with a flow rate of 0.25 ml/min. The eluent was monitored using a Thermo Finnigan TSQ Quantum 1.5 detector (Thermo Fisher Scientific). The instrument was equipped with an electrospray interface. The samples were ionized by the electrospray probe and analyzed in the positive ionization mode operating at a spray voltage of 4500 V for both elacridar and the internal standard. The spectrometer was programmed to allow the $[\text{MH}^+]$ ion of elacridar at m/z 564.6 and that of the internal standard at m/z 316.67 to pass through the first quadrupole (Q1) and into the collision cells (Q2). The collision energy was set at 39 V for elacridar and 9 V for tyrphostin. The product ions for elacridar (m/z 252.9) and the internal standard (m/z 300.9) were monitored through quadrupole 3 (Q3). The scan width and scan time for monitoring the two product ions were m/z 1.5 and 0.5 s, respectively. The assay was precise and linear over a range of 2.5 to 1500 ng/ml (CV was less than 10% over all concentrations).

Pharmacokinetic Calculations. Pharmacokinetic parameters from the concentration-time profile in plasma and brain were calculated by noncompartmental analysis using Phoenix WinNonlin 6.1 (Pharsight, Mountain View, CA). The terminal rate constants were determined using the last three data points for plasma and brain. The areas under the concentration-time curve for plasma ($\text{AUC}_{\text{plasma}}$) and brain ($\text{AUC}_{\text{brain}}$) from time 0 to infinity were calculated using the linear trapezoidal method with the AUC from last measured time point to infinity estimated by dividing the last measured concentration by the elimination rate constant. After intravenous dosing, the concentration at time zero (C_0) was back-extrapolated by log-linear regression of the first two data points. The $\text{AUC}_{\text{plasma}}$ from time zero to infinity after intravenous injection was then determined. The absolute bioavailability after oral and intraperitoneal administration was calculated as

$$F = \frac{\text{AUC}_{\text{plasma}} (\text{i.p. or p.o.})}{\text{AUC}_{\text{plasma}_{\text{intravenous}}}} \times \frac{\text{Dose}_{\text{intravenous}}}{\text{Dose} (\text{i.p. or p.o.})}$$

The brain-to-plasma partition coefficient ($K_{p,\text{brain}}$) of elacridar after different routes of administration was calculated as a ratio of AUC ($\text{AUC}_{\text{brain}}/\text{AUC}_{\text{plasma}}$). The brain-to-plasma concentration ratio at each time point was calculated as a ratio of brain concentration to plasma concentration ($C_{\text{brain}}/C_{\text{plasma}}$).

Statistical Analysis. SigmaPlot for Windows version 11.0 (Systat Software, Inc., San Jose, CA) was used to determine whether the difference between the two groups was statistically significant. One-way analysis of variance with the Holm-Sidak post hoc test was used for multiple comparisons at a significance level of 0.05.

Results

Intravenous Administration of Elacridar. The disposition of elacridar in plasma and brain was studied in FVB wild-type mice after an intravenous injection. The plasma concentrations showed a bi-exponential decline indicating distinct distribution and elimination

FIG. 1. A, elacridar concentrations in plasma and brain after a single intravenous dose of 2.5 mg/kg in FVB wild-type mice. Brain concentrations are higher at corresponding higher plasma concentration and decrease as plasma concentrations decrease. *, $p < 0.05$. Mean $\pm$ S.D. ($n = 4$ at each time point). B, brain-to-plasma concentration ratio for elacridar after a single intravenous dose of 2.5 mg/kg in FVB wild-type mice. The brain-to-plasma concentration ratio is greater than one at initial time points and then drops below unity as plasma concentrations decrease. Data are presented as mean $\pm$ S.D. ($n = 4$ at each time point).

phases (Fig. 1A). Concentrations rapidly reached peak levels in brain within 0.5 h after intravenous dosing with the maximal concentration ($C_{\max}$) observed at 0.5 h, the first measured time point. The brain-to-plasma concentration ratio was high at the initial time points (up to 2 h) and decreased thereafter as concentrations in brain declined more rapidly compared with those in plasma (Fig. 1B). This is consistent with the observed terminal $t_{1/2}$ of 4.4 h in plasma and 1.5 h in brain

(Table 1). The total plasma clearance was estimated to be 0.46 ml/min. The blood flow to the liver for a 20-g mouse is 1.8 ml/min (Davies and Morris, 1993), meaning elacridar has at most low to moderate hepatic extraction in the mouse. The AUC from time zero to infinity ($AUC_{0-\infty}$) was 161 $\mu\text{g} \cdot \text{min}/\text{ml}$ in plasma and 131 $\mu\text{g} \cdot \text{min}/\text{ml}$ in brain. The resulting brain K_p ratio was 0.82, indicating that

TABLE 1						
Plasma and brain pharmacokinetic parameters calculated by noncompartmental analysis after administration of a single dose of elacridar in FVB wild-type mice						
Route	$t_{1/2}$	$C_{\max}$	$T_{\max}$	$AUC_{0-\text{last}}$	$AUC_{0-\infty}$	K_p
	<i>h</i>	$\mu\text{g}/\text{ml}$	<i>h</i>	$\mu\text{g} \cdot \text{min}/\text{ml}$	$\mu\text{g} \cdot \text{min}/\text{ml}$	
2.5 mg/kg i.v.						
Plasma	4.4	0.564 ± 0.14	0.5	138.6 ± 6.5	161.4	0.82
Brain	1.5	1.05 ± 0.38	0.5	128.3 ± 19.6	131.3	
100 mg/kg i.p.						
Plasma	4.3	0.295 ± 0.06	0.5	63.5 ± 6.6	90.3	0.48
Brain	9.2	0.061 ± 0.02	4	19.9 ± 4.7	43.5	
100 mg/kg p.o.						
Plasma	20	0.78 ± 0.12	8	792.8 ± 67.9	1460	4.31
Brain	16	4.34 ± 0.79	8	3887 ± 410	6296	

after intravenous dosing at 2.5 mg/kg, there is approximately equal partitioning of elacridar into the brain as plasma.

Intraperitoneal Administration of Elacridar. The plasma and brain concentrations after an intraperitoneal dose of 100 mg/kg were measured in FVB wild-type mice. After intraperitoneal dosing, brain concentrations were significantly lower than plasma concentrations at all measured time points, except at 4 h after the dose (Fig. 2A). The corresponding brain-to-plasma concentration ratios remained less than one at all measured time points (Fig. 2B). It is important to note that the plasma concentrations after intravenous administration at which we observe a greater than one brain-to-plasma concentration ratio are higher than the maximal plasma concentrations seen after intraperitoneal dosing. The brain distribution ratio (i.e., the partition coefficient) of elacridar is likely to be dependent on its plasma levels. The observed $C_{\max}$ in plasma after the intraperitoneal dose was $0.295 \pm 0.06 \mu\text{g/ml}$, and that in brain was $0.061 \pm 0.024 \mu\text{g/ml}$. The apparent plasma clearance (Cl/F) was estimated to be 33 ml/min by noncompartmental analysis. The $AUC_{0-\infty}$ in plasma was 90.3 and 43.5 $\mu\text{g} \cdot \text{min/ml}$ in the brain. The elimination-phase $t_{1/2}$ of elacridar after noncompartmental analysis was estimated to be 4.3 in plasma and 9.2 h in brain. The K_p ratio was 0.48, indicating that the partitioning

into the brain was lower after intraperitoneal administration, even at a dose 40 times higher, compared with intravenous administration.

Oral Administration of Elacridar. The brain and plasma pharmacokinetics of elacridar were studied in FVB wild-type mice after a 100-mg/kg dose administered orally. The brain concentrations were lower than plasma concentrations until 1 h after the dose, after which the brain concentrations were several-fold higher than plasma concentrations (Fig. 3A). The brain-to-plasma ratio after oral administration was less than one for initial time points and then increased, reaching a maximum of approximately 6 at 4 h after the dose, before showing a slow decline (Fig. 3B). Noncompartmental analysis of plasma and brain concentration-time data showed that the plasma $AUC_{0-\infty}$ was $1460 \mu\text{g} \cdot \text{min/ml}$ and the brain $AUC_{0-\infty}$ was $6296 \mu\text{g} \cdot \text{min/ml}$ (Table 1). The $C_{\max}$ in brain after oral dosing was $4.34 \pm 0.79 \mu\text{g/ml}$, significantly higher than that seen after intraperitoneal dosing. The time to reach $C_{\max}$ in plasma was 4 h, indicating slow dissolution and absorption from the gut. After oral dosing, the brain K_p was found to be 4.31, suggesting that a high dose of elacridar administered orally gives high distribution in the brain. The $t_{1/2}$ of the drug in the brain after the oral dose mirrored its plasma $t_{1/2}$, with the values being 19.8 and 15.6 h, respectively.

FIG. 2. A, elacridar concentrations in plasma and brain after a single intraperitoneal dose of 100 mg/kg in FVB wild-type mice. Brain concentrations are significantly lower than plasma concentrations, indicating that brain delivery of elacridar after intraperitoneal administration is limited. *, $p < 0.05$. Mean $\pm$ S.D. ($n = 4$ at each time point). B, brain-to-plasma concentration ratio for elacridar after a single intraperitoneal dose of 100 mg/kg in FVB wild-type mice. The brain-to-plasma concentration ratio is less than unity at all time points, indicating poor brain distribution. Data are presented as mean $\pm$ S.D. ($n = 4$ at each time point).

FIG. 3. A, elacridar concentrations in plasma and brain after a single oral dose of 100 mg/kg in FVB wild-type mice. Brain concentrations are significantly lower than plasma concentrations at early time points and higher than plasma at later time points. *, $p < 0.05$. Mean $\pm$ S.D. ($n = 4$ at each time point). B, brain-to-plasma concentration ratios for elacridar after a single oral dose of 100 mg/kg in FVB wild-type mice. The brain-to-plasma concentration ratio is less than unity at initial times but increases with time. Data are presented as mean $\pm$ S.D. ($n = 4$ at each time point).

Determination of Bioavailability after Intraperitoneal and Oral Administration. Absolute bioavailability was determined as a ratio of dose-normalized AUC after intraperitoneal or oral administration to dose-normalized AUC after intravenous administration (Table 2). The bioavailability after intraperitoneal administration was 1.3%, and that after oral administration was 22% with the suspension. The bioavailability after intraperitoneal dosing was very poor, indicating that intraperitoneal may not be a favorable route of administration to get reproducible plasma and brain exposure of elacridar using this simple suspension formulation. The bioavailability of elacridar after oral administration was higher than that after an equivalent dose administered intraperitoneally; this could be due to enhanced dissolution in the gut through greater solvent availability or micellar effects of bile

salts. The $t_{1/2}$ of elacridar after oral administration was approximately 5 times longer than that after intravenous or intraperitoneal administration. There is a possibility that there is nonlinearity in the absorption, distribution, and possibly elimination of elacridar. However, the bioavailability has been calculated with the assumption that there is no change in the clearance of the drug with increases in plasma exposure of the drug.

Discussion

Elacridar is a third-generation inhibitor of the transporters P-gp and BCRP (Hyafil et al., 1993; Witherspoon et al., 1996). It has been used to determine the influence of P-gp and BCRP on the brain distribution of drugs that are substrates for P-gp and BCRP (Breedveld et al.,

TABLE 2						
Plasma pharmacokinetics and bioavailability calculated by noncompartmental analysis of data from intravenous, intraperitoneal, and oral administration						
Route	Dose	CL/F	Vd/F	$t_{1/2}$	AUC _(0-inf)	F
	mg/kg	ml/min	liter	h	$\mu\text{g} \cdot \text{min/ml}$	
Oral	100	2.05	3.5	20	1460	0.22
Intraperitoneal	100	33.2	12.3	4.3	90.3	0.013
Intravenous	2.5	0.46	0.17	4.4	161.4	1

2005; Bihorel et al., 2007; Chen et al., 2009; Agarwal et al., 2010, 2011b). It has also been suggested as a tool to improve the efficacy of drugs such as sunitinib in the treatment of glioma (Tang et al., 2012a). The objective of this study was to investigate the brain and plasma pharmacokinetics of elacridar in the mouse and determine its bioavailability after oral and intraperitoneal administration.

The physicochemical properties of elacridar such as poor solubility and high lipophilicity could be responsible for the variability that is observed in plasma concentrations in this study. This interindividual variability has been observed previously in humans upon oral administration of elacridar (Kuppens et al., 2007) and was attributed to variability in dissolution of elacridar. In a study done in mice, the rate of dissolution of elacridar was also found to be a factor in limiting the plasma exposure of orally administered elacridar, despite increasing its dose (Ward and Azzarano, 2004).

Oral dosing is a convenient route for drug administration in both rodents and humans, especially for chronic administration. It has been shown that brain penetration of the TKI sunitinib and its active metabolite is limited by P-gp and BCRP at the BBB. Oral administration of elacridar at a 100 mg/kg dose improved the brain penetration of sunitinib in wild-type mice by nearly 12-fold (Tang et al., 2012a,b). Both plasma and brain exposure of elacridar after a 100 mg/kg oral dose was significantly higher than that after intraperitoneal administration. The partition coefficient of elacridar in the brain after oral administration was 4.31, indicating good distribution of elacridar from systemic circulation into the brain after this oral dose. The elimination $t_{1/2}$ after an oral dose of 100 mg/kg was close to 20 h, approximately 5-fold greater than the $t_{1/2}$ after intraperitoneal or intravenous dosing. There could be several possible reasons for this phenomenon. The increased oral $t_{1/2}$ could be due to a slow dissolution rate (given the poor aqueous solubility), which limits the rate of absorption and thus leads to an extended-release period. Thus, the observed terminal $t_{1/2}$ might be a measure of a possible long absorption $t_{1/2}$ (flip-flop kinetics). The finding that the observed $T_{\max}$ in plasma was 8 h postdose supports this hypothesis. Another reason for the extended $t_{1/2}$ could be a change in hepatic or renal clearance of the drug due to the higher plasma exposures of elacridar. However, elacridar is not a potent inhibitor of any cytochrome P450 enzymes in vitro (Ward and Azzarano, 2004), with the IC_{50} values in the micromolar range. The AUC_{plasma} (dose-normalized) was greater after oral dosing versus intraperitoneal dosing, which resulted in a greater bioavailability. This may be explained by inhibition of intestinal P-gp by elacridar after its oral administration, which would also improve its absorption from the gastrointestinal tract, contributing to a higher bioavailability compared with intraperitoneal administration. Another possible explanation of this phenomenon could be enterohepatic recycling of elacridar upon oral administration, which could lead to a larger than expected AUC. Irrespective of these possibilities, the results show that high-dose administration of elacridar by the oral route can yield high exposures in both plasma and brain. Tang et al. (2012a) suggested that a 100-mg/kg dose could represent an effective dose in mice and indicated that there is no significant toxicity associated with administration of this dose. Although toxicity was not evaluated in the current study, there were no overt elacridar-related adverse effects after this single dose in mice. However, if a chronic dosing regimen is to be considered, the concerns for toxicity cannot be dismissed, especially considering the high plasma concentrations and the extended $t_{1/2}$ of elacridar (which could lead to significant accumulation at steady state) that are seen with this dose.

Intraperitoneal dosing is also an attractive option for chronic dosing, especially in preclinical studies. The mice that received elacridar intraperitoneally showed a much lower plasma exposure compared

with oral administration after an equivalent dose. This may most likely be due to poor dissolution and/or absorption of the drug from the peritoneal cavity. The plasma and brain concentrations also showed significant interanimal variability. The intraperitoneal route of administration, though convenient to dose chronically in mice, resulted in reduced plasma exposure of elacridar; therefore, the applications of an intraperitoneal dosing regimen with the current suspension formulation are limited. In the current study, the K_p ($AUC_{\text{brain}}/AUC_{\text{plasma}}$) ratio after an intraperitoneal dose of 100 mg/kg was 0.48. In a similar study by Padowski and Pollack (2010), an intraperitoneal dose of 10 mg/kg yielded a K_p of 0.0784. Taken together, these findings may indicate the presence of nonlinearity in plasma and brain exposures with increasing dose, especially after intraperitoneal dosing.

Exposure after an intravenous dose was used as the reference for calculating the absolute bioavailability after the other two routes of administration. After an intravenous dose of 2.5 mg/kg, the AUC in of elacridar in plasma and brain were approximately equal, yielding a K_p ratio of 0.82 (Table 1). Despite observing this substantial brain distribution of elacridar after intravenous administration, intravenous dosing is not a viable option for chronic dosing in noncatheterized mice because of difficulties in performing repeated injections. Moreover, the dosing solution prepared for intravenous administration is unstable because it is prone to precipitation.

The bioavailability of elacridar is limited by its poor physicochemical properties. The bioavailability of elacridar after oral administration of a high dose was approximately 22%, whereas that after intraperitoneal administration at the same dose was only approximately 1%. The higher AUC_{plasma} after oral administration versus intraperitoneal administration could be due to the solubilizing effect of bile salts in the gut. Oral administration of elacridar, when using this simple suspension formulation, appears to be the most effective way to achieve plasma exposures necessary to effectively inhibit P-gp and BCRP at the BBB.

There were significant findings in this study related to the brain penetration of elacridar after the different routes of administration. First, the K_p for elacridar (a measure of its brain distribution) was found to be dependent on its plasma exposure (see Fig. 4). When the plasma exposure was relatively high, as seen after oral and intravenous administration, the K_p ratio was greater than one, with the highest K_p ratio of ~ 5 seen after oral dosing, which yielded the highest plasma exposure. The K_p ratio after the intraperitoneal route of administration, which resulted in a relatively low plasma exposure, was less than one. Second, the brain-to-plasma concentration ratios plotted as a function of time for all routes of administration showed an increase to a maximal value followed by a decrease (see Figs. 1B, 2B, and 3B). This was unexpected because after reaching a steady state in the tissue of distribution (pseudodistributional equilibrium), the brain-to-plasma concentration ratio should remain constant. One explanation for both these findings can be the active efflux of elacridar from the brain by P-gp and BCRP. Elacridar inhibits both of these transporters at the BBB, and it is possible that the mechanism behind the inhibitory action might be competitive (because of it being a substrate for the two transporters). This has been shown to be true in a study that used positron emission tomographic imaging and transporter knockout mice to study the influence of P-gp and BCRP on elacridar distribution at the BBB (Kawamura et al., 2011a,b). This finding is currently being further investigated in another study. However, it does explain why brain exposures are dependent on plasma concentrations. As the plasma concentrations drop below levels that are required to saturate efflux at the BBB, the net efflux from the brain will become greater than the passive diffusion into the brain. This would result in the brain concentrations decreasing more rapidly than the corresponding

FIG. 4. K_p ratio ($AUC_{\text{brain}}/AUC_{\text{plasma}}$) of elacridar after different routes of administration as a function of the AUC_{plasma} . The brain penetration of elacridar in mice is a function of the plasma AUC; as the plasma AUC increases, the brain partition coefficient of elacridar increases to greater than one, indicating possible saturation of efflux at the BBB.

plasma concentration, ultimately resulting in a decreasing trend in brain-to-plasma ratios with respect to time.

Concurrent administration of elacridar with drugs that are substrates for P-gp and BCRP improves their distribution across the BBB and could lead to improved efficacy. The study of elacridar pharmacokinetics in the brain is important if we consider the issue of target cells that are present behind an intact BBB in the invasive rim of a brain tumor or the normal brain (Agarwal et al., 2011a). Distribution of elacridar in the brain tissue could possibly address the issue of target cells that express BCRP and P-gp and are therefore resistant to chemotherapy (Lu and Shervington, 2008). Elacridar in the brain could be effective in inhibiting P-gp and BCRP present on these cells, allowing the chemotherapeutic agents to act on them, possibly preventing recurrence of the tumor. A targeted approach for central nervous system delivery that employs elacridar could also reduce the dose required to achieve effective brain concentrations, thus reducing systemic toxicity.

The use of elacridar has been limited in both preclinical and clinical situations partly because of its poor solubility and poor bioavailability. It is an unmet need to improve the bioavailability and solubility of elacridar. There are several possible methods that could be explored, including synthesis of water-soluble prodrugs, preparation of solid dispersions, and use of surfactants, cyclodextrins, and permeation enhancers.

In summary, this study has examined the pharmacokinetics of elacridar in plasma and brain in mice and determined its bioavailability after different routes of administration. The results from these experiments can be used to help guide the selection of doses and routes of administration of elacridar for future studies.

Authorship Contributions

Participated in research design: Sane and Elmquist.

Conducted experiments: Sane and Agarwal.

Contributed new reagents or analytic tools: Sane and Elmquist.

Performed data analysis: Sane and Elmquist.

Wrote or contributed to the writing of the manuscript: Sane, Agarwal, and Elmquist.

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