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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of valproic acid after oral administration in non-human primates

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Abstract
Purpose Valproic acid (VPA), a widely used antiepileptic, also inhibits histone deacetylase (HDAC), and is undergoing evaluation as an anti-cancer agent. We studied the pharmacokinetics of VPA in the plasma and cerebrospinal fluid (CSF) in a non-human primate model that is highly predictive of human CSF penetration to determine if levels of VPA required to inhibit HDAC in in vivo models can be attained.
Methods Oral VPA, 75 mg/kg, was administered to four non-human primates. Serial samples of blood ($n = 4$) and CSF ($n = 3$) were obtained for pharmacokinetic studies of total and free VPA. Plasma and CSF VPA concentrations were measured using the commercially available Abbott AxSYM VPA assay reagent system (Abbott Laboratories, Abbott Park, IL, USA). The resultant plasma and CSF data were evaluated using pharmacokinetic modeling methods.
Results At a dose of 75 mg/kg, the maximum plasma concentration of VPA was 130.1 ± 70.6 μg/ml (mean ± standard deviation) for total drug and 53.3 ± 44.4 μg/ml for free drug. The mean plasma area under the curve (AUC) for total drug was 680 ± 233 μg/ml h and for free drug 146 ± 89 μg/ml hr. The maximum CSF concentration occurred 2–3 h after administration and was 28.2 ± 18.6 μg/ml. The CSF AUC for VPA was 108 ± 52 μg/ml h. The CSF penetration of VPA was 12.9 ± 5.1% for total drug and 57.0 ± 8.7% for free drug. Disappearance from the plasma followed non-linear kinetics with a V_{max} of 321.2 ± 65.6 μg/kg/min and a K_m of 17.2 ± 13.7 mg/l.
Conclusion Valproic acid deserves further study for the treatment of CNS tumors given its high CSF penetration after oral dosing coupled with the anti-tumor activity observed in preclinical studies.

Keywords Valproic acid · Histone deacetylase inhibitor · CSF penetration · Pharmacokinetics

Introduction

Aberrant histone deacetylase (HDAC) activity has been linked with cancer. Valproic acid (VPA) (2-propylpentanoic acid), one of the many available HDAC inhibitors, has in vitro and in vivo anti-tumor activity against a wide variety of cancers, including tumors of the central nervous system [2, 3, 5, 6]. Valproic acid is a carboxylic short chain fatty acid that has been used as an antiepileptic drug for many decades, and the anti-cancer activity is believed to be modulated through the inhibition of HDAC.

We wanted to determine if the VPA concentrations (free and total) that inhibit HDAC in in vivo orthotopic CNS xenografts could be achieved following oral dosing. We used a non-human primate model that has previously been shown to be predictive of cerebrospinal fluid (CSF) penetration in humans [1]. The CSF penetration and pharmacokinetics of free and total VPA administered orally at these doses have not previously been described.

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Materials and methods

Drug

Valproic acid was purchased from Abbott Laboratories as a 50 mg/ml suspension. The appropriate dose of drug was administered orally without further dilution. A dose of 75 mg/kg was chosen in this experiment based on prior published literature that this dose would result in a serum total VPA concentration of 150 μg/ml [2] and a minimum CSF concentration of 50 μg/ml, based on the percentage protein binding of VPA at this concentration (54–70%) [3] and assuming that 70% of the unbound fraction would cross into the CNS [4]. This target concentration was based on the VPA exposure associated with inhibition of HDAC in in vivo models [5].

Animals

Four adult rhesus monkeys (*Macaca mulatta*) weighing 10.5–15.9 kg with normal organ function were used for this study. The animals were fed Lab Diet 5045 twice daily and were group housed in accordance with the Guide for the Care and Use of Laboratory Animals [6]. Blood samples were obtained through a central venous catheter. CSF samples were obtained from a chronically indwelling subcutaneously placed Ommaya reservoir attached to a Pudenz catheter with its tip in the fourth ventricle. The reservoir was pumped three times before each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Valproic acid was administered orally to the animals after fasting for a minimum of 14 h with a syringe at a dose of 75 mg/kg. Blood and CSF samples were obtained prior to the dose, and at 30, 60 and 90 min, and 2, 3, 4, 6, 8, and 24 h after the dose. Blood was placed into a polypropelene tube and plasma was immediately separated by centrifugation for 10 min at 4,400 RPM at 5°C. Samples were stored for maximum of 20 h at 2–8°C without freezing. Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained prior to the first dose and on a weekly basis for a minimum of 3 weeks after each dose of VPA. Animals were also observed on a daily basis for a minimum of 3 weeks after each dose for any evidence of clinical toxicity.

Sample analysis

Total VPA concentrations in plasma were measured using the Abbott AxSYM VPA assay reagent system (Abbott Laboratories, Abbott Park, IL, USA). Free VPA concentrations in the plasma and CSF were determined using the same reagent system after separating free from bound drug using an ultrafiltration membrane (Centrifree Micropartition System, Millipore Corporation, Bedford, MA, USA). The lower limit of quantitation (LLQ) of the assay is 7 μg/ml and the linear range is 0–150 μg/ml.

Pharmacokinetic analysis

The area under the concentration versus time curve (AUC) was determined using the linear trapezoidal method and was extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. Penetration of drug into the CSF was calculated by the ratio of the AUC of CSF to the AUC of plasma.

Subsequently we developed a pharmacokinetic model that could be simultaneously fit to each animal's plasma and CSF concentration-time data using ADAPT II [7]. A schematic of the model is shown in Fig. 1 and the model equations and variables and shown in Fig. 2.

Results

Valproic acid was well tolerated by all four animals without clinical or laboratory evidence of toxicity.

The mean plasma C_{max} was 130.1 ± 70.6 μg/ml for total drug and 53.3 ± 44.4 μg/ml for free drug (Table 1). The mean plasma $AUC_{0\rightarrow\infty}$ for total drug was 680 ± 233 μg/ml h and for free drug was 146 ± 89 μg/ml h, with over 94% of the AUC measured ($AUC_{0\rightarrow t\text{-last}}$).

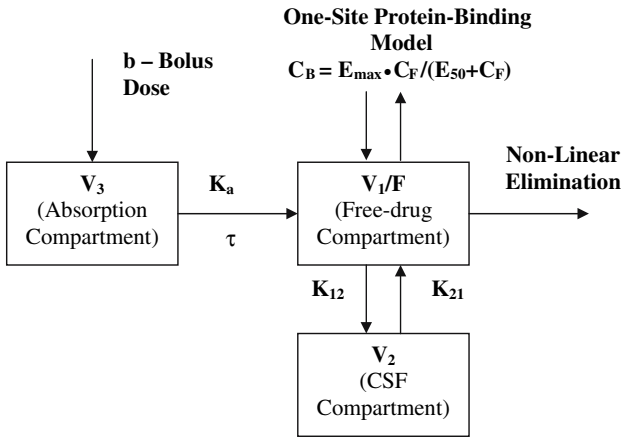

Fig. 1 Schematic of the pharmacokinetic model for valproic acid in non-human primates. The pharmacokinetic model for describing the plasma and CSF disposition of valproic acid consists of an absorption compartment, a free-drug compartment, and a CSF compartment. The model uses first order absorption with a 15 min lag time, non-linear irreversible elimination from the free-drug compartment, linear transfer between free-drug and CSF, and a one-site protein binding model for the relationship between free and bound drug

Fig. 2 Equations for the pharmacokinetic model of valproic acid in non-human primates

Differential Equations

$dX_1/dt = K_a \bullet X_3(t) - K_{12} \bullet X_1(t) + K_{21} \bullet X_2(t) - [V_{max}/(V_1 \bullet K_m + X_1(t))] \bullet X_1(t)$

$dX_2/dt = K_{12} \bullet X_1(t) - K_{21} \bullet X_2(t)$

$dX_3/dt = - K_a \bullet X_3(t)$

All three differential equations have a time delay (τ) incorporated to account for lag-time in absorption.

Output Equations

$C_1(t) = X_1(t)/ V_1$

$C_2(t) = X_2(t)/ V_2 \bullet M$

Protein Binding

$Cb(t) = E_{max} \bullet C_1(t)/(E_{50} + C_1(t))$

Variables

t = time; (min)

b = bolus dose; (mg/kg)

τ = time delay; (min)

M = mass of NHP; (kg)

$X_1(t)$ = mass of free drug in plasma (compartment 1); (mg/kg)

$X_2(t)$ = mass of free drug in CSF (compartment 2); (mg/kg)

$X_3(t)$ = mass of free drug in absorption compartment (compartment 3); (mg/kg)

V_1/F = apparent volume of distribution of free-drug compartment 1; (L/kg)

V_2 = volume of distribution of CSF (compartment 2); (L) – this is fixed at 10 cc in the model, the approximate volume of CSF in a non-human primate.

$C_1(t)$ = concentration of free drug in plasma (compartment 1); (mg/L=µg/mL)

$C_2(t)$ = concentration of free drug in CSF (compartment 2); (mg/L=µg/mL)

$C_b(t)$ = concentration of bound drug in plasma; (mg/L=µg/mL)

K_a = absorption rate constant; (min-1)

K_{12}= rate constant; (min-1)

K_{21}= rate constant; (min-1)

V_{max} = maximum velocity – Michaelis-Menten PK parameter; (mg/kg/min)

K_m = half-saturation constant – Michaelis-Menten PK parameter; (mg/L=µg/mL)

E_m = protein binding constant; (mg/L=µg/mL)

E_{50} = protein binding constant; (mg/L=µg/mL)

Table 2 shows the CSF pharmacokinetic results. Peak measured CSF concentrations occurred 116–180 min after administration of the drug and were 28.2 ± 18.6 µg/ml (range 12.8–48.2 µg/ml). The ratio of CSF AUC to plasma AUC was 12.9 ± 5.1% for total drug and 57.1 ± 8.7% for free drug in all three monkeys from which CSF samples were available. Estimated clearance from the CSF was 0.59 ± 0.31 ml/h.

Table 1 Model independent pharmacokinetic parameters of free and total valproic acid in plasma after a single oral dose of 75 mg/kg to non-human primates

Animal	Total valproic acid		Free valproic acid	
	C_{max} (µg/ml)	$AUC_{0\rightarrow\infty}$ (µg/ml h)	C_{max} (µg/ml)	$AUC_{0\rightarrow\infty}$ (µg/ml h)
L976	66.6	332	10.1	38
J128	128.8	700	46.0	146
J124	96.1	699	41.7	117
L962	229.0	989	115.5	285
Mean ± SD	130.1 ± 70.6	680 ± 233	53.3 ± 44.4	146 ± 89

The modeling results are shown in Table 3. The absorption half-life ($t_{1/2\text{-abs}}$) was 1.5 ± 2.0 h. The apparent volume of distribution of the free-drug compartment (V_1/F) was 0.87 ± 0.53 l/kg. Disappearance from the plasma was described by non-linear (Michaelis–Menten) kinetics with a V_{max} of 321 ± 66 µg/kg/min and a K_m of 17.2 ± 13.7 mg/l. For the protein binding model relating free to bound drug, E_{max} was 109 ± 50 µg/ml and E_{50} was 11.3 ± 7.7 µg/ml. Figure 3a–c shows the model predicted versus measured concentrations in plasma and CSF in the three animals that had CSF sampling. The plots show concentration data to 8 h since CSF and free plasma levels were below the LLQ at 24 h.

Discussion

The results of our pharmacokinetic modeling are consistent with studies of VPA pharmacokinetics in other animals, including sheep and lambs. The V_{max} was 445.0 µg/kg/min

Table 2 CSF pharmacokinetic parameters of valproic acid for total drug after a single oral dose of 75 mg/kg to non-human primates

Animal	C_{max} (μg/ml)	$AUC_{0\rightarrow\infty}$ (μg/ml h)	$AUC_{csf}/AUC_{Plasma-Total}$ (%)	$AUC_{csf}/AUC_{Plasma-Free}$ (%)	Cl_{csf} (ml/h)
L976	N/A	N/A	N/A	N/A	N/A
J128	22.8	89	12.7	61.1	0.94
J124	12.8	55	7.8	47.0	0.39
L962	48.9	179	18.1	62.9	0.43
Mean ± SD	28.2 ± 18.6	108 ± 52	12.9 ± 5.1	57.0 ± 8.7	0.59 ± 0.31

Table 3 Model-dependent pharmacokinetic parameters of free and bound valproic acid in plasma and CSF after a single oral dose of 75 mg/kg to non-human primates

Animal	$t_{1/2\text{-abs}}$ (h)	V_{max} (μg/kg/min)	K_m (mg/l)	V_1/F (l/kg)	k_{12} (h^{-1})	k_{21} (h^{-1})	V_2 (l)	E_{max} (μg/ml)	E_{50} (μg/ml)
L976	4.50	252	5.7	0.36	N/A	N/A	N/A	175	22.6
J128	0.34	291	11.6	1.45	0.11	1.57	0.01	77	6.0
J124	0.88	337	14.6	1.19	0.03	0.65	0.01	65	7.3
L962	0.15	404	37.0	0.49	0.13	0.72	0.01	120	9.3
Mean ± SD	1.47 ± 2.04	321 ± 66	17.2 ± 13.7	0.87 ± 0.53	0.09 ± 0.05	0.98 ± 0.51	N/A (fixed)	109 ± 50	11.3 ± 7.7

in adult sheep and 429.9 μg/kg/min in newborns lamb; E_{max} was 91.8 μg/ml in sheep and 429.9 μg/ml in lambs; and K_m was 30.0 μg/ml in sheep and 69.9 μg/ml in lambs [8]. These data suggest that VPA pharmacokinetics are relatively consistent across large animal species.

The CSF penetration of VPA in our study, 12.9 ± 5.1% for total drug and 57.1 ± 8.7% for free drug, is similar to results from studies in humans. The CSF:total plasma drug ratio in humans at steady state with plasma concentrations of 50–80 μg/ml was 8–25% [9]. The ratio of subdural CSF:free plasma drug in humans ranged from 70 to 124% after either a single dose of 600 or 900 mg or chronic dosing of 600 mg t.i.d. [4]. Estimated clearance from the CSF was 0.59 ± 0.31 ml/h, which is slower than expected based on bulk flow (2–4 ml/h) [10].

The higher penetration of free drug is expected since protein bound drug does not penetrate well into CSF [11, 12]. Our pharmacokinetic model predicts that protein binding is approximately 80% saturated (free drug = 28%) at a total plasma concentration of 110 μg/ml and approaches 95% saturation (free drug = 50%) at 220 μg/ml (Fig. 4). At standard "therapeutic" antiepileptic levels (50–100 μg/ml), our model predicts that protein binding is approximately 33% saturated (free drug = 13%). The data in humans is similar at the lower concentrations, and data is varied at higher concentrations. Higher doses of VPA, which would be expected to produce concentrations that saturate protein binding, should increase the amount of unbound VPA available to penetrate into the CSF. However it should be noted free drug undergoes more rapid elimination than bound drug [13]. Therefore human clinical studies are needed to determine the optimal dosing regimen. Higher doses may be advantageous as an approach to the treatment of CNS tumors if they can be administered without unacceptable toxicity.

The maximum CSF VPA concentrations in the non-human primate in our study were 28.2 ± 18.6 μg/ml (range 12.8–48.2 μg/ml) following a single dose of 75 mg/kg, In in vitro studies, the IC_{50} for VPA for two medulloblastoma cell lines D283 and DAOY was 0.2 mmol/l (28.8 μg/ml) for a 14-day exposure and 0.6 mmol/l (86.4 μg/ml) for a 21-day exposure, respectively [5]. Thus the concentrations obtained in the non-human primates were approximately within the range needed to obtain cytotoxicity. We were able to obtain the target concentration for up to 3 h in one animal, however, it should be noted this was after only a single dose (steady-state was not obtained); therefore higher exposures of sustained duration would likely be obtainable with chronic dosing. The safety of delivering this dose to humans chronically is currently being evaluated in a phase I study.

The standard deviations in this experiment are likely related to the small size of the study and the inter-subject variability. In humans, wide variations in plasma and CFS levels have been reported [14].

Valproic acid represents a new and potentially useful class of anticancer agents. The advantages of this particular drug include low cost, favorable safety profile, and oral dosing. In addition, as demonstrated in this preclinical study, the CSF penetration of VPA in non-human primates

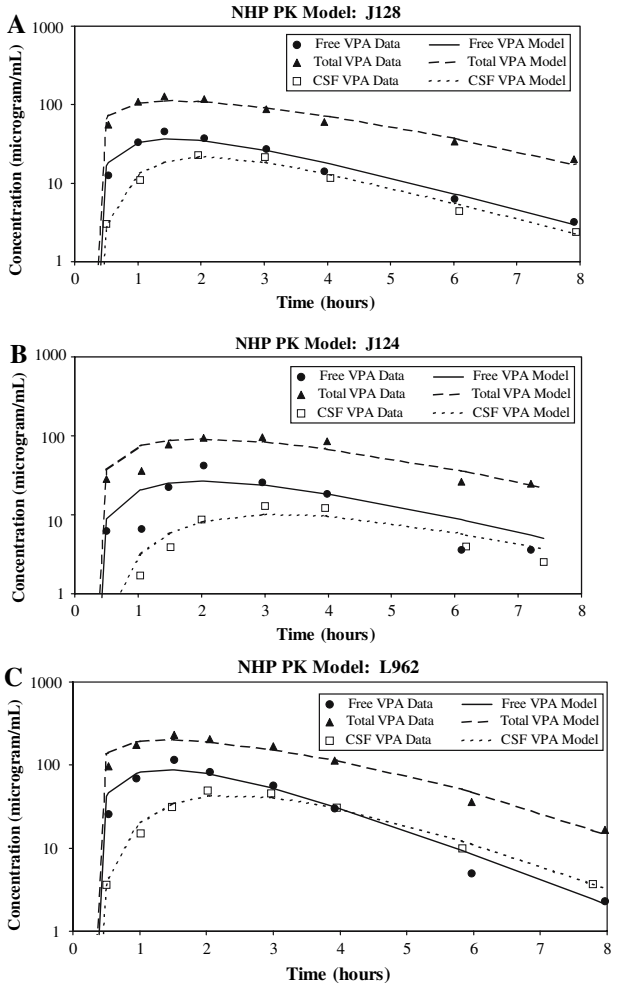

Fig. 3 Model predicted versus measured valproic acid concentrations in plasma and CSF for the non-human primates J128, J124, and L962. Free and total plasma and total CSF concentrations of valproic acid are compared to measured concentrations for each of the three animals with simultaneous plasma and CSF pharmacokinetics. The *lines* represent the model predictions and the *symbols* the data points. The *solid line* represents the free plasma VPA model estimates, the *coarse dashed-line* the total plasma VPA estimates, and the *fine dashed line* the CSF VPA estimate. The *circles* represent the measured free VPA, the *triangles* the total plasma VPA, and the *open squares* the CSF VPA

was high. The combined plasma and CSF pharmacokinetic model described here may be useful in developing dosing schemes which maximize CSF penetration, and potentially optimize VPA's role in treating CNS tumors. There is one published case report of a complete response of a glioblastoma multiforme in a child with chronic VPA monotherapy in which the dosage aim was plasma trough levels two to threefold greater than therapeutic antiepileptic levels [15]. In the United States, there is a Children's Oncology Group phase I trial of VPA for pediatric patients with recurrent or progressive CNS or other solid tumors. The goal of this trial is to determine if steady state trough concentrations in the range of 100–150 μg/ml or 150–200 μg/ml can be

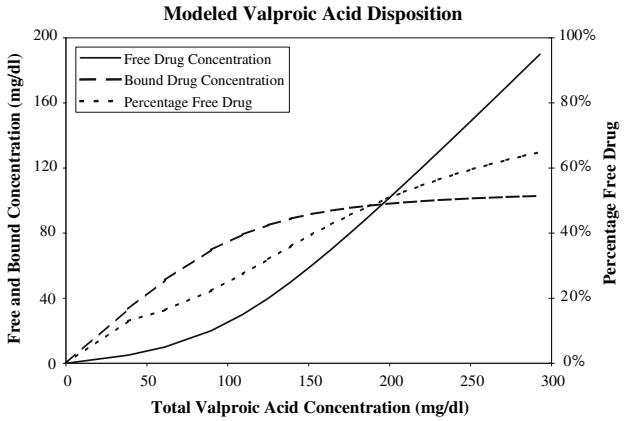

Fig. 4 Modeled data predicting bound concentration is saturable due to protein binding, while free concentration increases with total concentration

achieved without dose-limiting toxicity. In addition, plasma pharmacokinetics of total and free VPA will also be obtained.

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