Assessment of Tear Concentrations on Therapeutic Drug Monitoring. II. Pharmacokinetic Analysis of Valproic Acid in Guinea Pig Serum, Cerebrospinal Fluid, and Tears

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Purpose. To quantitatively describe the pharmacokinetics of valproic acid (VPA) in guinea pig serum (total [Cf+b] and free [Cf]), cerebrospinal fluid (CSF) $[C]_{CSF}$ and tears $[C]_T$ using a simple kinetic model, and to examine whether [Cf] and $[C]_{CSF}$ can be predicted by $[C]_T$ using the resulting pharmacokinetic parameters.
Methods. [Cf+b], [Cf], $[C]_{CSF}$ and $[C]_T$ were determined after bolus i.v. injection of 10 or 20 mg/kg VPA using GC/ECNCI/MS.
Results. [Cf+b] could be quantitatively described by a two compartment model with linear elimination kinetics. [Cf] was separately analyzed using multi-exponential equations. $[C]_{CSF}$ was analyzed using a simple kinetic model in which the CSF compartment is independently connected with the serum compartment by the apparent diffusion constants (K_{INCSF} and K_{OUTCSF}). $[C]_T$ was analyzed using the same simple kinetic model used for $[C]_{CSF}$. The values of $[C]_{CSF}$ and [Cf] in the steady state can be represented by the following equations; $[C]_{CSF} = K_{INCSF}/K_{OUTCSF} \times [Cf]$, $[Cf] = K_{OUTT}/K_{INT} \times [C]_T$, and indicating that [Cf] and $[C]_{CSF}$ can be predicted by $[C]_T$ using the resulting pharmacokinetic parameters.
Conclusions. The measurement of $[C]_T$ which can be collected noninvasively and estimated the pharmacokinetic parameters for [Cf], $[C]_{CSF}$, and $[C]_T$ might be a very useful method for TDM of VPA.

KEY WORDS: valproic acid; cerebrospinal fluid; tears; pharmacokinetics; guinea pig.

INTRODUCTION

Valproic acid (VPA) has been widely used for the treatment of different types of epilepsy. The plasma total concentration of VPA [Cf+b] is routinely monitored in patients. However, only the unbound (free) drug in plasma [Cf] is believed to be responsible for therapeutic effects and adverse reactions. The protein binding of VPA is nonlinear (1), and is modified by different physiological variables such as hepatic function, plasma albumin, pH and fatty acids (2). Moreover, the protein binding of VPA exhibits great intra- and intersubject variability, and the free fraction of VPA in healthy subjects and patients with renal disease ranges from 5.2 to 12.3% and from 6.6 to 28.9%, respectively (3). Measuring [Cf] is considered to be very useful for therapeutic drug monitoring (TDM) of VPA. Although [Cf] can be measured by equilibrium dialysis or ultra-filtration, these operations are tedious and time consuming for routine TDM.

Recently, we developed a simple and sensitive method for the quantification of VPA in epilepsy patient tears using gas chromatography/electron capture negative chemical ionization/mass spectrometry (GC/ECNCI/MS). Using this method we demonstrated statistically positive correlation between $[C]_T$ and [Cf] (4), while Monaco *et al.*, had shown that $[C]_T$ significantly correlates with both [Cf+b] and $[C]_{CSF}$ (5). These results suggested that $[C]_T$ might be an excellent indicator for [Cf] and biological fluids of the central nervous system (CNS) including $[C]_{CSF}$. The penetration of VPA into the CNS is very rapid in spite of the high degree of ionization of VPA (99.84%, pKa = 4.6, pH = 7.4) (6). The rapid penetration rate of VPA into the CNS may be mediated in part by active transport systems such as monocarboxylic acid transport. The uptake of N,N-dimethyl-2-phenylethylene into acini cells removed from rabbit lacrimal glands is primarily accomplished by a carrier mediated transport system (7), and a Na^+-dependent monocarboxylate transport process has been detected on the mucosal side of pigmented rabbit conjunctiva (8). Thus, the transport of VPA between serum and CSF, and serum and tears may be mediated by active transport systems. However, no comprehensive pharmacokinetic studies of [Cf+b], [Cf], $[C]_{CSF}$ and $[C]_T$ have been published to date. Our previous study showed that the time course of chlorpromazine in rat CSF can be described quantitatively by a basic physiological model in which the CSF compartment is connected with the serum compartment (free drug) by apparent diffusion clearance (9). In the present study, we found that the time courses of [Cf+b], [Cf], $[C]_{CSF}$ and $[C]_T$ in guinea pig after VPA administration can be quantitatively described using a basic physiological model, and that the values of [Cf] and $[C]_{CSF}$ can be predicted by the values of $[C]_T$ via use of the resulting pharmacokinetic parameters.

EXPERIMENTAL SECTION

Materials

Sodium valproate was generously supplied by Kyowa Hakkou Kougiyou Co., Ltd. (Tokyo, Japan) and was used without further purification, and was dissolved in isotonic sodium chloride solution (Otsuka Pharmaceuticals, Tokyo, Japan). All other chemicals were of reagent grade and were obtained commercially.

Animal Experiments for [Cf+b], [Cf], $[C]_{CSF}$ and $[C]_T$

Male Hartley guinea pigs (270 ± 17 g, Sankyo Lab Service Corporation, Inc., Shizuoka, Japan) were used. The guinea pigs were housed individually in metal cages in an environment of controlled temperature (22–24°C) and alternating 12 hr light (7 a.m.–7 p.m.) and dark cycles. Food and

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water were withdrawn in the morning on the day of the experiment, and the guinea pigs were then placed in individual plastic metabolic cages. The animals had an indwelling cannula implanted in the right jugular vein 1 day before the experiments. To characterize changes to [Cf+b], [Cf], $[C]_{CSF}$ and $[C]_{T}$, VPA (10 mg/kg as free acid) was given in a bolus injection into the right jugular vein. At 5, 10, 15, 30, 45, 60, 120, 240, and 360 min after administration, guinea pigs were lightly anesthetized with ether and then tear, CSF and blood samples taken. Tears were collected from both eyes using a Schirmer tears test strip (Alcon Laboratories, TX), CSF was obtained by cisternal puncture, and as much blood as possible was obtained from the abdominal aorta (about 4 ml). The volume of tears was first estimated using a 5 × 5 mm Schirmer tears test strip (3.37 ± 0.41 μl, $n = 10$). The sample volume of tears was determined by weighing and the pH measured from both eyes after tear sampling using pH indicator paper (TB, Advantec, Tokyo). The collected blood was centrifuged (10 min, 3000 rpm) and the separated serum used for determination of drug concentration and the binding experiment. Serum protein binding of VPA was determined using the ultrafiltration method (Centrifree MPS-3, Amicon, MA). The tear, CSF and serum samples were stored at −80°C until analysis of VPA concentration. To determine the effect of dose on the VPA disposition in serum and tears, VPA (20 mg/kg) was given in a bolus injection into the right jugular vein cannula, and then saline run through the cannula. Blood (0.5 ml) and tear samples were collected from the same right jugular vein cannula and from both eyes using Schirmer tears test strips, respectively, at 5, 10, 15, 30, 45, 60, 120, 240, and 360 min after VPA injection. The blood was then replaced by injection of an equal volume of citrated blood from a donor guinea pig. The tear and serum samples were stored at −80°C until analysis of VPA concentration.

Quantification of [Cf+b], [Cf], $[C]_{CSF}$, and $[C]_{T}$

[Cf+b], [Cf], $[C]_{CSF}$, and $[C]_{T}$ were determined using the GC/ECNCI/MS method which was developed in previous studies with $[^2H_{14}]$-VPA as the internal standard (4). In this method, VPA was converted directly into its pentafluorobenzyl ester derivative without the need to perform any concentration from the biologic fluid. For the serum, serum filtrate and CSF samples, 2 μl of each samples was pipetted on a Schirmer tears test strip (5 × 5 mm). For the tear samples, the 5 × 5 mm Schirmer tears test strip after use was cut into pieces which were then placed into a screw cap test tube. To this tube, 2 μl of the internal standard solution ($[^2H_{14}]$-VPA), 100 μl of N,N-dimethylformamide, 10 μl of pentafluorobenzyl bromide (Aldrich Chemicals, Milwaukee, WI) and 20 μl of N,N-diisipropylethylamine (Aldrich Chemicals) were added to convert VPA to its pentafluorobenzyl (PFB) derivative. The mixture was incubated for 1 h at room temperature, then extracted with 200 μl of n-hexane and centrifuged at room temperature at 2000 rpm for 2 min. The upper organic layer was transferred into another glass tube containing 300 μl of water, and the organic layer then washed to remove excess regent. After centrifugation (2000 rpm, 2 min), 1 μl of the n-hexane solution was injected into the GC/ECNCI/MS system.

The assay was performed on a Thermo-Quest GCQ plus system (San Jose, CA). The instrument was operated in ECNCI mode using methane as the reagent gas with the source and transfer line at 200°C and 275°C, respectively. The chromatographic column was a capillary column, DB-5ms (J & W Scientific, CA) 30 m × 0.25 mm I.D. The injector port temperature was 220°C and the helium carrier gas flow rate was 1.5 ml/min. The initial column oven temperature was 50°C, and at 2 min after injection the oven temperature was increased at 30°C/min to 220°C. The ionization potential was 70 eV. The monitoring ions at m/z 143 $[M\text{-}PFB]^-$ for VPA-PFB derivative and m/z 157 $[M\text{-}PFB]^-$ for $[^2H_{14}]$-VPA-PFB derivative, as an internal standard were used. The limit of VPA quantitation was 1 pg per 1 μl injection which corresponds to 100 ng/ml in this study. Because 1 μl of 200 μl n-hexan layer extracted from 2 μl of biological fluids was subjected into GC/MS without concentration.

Pharmacokinetic Analysis of [Cf+b], [Cf], $[C]_{CSF}$, and $[C]_{T}$

In order to quantitatively describe the time course of [Cf+b], [Cf], $[C]_{CSF}$, and $[C]_{T}$ after i.v. injection of VPA, a pharmacokinetic model for VPA was constructed as shown in Fig. 1. Assuming that the time course of [Cf+b] can be described by a two compartment model with linear elimination kinetics in the dose range of this study, a two-exponential equation as follows was applied to elucidate the time course of [Cf+b].

$$[Cf+b] = \text{Dose}\,[A \exp(-\alpha t) + B \exp(-\beta t)] \tag{1}$$

Definitions of the symbols used in all the equations are listed in Table I. The time courses of [Cf] did not parallel those of [Cf+b]: the free fraction of VPA in serum increased with the elapse of time and the enlargement of dose. Consequently, the time courses of [Cf] after 10 and 20 mg/kg administration were separately analyzed using the two-exponential and three-exponential equations, respectively, as follows.

$$[Cf] = fA \exp(-f\alpha\, t) + fB \exp(-f\beta\, t) \tag{2}$$

$$[Cf] = fA \exp(-f\alpha\, t) + fB \exp(-f\beta\, t) + fC \exp(-f\gamma\, t) \tag{3}$$

In order to quantitatively describe the time courses of $[C]_{CSF}$ and $[C]_{T}$, a basic physiological model that conformed with the CSF model (Fig. 1) was constructed (9). The time courses of $[C]_{CSF}$ and $[C]_{T}$ could be expressed by the following equations.

$$V_{CSF} \frac{d\,[C]_{CSF}}{dt} = CL_{INCSF}\,[Cf] - CL_{OUTCSF}\,[C]_{CSF} \tag{4}$$

$$V_{T} \frac{d\,[C]_{T}}{dt} = CL_{INT}\,[Cf] - CL_{OUTT}\,[C]_{T} \tag{5}$$

$CL_{INCSF} = Q_{CSF} + PA_{CSF}$, $CL_{OUTCSF} = Q_{CSF} + PA_{CSF} + CL_{EFFCSF}$, $CL_{INT} = Q_{T} + PA_{T}$, $CL_{OUTT} = Q_{T} + PA_{T} + CL_{EFFT}$. Substitution of Eq. 2 or 3 into 4 or 5, followed by integration, gives the following equations.

$$[C]_{CSF\text{ or }T} = K_{INCSF\text{ or }INT}\left[\frac{fA}{f\alpha - K_{OUTCSF\text{ or }OUTT}}\{\exp(-K_{OUTCSF\text{ or }OUTT}\,t) - \exp(-f\alpha\, t)\} + \frac{fB}{f\beta - K_{OUTCSF\text{ or }OUTT}}\{\exp(-K_{OUTCSF\text{ or }OUTT}\,t) - \exp(-f\beta\, t)\}\right] \tag{6}$$

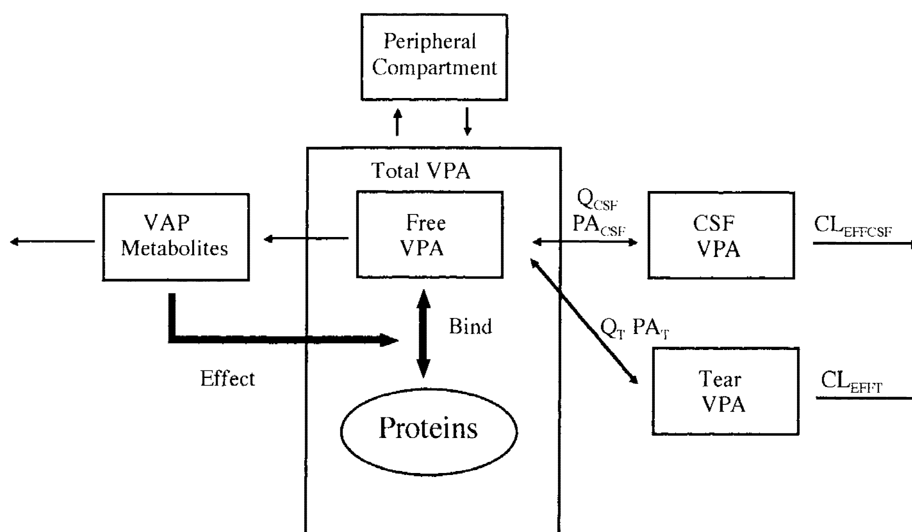

Fig. 1. Schematic representation of pharmacokinetic model for VPA in guinea pig serum, CSF, and tears.

$$[C]_{CSF\ or\ T} = K_{INCSF\ or\ INT}\left[\frac{fA}{f\alpha - K_{OUTCSF\ or\ OUTT}}\{\exp(-K_{OUTCSF\ or\ OUTT}\, t) - \exp(-f\alpha\, t)\} + \frac{fB}{f\beta - K_{OUTCSF\ or\ OUTT}}\{\exp(-K_{OUTCSF\ or\ OUTT}\, t) - \exp(-f\beta\, t)\} + \frac{fC}{f\gamma - K_{OUTCSF\ or\ OUTT}}\{\exp(-K_{OUTCSF\ or\ OUTT}\, t) - \exp(-f\gamma\, t)\}\right] \quad (7)$$

The time courses of $[C]_{CSF}$ and $[C]_T$ after i.v. administration of VPA were analyzed using Eq. 6 (10 mg/kg i.v.) and Eq. 7 (20 mg/kg i.v.).

Least Squares Model Adaptation

In order to estimate the pharmacokinetic parameters of VPA, the data on the concentrations of VPA were fitted to Eqs. 1, 2, 3, 6, 7, 8, and 9 using a nonlinear least squares regression program, FKDM (10), and a digital computer (PC-9821 Ae, NEC, Tokyo, Japan). The inverse value of each datum was used as the weighting value of the least squares method.

Statistical Analysis

Differences in the free fraction of VPA at 5, 10, 15, 30, 45, 60, 120, 240, and 360 min after VPA administration were evaluated statistically using one-way analysis of variance followed by the Tukey test. Logarithmic correlation between [Cf] and $[C]_{CSF}$, and [Cf] and $[C]_T$ was evaluated statistically using the simple linear regression equation followed by Student's *t*-test. The differences between the population regression coefficients of 10 and 20 mg/kg i.v. administration studies on [Cf+b] and $[C]_T$ were evaluated statistically by comparison of the simple linear regression equations followed by Student's *t*-test. Statistical significance was established at the $p < 0.05$ level (11).

RESULTS AND DISCUSSION

Total VPA Concentration in Serum [Cf+b]

The time courses of [Cf+b] after i.v. administration of VPA at 10 or 20 mg/kg in the guinea pigs are shown in Fig. 2a. The data are plotted semi-logarithmically as a function of time. The disappearance of [Cf+b] followed two-exponential curves. The solid lines represent the calculated values using Eq. 1. The pharmacokinetics of [Cf+b] was able to be described quantitatively using the two compartment model with linear elimination kinetics. The pharmacokinetic parameters were estimated using the nonlinear least squares method, and these resulting parameters are listed in Table II.

Free VPA Concentration in Serum [Cf]

The time courses of [Cf] after i.v. administration of VPA (10 and 20 mg/kg) are shown in Fig. 2b. The time courses of [Cf] did not parallel those of [Cf+b]. The dotted line represents the calculated VPA concentration after the 20 mg/kg i.v. dose, and was obtained using the fixed value of the free fraction of VPA (15%). The time course of [Cf] after the 20 mg/kg i.v. dose could not be described when assuming linear protein binding behavior of VPA. Figure 3a shows the changes in the free fraction of VPA in serum. The free fraction of VPA in serum increased with the elapse of time (10 mg/kg: from 13% to 29%) and increase in dose (20 mg/kg: 30%). Although not shown in Fig. 3a, the free fraction of VPA at 360 min was statistically greater than that at 10, 15, 30, or 45 min after administration of the 10 mg/kg dose.

The protein binding of VPA has been shown to be nonlinear (1), and altered by different physiological variables such as hepatic function, plasma albumin, pH and fatty acids (2). Yu and Shen have demonstrated that the free fraction of VPA increases from 14 to 79% with an increase in added VPA plasma total concentrations *in vitro* (from 10 to 1000 μg/ml), and that the free fraction of VPA increases from 25 ± 2 to 95 ± 1% with an increase in the steady state guinea pig plasma concentration of VPA (from 11.3 ± 2.6 to 1303.3 ±

Table I. List of Symbols and Definitions

Symbol	Definition
A, B	(μg/ml)/(mg/kg) Coefficient for total concentration of VPA
α, β	(h^{-1}) Disposition constant for total concentration of VPA
[Cf]	(μg/ml) Free concentration of VPA in serum
[Cf + b]	(μg/ml) Total concentration of VPA in serum
$[C]_{CSF}$	(μg/ml) VPA concentration in CSF
$[C]_T$	(μg/ml) VPA concentration in tears
CI_{EFFCSF}	(ml/h) Efflux clearance of VPA from CSF to serum and others
CL_{EFFT}	(ml/h) Efflux clearance of VPA from tears to serum and others
CL_{INCSF}	(ml/h) Apparent diffusion clearance of VPA from serum to CSF including the bulk flow rate of CSF (Q_{CSF}) and the diffusion clearance of VPA between serum and CSF (PA_{CSF})
CL_{INT}	(ml/h) Apparent diffusion clearance of VPA from serum to tears including the bulk flow of tears (Q_T) and the diffusion clearance of VPA between serum and tears (PA_T)
CL_{OUTCSF}	(ml/h) Apparent diffusion clearance of VPA from serum to CSF including the bulk flow rate of CSF (Q_{CSF}), the diffusion clearance of VPA between serum and CSF (PA_{CSF}) and the efflux clearance from CSF to serum (CL_{EFFCSF})
CL_{OUTT}	(ml/h) Apparent diffusion clearance of VPA from serum to tears including the bulk flow rate of tears (Q_T) and the diffusion clearance of VPA between serum and tears (PA_T)
Dose	(mg/kg) Dose of VPA
fA, fB, fC	(μg/ml) Zero time intercept for free concentration of VPA
$f\alpha$, $f\beta$, $f\gamma$	(h^{-1}) Disposition constant for free concentration of VPA
K_{INCSF}	(h^{-1}) Apparent first-order constant from serum to CSF
K_{INT}	(h^{-1}) Apparent first-order constant from serum to tears
K_{OUTCSF}	(h^{-1}) Apparent first-order constant from CSF to serum
K_{OUTT}	(h^{-1}) Apparent first-order constant from tears to serum
PA_{CSF}	(ml/h) Diffusion clearance of VPA between serum and CSF
PA_T	(ml/h) Diffusion clearance of VPA between serum and tears
Q_{CSF}	(ml/h) Bulk flow rate of CSF
Q_T	(ml/h) Bulk flow rate of tears
t	(h) Time after VPA administration
UIF_S	The unionized form fraction of VPA in serum
UIF_T	The unionized form fraction of VPA in tears
V_{CSF}	(ml) CSF volume
V_T	(ml) Tears volume

122.9 μg/ml) *in vivo* by constant intravenous infusion of VPA (12). These results indicate that the protein binding behavior of VPA *in vitro* does not parallel that *in vivo,* thereby suggesting that the protein binding of VPA is affected by VPA metabolites produced after administration of VPA. In the present study, after i.v. administration of VPA, the free fraction of VPA changed from 13.3 ± 1.5 to 36.6 ± 3.0% as the serum total concentration of VPA changed (1.1 ± 0.7 to 72.5 ± 7.9 μg/ml). These results are consistent with those of results of Yu and Shen (12).

In order to describe the time course of [Cf] after i.v. administration of VPA, it was assumed that the serum protein binding behavior of VPA is nonlinear and is affected by the metabolites of VPA. The time courses of [Cf] after 10 and 20 mg/kg VPA administration were separately analyzed using two exponential equation (Eq. 2). The solid line (10 mg/kg) and the broken line (20 mg/kg) in Fig. 2b represent the calculated values using the two exponential equation. The time course of [Cf] after 10 mg/kg i.v. VPA administration was able to be quantitatively described by the two exponential equation, whereas the time course of [Cf] after 20 mg/kg i.v. VPA administration could not be described using this equation. The three exponential equation (Eq. 3) was then successfully used to describe the time course of [Cf] after 20 mg/kg i.v. VPA administration. The pharmacokinetic parameters were simultaneously estimated by fitting the data of the serum free, CSF and tears concentrations of VPA to Eqs. 2, 3, 6, and 7 using the nonlinear least squares method, and these resulting parameters and the values of the sum of squares (SS) and Akaike's information criterion (AIC) are listed in Table II.

In previous studies, we have demonstrated that the characteristics of chlorpromazine protein binding are altered by chlorpromazine metabolites, such as chlorpromazine S-oxide and chlorpromazine N-oxide, and that the time course of free chlorpromazine concentration in serum after i.v. chlorpromazine administration can be predicted by the correlation between the ratio of chlorpromazine metabolites (S-oxide and N-oxide)/chlorpromazine and the free fraction of chlorpromazine in serum (13). Similarly, the time courses of [Cf] may be predicted using the correlation between the ratio of VPA metabolites/VPA and the free fraction of VPA in serum, given that the effect of VPA metabolites on the protein binding of VPA has been demonstrated.

VPA Concentration in CSF $[C]_{CSF}$

The time course of $[C]_{CSF}$ after i.v. administration of 10 mg/kg VPA was similar to that of [Cf] and is shown in Fig. 2c. Several kinetic models have been used to elucidate the disposition of drugs in the CSF (14–16). In a previous study, the time course of chlorpromazine concentration in CSF was able to be described quantitatively using a basic physiological model in which the CSF compartment was connected to the serum compartment (free drug) by apparent diffusion clearance (9). The time course of $[C]_{CSF}$ was analyzed using this basic physiological model (Fig. 1). The solid line in Fig. 2c represents the calculated values of $[C]_{CSF}$, and indicates that the time course of $[C]_{CSF}$ can be described by the basic physiological model. The pharmacokinetic parameter (K_{INCSF} and K_{OUTCSF}) were computed using the nonlinear least squares method, and other parameters (CL_{INCSF}, CL_{OUTCSF}, PA_{CSF}, and CL_{EFFCSF}) were calculated using published data (Table 2), where the values of the CSF volume (V_{CSF}) and the CSF flow rate (Q_{CSF}) were estimated from an allometric relationship between the body weight of animals and the values of V_{CSF} and Q_{CSF} previously reported for rats and humans (14–15).

Many reports have suggested that the translocation of VPA between serum and the CNS including the CSF is very complex. Although VPA is almost completely ionized at physiological pH (99.84%: pHs 7.4, pKa 4.6), the penetration of VPA into the CNS is very rapid (6). The uptake of VPA

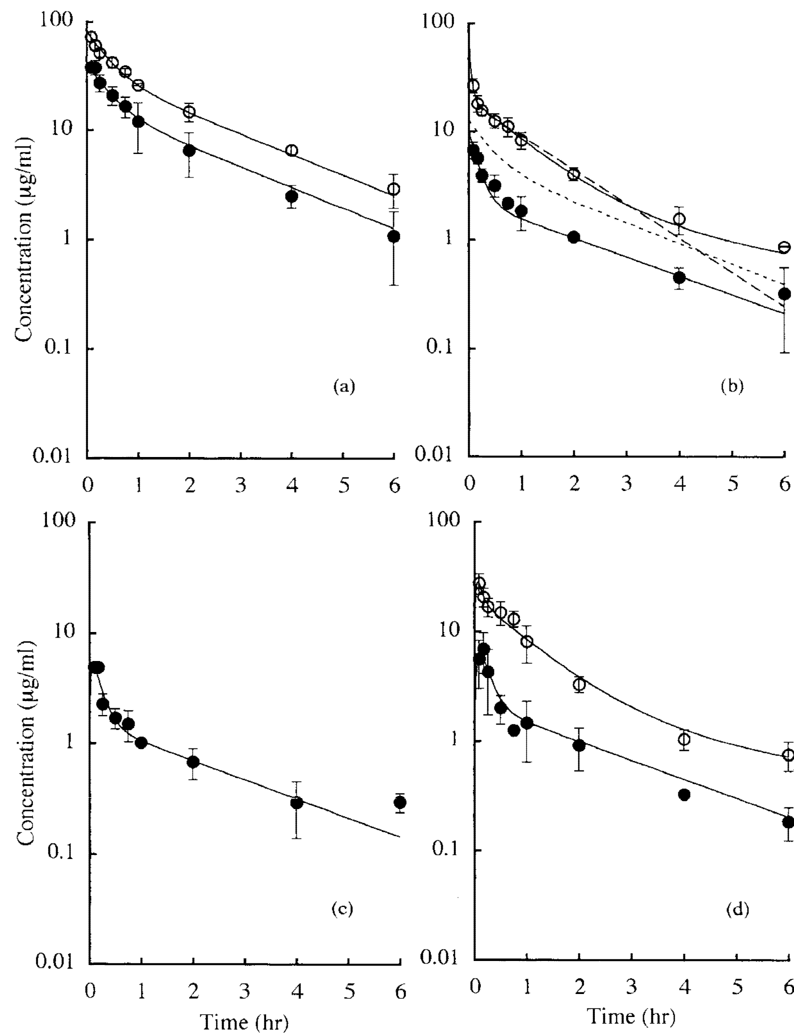

Fig. 2. Time courses of [Cf+b] (a), [Cf] (b), $[C]_{CSF}$ (c) and $[C]_T$ (d) after i.v. administration of VPA 10 mg/kg (●) and 20 mg/kg (○). The plotted points represent the observed data (n = 3). Each experimental point represents the mean ± S.D. The solid lines represent the calculated values using Eqs. 1, 2, 3, 6, and 7 in the text. The dotted line (----) represents the calculated values which were obtained using the constant value of the free fraction of VPA (15%). The broken line (– – – –) represents the calculated values using the two exponential equation (Eq. 2) for [Cf] after the 20 mg/kg VPA dose. The calculated values for $[C]_{CSF}$ and $[C]_T$ which were obtained using the two exponential equation for [Cf] after the 20 mg/kg dose are not shown.

into the CNS appears to be saturable (17). The rapid penetration rate of VPA into the CNS may be mediated in part by active transport systems such as monocarboxylic acid transport. On the other hand, the translocation of VPA from serum to CSF in dogs increases approximately 50% with a decrease in serum pH from 7.4 to 6.9 (18). This result indicates that both active transport and passive diffusion may contribute to the transfer of VPA between serum and CSF. Lucke *et al.,* have investigated the relationship between the free concentration of VPA in blood and the VPA concentration in the extracellular space of the cerebral cortex of rats using VPA selective microelectrodes. They found that the VPA concentration in the extracellular space of the cerebral cortex immediately equilibrated with the blood concentration of VPA after i.v. injection of VPA, and suggested that VPA can freely diffuse through the blood brain barrier (19). However, the brain concentration of VPA in humans is lower than the free concentration of VPA in serum (20). Furthermore, the efflux of VPA from CSF in dogs is decreased by probenecid (18). These lower VPA concentrations in brain are considered to be caused by asymmetric blood brain barrier transport of VPA (21). In the present study, $[C]_{CSF}$ peaked within 5 min after i.v. administration of VPA (Fig. 2c), which is in agreement with the results of Lucke *et al.* (19) and suggests that [Cf] might be able to be freely diffused through the guinea pig blood CSF barrier. Moreover, in the present study, $[C]_{CSF}$ was lower than [Cf], and $[C]_{CSF}$/[Cf] was 0.72 ± 0.25. The value of the efflux constant (K_{OUTCSF}) from CSF to serum was greater than the corresponding value of the influx constant (K_{INCSF}), and the value of the constant ratio K_{INCSF}/K_{OUTCSF} was 0.67 (Table II). These results suggest that the efflux of VPA from CSF is mediated by additional active

Table II. Values of Pharmacokinetic Parameters of VPA in Guinea Pig Obtained from Computer Fitting of [Cf + b], [Cf], $[C]_{CSF}$, and $[C]_T$ After i.v. Administration of VPA

Parameters		10 mg/kg	20 mg/kg
Serum Total [Cf + b][a]			
A	(μg/ml)/(mg/kg)	2.41 ± 2.85	←
B	(μg/ml)/(mg/kg)	1.69 ± 3.24	←
α	(h^{-1})	2.48 ± 0.61	←
β	(h^{-1})	0.43 ± 0.06	←
Serum Free [Cf][b]			
fA	(μg/ml)	8.10 ± 1.58	59.52 ± 31.97
		(8.10 ± 1.80)[c]	(54.97 ± 30.61)[c]
fB	(μg/ml)	2.27 ± 0.38	18.99 ± 1.14
		(2.27 ± 0.45)[c]	(18.81 ± 1.13)[c]
fC	(μg/ml)	—	1.91 ± 0.45
$f\alpha$	(h^{-1})	5.80 ± 1.65	26.66 ± 8.45
		(5.80 ± 1.91)[c]	(22.21 ± 7.90)[c]
$f\beta$	(h^{-1})	0.40 ± 0.07	1.00 ± 0.11
		(0.40 ± 0.08)[c]	(0.72 ± 0.05)[c]
$f\gamma$	(h^{-1})	—	0.17 ± 0.23
CSF $[C]_{CSF}$[b]			
K_{INCSF}	(h^{-1})	21.37 ± 18.27	←
		(21.38 ± 21.53)[c]	
K_{OUTCSF}	(h^{-1})	31.85 ± 26.50	←
		(31.84 ± 31.20)[c]	
V_{CSF}	(ml)	0.18[d]	←
Q_{CSF}	(ml/h)	0.16[d]	←
PA_{CSF}	(ml/h)	3.69[e]	←
CL_{EFFCSF}	(ml/h)	1.89[e]	←
Tears $[C]_T$[b]			
K_{INT}	(h^{-1})	16.44 ± 5.52	←
		(16.44 ± 6.18)[c]	
K_{OUTT}	(h^{-1})	17.47 ± 5.42	←
		(17.44 ± 6.07)[c]	
V_T	(ml)	5.4×10^{-3} [f]	←
Q_T	(ml/h)	15.0×10^{-3} [f]	←
PA_T	(ml/h)	73.8×10^{-3} [g]	←
CL_{EFFT}	(ml/h)	5.6×10^{-3} [g]	←
$[C]_T$ (unionized form)[h]			
K_{INT}	(h^{-1})	2.61 ± 0.32	←
K_{OUTT}	(h^{-1})	17.45 ± 2.21	←
$K_{INT} \times V_T$	(ml/h)	0.01	←

[a] The pharmacokinetic parameters for [Cf + b] were estimated by fitting the data of the serum total concentration of VPA to Eq. 1 using the computer program FKDM. All data express mean ± S.D. of the estimated parameter.

[b] The pharmacokinetic parameters for [Cf], $[C]_{CSF}$ and $[C]_T$ were simultaneously estimated by fitting the data of the serum free, CSF and tears concentration of VPA to Eq. 2, 3, 6, and 7. The values of sum of squares (SS) and Akaike's information criterion (AIC) are as follows; SS = 2.72, AIC = 70.99.

[c] The values of these parameters were obtained using only the two exponential equation (Eq. 2) for serum free concentration of VPA. These pharmacokinetic parameters for [Cf], $[C]_{CSF}$ and $[C]_T$ were simultaneously estimated by fitting the data of the serum free, CSF and tears concentration of VPA to Eq. 2 and 6. The values of SS and AIC are 3.84 and 84.57, respectively.

[d] The values of these parameters were estimated from the allometric relationships between the body weight of animals and the values of CSF volume (rats: 0.15 ml, humans: 65 ml) and CSF flow rate (rats: 2.2 μl/min, humans: 350 μl/min) (14–15).

[e] The values of these parameters were calculated using the following equation: $PA_{CSF} = K_{INCSF} \times V_{CSF} - Q_{CSF}$, $CL_{EFFCSF} = K_{OUTCSF} \times V_{CSF} - Q_{CSF} - PA_{CSF}$

[f] The values of these parameters were estimated from the allometric relationships between the body weight of animals and the values of the tears volume (rabbits: 7.5 μl, humans: 12.4 μl) and the tears flow rate (rabbits: 0.66 μl/min, humans: 2.98 μl/min) (22–24).

[g] The values of these parameters were calculated using the following equation: $PA_T = K_{INT} \times V_T - Q_T$, $CL_{EFFT} = K_{OUTT} \times V_T - Q_T - PA_T$

[h] The values of these parameters were obtained using the assumption that only the unionized form of VPA can diffuse between serum and tears. These pharmacokinetic parameters were estimated by fitting the data to Eq. 8 and 9.

efflux systems. Thus, the translocation of VPA into the CNS appears to be very complicated and the parameter PA_{CSF} may take into account active transport clearances. Further research will be required to clarify the contribution of carrier mediated systems to the transfer of VPA between serum and CSF.

VPA Concentration in Tears $[C]_T$

The time courses of $[C]_T$ after i.v. administration of VPA at 10 or 20 mg/kg were similar to those of [Cf] and are shown in Fig. 2d. $[C]_T$ was practically identical to [Cf], and $[C]_T$/[Cf]

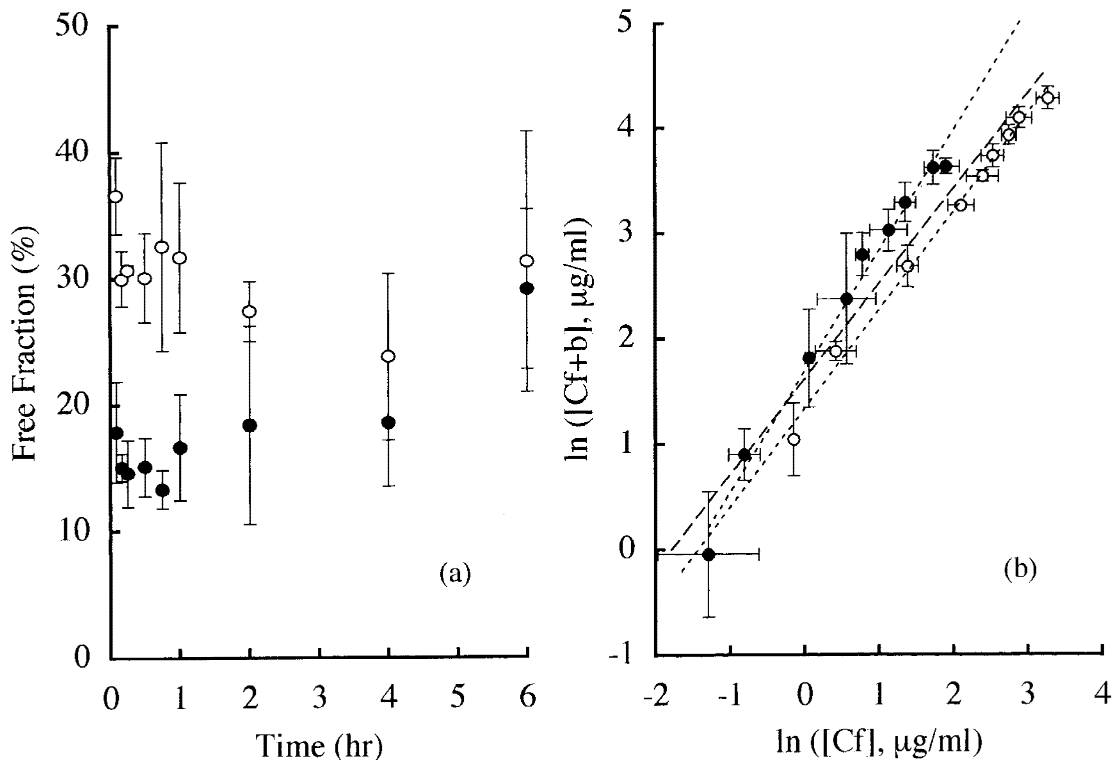

Fig. 3. Time courses of the free fraction of VPA in guinea pig serum (a) and both logarithmic relationships between [Cf] and [Cf+b] (b) after i.v. administration of VPA 10 mg/kg (●) and 20 mg/kg (○). The plotted points represent the observed data (n = 3). Each experimental point represent the mean ± S.D. Although not shown in (a), the free fraction of VPA at 360 min was statistically higher than that at 10, 15, 30, and 45 min after the 10 mg/kg dose, $p < 0.05$, by one-way analysis of variance followed by the Tukey test (11). The broken lines (– – – –) in (b) represent the regression lines. There is statistically significant positive correlation between [Cf] and [Cf+b], $p < 0.05$, by Student's *t*-test (11). The values of the slope, intercept and the correlation coefficient are shown in Table 3. The dotted lines (----) represent the regression lines for the 10 and 20 mg/kg doses. There was a statistically significant difference between the population regression coefficients for the 10 and 20 mg/kg doses for the relationship between [Cf] and [Cf+b].

was 0.94 ± 0.37. The maximum concentration of $[C]_T$ was attained immediately after i.v. administration of VPA. The same basic physiological model for CSF was successfully used to quantitatively describe the time courses of $[C]_T$ (solid lines in Fig. 2d represent the calculated values of $[C]_T$). The pharmacokinetic parameters (K_{INT} and K_{OUTT}) were computed using the nonlinear least squares method, and other parameters (CL_{INT}, CL_{OUTT}, PA_T, and CL_{EFFT}) were calculated using published data (resulting parameters are listed in Table 2), where the values of tear volume (V_T) and tear flow rate (Q_T) were also estimated from an allometric relationship between the weights of animals and the values of V_T and Q_T previously reported for rabbits and humans (22–24).

Haeringen has suggested that the correlation between the free concentration of drugs (acidic and basic drugs) in plasma and the concentration of drugs in tears might be altered by fluctuation in tear pH, and consequently the measurement of drug concentration in tears had been thought an unusual method for TDM (25). The pH of serum and tear were measured using pH indicator paper, and were found to be 7.4 and 8.2, respectively. The pH of tears was consistent with the report of Baeyens and Gurny (26). The theoretical values of the percentages of the unionized forms of VPA in serum and tears were calculated to be 0.158% and 0.025%, respectively. The theoretical value of $[C]_T/[Cf]$ was calculated to be 6.3 by the following equation; $[C]_T/[Cf] = (1 + 10^{pHt-pKa})/(1 + 10^{pHs-pKa})$, tears pH (pHt) = 8.2. This calculated value (6.3) was markedly higher than the observed value (0.94 ± 0.37). These results suggest that the transfer of VPA between serum and tears might not conform to the pH-partition theory.

In order to examine whether the time courses of $[C]_T$ can be quantitatively described using the assumption that only the unionized form of VPA can diffuse between serum and tears, the time courses of $[C]_T$ were analyzed using the following equations.

$$[C]_T = UIF_S/UIF_T\ Z \quad (8)$$

$$[C]_T = UIF_S/UIF_T\ ZZ \quad (9)$$

where UIF_S and UIF_T are the unionized form fractions of VPA in serum and tears, respectively, and Z and ZZ are all the rest of the right-hand sides of Eq. 6 and 7, respectively. The calculated values of $[C]_T$ agreed reasonably well with the observed data (solid lines in Fig. 2d, the same calculated values). However, the value of $K_{INT} \times V_T$ was smaller than that of Q_T (Table 2), and the value of PA_T could not be calculated. These results indicate that the time course of $[C]_T$ after VPA dosing cannot be quantitatively described using the assumption that only the unionized form of VPA can diffuse between serum and tears. From the results of these analyses, we be-

lieve that an additional assumption is necessary to describe the time course of $[C]_T$, such as that both unionized and ionized forms of VPA can diffuse between serum and tears, or that transfer of VPA between serum and tears is mediated by the active transport systems.

Corneal epithelium is covered by a tears film which is composed of three layers, namely a lower mucus layer, a middle aqueous layer and an upper lipid layer. The aqueous layer, which is most of the thickness of the tears film, is produced by an isotonic ultrafiltrate of plasma on the main and accessory lacrimal glands (27). The rate of tears secretion by the lacrimal gland is controlled primarily by parasympathetic innervation and is modulated by sympathetic innervation (27). The production of a primary NaCl rich fluid of tears in the lacrimal acini is believed to be driven by Na^+/K^+-ATPase pump units localized in the acinar cell basolateral plasma membrane (28). Cl^--selective channels are present in the apical membrane and Na^+/H^+ antiporters, Cl^-/HCO_3^--antiporters, K^+-channels and Na^+/K^+-ATPase are present in the basolateral plasma membrane (27). The uptake of N,N-dimethyl-2-phenylethylene into acini cells removed from rabbit lacrimal glands has been shown to be primarily accomplished by a carrier mediated transport system (7). These reports suggest that the transport of VPA from serum to tears is mediated by active transport systems. The collecting portion of tears is composed of the canaliculi, the lacrimal sac and the nasolacrimal duct. Tears enter into the canaliculi through the puncta and then passes into the lacrimal sac and through the nasolacrimal canal. The epithelium of the conjunctiva is continuous with the lacrimal drainage system through the puncta. Horibe *et al.* have demonstrated that an Na^+-dependent monocarboxylate transport process is present on the mucosal side of the pigmented rabbit conjunctiva (8). This result suggests that the transport of VPA from tears to serum on the guinea pig conjunctiva is mediated by the Na^+-dependent monocarboxylate transport systems. Mitra *et al.,* indicated that the unionized and ionized pilocarpine species can permeate the corneal membrane and that the transport of pilocarpinium cations across the lipoidal epithelium may occur as tightly bound ion pairs with dihydrogen phosphate and (or) nitrate counter ions (29). Moreover, a small amount of ionized compounds permeate the paracellular pathway in the corneal membrane layers (30). Thus, the transfer of VPA between serum and tears is very complicated. Although the kinetics of $[C]_T$ were able to be quantitatively described using the simple kinetic model, the resulting parameter PA_T may also take into account active transport clearances. On the other hand, in the present study, the maximum concentration of $[C]_T$ was attained immediately after i.v. administration of VPA (Fig. 2d) and $[C]_T/[Cf]$ was almost 1 (0.94 ± 0.37). These results suggest that the ionized form of VPA is able to diffuse easily between serum and tears. The contribution of carrier mediated transport will be elucidated by clarifying the mechanism for the transfer of VPA between serum and tears.

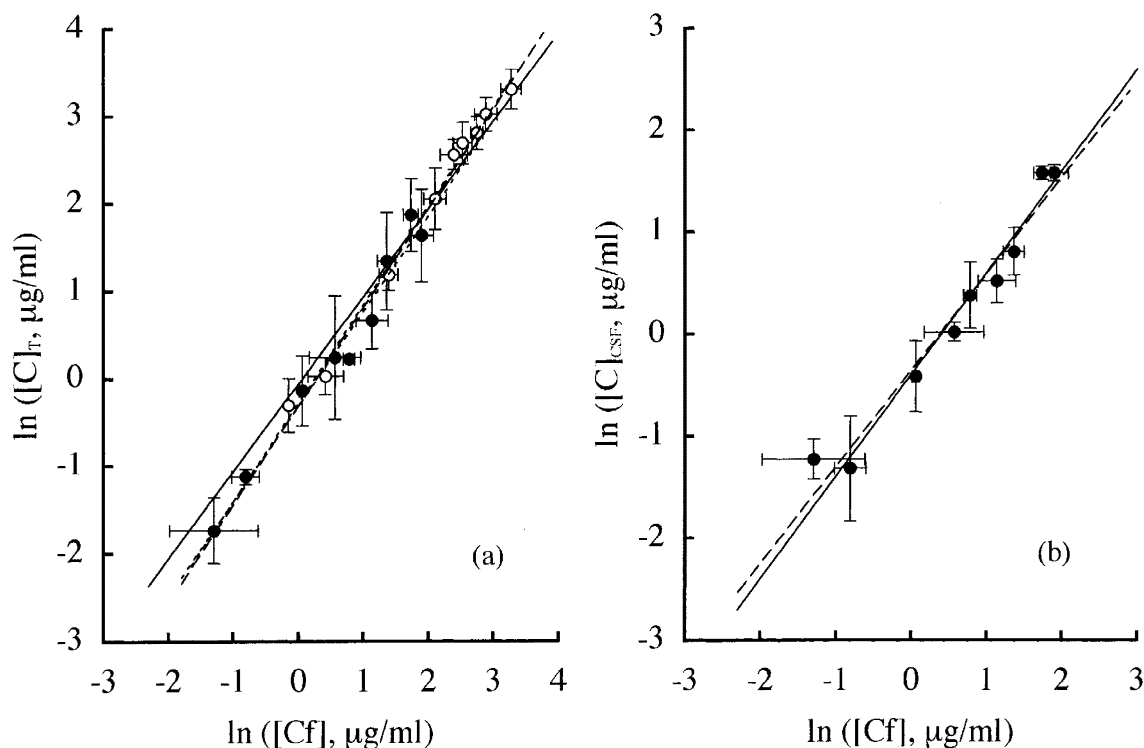

Fig. 4. Both logarithmic relationships between [Cf] and $[C]_T$ (a), and [Cf] and $[C]_{CSF}$ (b) after i.v. administration of VPA 10 (●) and 20 (○) mg/kg. The plotted points represent the observed data (n = 3). Each experimental point represents the mean ± S.D. The broken lines (– – – –) represent the regression lines. There is statistically significant positive correlation between [Cf] and $[C]_T$, and [Cf] and $[C]_{CSF}$, $p < 0.05$, by Student's *t*-test (11). The values of the slope, intercept and the correlation coefficient are shown in Table 3. The dotted lines (----) represent the regression lines for the 10 and 20 mg/kg doses. There was no statistically significant difference between the population regression coefficients for the 10 and 20 mg/kg doses for the relationship between [Cf] and $[C]_T$ (a). The solid lines represent the calculated value using Eq. 10 and 11 in the text. The calculated values were able to describe the correlation between [Cf] and $[C]_T$, and [Cf] and $[C]_{CSF}$.

Relationships Between [Cf] and [Cf+b], [Cf] and $[C]_{CSF}$, and [Cf] and $[C]_T$

Both X and Y logarithmic correlation between [Cf] and [Cf+b], [Cf] and $[C]_{CSF}$, and [Cf] and $[C]_T$ was examined (Fig. 3b and 4). The values of the slope, the intercept and the correlation coefficient are shown in Table III. Figure 3b shows the both logarithmic correlation between [Cf] and [Cf+b]. Although statistically significant positive correlation between [Cf] and [Cf+b] was observed, there was a statistically significant difference between the population regression coefficients of the 10 and 20 mg/kg i.v. administration studies. This difference is thought to be caused by the nonlinear protein binding of VPA affecting the amounts of VPA metabolites. These results indicate that it would be difficult to predict the values of [Cf] from the values of [Cf+b]. On the other hand, there was no statistically significant difference between the population regression coefficients of ln [Cf] and ln $[C]_T$ for both 10 and 20 mg/kg i.v. VPA administration, as shown in Fig. 4a. This result indicates that [Cf] can be predicted by $[C]_T$. Figure 4b shows statistically significant positive correlation between ln [Cf] and ln $[C]_{CSF}$. These results are consistent with the results of Monaco *et al.* (5), indicating that the values of [Cf] and $[C]_{CSF}$ may be able to be predicted by the values of $[C]_T$.

The time courses of $[C]_{CSF}$ and $[C]_T$ could be quantitatively described using the constructed simple kinetic models (Eq. 4 and 5). In these models, $[C]_{CSF}$ and $[C]_T$ at steady state could be represented by the following equations.

$$[C]_{CSF} = K_{INCSF}/K_{OUTCSF} \times [Cf] \quad (10)$$

$$[C]_T = K_{INT}/K_{OUTT} \times [Cf] \quad (11)$$

The calculated values of the constant ratios K_{INCSF}/K_{OUTCSF} and K_{INT}/K_{OUTT} were 0.67 and 0.94, respectively. These values are in good agreement with the corresponding values of the observed $[C]_{CSF}$/[Cf] (0.72 ± 0.25) and $[C]_T$/[Cf] (0.94 ± 0.37). The solid lines in Fig. 4 represent the calculated regression patterns between [Cf] and $[C]_T$, and [Cf] and $[C]_{CSF}$ using Eq. 10 and 11. These results indicate that [Cf] and $[C]_{CSF}$ at steady state can be predicted by $[C]_T$ using the resulting pharmacokinetic parameters. Thus, the measurement of $[C]_T$, which requires non-invasive collection of tears, may be a very useful method for TDM, since the values of [Cf] and $[C]_{CSF}$ could be predicted by the values of $[C]_T$ using the resulting pharmacokinetic parameters.

Table III. Values of Slope, Intercept and Correlation Coefficient for Both Logarithmic Relationships Between [Cf] and [Cf + b], [Cf] and $[C]_T$, and [Cf] and $[C]_{CSF}$

	Slope	Intercept	Correlation coefficient
[Cf] *Vs* [Cf + b]	0.90	1.62	0.93[a]
10 mg/kg	1.15	1.69	0.99[b]
20 mg/kg	0.93	1.34	0.99[b]
[Cf] *Vs* $[C]_T$	1.13	–0.31	0.99[a]
10 mg/kg	1.09	–0.32	0.97[c]
20 mg/kg	1.13	–0.28	0.99[c]
[Cf] *Vs* $[C]_{CSF}$	0.95	–0.36	0.96[a]

[a] Statistically significant positive correlation between [Cf] and [Cf + b] or [Cf] and $[C]_T$ or [Cf] and $[C]_{CSF}$.

[b] Statistically significant difference between two population regression coefficients for 10 and 20 mg/kg dose studies, $p < 0.05$, by Student's *t*-test.

[c] No statistically significant difference between two population regression coefficients for 10 and 20 mg/kg dose studies, $p < 0.05$, by Student's *t*-test.

CONCLUSIONS

The time courses of [Cf+b], [Cf], $[C]_{CSF}$, and $[C]_T$ in guinea pig after i.v. administration of VPA were quantitatively described using a basic physiological model. Our results also suggest that the transfer of VPA between serum and CSF, and serum and tears is mediated by active transport systems. There were statistically significant positive correlations between [Cf] and $[C]_T$, and [Cf] and $[C]_{CSF}$. These correlations were corresponded with the results of Monaco *et al.* (5). The values of [Cf] and $[C]_{CSF}$ at steady state can be predicted by the values of $[C]_T$ using the resulting pharmacokinetic parameters. These results indicate that the measurement of $[C]_T$, using tears which can be collected non-invasively, and the estimation of pharmacokinetic parameters for $[C]_T$ might be a very useful method for TDM of VPA.

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