Analysis of Tissue Distribution of Valproate in Guinea-Pigs: Evidence for Its Capacity-Limited Tissue Distribution

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Received March 28, 1994; accepted October 12, 1994

The tissue distribution of valproic acid (VPA) was investigated over a wide range of steady-state plasma levels (C_{ss}) in guinea-pigs. The VPA concentrations in various tissues, except the kidney, were all lower than in plasma. Tissue-to-unbound plasma concentration ratios (K_{pu}) of VPA for adipose, heart, kidney, liver, lung, muscle, pancreas and skin all decreased significantly with increasing unbound plasma concentration (C_{uss}). The K_{pu} for brain (0.5–0.9), intestine, spleen and stomach failed to show significant change with C_{uss}. The disposition of VPA in tissues is adequately described by a model in which VPA was distributed in interstitial and intracellular fluid and bound to interstitial albumin, with limited tissue binding. Tissue binding was extensive only in the kidney. Most of the measured apparent K_{pu} values agreed well with simulated K_{pu} values. Steady-state tissue concentration of VPA can be predicted from C_{ss} and C_{uss} when reference data for interstitial albumin and tissue total water are available.

KEY WORDS: valproate; pharmacokinetic tissue model; tissue distribution; tissue-to-plasma partition; tissue binding.

INTRODUCTION

The anticonvulsant valproic acid (VPA) is highly bound to plasma proteins, predominately albumin. Plasma protein binding can affect tissue distribution (1). The proportional relationship between tissue- and plasma-concentrations may not always be linear, especially when plasma protein binding of a drug is nonlinear. Nonlinear (2–4) and variable binding (5) of VPA to plasma has been reported, and tissue binding was proposed to be concentration-dependent, based on pharmacokinetic analysis of plasma concentration data (6). However, tissue concentrations resulting from nonlinear plasma protein binding of VPA had not been reported.

In this study we investigated the partition of VPA into various tissues at different steady-state plasma levels to determine the relationship between tissue and unbound plasma concentrations of VPA, and to estimate the tissue binding of VPA. The results were fitted to a model describing the characteristics of VPA tissue distribution for predicting tissue concentrations from plasma VPA concentration.

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MATERIALS AND METHODS

Animals and Treatment

Male adult guinea-pigs (Experimental Animal Center, College of Medicine, National Taiwan University), body weight 230–300 g, were dosed with VPA by intravenous (i.v.) bolus injection followed by constant infusions to achieve steady-state plasma concentrations. The infusion doses ranged from 50 to 2000 ug/min/kg. Entero-hepatic recycling of VPA (7) was excluded by bile duct cannula. At steady-states, blood samples were collected from the carotid artery cannula and immediately analyzed for total (C_{ss}) and unbound (C_{uss}) VPA. Animals were then sacrificed by exsanguination, and tissues were isolated and homogenized with an equal part of normal saline to determine VPA concentration.

Determination of Drug Concentrations

The concentrations of VPA were determined by gas chromatography (GC) (8). The plasma unbound drug was separated by a CF25 Centriflo Membrane Cone (Amicon, Lexington, MA) (8). The contamination of capillary blood VPA in tissue homogenates was corrected as reported elsewhere (9).

Calculation

K_p or K_{pu} value of a drug is defined as the ratio of tissue drug concentration (C_T) to total- (for K_p) or unbound- (for K_{pu}) drug concentration in plasma of the blood flowing out of tissue. Since VPA is almost totally eliminated from the body by hepatic metabolism, the apparent C_{ss} and C_{uss} were applied in calculating the K_p and the K_{pu} for all tissues except the liver, where the difference in drug concentration between flow-in and flow-out plasma was corrected by using hepatic blood flow rate Q = 51.7 ml/min/kg (10), blood-to-plasma concentration ratio (R), apparent clearance CL = dose/Css and plasma unbound fraction $f_u = C_{uss}/C_{ss}$ as follows:

$$K_p,\text{corrected} = K_p,\text{apparent} \times Q \times R/(Q \times R - CL) \quad \text{eq. 1}$$

$$K_{pu},\text{corrected} = K_p,\text{corrected}/f_u \quad \text{eq. 2}$$

Model Fitting

Three models were considered in describing the disposition of VPA in a tissue (Fig. 1).

A. Tissue drug distribution is only in the interstitial space, and can bind only with albumin in this space (11), then the homogenized total tissue concentration (C_T) at steady-state can be expressed by equation 3.

$$C_T = (C_u + C_b \times AR) \times IS \quad \text{eq. 3}$$

$$K_{pu} = C_T/C_u = [1 + (C_b/C_u) \times AR] \times IS \quad \text{eq. 4}$$

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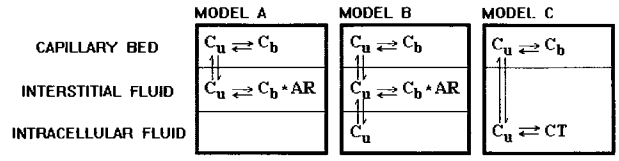

Fig. 1. Tissue cell models for the description of the tissue distribution of valproate. C_u and C_b represent unbound and bound concentrations in plasma, AR, interstitial-to-plasma albumin ratio, CT, bound concentration in tissue, respectively.

where C_u, C_b, AR and IS represent plasma-unbound and plasma-bound drug concentrations, interstitial-to-plasma albumin ratio and interstitial space in terms of tissue volume fraction, respectively. Theoretically, eq. 3 can be derived to be a single function of C_u by replacing Cb with C_u and binding parameters. However, apparent C_b was used for model simulation because the plasma protein binding of VPA after continuous infusion of high dose does not follow the simple binding theory (3).

B. The drug distribution in tissue is present in the tissue fluid (including interstitial fluid) without substantial tissue binding (12), as expressed by the following equations.

$$C_T = TW \times C_u + C_b \times AR \times IS \qquad \text{eq. 5}$$

$$K_{pu} = C_T/C_u = (C_b/C_u) \times AR \times IS + TW \qquad \text{eq. 6}$$

where TW is the tissue total (including tissue interstitial and intracellular) fluid fraction.

C. The tissue drug concentration includes the unbound in the tissue total fluid and the tissue-bound drug (13). The C_T and K_{pu} are then expressed as follows.

$$C_T = T_{max} \times C_u/(K_{dt} + C_u) + VF \times C_u \qquad \text{eq. 7}$$

$$K_{pu} = T_{max}/(K_{dt} + C_u) + VF \qquad \text{eq. 8}$$

where T_{max} is the theoretical maximum capacity of the tissue binding, K_{dt} is the dissociation constant of the binding, and VF is the tissue volume fraction where unbound drug exists. If $K_{dt} <<< C_u$, then eqs. 7 and 8 can be simplified to eqs. 9 and 10.

$$C_T = T_{max} + VF \times C_u \qquad \text{eq. 9}$$

$$K_{pu} = T_{max}/C_u + VF \qquad \text{eq. 10}$$

Simulation was performed by a PCNONLIN (14). The literature data of AR, IS (15) and TW (12) of rat were applied under the assumption that these physiological values are equivalent between guinea-pigs and rats. Coefficients of correlation and AIC (16) values were considered in judging model suitability between eqs. 8 and 10.

RESULTS

The K_p (Table I) values of VPA demonstrated that the tissue concentrations of VPA were all lower than plasma concentration ($K_p < 1$), except the kidney, at the low plasma concentration region. The K_{pu} values of VPA (Table II) for kidney and liver decreased significantly with increasing C_{uss}. For the other tissues, except brain, the K_{pu} values were nearly constant over a wide C_{uss} range with an increase only at the lowest C_{uss} region; and the increase was significant for adipose, heart, lung and muscle. It is worthy of note that the K_{pu}, but not the K_p value of VPA for brain, the target organ, was not significantly changed over a wide plasma concentration range. This result indicated that the brain concentration of VPA was in a constant ratio to the unbound, but not to the total, plasma concentrations.

The apparent K_{pu} values for all the tissues studied agreed fairly with the simulated K_{pu} values based on model B or C. Higher apparent than simulated K_{pu} values were observed in kidney, as well as in heart, liver and lung at the low C_{uss} region. The simulated K_{pu} versus C_{uss} for some tissues is illustrated in Fig. 2.

To estimate the apparent tissue binding parameters of VPA, experimental data of C_u and K_{pu} were fitted to eqs. 8 and 10. A pilot fitting of eq. 8 to the experimental data obtained a very low K_{dt} value (less than 0.6 ug/ml), a much higher AIC value (higher than 41.0) than fitting to eq. 10, and a poor correlation of coefficient for each of the tissues, except kidney. Consequently, eq. 10 was selected for estimation of VPA tissue binding. The results are presented in Table III. The T_{max} of all the tissues except kidney was less than 7.5 ug/ml, and the VF values for each tissue thus obtained were comparable to the TW value of rats (12).

DISCUSSION

According to the equations of the three models in which K_{pu} is a function of C_b/C_u or $1/C_u$, the nonlinear plasma protein (albumin) binding of a drug should cause the K_{pu} to decrease with increasing plasma concentration. However, the K_{pu} of VPA for the brain was not significantly decreased, but remained rather constant with increasing C_{uss}. The brain contains cerebrospinal fluid (CSF) instead of ISF; and the CSF contains so little protein (30 mg/100 ml) (17) that albumin, and thus its drug binding, is tenuous. Consequently, the VPA concentration in brain should be in a constant proportion to C_{uss} when brain-binding of the drug is not detectable. Either the VF value (0.68) estimated by eq. 9 or the apparent K_{pu} value (0.5–0.9) is comparable to the TW of brain in humans, rats and other animals (0.75–0.78) (18). This result implies that the distribution of VPA in brain can be described by any equation among eqs. 3 to 10, with the C_b or T_{max} equal to zero. Therefore, the consistent K_{pu} value with TW for brain obtained from the present experiment is reasonable. The result also suggests that application of physiological data between different animals for pharmacokinetic model simulations is feasible.

The higher plasma than tissue concentration of VPA for all tissues (except kidney) over the wide plasma concentration range studied may attribute to high plasma protein binding, low tissue binding and/or restricted localization of the drug within tissues. Theoretically, the unbound drug concentration should be equilibrated over all the body fluid. Nevertheless, a lower apparent tissue (homogenate) total concentration than (plasma) unbound concentration of VPA was

Table I. The Tissue-to-Plasma Concentration Ratio (K_p) of Valproate at Steady-State in Guinea-Pigs

Dose μg/min/kg	50	100	200	500	640	1280	2000	
C_{ss}, μg/ml	11 ± 2.6	28 ± 3.8	88 ± 7.3	219 ± 16	345 ± 53	659 ± 52	1303 ± 123	
	K_p							F(ANOVA)
Blood	0.82 ± 0.06	0.73 ± 0.12	0.73 ± 0.05	0.77 ± 0.03	0.92 ± 0.12	0.80 ± 0.07	0.93 ± 0.01	
Adipose	0.69 ± 0.12	0.33 ± 0.08	0.23 ± 0.06	0.21 ± 0.01	0.31 ± 0.05	0.40 ± 0.05	0.34 ± 0.03	5.06
Brain	0.18 ± 0.04	0.35 ± 0.04	0.51 ± 0.02	0.21 ± 0.04	0.14 ± 0.03	0.70 ± 0.07	0.50 ± 0.07	20.01
Heart	0.58 ± 0.06	0.72 ± 0.20	0.48 ± 0.12	0.41 ± 0.03	0.54 ± 0.09	0.75 ± 0.12	0.70 ± 0.06	6.04
Intestine	0.29 ± 0.08	0.21 ± 0.04	0.23 ± 0.07	0.39 ± 0.05	0.47 ± 0.04	0.72 ± 0.07	0.57 ± 0.08	9.04
Kidney	3.34 ± 0.58	2.10 ± 0.22	0.81 ± 0.16	0.65 ± 0.10	0.60 ± 0.07	0.77 ± 0.11	0.61 ± 0.06	18.22
Liver	0.91 ± 0.14	0.49 ± 0.11	0.30 ± 0.07	0.42 ± 0.03	0.50 ± 0.05	0.63 ± 0.10	0.66 ± 0.06	5.45
Lung	0.73 ± 0.16	0.40 ± 0.06	0.57 ± 0.57	0.48 ± 0.11	0.30 ± 0.06	0.86 ± 0.09	0.98 ± 0.17	4.98
Muscle	0.30 ± 0.07	0.36 ± 0.05	0.33 ± 0.07	0.35 ± 0.02	0.41 ± 0.03	0.56 ± 0.05	0.43 ± 0.08	2.30 NS
Pancreas	0.45 ± 0.14	0.44 ± 0.13	0.23 ± 0.06	0.36 ± 0.02	0.44 ± 0.04	0.57 ± 0.09	0.59 ± 0.07	1.97 NS
Skin	0.44 ± 0.12	0.34 ± 0.03	0.22 ± 0.05	0.30 ± 0.02	0.35 ± 0.03	0.50 ± 0.05	0.41 ± 0.06	2.30 NS
Spleen	0.43 ± 0.10	0.23 ± 0.05	0.30 ± 0.10	0.41 ± 0.06	0.52 ± 0.11	0.60 ± 0.10	0.47 ± 0.08	2.15 NS
Stomach	0.45 ± 0.06	0.31 ± 0.06	0.25 ± 0.06	0.40 ± 0.03	0.54 ± 0.05	0.66 ± 0.07	0.57 ± 0.06	4.55

Data are means ± SE of five determinations.
F(ANOVA), df1 = 6, df2 = 28, F(0.05) = 2.44, F(0.01) = 3.53.
NS, Not significantly different.

found, in contradiction to the pharmacokinetic fundamental concept. Such a result could occur when tissue binding is minimal, and the unbound drug is present only in tissue water, which could become diluted by non-water tissue fraction upon the homogenizing of a whole organ.

Model C is a modification of model B. The only difference is that the ISF in model B is included in tissue-as-a-whole in model C. In model B the binding of a drug by interstitial albumin and by substantial tissue cells can be clearly discriminated. Model C is a direct fitting of the experimental data without extra factors of AR and IS in the calculation, and thus shows the best agreement, but the tissue binding data thus obtained include albumin binding in ISF.

The VF values for each tissue obtained from eq. 10 are comparable to that of TW value of rat (12), which confirms that the VF value represents the TW of guinea-pigs. The fitness of apparent K_{pu} values to both models B and C de-

Table II. The Tissue-to-Unbound Plasma Concentration Ratio (K_{pu}) of Valproate at Steady-State in Guinea-Pigs

Dose μg/min/kg	A 50	B 100	C 200	D 500	E 640	F 1280	G 2000	
Cuss μg/ml	2.9 ± 0.7	7.4 ± 1.5	30 ± 5	116 ± 14	192 ± 40	561 ± 31	1230 ± 115	
	K_{pu}							F(ANOVA)
Adipose	2.6[a] ± 0.4	1.2 ± 0.2	0.60 ± 0.12	0.39 ± 0.03	0.50 ± 0.02	0.45 ± 0.03	0.36 ± 0.04	20.3
Brain	0.66 ± 0.11	0.89 ± 0.25	0.58 ± 0.05	0.67 ± 0.09	0.90 ± 0.10	0.81 ± 0.09	0.51 ± 0.05	2.37 NS
Heart	2.4[a] ± 0.5	2.8[b] ± 0.2	1.3 ± 0.2	0.78 ± 0.10	0.90 ± 0.08	0.88 ± 0.12	0.73 ± 0.07	13.56
Intestine	1.0 ± 0.3	0.87 ± 0.21	0.63 ± 0.11	0.72 ± 0.06	0.83 ± 0.10	0.84 ± 0.08	0.56 ± 0.03	1.80 NS
Kidney	15[a] ± 5	7.5 ± 0.8	1.9 ± 0.3	1.2 ± 0.1	1.0 ± 0.09	0.88 ± 0.07	0.61 ± 0.04	8.79
Liver	6.5[a] ± 0.6	3.3[a] ± 0.6	1.0 ± 0.1	0.89 ± 0.05	0.95 ± 0.07	0.77 ± 0.09	0.65 ± 0.06	30.72
Lung	2.6[a] ± 0.2	1.9[a] ± 0.4	0.86 ± 0.13	0.76 ± 0.10	0.97 ± 0.11	0.99 ± 0.08	0.79 ± 0.04	30.74
Muscle	1.1[b] ± 0.2	1.2[b] ± 0.2	0.91 ± 0.15	0.67 ± 0.05	0.72 ± 0.09	0.64 ± 0.03	0.45 ± 0.03	4.37
Pancreas	1.6 ± 0.3	1.6 ± 0.3	0.63 ± 0.09	0.67 ± 0.03	0.76 ± 0.07	0.64 ± 0.07	0.61 ± 0.06	2.84
Skin	1.7 ± 0.5	1.3 ± 0.2	0.60 ± 0.07	0.57 ± 0.05	0.60 ± 0.04	0.56 ± 0.03	0.41 ± 0.04	3.85
Spleen	1.6 ± 0.2	0.9 ± 0.1	0.78 ± 0.14	0.76 ± 0.09	0.86 ± 0.07	0.77 ± 0.11	0.46 ± 0.02	1.76 NS
Stomach	1.9 ± 0.6	1.2 ± 0.2	0.68 ± 0.12	0.74 ± 0.06	0.93 ± 0.10	0.76 ± 0.06	0.62 ± 0.06	2.15 NS

[a] Significantly different $p < 0.05$) from other groups by Sheffe test.
[b] Significantly different ($p < 0.05$) from group G by Sheffe test.
Data are mean ± SE of five determinations.
F(ANOVA), df1 = 6, df2 = 28, F(0.05) = 2.44, F(0.01) = 3.53.
SHEFFE: 0.05F(6,28) = 2.44; R = 3.826
NS, Not significantly different.

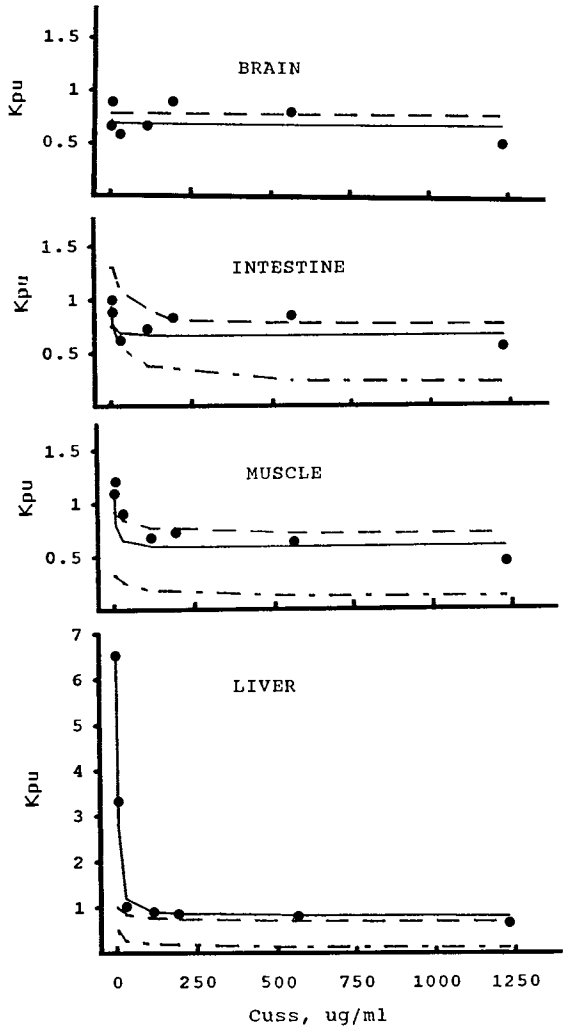

Fig. 2. The theoretical K_{pu} values based on model A (—·—), B (– – –) and C (——), and the apparent K_{pu} (●); each point represents the mean of five experiments. SD not shown.

scribed the same characteristics of VPA tissue distributions: VPA in tissue is mainly in ISF and tissue water.

VPA is an organic anion. Ligandin, an important anion-binding protein in tissues (19), is found in rat liver and kidney tubule cells, but has not been detected in plasma and the brain (20). The binding of VPA in liver and kidney, as observed from this study, may be partly ascribed to intracellular ligandin. This hypothesis requires further investigation.

In conclusion, tissue distribution of valproate is mainly in unbound form in tissue fluid. The concentration of VPA in each tissue can be adequately predicted from total and unbound plasma concentration when physiological data of ISF and tissue water are available for reference.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Y. Sugiyama, University of Tokyo, Japan, for his valuable discussion of this article. Laboratory assistance by technician Miss M. S. Wu is appreciated. Sodium valproate crystalline powder was a generous gift from Sanofi, France.

Table III. Tissue Binding Parameters[1] of VPA in Guinea-Pigs

	T_{max} (μg/ml)	VF	r	TW[2]
Adipose	0.45 ± 0.19	0.39 ± 0.03	0.998	
Brain	0.06 ± 0.68	0.68 ± 0.10	0.054	0.78[3]
Heart	4.3 ± 0.62	0.86 ± 0.09	0.951	0.77
Intestine	0.80 ± 0.63	0.66 ± 0.09	0.488	0.75
Kidney	41 ± 0.7	0.68 ± 0.09	0.999	0.78
Liver	7.5 ± 0.57	0.63 ± 0.08	0.986	0.70
Lung	4.9 ± 0.7	0.78 ± 0.10	0.948	0.78
Muscle	1.6 ± 0.4	0.58 ± 0.06	0.853	0.71
Pancreas	2.2 ± 0.6	0.59 ± 0.08	0.905	
Skin	3.3 ± 0.5	0.48 ± 0.06	0.956	0.60
Spleen	2.5 ± 0.6	0.65 ± 0.08	0.891	
Stomach	3.1 ± 0.6	0.68 ± 0.09	0.911	

[1] Estimated by eq. 10, $K_{pu} = T_{max}/C_u + VF$, with SE of linear fitting.
[2] Ref. 12 (except brain).
[3] Ref. 18.
r, coefficient of correlation.

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