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Physiologically based pharmacokinetics of valproic acid in rabbits

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Summary

Protein-binding parameters of valproic acid (VPA) in rabbit serum were determined. Due to the non-linear binding, the binding percentage decreased from 91 to 41% when the serum concentration of VPA rose from 10 to 1000 μg/ml. The hepatic clearance of VPA as unbound drug followed Michaelis-Menten kinetics. A physiologically based pharmacokinetics model was adopted to interpret the overall disposition of VPA in rabbits which incorporated the non-linear plasma protein binding and non-linear intrinsic hepatic clearance. The predicted plasma and tissue concentrations were found to be in good agreement with the observed concentrations. The reason why the plasma concentration versus time curves appear to be apparently parallel in spite of remarkable changes in the unbound concentration among three different doses could be explained by the association effects in which an increase in the total plasma concentration may produce a decrease in intrinsic hepatic clearance consistent with Michaelis-Menten kinetics resulting in an increase in the unbound fraction of VPA in plasma.

Introduction

Valproic acid (VPA) is a fatty acid with a simple branched chain and is used clinically in the treatment of both generalized and partial seizures, particularly in children (Simon and Penny, 1975).

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Several studies on the pharmacokinetics of VPA have been reported in man (Klotz and Antonin, 1977; Klotz et al., 1978; Gugler and Unruch, 1980) and animals (Lösher, 1977; Lösher and Esenwein, 1978; Viswanathan and Levy, 1981). Gram et al. (1979) found that the protein binding of VPA was reduced at high therapeutic concentrations in humans. In addition, Dickinson et al. reported that the elimination behavior in the liver exhibited a dose-dependency in the rat (1979) and monkey (1980). However, few reports have dealt kinetically with how the non-linearity of the plasma protein binding and non-linearity of the elimination process of VPA mutually affect the overall disposition of the drug in the body.

In recent years, anatomically and physiologically realistic pharmacokinetic models have been developed (Bischoff et al., 1971; Harrison and Gibaldi, 1977; Chen et al., 1978; Igari et al., 1982; Tsuji et al., 1983; Ichimura et al., 1984) and these have made it possible to predict the concentration in the target region, other tissues or organs as well as plasma or blood. The present study concerns a physiologically based pharmacokinetic model and its use to interpret the overall disposition of VPA in rabbits including the non-linear elimination process in the liver and non-linear plasma protein binding.

Materials and Methods

Materials

Sodium valproate was kindly supplied by Kyowa Hakko (Tokyo). All other chemicals were of reagent grade and were used without further purification.

Animals

Male albino rabbits weighing between 2.7 and 3.3 kg were housed in a well-ventilated room and maintained on a normal laboratory animal diet and water ad libitum. The animals were fasted for 12 h prior to the experiments, but water was given freely.

Intravenous bolus dose studies

Rabbits were catheterized into the femoral artery under light ether anesthesia. After recovery from the anesthesia, the animals were injected for 30 s through the ear vein with various doses of sodium valproate dissolved in the appropriate volume of physiological saline.

After the intravenous injection, 1-ml blood samples were collected from the arterial cannula at designated times into centrifuge tubes. The blood was centrifuged and the plasma was separated for GLC assay.

At 0.5, 2, 4 and 6 h after the administration of VPA, the animals were sacrificed by injection of saturated KCl solution into the femoral artery. Each tissue was immediately excised, rinsed with physiological saline, blotted, and weighed. The visceral organs except the skin and bone were homogenized with a 2–4-fold excess of physiological saline in a grinder.

Sections of the skin and bone from the femoral region were cut into small pieces

and crushed, respectively. One gram of these tissues was then extracted with 10 ml of physiological saline by shaking for over 12 h. The tissue samples were assayed as described below.

Determination of urinary and biliary recoveries

Rabbits were catheterized into the femoral artery as described above. Under light ether anesthesia, a midline abdominal incision was made, the bile duct and the urinary bladder were cannulated with polyethylene tubes, and the incision was closed with surgical adhesive. After bolus injection of 300 mg of NaVPA per kg, bile was collected into glass tubes at appropriate intervals until 24 h and urine was collected continuously for 24 h. Samples of the bile and urine were assayed, and the cumulative amount of excreted drug was determined.

Serum protein binding experiments

Equilibrium dialysis studies were performed to estimate the kinetic parameters for serum protein binding with an acrylic resin plate apparatus having eight compartments of depth 5 mm and diameter 25 mm. Two plates were joined tightly, holding a cellulose membrane between them.

One ml of rabbit serum was placed into one compartment and the same volume of pH 7.4 isotonic phosphate buffer containing a wide range (0.8–4000 μg/ml) of NaVPA concentrations was placed into the other compartment. The plates were incubated for 4 h at 37°C. The VPA concentrations in both compartments were measured. Equilibration was established within 3 h.

Albumin concentration in serum was determined by colorimetric assay with bromcresol green using a commercially available reagent.

Intravenous infusion studies and determination of hepatic clearance

Animals were catheterized into the femoral artery and were cannulated into the bile duct and the urinary bladder under anesthesia. Intravenous infusions were performed to achieve appropriate steady-state plasma concentration levels and to estimate the hepatic clearance of VPA at steady-state. An intravenous bolus loading dose of VPA was administered via the ear vein and was followed immediately by constant-rate infusion of VPA into the opposite site. The intravenous bolus dose and infusion rate were calculated from preliminary experiments because of the concentration-dependent elimination of VPA in the liver. Two hours after the infusion was started, blood samples were collected every 30 min. The plasma concentration of VPA was immediately monitored by GLC assay to confirm whether the steady-state concentration designed was achieved or not. After establishment of the steady-state, the plasma concentration of VPA at steady-state was determined as a mean value of two or three samples collected for all collections after 30 min. The bile and urine were collected for appropriate intervals in the steady-state.

The intrinsic hepatic clearance was approximated by the value obtained from the ratio of the infusion rate and unbound arterial plasma concentration at steady-state. In this calculation, the unbound concentration of VPA at steady-state was obtained using the total plasma concentration and the parameters estimated from in vitro serum protein binding studies.

Distribution of VPA into red blood cells

The ratio of the blood concentration to plasma concentration of VPA was determined in vitro. After administration of heparin at a dose of 0.1 ml/100 g body weight (100 units), the whole blood was collected via the femoral artery. Aliquots (0.1 ml) of isotonic buffer solution containing various amounts of VPA were added to 5 ml of whole blood. The samples were incubated with shaking (very slowly) for 30 min at 37°C. The concentrations of VPA in the plasma after separation by centrifugation and in the whole blood were then assayed.

Analytical procedures

The VPA in biological fluid was assayed by a GLC method using FID detection. For assay of the VPA in plasma or serum, blood, urine and bile, an aliquot (0.2 ml) of the biological samples was added to a centrifuge tube (10 ml) containing 0.4 ml of 2 N HCl solution and 0.2 ml of chloroform which contained diphenyl as an internal standard. The mixture was shaken with a mixer for 1 min, centrifuged and 5–15 μl of the chloroform layer was injected into a gas chromatograph. For determination of the content in visceral organs except the skin and bone, an aliquot (1 ml) of homogenized tissues was added to a centrifuge tube (50 ml) containing 1 ml of 0.25 M NaH_2PO_4 buffer and 20 ml of 1-chlorbutane. The mixture was shaken for 10 min and centrifuged at 4000 rpm for 20 min. Then, 15 ml of the organic layer was shaken with 3 ml of 0.5 M NaOH and centrifuged, and the organic solvent was aspirated off. Two ml of the alkaline extract was pipetted into a 10-ml tube containing 2 ml of aqueous solution (4 M NaCl–1 M HCl) and 0.2 ml of chloroform which contained nonanic acid as an internal standard. The mixture was treated in the same manner as described above. For skin and bone, the solution extracted with physiological saline was added to a tube containing 1 ml of 2 N HCl and 25 ml of chlorbutane. The same procedures described above were then followed. Calibration curves were obtained by the same method for each biological sample. The detection limit of this method was 0.2 μg/ml for the biological samples except tissues and 0.2 μg/g for tissues.

GLC method

The gas chromatograph (Model GC-7AG, Shimadzu, Japan) was equipped with a flame ionization detecter and a 2.1 m × 3 mm glass column packed with DEGA on 60–80 mesh Chromosorb W for fluid samples and with Termon-1000 ($+H_3PO_4$: 5 + 0.5%) on 80–100 mesh Chromosorb W for tissue samples. Gas chromatography was carried out under the following conditions: temperature of injection ports, 230°C; column oven temperature, 190°C; and nitrogen flow rate, 80 ml/min.

Results and Discussion

Serum protein binding of VPA

Non-linear serum protein binding was observed for VPA as shown in Fig. 1. The binding percentages decreased from 91 to 41% with increases in the total VPA concentration in the serum from 10 to 1000 μg/ml.

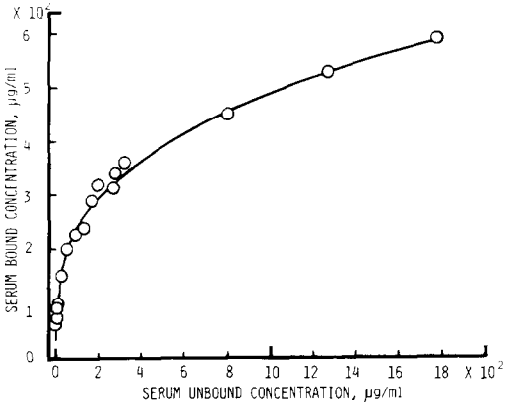


Fig. 1. Serum protein binding profiles for VPA in rabbits as a function of the postdialysis bound and unbound VPA concentrations at 37°C. The points represent experimental values, and the solid line was generated from Eqn. 1.

As shown in Fig. 2, the Scatchard plots of the binding data suggested two independent classes of binding site. The results were then analyzed according to the following equation (see Appendix I),

$$C_{p,b} = \frac{n_1 K_1 P C_{p,f}}{1 + K_1 C_{p,f}} + \frac{n_2 K_2 P C_{p,f}}{1 + K_2 C_{p,f}} \quad (1)$$

All parameters given in Table 1 were estimated by the non-linear least-squares method using a NONLIN program (Metzler, 1969). As a result, it was shown that the binding behavior of VPA could be explained by the non-linear binding equation (Eqn. 1).

Löscher (1977) demonstrated that the serum protein binding ratio of VPA varied

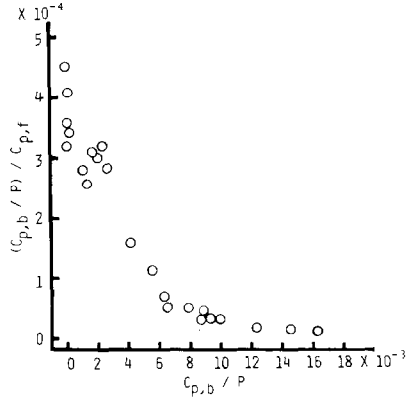


Fig. 2. Scatchard plots of data for the binding of VPA to serum protein.

TABLE 1

PARAMETERS [a] OF SERUM PROTEIN BINDING OF VPA IN RABBITS

Parameter	Estimated value
n_1	3.14
$K_1 \times 10^{-3}$, M^{-1}	8.63
n_2	7.90
$K_2 \times 10^{-3}$, M^{-1}	0.11

[a] An albumin concentration of 5.0×10^{-4} M was used for parameter estimation. Each point represents the mean value estimated by the non-linear least-squares method.

with the species of animal and decreased significantly at higher concentrations in the dog. Gram et al. (1979) also reported that the serum protein binding ratio of VPA was reduced at high therapeutic concentrations in man. It is generally considered that such binding behaviors of VPA remarkably affect the organ clearance (Wilkinson and Shand, 1975; Shand et al., 1976) and the tissue distribution (McNamara et al., 1979) in several species of animals and in man.

Intravenous bolus and infusion administration

Time courses of the cumulative amounts of VPA excreted into the urine and bile in rabbits given a 300 mg/kg i.v. dose of VPA are shown in Fig. 3. Only less than 3% of the administered dose was excreted into the urine and bile until 24 h. Thus, in rabbits, renal and bile excretion appears not to represent the main elimination route of VPA. A similar result in man has been reported by Gugler et al. (1977), with only 1.8 to 3.2% of the dose being excreted in the urine. Previous papers (Dickinson et al., 1979, 1980) have indicated that the main means of elimination of VPA in the rats and monkey is through metabolism in the liver. It is considered therefore that the main elimination route in rabbits may also be hepatic metabolism.

Data for plasma concentration of VPA versus time in rabbits after various i.v.

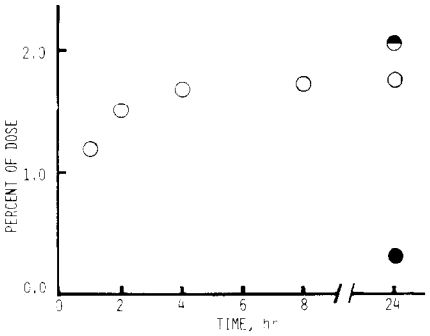


Fig. 3. Cumulative urinary and biliary excretion time courses after a 300 mg/kg i.v. bolus dose of VPA in rabbits. Each of the points represents the mean value. Key: ●, urinary excreted amount; ○, biliary excreted amount; ◓, total excreted amount.

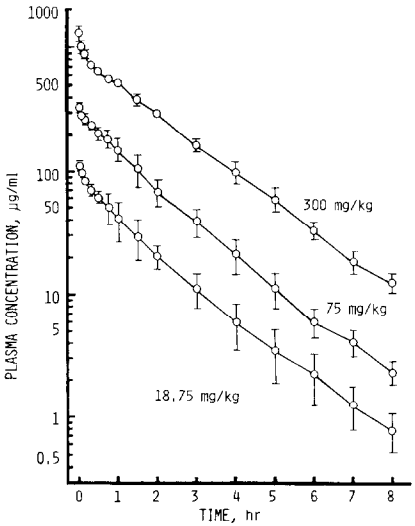


Fig. 4. Plasma concentration–time courses of VPA in rabbits following various i.v. bolus doses. Each of the data points represents the mean of three to four rabbits, and the vertical bars show the standard deviations.

doses are shown in Fig. 4. The plasma concentration data were analyzed using the non-compartmental method. The important parameters estimated are listed in Table 2. The value of the total plasma clearance (CL_{tot}) was very small compared to the liver plasma flow, so that the disappearance of VPA from the liver in rabbits is expected to depend on the intrinsic hepatic clearance (CL_{int}). Löscher (1977) reported similar results in man, the dog, rat and mouse indicating that VPA belonged to the group of drugs with restricted and flow-independent elimination.

TABLE 2

PHARMACOKINETIC PARAMETERS AFTER INTRAVENOUS ADMINISTRATION OF VARIOUS DOSES OF VPA IN RABBITS

Parameter	Dose (mg/kg)		
	300	75	18.75
$10^2\lambda n$ [a] (min^{-1})	0.868 ± 0.014	1.020 ± 0.022	0.939 ± 0.035
AUC [b] ($\mu g \cdot h/ml$)	1656.4 ± 71.2	447.9 ± 81.4	130.2 ± 28.3
AUMC [c] ($\mu g \cdot h^2/ml$)	2990.4 ± 312.5	686.5 ± 160.0	204.0 ± 62.1
CL_{tot} [d] (ml/min)	9.07 ± 0.4	8.54 ± 1.54	7.41 ± 1.34
V_{dss} [e] (ml)	978.6 ± 20.8	778.3 ± 104.7	682.7 ± 101.9

[a] λn represents the slope of the terminal exponential phase of a plot of ln drug concentration versus time.
[b] AUC represents the area under the drug concentration in plasma versus time curve from zero time to infinity.
[c] AUMC represents the area under the first moment curve from zero to infinity.
[d] CL_{tot} represents the total plasma clearance.
[e] V_{dss} represents the volume of distribution in the steady state.
Each value represents the mean ± S.D. of results for 3–4 rabbits (mean weight, 3.0 kg).

TABLE 3

ESTIMATION OF HEPATIC CLEARANCE AT STEADY-STATE

$C^{T}_{p,ss}$ [a] (μg/ml)	$C^{f}_{p,ss}$ [b] (μg/ml)	f_p [c]	I_0/W [d] (μg/min/kg)	CL^{ss}_{tot} [e] (ml/min/kg)	CL^{ss}_{int} [f] (ml/min/kg)
61.0	6.3	0.103	135.9	2.23	21.63
133.0	17.2	0.129	335.7	2.52	19.53
233.9	43.3	0.186	868.3	3.71	19.96
406.1	130.1	0.320	1283.3	3.16	9.86
825.0	447.4	0.542	1770.0	2.15	3.96
1016.5	605.8	0.596	2343.3	2.31	3.88
1100.0	676.0	0.615	2038.0	1.85	3.01
1287.3	835.3	0.648	2284.0	1.77	2.73

[a] $C^{T}_{p,ss}$ represents the total plasma concentration of VPA at steady-state.
[b] $C^{f}_{p,ss}$ represents the unbound plasma concentration of VPA at steady-state, and calculated from in vitro serum protein binding study.
[c] f_p represents the unbound fraction of VPA in plasma.
[d] I_0/W represents the infusion rate per animal body weight.
[e] CL^{ss}_{tot} represents the total clearance at steady-state.
[f] CL^{ss}_{int} represents the intrinsic clearance at steady-state.

Total plasma clearance (CL^{ss}_{tot}) and intrinsic clearance (CL^{ss}_{int}) at steady-state obtained in the intravenous infusion study are given in Table 3. At the various steady-states, the extent of changes in CL^{ss}_{tot} appears to be small as compared to the considerable changes in the unbound fraction. If CL^{ss}_{tot} is in proportion to the unbound fraction in the plasma (i.e. $CL^{ss}_{tot} = fpCL^{ss}_{int}$; see Appendix I), CL^{ss}_{tot} should increase remarkably with increases in the administered infusion dose of VPA in rabbits.

Dickinson et al. found that the metabolism of VPA in the liver revealed a dose-dependency in the rat (1979) and monkey (1980). Therefore, one possible reason for the discrepancy involving CL^{ss}_{tot} may be considered to be that the intrinsic clearance decreases with increases in the unbound concentration of VPA in the plasma. As listed in Table 3, our results obtained in this steady-state experiment agree with the above finding by Dickinson et al.

Hepatic clearance

The steady-state clearance concept was adopted for evaluating the hepatic clearance. Since the urinary and biliary excretion of unchanged VPA was negligible (data not shown), the following relationship may be written:

$$I_0 = \left(\frac{dX_{hep}}{dt} \right)_{ss} \tag{2}$$

If the hepatic elimination of VPA in rabbits is also assumed to follow a capacity-limited process similar to previous data as mentioned above, the following equation may be written, because the extremely small value of CL^{ss}_{tot} (1.77–3.71 ml/min/kg)

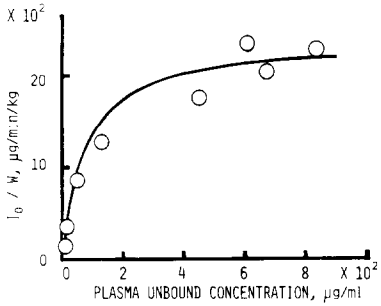


Fig. 5. Plots of steady-state rates for hepatic elimination of VPA against the steady-state plasma unbound concentration in rabbits. The solid line represents the theoretical values according to Eqn. 3.

compared to the product of RBP and hepatic blood flow (47.67 ml/min/kg) would provide the plasma unbound concentration in artery ($C_{p,f}$) approximately equal with that in hepatic venous ($C^{v}_{p,f}$).

$$\frac{I_0}{W} = \frac{V_m C_{p.f}}{K_m + C_{p.f}} \tag{3}$$

where I_0 is the infusion rate and W is the weight of the animals used in this experiment.

Fig. 5 shows the experimental results. The kinetic parameters of V_m and K_m were evaluated by a NONLIN program and are listed in Table 4. The intrinsic hepatic clearance could be explained by the Michaelis-Menten equation and was found to decrease with increases in the unbound concentration of VPA in the plasma. This suggests that the total plasma clearance remains apparently unchanged because increases in total concentration are associated with an increase in unbound fraction as well as a decrease in intrinsic clearance.

Tissue-to-plasma concentration ratios of VPA

Apparent tissue-to-plasma concentration ratios ($K_{p,app}$) obtained from in vivo experiments were calculated from the drug concentrations in the tissues and arterial plasma at 2, 4 and 6 h after i.v. bolus administration. Since pseudo-equilibrium

TABLE 4

MICHAELIS-MENTEN PARAMETERS FOR HEPATIC ELIMINATION OF VPA IN RABBITS

Parameter	Estimated value
K_m (μg/ml)	71.0 ± 18.7
10^{-3} V_m (μg/min/kg)	2.40 ± 0.15

Each point represents the mean ± S.D. estimated by the non-linear least-squares method using a NONLIN program.

TABLE 5

TISSUE TO PLASMA CONCENTRATION RATIOS OF VPA IN RABBITS

Tissues	K_p value [a]
Lung	0.28 ± 0.03
Brain	0.23 ± 0.04
Heart	0.52 ± 0.20
Liver	0.52 ± 0.03
G.I. tract	0.55 ± 0.04
Kidney	2.01 ± 0.25
Muscle	0.22 ± 0.01
Skin	0.55 ± 0.06
Fat tissue	0.61 ± 0.28
Bone	0.20 ± 0.02

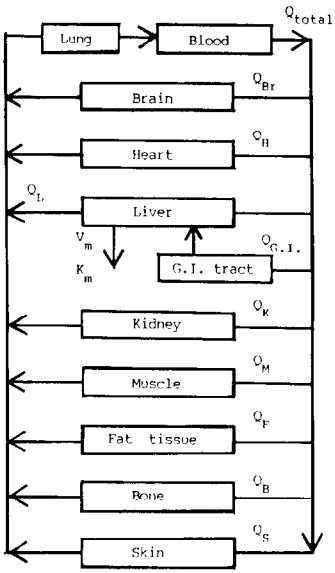
[a] These values were calculated using data for 2, 4 and 6 h after i.v. injection and have been corrected following the method of Terasaki et al. (1982).
Each value represents the mean ± S.E.

between the tissue and plasma was completely achieved within 2 h, the values of $K_{p,app}$ were corrected to tissue-to-plasma concentration ratios (K_p) according to the method of Terasaki et al. (1982). In addition, because the values of K_p estimated for the different times were almost identical, the mean value of K_p obtained with the three different times was adopted. These K_p values are listed in Table 5.

Within the limits of the concentrations for which the values of K_p were determined, the unbound fraction in the plasma varied (0.22–0.094). Nevertheless, the K_p values obtained remained apparently unchanged. Recently, McNamara et al. reported that the lack of distribution changes occurring with ceftriaxone despite dramatic changes in the unbound fraction in the plasma could be explained theoretically, by assuming that the antibiotic in the interstitial fluid could bind with interstitial albumin in a manner resembling that with plasma albumin (1983). Similarly, although entrance of VPA into the intracellular water is considered to take place, the lack of changes in K_p value occurring with VPA despite the twice as large as changes in unbound fraction in the plasma might possibly be explained by assuming that the binding of VPA to the constituents in tissues and organs takes place in a manner resembling that with plasma protein.

Physiological model and data prediction

To confirm rationally the overall disposition of VPA in rabbits incorporating the non-linear plasma protein binding and concentration-dependent hepatic clearance, a physiologically based pharmacokinetic model was adopted. The model consisted of eleven tissue and blood compartments including the target organ of VPA, the brain. The following assumptions were made: (a) each tissue acts as a well-stirred compartment; (b) the drug distribution is blood flow limited; (c) elimination of VPA occurs only from the liver in a non-linear manner; and (d) VPA binds to plasma proteins in a non-linear manner. The flow diagram of the model is shown in Scheme I. The model assumes that the K_p value of each tissue is apparently independent of the unbound concentration of drug in the plasma.



Scheme I.

TABLE 6

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS FOR RABBITS (BODY WEIGHT = 3.0 kg)

Tissues	Volume [a] (ml)	Blood flow' [a] (ml/min)
Blood	237.6	(total)
Lung	21.8	(total)
Brain	8.0	8.1
Heart	7.7	16.3
Kidney	19.3	67.4
Muscle	1440.0	104.3
Fat tissue	154.6	36.6
Liver	128.4	204.3
G.I. tract	154.6	106.3
Skin	300.0	19.3
Bone	428.6 [b]	34.4 [c]

Blood-to-plasma partition coefficient, RBP 0.7 [d]

[a] Taken from Lin et al. (1982) and estimated through an appropriate scaling of the data.
[b] Estimated through an appropriate scaling down from Mapleson (1963).
[c] 7% of cardiac output (Jones, 1950).
[d] This value was given in this experiment.

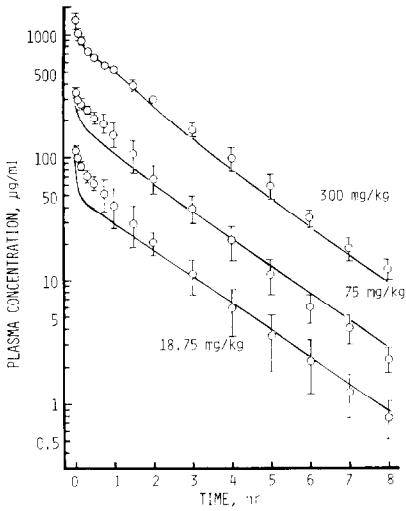


Fig. 6. Predicted lines versus observed plasma concentrations of VPA after 300, 75 and 18.75 mg/kg i.v. bolus injection doses in rabbits.

The mass balance equations developed according to the flow diagram in Scheme I are given in Appendix II. For simplification of the calculation, the term representing the intrinsic clearance in the liver compartment was multiplied by the unbound concentration in the arterial plasma, based on the concept that no great error would arise in the calculated results in the case where the K_p value was less than unity.

The differential equations were simultaneously solved numerically by the Runge-Kutta method using a FACOM M-170F digital computer. The physiological and anatomical parameters for 3.0 kg rabbits are listed in Table 6. The model predicted the VPA concentrations in the plasma and various tissues shown in Figs. 6 and 7A and B. The results predicted by this model agreed reasonably well with the corresponding observed data.

Sugita et al. (1982) reported that the interaction of tolbutamide and sulfonamide could be successfully predicted using the tissue-to-plasma unbound concentration ratios ($K_{p,f}$) in a physiologically based pharmacokinetic model. If the tissue binding of VPA did not depend on the plasma VPA concentration and the tissue distribution of VPA increase in proportion to the unbound fraction of VPA in the plasma, the value of $K_{p,f}$ might be available for elucidating the disposition of VPA. Prediction by the physiological model for VPA in rabbits was therefore attempted using the value of $K_{p,f}$ as the distribution parameter. However, the simulation results were not in agreement with the observed data, since in the initial distribution phase of the plasma concentration versus time curve after 300 mg/kg i.v. administration, the predicted line considerably underestimated the experimental values.

The following conclusions can be drawn from the present simulation results. The concentration of VPA in all tissues and organs including the brain is rapidly equilibrated with the concentration in the plasma. The reason why the plasma

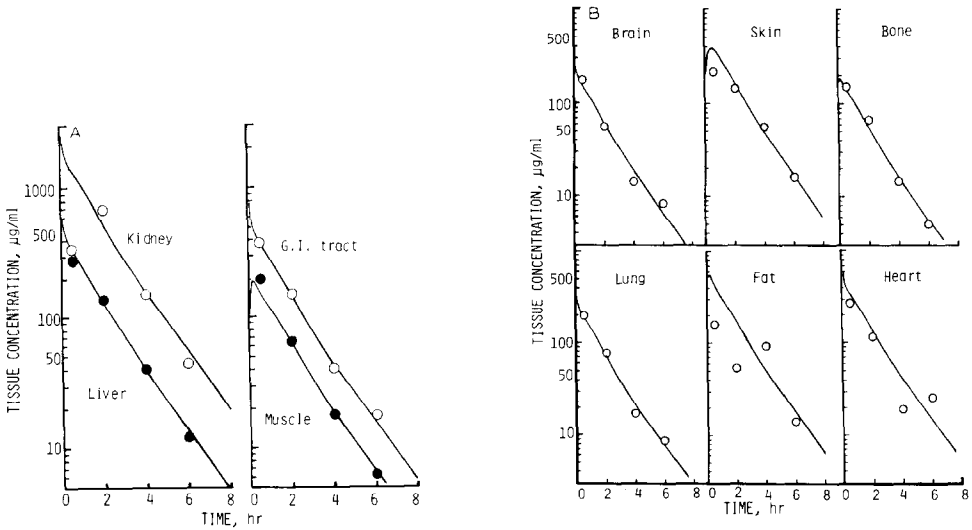


Fig. 7. A: predicted lines versus observed data for various tissues or organs after a 300 mg/kg i.v. bolus injection dose of VPA. B: predicted lines versus observed data for various tissues or organs including the target organ, the brain, after a 300 mg/kg i.v. bolus dose of VPA.

concentrations versus time curves appear to be parallel in spite of remarkable changes in the unbound concentration among three different doses can be explained by the association effect that increase of the total plasma concentration may produce the decrease of intrinsic hepatic clearance following Michaelis-Menten equation and increase of the unbound fraction of VPA in plasma.

In this study, we elucidated the disposition of VPA in rabbits using a physiologically based pharmacokinetic model including the non-linear plasma protein binding and non-linear intrinsic hepatic clearance. However, a number of deficiencies still remain. Strictly speaking, it is not adequate to assume that the tissue-to-plasma concentration ratios (K_p) remain unchanged over a wide range of total plasma concentration. Such an assumption cannot explain the slight increase in values of V_{dss} with increasing administered dose as illustrated in Table 2. It is necessary to develop a precise model for the distribution to tissues or organs which includes the binding to interstitial albumin (Tsuji et al., 1983) and to tissue constituents.

Appendix I

General

V	volume of tissue, ml
Q	blood flow rate through tissue, ml/min
C	tissue or blood concentration of VPA, μg/ml
$C_{p,f}$	unbound concentration of VPA in plasma, μg/ml
$C_{p,b}$	bound concentration of VPA in plasma, μg/ml
f_p	unbound fraction in plasma

f_t	unbound fraction in tissue
K_p	tissue-to-plasma partition coefficient of VPA
RBP	blood-to-plasma concentration ratio of VPA
n_1, n_2	number of binding sites
K_1, K_2	dissociation constant of serum protein binding, M^{-1}
P	concentration of serum protein, 5.0×10^{-4} M
V_m	maximum velocity of hepatic clearance, μg/min/kg
K_m	Michaelis constant, μg/ml
X_{hep}	amount of drug for hepatic elimination

Subscripts

B,	blood
K,	kidney
Lu,	lung
Br,	brain
Bo,	bone
T,	tissue
p,	plasma
G.I.,	gastrointestinal tract
H,	heart
F,	fat tissue
v,	venous
L,	liver
M,	muscle
S,	skin

Appendix II: Equations

Non-eliminating organ or tissue

$$V_i \frac{dC_{T,i}}{dt} = RBP\, Q_{T,i}\left[(1+\psi_p)C_{p,f} - \frac{C_{T,i}}{K_p^{T,i}}\right]$$

$$\psi_p = \frac{n_1 K_1 P}{1 + K_1 C_{p,f}} + \frac{n_2 K_2 P}{1 + K_2 C_{p,f}}$$

Blood compartment

$$V_B(1+\psi_d)\frac{dC_{p,f}}{dt} = Q_B \frac{C_{LU}}{K_p^{LU}} - Q_B(1+\psi_p)C_{p,f} + \frac{DI(t)}{RBP}$$

$$\psi_d = \frac{n_1 K_1 P}{(1 + K_1 C_{p,f})^2} + \frac{n_2 K_2 P}{(1 + K_2 C_{p,f})^2}$$

$$Q_B = Q_{Br} + Q_H + Q_K + Q_M + Q_F + Q_L + Q_S + Q_{BO}$$

where DI(t) is the infusion rate of the total VPA dose (μg/min).

Lung

$$V_{LU}\frac{dC_{LU}}{dt} = RBP\left(\sum Q_{T,i}\frac{C_{T,i}}{K_p^{T,i}} - Q_B\frac{C_{LU}}{K_p^{LU}}\right)$$

Liver

$$V_L\frac{dC_L}{dt} = RBP\left[(Q_L - Q_{G.I.})(1+\psi_p)C_{p,f} + Q_{G.I.}\frac{C_{G.I.}}{K_p^{G.I.}} - Q_l\frac{C_L}{K_p^L}\right] - \frac{V_m W C_{p,f}}{K_m + C_{p,f}}$$

where W is the body weight of the animal.

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