Use of Hexadeuterated Valproic Acid and Gas Chromatography–Mass Spectrometry to Determine the Pharmacokinetics of Valproic Acid

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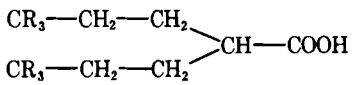
Abstract □ Di-([3,3,3-$^2H_3$]propyl)acetic acid, a hexadeuterated analogue of valproic acid, was synthesized and its pharmacokinetic properties compared with valproic acid. Concentrations of valproic acid and [^{2}H]valproic acid in serum and saliva were determined by GC–MS using selected-ion monitoring. Saliva drug levels were measured with good precision down to 0.1 μg/mL. Kinetic equivalence of valproic acid and [^{2}H]valproic acid was demonstrated in a single-dose study in a human volunteer. An isotope effect was observed for ω-oxidation, but the difference in metabolism was not sufficient to make [^{2}H]valproic acid biologically nonequivalent. The application of [^{2}H]valproic acid to determine the kinetics of valproic acid under steady-state concentrations was evaluated in the same volunteer. The kinetic data obtained with [^{2}H]valproic acid was consistent with previously reported values for valproic acid including kinetic differences observed between single-dose and steady-state experiments. Saliva levels of valproic acid were found to give a good correlation (r = 0.953) with total serum valproic acid under multiple-dose conditions. A concentration dependence was found for the ratio of saliva valproic acid to free valproic acid in serum, low ratios being observed at high serum concentrations of valproic acid.

Keyphrases □ Valproic acid—hexadeuterated analogue, gas chromatography–mass spectrometry, pharmacokinetics single- and multiple-dose regimes □ Pharmacokinetics—valproic acid, hexadeuterated, analogue, single- and multiple-dose regimens, gas chromatography–mass spectrometry

Valproic acid (I), an anticonvulsant drug that is effective in the treatment of absence seizures, is also used to treat primary and generalized tonic-clonic seizures (1, 2). The pharmacokinetics of valproic acid have been studied extensively in patients who also receive other antiepileptic drugs (3–6). As a consequence, little is known about the basic elimination kinetics of valproic acid during multiple dosing with valproic acid alone. Only a few studies have reported multiple-dose kinetics of valproic acid given as a single agent to normal volunteers (7–9).



I R = H
II R = ^{2}H

One method of determining changes in the kinetic parameters of a drug in patients on multiple-dose therapy is the use of a pulse-dose of a stable-isotope-labeled drug (10, 11). Application of this method to antiepileptic drug studies allows the elimination kinetics of the drug to be determined without discontinuing therapy and risking the exacerbation of seizures. The labeled-drug elimination phase can be followed for 3–4 half-lives during subsequent uninterrupted multiple dosing of unlabeled drug. This technique has been used by Von Unruh *et al.* (6) to study the elimination kinetics of valproic acid under steady-state conditions in patients on combined antiepileptic drug therapy using di-([2,3-$^2H_2$]propyl)acetic acid.

The purpose of this study was to synthesize a deuterium analogue of valproic acid, namely, di-([3,3,3-$^2H_3$]-propyl)-acetic acid (II) and to undertake a preliminary evaluation of its pharmacokinetics. The location of the deuterium atoms only on the terminal carbons of the propyl chains of II was thought to be ideal since ω-oxidation of valproic acid is a minor metabolic pathway (3).

To demonstrate the applicability of labeled valproic acid for determining the steady-state kinetics of valproic acid without interruption of drug administration, the pharmacokinetics of II were compared with I under both single- and multiple-dose situations in a healthy human volunteer. To achieve this, a selective and sensitive assay for II acid using GC–MS was required. The application of this technique to the measurement of salivary levels of valproic acid and [^{2}H]valproic acid was also investigated.

EXPERIMENTAL

Chemicals and Reagents—Valproic acid[1] and 1-bromo[3,3,3-$^2H_3$]propane[2] were obtained commercially. Octanoic acid[3] and diethyl malonate[4] were redistilled before use. Ethyl acetate[5], hexane[5], and methanol[5] were distilled-in-glass grade. All other chemicals were analytical grade.

Synthesis of Di-([3,3,3-$^2H_3$]propyl)acetic Acid (II)—Diethyl malonate (2.88 g, 0.018 mol) was added dropwise to a solution of sodium ethoxide (0.44 g of sodium in 11.0 mL of absolute ethanol). The mixture was heated at reflux for 20 min, cooled, and then 1-bromo[3,3,3-$^2H_3$]propane (2.5 g, 0.02 mol) was introduced in a dropwise manner. The mixture was stirred at reflux for 2 h. For the second alkylation step, the same amounts, of sodium ethoxide solution and 1-bromo[3,3,3-$^2H_3$]propane as used previously were added, and the reaction mixture was heated at reflux for an additional 3 h.

Sodium hydroxide solution (5.5 g in 16 mL of water) was added to the reaction mixture containing diethyl [$^2H_6$]dipropylmalonate and the mixture was refluxed for 8 h. After removing the ethanol by distillation at atmospheric pressure, the solution was diluted and cooled in an ice-water bath. A solution of 9 M sulfuric acid was added in a dropwise manner until the pH of the resulting solution fell to 2.5 (monitored with a pH meter[6]). A white precipitate of [^{2}H]dipropylmalonic acid was collected by filtration, washed with water, and dried *in vacuo* for 6 h (mp[7] 156–158°C). The [^{2}H]dipropylmalonic acid was heated at 190–200°C until evolution of carbon dioxide had ceased. The residue was distilled *in vacuo* to give 1.39 g of [^{2}H]valproic acid (50% yield based on diethyl malonate), bp 71–72°C, 0.5 mm Hg [lit. (12) bp 221°C, 760 mm Hg for valproic acid]. ^{1}H-NMR[8] ($CDCl_3$) relative to tetramethylsilane: δ 1.1–1.9 (m, 8), 2.2–2.5 (m, 1), and 10.5–11.5 ppm (br s, 1, COOH). The deuterium label was >97% as determined by GC–MS analysis of the *tert*-butyldimethylsilyl ester of the product as compared with derivatized unlabeled valproic acid.

Gas Chromatography–Mass Spectrometry— The instrument used for the ion-monitoring assay was a gas chromatograph[9] interfaced to a mass spec-

[1] K and K Fine Chemicals, ICN Pharmaceuticals, Plainview, N.Y.
[2] Purity 98 atom %D; Merck Sharp and Dohme Canada Ltd.
[3] Nutritional Biochemical Corp., Cleveland, Ohio.
[4] British Drug House Ltd, Poole, U.K.
[5] Caledone, Georgetown, Canada.
[6] Model 220 pH meter; Fisher Scientific, Fair Lawn, N.J.
[7] Thomas Hoover Apparatus; Arthur H. Thomas Co., Philadelphia, Pa.
[8] Spectrum recorded with an XL-100 spectrometer; Varian Associates, Palo Alto, Calif.
[9] Model 5700A; Hewlett-Packard, Avondale, Pa.

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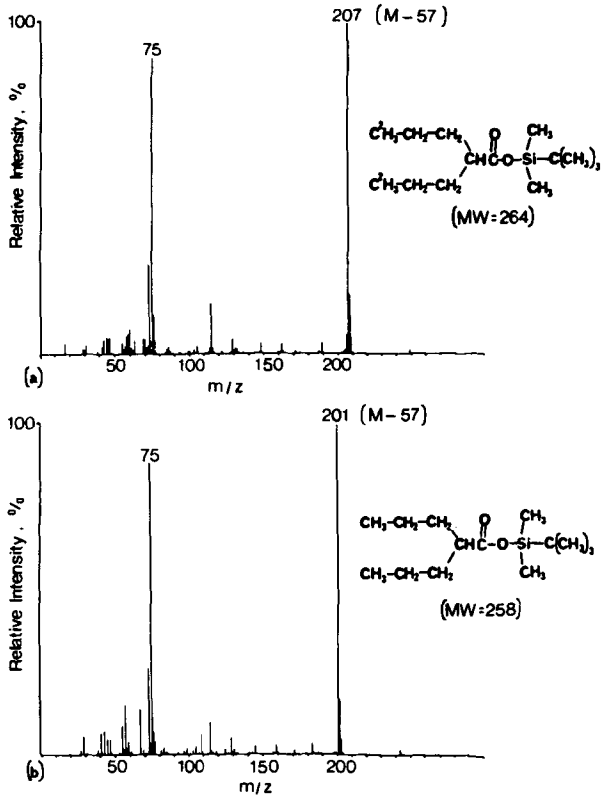

Figure 1—*Electron-impact mass spectra of the* tert-*butyldimethylsilyl esters of (a) [²H]valproic acid and (b) valproic acid.*

trometer[10] *via* a variable-slit separator and connected to an on-line data system[11]. The 1.8-m × 2-mm i.d. glass column was packed with 3% Dexsil 300 on 100–120 mesh Supelcoport[12]. Injection into the system was accomplished with an automatic sampler[13]. Operating conditions were: injection port temperature, 250°C; inlet line temperature, 250°C; column temperature, 135°C; and helium (carrier gas) flow rate of 25 mL/min. The mass spectrometer was operated with an electron-ionization energy of 80 eV, trap current of 300 μA, and ion source pressure of 5.0×10^{-6} Torr.

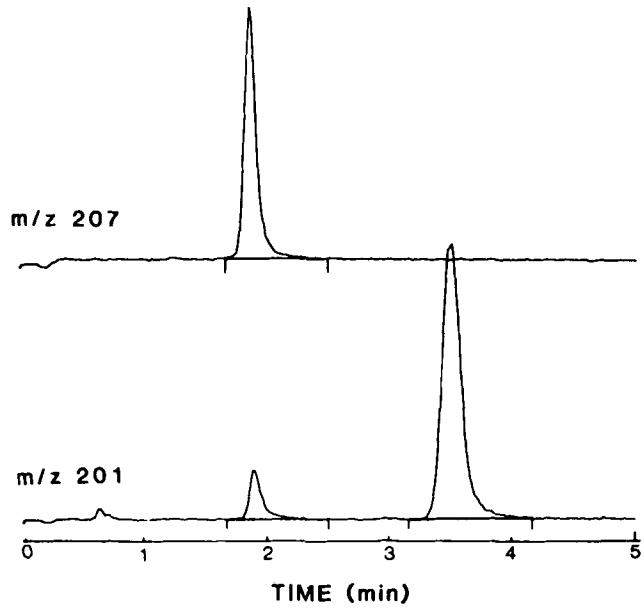

Figure 2—*Selected-ion chromatograms of an extracted saliva sample. Key: (m/z 207) [²H]valproic acid; (m/z 201) valproic (1.90 min) and octanoic (3.55 min) acids.*

[10] Model MAT-111; Varian Associates.
[11] Model 620L Computer; Varian Associates.
[12] Supelco, Inc., Bellefonte, Pa.
[13] Model 761A; Hewlett-Packard.

Table I—Calibration Curve Data of the *tert*-Butyldimethylsilyl Ester of [²H]Valproic Acid in Biological Samples

Concentration, μg/mL	Mean Peak Area Ratio[a] (*RSD*)	Linear Regression Parameters[b]
	Saliva	
0.10	0.20 (6.67%)	
0.25	0.045 (3.77%)	$a^0 = -0.008$
0.50	0.085 (0.79%)	
1.00	0.186 (1.89%)	$a_1 = 0.185$
1.50	0.238 (0.92%)	
3.00	0.546 (1.02%)	$r^2 = 0.9986$
6.00	1.108 (1.45%)	
	Serum	
10	0.350 (1.75%)	$a^0 = -0.0349$
20	0.705 (2.68%)	
40	1.295 (0.77%)	$a_1 = 0.0358$
60	2.130 (0.57%)	
80	2.880 (0.31%)	$r^2 = 0.9981$
100	3.525 (0.44%)	

[a] $n = 4$. Peak area ratio is the ratio of *m/z* 207 of hexadeuterated valproic acid ester to *m/z* 201 of octanoic acid ester. [b] r^2 is the coefficient of determination, a_1 is the slope, and a^0 is the intercept. Equation for the line is $y = a_1x + a^0$ where y is the peak area ratio mean and x is drug concentration.

Single-Dose Study—A healthy male volunteer, 41 years of age (weight, 63.64 kg), who had not taken any drugs during the month prior to the experiment, participated in both the single- and multiple-dose studies. A single oral dose was prepared by dissolving 372 mg of valproic acid (I) and 182 mg of [²H]valproic acid (II) in 100 mL of water, and the pH of the resulting solution was adjusted to 7.4 using 1 M NaOH. The oral dose was taken in the morning after an overnight fast; food was not allowed until 3 h after dosing. Blood samples (5 mL) were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12, 24, and 48 h, using an indwelling catheter (flushed with heparin solution) for the first 4 h and thereafter by venipuncture. Serum was prepared immediately after blood sampling. Saliva samples (2–5 mL) were obtained coincident to blood sample collections *via* expectoration into clean scintillation vials. Urine was collected at the following times: 0, 1, 2, 3, 4, 5, 6, 7, 8–12, 12–24, and 24–48 h after dosing. The serum, saliva, and urine samples were kept at −20°C until they were analyzed.

Multiple-Dose Study—A 600-mg oral dose of valproic acid syrup[14] (50 mg/mL) was given to the volunteer, who participated in both dosing studies, at 8 p.m. on day 1. The same dose of valproic acid was administered twice a day 12 h apart for the next 3 d. On day 5 (84 h after the initial dose) 600 mg of [²H]valproic acid dissolved in 100 mL of water (pH 7.4) was substituted for the valproic acid as the eighth dose. This dose was taken at the usual time in the morning after an overnight fast (all morning doses were following an

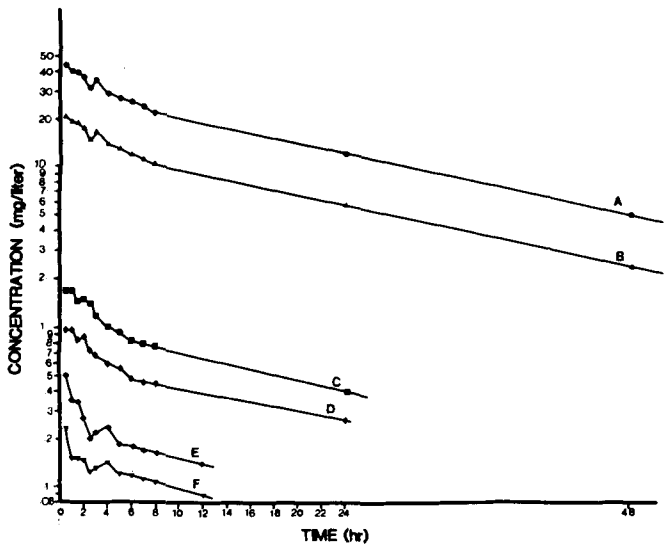

Figure 3—*Time course of valproic acid (I) and [²H]valproic acid (II) in saliva and serum following a single oral dose of 372 mg of I plus 182 mg of II. Key: (A) serum total I; (B) serum total II; (C) serum free I; (D) serum free II; (E) saliva I; (F) saliva II.*

[14] Depakene; Abbott Laboratories Ltd., Montreal, Canada.

Table II—Pharmacokinetic Parameters for Valproic Acid (I) and [^{2}H]-Valproic Acid (II) Under Single-Dose and Steady-State Conditions in a Healthy Male Volunteer [a]

Biophase	$t_{1/2}$,h	K_E, h^{-1}	CL[b], L/h/kg	Vd (area)	AUC, mg·h/L
Single-Dose Conditions (372 mg of I and 182 mg of II)					
Serum					
Total I	18.6	0.0372	0.0070	0.188	841.3
Total II	18.7	0.0371	0.0075	0.202	379.1
Free I	16.8	0.0413	0.20	4.84	29.27
Free II	18.3	0.0378	0.17	4.50	16.83
Saliva					
I	17.4	0.0398	1.1	27.6	5.23
II	15.4	0.0451	0.88	19.5	3.25
Steady-State Conditions (Multiple Doses of I, 600 mg of II)					
Serum					
Total II[c]	14.3	0.0485	0.010	0.206	900
Total (I + II)[d]	13.5	0.0513	0.011	0.214	872
Free II	13.2	0.0525	0.12	2.29	79.48
Free (I + II)	14.8	0.0468	0.13	2.78	72.52
Saliva					
II	16.5	0.0420	0.88	20.9	10.66
(I + II)	13.1	0.0529	1.1	20.8	8.86

[a] Weight = 63.64 kg. [b] Oral bioavailability is assumed to be unity. [c] Parameters for II are calculated from $t = 0$–∞. [d] Parameters for (I + II) are calculated from steady-state I and II concentrations summed during the dosing interval of 12 h following the dose of II.

overnight fast). Thereafter, valproic acid syrup (600 mg) was administered every 12 h for a further period of 3 d.

During the multiple-dose study, blood samples (5 mL) were withdrawn periodically (usually at the trough levels) prior to the administration of the labeled valproic acid. Following the [^{2}H]valproic acid dose, blood samples were taken at various times. Serum was prepared from each sample, and the pH was measured immediately using a pH meter[6]. Serum samples were stored at −20°C until analyzed.

Saliva samples (2–5 mL) were obtained with and without stimulation with sucrose. For the correlation study of saliva and serum drug levels, unstimulated saliva samples were collected 5 min before each blood sampling time and a stimulated saliva sample was obtained 5 min following the blood sample collection. Saliva samples were also obtained at times not coincident with blood sampling times. The pH of the saliva sample was determined immediately following collection using a pH meter[6]. Saliva samples were stored at −20°C until analyzed.

Serum and Saliva Standards—The valproic acid assay used has been described previously in detail (13). Serum standards of labeled and unlabeled valproic acid were prepared by the addition of 0.1 mL of appropriate stock solutions in methanol into 5-mL volumetric flasks. Drug-free serum was added to the 5-mL mark to provide [^{2}H]valproic acid concentrations of 10, 20, 40, 60, 80, and 100 μg/mL and valproic acid concentrations of 20, 40, 60, 80, 100, and 120 μg/mL.

Saliva standards were prepared similarly, but with [^{2}H]valproic acid concentrations of 0.1, 0.25, 0.5, 1.0, 1.5, 3.0, and 6.0 μg/mL and valproic acid concentrations of 0.25, 0.5, 1.0, 1.5, 3.0, and 6.0 μg/mL.

Extraction and Derivatization—Serum standard or sample (50 μL) was pipetted into a 1.0-mL conical reaction vial[15]. Internal standard (200 μL of a solution of 10 mg/L octanoic acid in 1 M HCl) was added, followed by 200 μL of solvent (10% ethyl acetate in *n*-hexane), and the mixture was vortex mixed for 60 s. After centrifuging at 1000×*g* for 20 min, 150 μL of the solvent layer was transferred to a second vial and 7 μL of *tert*-butyldimethylchlorosilane reagent[16] was added. The mixture was vortex mixed for 30 s and then centrifuged for 5 min. A 5-μL aliquot of the organic layer was injected into the GC–MS system.

For saliva drug analysis, 200-μL saliva samples were treated in a manner similar to the serum samples, except that the internal standard concentration was 6.5 mg/L in 1 M HCl.

Selected-Ion Monitoring—Labeled and unlabeled valproic acids were quantitated in serum and saliva by selected-ion monitoring with the GC–MS system operated in the EI-mode. The very intense ions *m/z* 201 from the *tert*-butyldimethylsilyl derivatives of valproic acid and octanoic acid and the corresponding *m/z* 207 from the derivative of [^{2}H]valproic acid were monitored continuously. The retention times for the *tert*-butyldimethylsilyl derivatives of valproic acid (or [^{2}H]valproic acid) and octanoic acid on the Dexsil 300 column were 2.0 and 3.6 min, respectively.

[15] Reacti-vial with teflon-lined cap; Pierce Chemical Co., Rockford, Ill.

[16] 1 mmol of *tert*-butyldimethylchlorosilane and 2.5 mmol of imidazole dissolved in 1 mL of dimethylformamide; Applied Science Lab. Inc., State College, Pa.

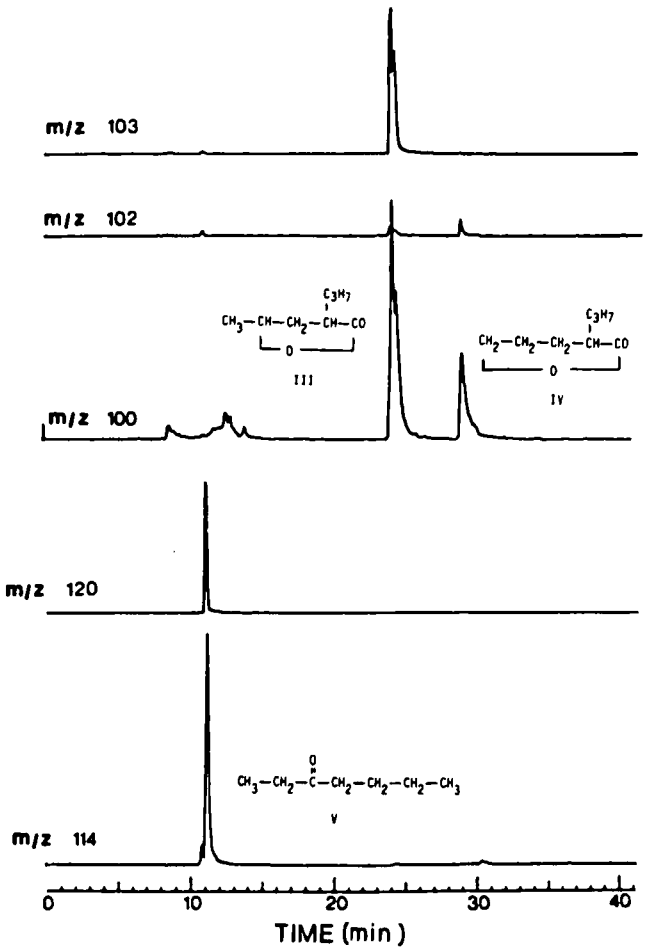

Figure 4—*Mass chromatograms of metabolites isolated from a urine sample following a single oral dose containing both valproic acid and [^{2}H]valproic acid. The* m/z *100 ion fragment peaks at 24 and 29 min are from the lactones of 4-hydroxyvalproic acid (III) and 5-hydroxyvalproic acid (IV), respectively. Corresponding peaks at* m/z *103 and* m/z *102 are the tri- and di- deuterated-analogues of the lactones. The lactones are formed during the acid extraction of the urine. Isomer separation is observed for the 4-hydroxyvalproic acid lactone (*m/z *100,* m/z *103 peaks);* m/z *114 and* m/z *120 peaks are 3-heptanone (V) and 3-[^{2}H]heptanone, respectively. The heptanone arises from acidic decarboxylation of 3-ketovalproic acid during the extraction.*

Calibration curves for valproic acid and [^{2}H]valproic acid were prepared by plotting the ion area ratios of drug to internal standard against the known drug concentrations. For valproic acid, the *m/z* 201 area ratios were used while the *m/z* 207 to *m/z* 201 area ratios were used for [^{2}H]valproic acid.

Plasma Protein Binding Studies—Free drug levels in serum were determined by equilibrium dialysis. The procedure has been described in a previous study (13). The serum and buffer samples from the two compartments were analyzed by GC–MS for free levels of valproic acid and [^{2}H]valproic acid. The extraction and derivatization procedures used were the same as for total drug quantitation in serum. The concentrations of free drug were determined using standard curves (1–10 μg/mL) prepared from buffer solutions to which valproic acid or [^{2}H]valproic acid had been added.

Pharmacokinetic Analysis—The elimination rate constants (K_E) of both valproic acid and [^{2}H]valproic acid were determined by least-squares regression analysis of the terminal log-linear part of the decay curves. The kinetic parameters, elimination half-life ($t_{1/2}$), area under the curve (AUC), total body clearance (CL), and apparent volume of distribution (Vd) were calculated by standard methods (14).

RESULTS AND DISCUSSION

Synthesis—[^{2}H]Valproic acid (II) was obtained *via* malonic ester synthesis according to the method of Adams and Kamm (15). The deuterated product had a high isotopic purity, as shown by comparison of the mass spectra of the

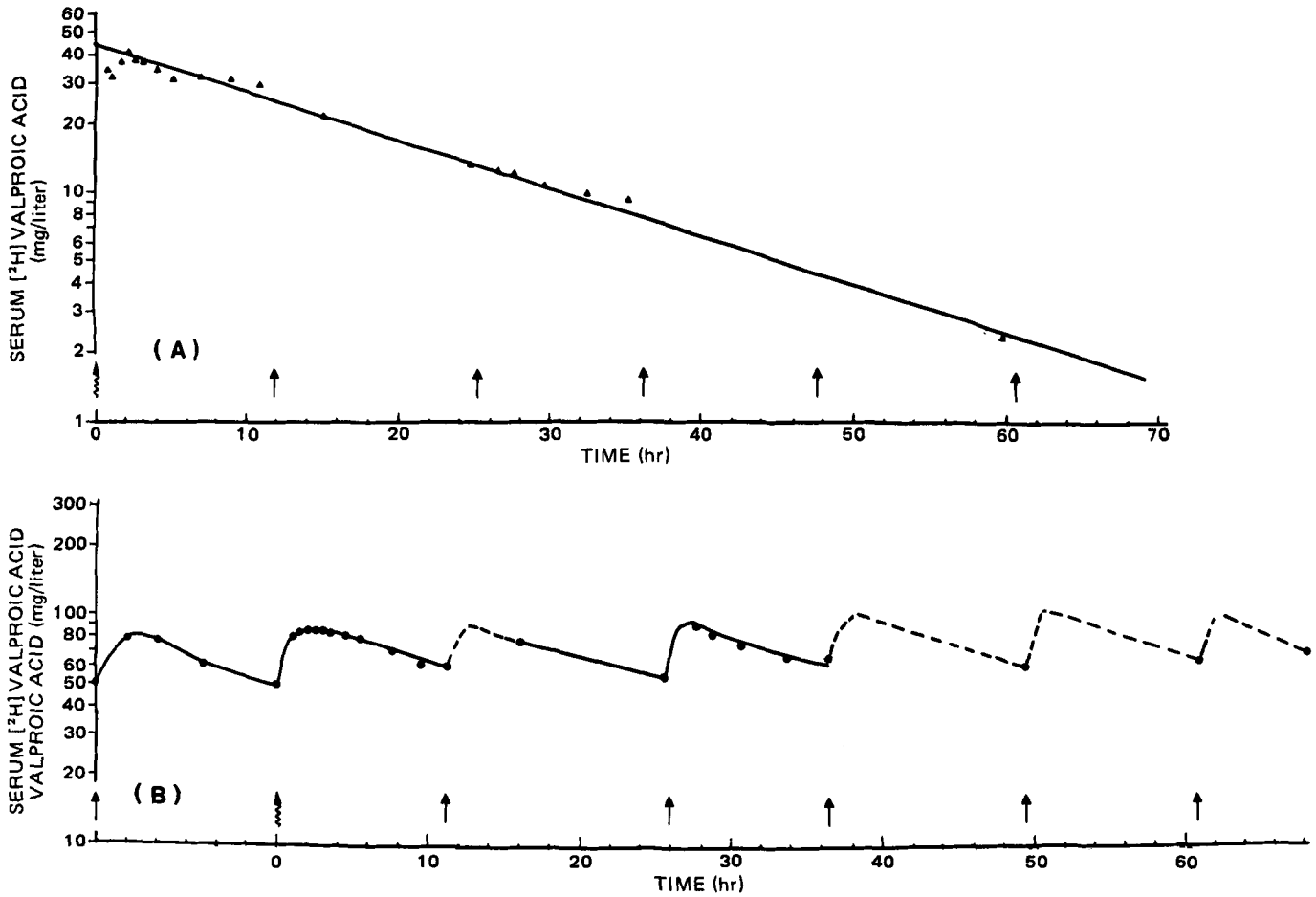

Figure 5—*(A) Time course of serum total [²H]valproic acid given as a substitute dose (600 mg) during multiple dosing with valproic acid. (B) Time course of serum total valproic acid + [²H]valproic acid beginning 12 h prior to and continuing for 60 h after the [²H]valproic acid dose. Key: (↕) time of dose of [²H]valproic acid; (↑) times when valproic acid (600 mg) was given.*

tert-butyldimethylsilyl derivatives of [²H]valproic acid and valproic acid (Fig. 1).

Assay—In this study it was imperative to develop a specific and sensitive assay to measure both valproic acid and [²H]valproic acid in serum and saliva. Quantitation was accomplished by monitoring the highly intense $[M-57]^+$ ions of the *tert*-butyldimethylsilyl derivatives (Fig. 1). The advantages of using the *tert*-butyldimethylsilyl derivatives compared with the trimethylsilyl and methyl esters of valproic acid have been discussed previously (13). The assay procedure used has resulted in clean mass chromatograms. Figure 2 shows a typical selected-ion chromatogram from a saliva sample. The recoveries of [²H]valproic acid from spiked serum and saliva samples were found to be 100%. The calibration curves constructed for valproic acid and [²H]valproic acid in serum and saliva were linear within the concentration ranges used and gave high correlation coefficients. Table I shows typical standard curves for [²H]valproic acid in serum and saliva. Calibration curves for valproic acid in serum and saliva were similar to that of a previous study (13). The relative standard deviations were generally <5%, attesting to the reproducibility of the assay.

Table III—Relationship of Saliva Concentrations of Valproic Acid and [²H]Valproic Acid and Serum Total or Serum Free Drug Levels in the Single- and Multiple-Dose Studies

Parameters[a]	Valproic Acid	[²H]Valproic Acid
Single-Dose Conditions		
Mean ratio, saliva/total serum	0.007	0.009
SD	0.001	0.001
r	0.926	0.901
Mean ratio, saliva/free serum	0.196	0.200
SD	0.027	0.033
r	0.863	0.850
Steady-State Conditions		
Mean ratio, saliva/total serum	0.009	0.011
SD	0.002	0.002
r	0.953	0.956
Mean ratio, saliva/free serum	0.112	0.138
SD	0.021	0.031
r	0.939	0.916

[a] Steady-state concentrations were measured during the first three dosing intervals following labeled-drug administration ($n = 17$); single-dose concentrations cover a period of 24 h ($n = 10$).

Pharmacokinetics of the Single- and Multiple-Dose Studies—Stable-isotope-labeled drugs have been used in different areas of pharmacokinetics including steady-state kinetics (10, 11), bioavailability studies (16), drug interaction studies (6), and drug metabolism studies (17). A stable-isotope-labeled drug which shows a significant biological isotope effect is not appropriate for use in a pharmacokinetic study. To examine the suitability of [²H]valproic acid for pharmacokinetic studies, (*i.e.*, lack of significant isotope effects), a single oral dose consisting of a 2:1 molar ratio of valproic acid to [²H]valproic acid was administered to a healthy human subject. The concentration–time curves of valproic acid and [²H]valproic acid for both serum and saliva describe the same constant 2:1 ratio of areas (Table II) as in the dose. The parallelism between the elimination phases of serum total valproic acid and serum total [²H]valproic acid (Fig. 3) strongly demonstrated that there was no significant isotope effect on the elimination kinetics of valproic acid after incorporation of six deuterium atoms onto the terminal carbon atoms of the propyl chains.

Additional evidence for the absence of a major isotope effect came from the analysis of urinary metabolites, where the major metabolites [valproic acid glucuronide, 3-ketovalproic acid, and 4-hydroxyvalproic acid (3)] showed the consistent 2:1 molar ratio of unlabeled to labeled metabolite (Fig. 4). An isotope effect was observed for the formation of the minor metabolite, 5-hydroxyvalproic acid. Because of the small proportion of 5-hydroxyvalproic acid relative to the other metabolites, a decrease in the formation of 5-hydroxy[$^2H_5$]valproic acid does not markedly affect the elimination kinetics of [²H]valproic acid.

The serum concentration–time curves of both valproic acid and [²H]valproic

acid after a single oral dose were biphasic, similar to those described by Gugler *et al.* (7) and as previously reported by Abbott *et al.* (13). Peak levels of valproic acid and [^{2}H]valproic acid were observed at 0.5 h in serum and saliva.

Following the dose of [^{2}H]valproic acid at steady state, absorption of this deuterated analogue was rapid with peak serum levels appearing within 2–3 h (Fig. 5). Biphasic elimination was not evident for either valproic acid in the 12-h dosing intervals or for [^{2}H]valproic acid as observed over 72 h. All decay curves appeared to be monoexponential.

Table II summarizes the pharmacokinetic data derived from the single-dose and steady-state experiments. The single-dose serum elimination half-life values observed for valproic acid and [^{2}H]valproic acid are practically identical and are comparable with the 15.9 ± 2.6 h value reported by Gugler *et al.* (7). Under multiple-dose conditions, the similar half-life values observed for serum total [^{2}H]valproic acid (14.3 h) and serum total valproic acid plus [^{2}H]valproic acid (13.5 h) was further evidence that [^{2}H]valproic acid is assimilated by the body in a manner identical to the unlabeled valproic acid. As an additional test, the serum total half-life values of valproic acid and valproic acid plus [^{2}H]valproic acid were compared over three consecutive days beginning the day prior to the deuterated dose. No significant change in half-life values was observed. Identical kinetic behavior of the two valproic acid species under multiple-dose conditions is also evident from the AUC data; the serum total $AUC_{0-\infty}$ for [^{2}H]valproic acid (900 mg·h/L) is essentially identical to the valproic acid plus [^{2}H]valproic acid $AUC_{\tau 1-\tau 2}$ (872 mg·h/L) as is expected from application of the principle of superposition where $AUC_{0-\infty}$ after first dose is equivalent to $AUC_{\tau 1-\tau 2}$ at steady state (14).

The elimination half-life of serum total [^{2}H]valproic acid of 14.3 h in this individual under multiple-dose conditions was shorter than the half-life observed in the single-dose study. The apparent volume of distribution of [^{2}H]valproic acid at steady state was not different from the single-dose value. However, the total body clearance of [^{2}H]valproic acid increased by 33% over the single-dose value. The observed increase in total body clearance may better reflect the change in valproic acid elimination kinetics from single- to multiple-dose conditions, since it is a parameter that is model independent (18). The change in total body clearance is not likely due to a reduction in the amount of drug absorbed, since the oral bioavailability is nearly 100% (19, 20). There is the possibility that the 33% increase in total body clearance is due to either an enzyme-inducing effect of valproic acid or a change in binding of valproic acid to serum proteins or both.

On the enzyme-inducing property of valproic acid, several investigators (21, 22) have reported that the half-life of antipyrine and D-glucaric acid urinary excretion in patients treated with sodium valproate were not different from those in drug-free controls. There has been one study (23) that reported that valproic acid increased D-glucaric acid urinary excretion significantly but not serum γ-glutamyltranspeptidase activity in epileptic children.

Valproic acid is cleared by metabolism and has a low extraction ratio (19); its rate of metabolism is proportional to the free serum concentrations. Total body clearance (CL_T) is a function of the free fraction of valproic acid (fu) and the intrinsic clearance (CL'_{int}) through the expression:

$$CL_T = CL'_{int} \cdot fu$$

where fu is the ratio of free drug to the total drug in serum and CL'_{int} is the inherent activity of the liver to metabolize unbound valproic acid (free clearance of valproic acid). In theory, a decrease in plasma protein binding (increase in fu) should increase total body clearance and decrease total serum concentrations of valproic acid or [^{2}H]valproic acid, but should have no effect on the free serum concentration of valproic acid or [^{2}H]valproic acid (18, 24).

In the present study total body clearance increased at steady state compared with the single-dose study (Table II), while the average free fraction in serum (fu) increased from 0.05 (±0.01 *SD*) under single-dose conditions to 0.09 (±0.01 *SD*) at steady-state serum total valproic acid plus [^{2}H]valproic acid concentrations of 50–100 μg/mL. Unless the liver enzyme activity was affected, one would not expect CL_{free} (CL'_{int}) to change between single-dose and steady-state conditions. The clearance of the free drug, however, was seen to decrease (*e.g.*, single dose for [^{2}H]valproic acid = 0.17 L/h/kg; steady state for [^{2}H]valproic acid = 0.12 L/h/kg; see Table II) under steady-state conditions, suggesting possible inhibition of metabolism of valproic acid or saturation of drug-metabolizing enzymes in this subject. Similar decreases in clearance of the free drug (CL_{free}) at steady state were observed by Bowdle *et al.* (8) for a group of normal volunteers on a multiple-dose schedule with valproic acid.

Saliva Valproic Acid Measurement—The saliva levels of valproic acid and [^{2}H]valproic acid were measured to determine if a significant correlation exists between serum total or serum free drug concentrations and saliva drug concentrations. The GC–MS method used here for measuring saliva valproic acid levels is highly sensitive and proved more than adequate to obtain precise data for the saliva concentrations in pharmacokinetic studies.

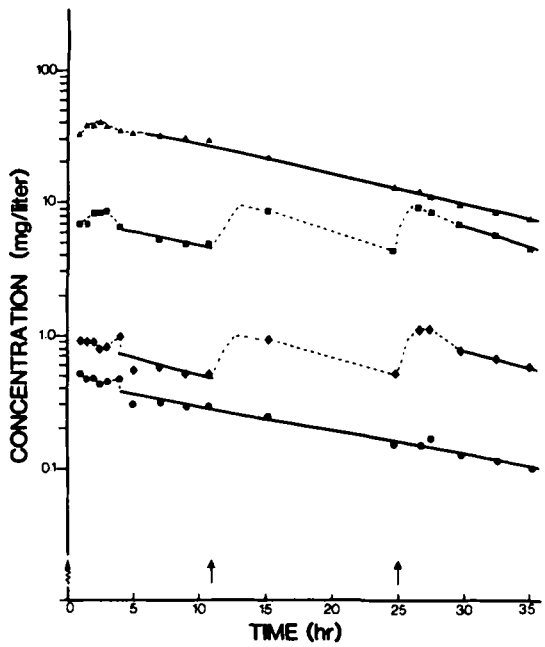

Figure 6—*Concentration–time curves of valproic acid (I) and [^{2}H]valproic acid (II) in serum and saliva following a 600-mg dose of II during the multiple-dose study. Key: (▲) serum total II; (■) serum free (I + II); (◆) saliva (I + II); (●) saliva II; (‡) time of dose of II; (↑) times when I (600 mg) was given.*

Sucrose-stimulated saliva samples had consistently lower pH (5.85 ± 0.48, n = 25) than unstimulated saliva samples (6.98 ± 0.29, n = 25) at similar sampling times. The greater variation in stimulated saliva pH may explain the greater fluctuations observed in the stimulated saliva drug concentrations compared with unstimulated saliva drug concentrations. Sucrose-stimulated saliva valproate levels during the multiple-dose study were higher (mean = 0.684 mg/L) than unstimulated saliva valproic acid levels (mean = 0.609 mg/L), and the difference was significant (pairwise t test, p = 0.013, n = 22).

The time course of unstimulated saliva valproic acid and [^{2}H]valproic acid concentrations at steady state are presented in Fig. 6 together with that of serum total [^{2}H]valproic acid concentrations and serum free valproic acid plus [^{2}H]valproic acid concentrations. Table III summarizes the relationship of drug levels found in saliva and serum.

The mean saliva to serum drug ratios are comparable with values obtained by Gugler *et al.* (7, 25). The correlation between saliva and serum total drug levels was fairly high (Table III) and is, in part, due to the precision of the assay (13). The low correlation values (0.11–0.64) reported in previous studies (9, 26, 27) may be due in part to the GC assays used in measuring saliva levels <1 μg/mL (7, 27). Another factor is the pooled human data analyzed in these studies, since interindividual variability is greater than intraindividual variability (7, 20).

The saliva levels of valproic acid were 20% of the serum free valproic acid concentration under single-dose conditions. Under a multiple-dose regimen, saliva valproate decreased to ~12% of the free serum drug concentrations (Table III). The increase in the serum free fraction under the multiple-dose conditions accounts for the observed decrease in the saliva to free serum valproate ratio.

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Pharmacokinetics of Nicorandil, a New Coronary Vasodilator, in Dogs

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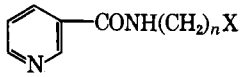
Abstract □ The kinetic disposition of nicorandil, *N*-[2-(nitroxy)ethyl]-3-pyridinecarboxamide (I), and its main metabolic product, *N*-[2-(hydroxy)-ethyl]-3-pyridinecarboxamide (II), was studied after administering intravenous and oral doses (2.5 mg/kg) of nicorandil to the same beagle dogs. The plasma concentrations were measured using a high-performance liquid chromatographic method. The pharmacokinetic data derived from intravenous administration of nicorandil were: $t_{1/2}$, 0.73 ± 0.11 h; Vd_{area}, 0.67 ± 0.04 L/kg; and total plasma clearance, 13.50 ± 1.05 mL/min/kg. After oral administration, nicorandil was rapidly absorbed (t_{max}, 0.58 ± 0.11 h). The oral bioavailability was calculated as 0.72 ± 0.07. The metabolic formation of the corresponding alcohol after intravenous and oral administration of the parent compound appeared to occur quite efficiently, and its elimination half-life (3.09 ± 0.25 and 3.69 ± 0.88 h after intravenous and oral administration of nicorandil, respectively) was longer than that of the parent compound. Since the dose employed in this study was much higher than the expected therapeutic doses, whether such a good bioavailability after a lower dose of the drug would be obtained in humans remains unanswered.

Keyphrases □ Nicorandil—metabolism in dogs, pharmacokinetics, detection in plasma by HPLC □ Vasodilators, coronary—nicorandil, metabolism in dogs, pharmacokinetics, detection in plasma by HPLC □ Bioavailability—nicorandil in dogs, oral and intravenous administration, pharmacokinetics, detection in plasma by HPLC

Nicorandil[1] (*N*-[2-(nitroxy)ethyl]-3-pyridinecarboxamide, I) a new coronary vasodilator has been shown to produce a potent coronary vasodilating effect, virtually without affecting cardiac contraction, heart rate, or myocardial oxygen consumption in anesthetized dogs (1–3). The pharmacological profile appears to be somewhat similar to that of nitroglycerin (2–4). However, except for one study where the metabolic disposition of nicorandil was qualitatively examined in rats (5), the pharmacokinetics and bioavailability of this drug have not been investigated. The lack of a sensitive and specific method for measurement of nicorandil in the blood has precluded any quantitative pharmacokinetic analysis.

In this study we describe a high-performance liquid chromatographic (HPLC) method for determining nicorandil levels in canine plasma. Using this method, we attempted to study the kinetic disposition of nicorandil (I) administered intravenously and orally on two separate occasions in the same dogs. Some pharmacokinetic variables of *N*-[2-(hydroxy)-ethyl]-3-pyridinecarboxamide (II), a primary metabolite of nicorandil, were also estimated.



I X = ONO_2, n = 2
II X = OH, n = 2
III X = ONO_2, n = 3

EXPERIMENTAL

Materials and Procedures—Six adult beagle dogs weighing 8.2–9.9 kg were used for the study. They were fasted overnight; water was supplied *ad libitum*.

[1] The generic name was published in the *Supplement to WHO Chronicle*, **34**(9), 18 (1980). Lot R9B09, synthesized at the Organic Chemistry Research Laboratory, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, was used throughout.

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