Multicompartment Pharmacokinetic Model of 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-Ethylidene-β-D-glucopyranoside) in Humans

FRANCIS R. PELSOR ˣ, LARRY M. ALLEN *, and PATRICK J. CREAVEN ‡

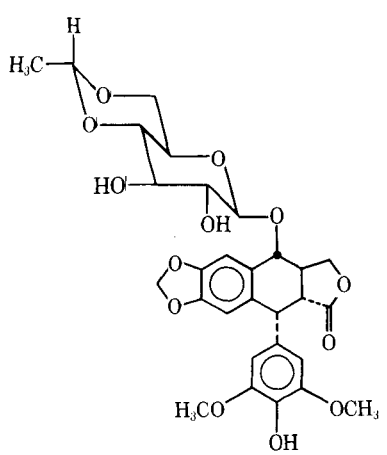
Received November 13, 1975, from the *National Cancer Institute–Veterans Administration Medical Oncology Branch, Veterans Administration Hospital, Washington, DC 20422.* Accepted for publication November 30, 1977. *Present address: Comprehensive Cancer Center, Division of Oncological Pharmacology, University of Miami School of Medicine, Jackson Memorial Medical Center, Miami, FL 33152. ‡Present address: Grace Cancer Drug Center, Division of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, NY 14263.

Abstract □ The formation and elimination of the metabolite of 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (I) were studied in seven patients with advanced cancer who received I intravenously. The plasma concentration–time data best fit a triexponential equation. The volume of the metabolite compartment (27.5 liters) was calculated as a fraction of the extrapolated volume. A larger body clearance (111.7 ml/min) of metabolite as compared to the renal clearance (31.3 ml/min) indicates that the metabolite is lost from the plasma equivalent space by another elimination route. The combination of metabolite data presented here with previously published data for unchanged I leads to a multicompartment model for the distribution, metabolism, and excretion of I and its metabolite. A comparison of algebraically derived model transfer constants with those evaluated by computer fitting the system of differential equations is presented.

Keyphrases □ 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside)—and metabolite, pharmacokinetics in humans □ Pharmacokinetics—podophyllotoxin derivative and metabolite in humans □ Antineoplastic agents, potential—podophyllotoxin derivative and metabolite, pharmacokinetics in humans □ Podophyllotoxin derivative and metabolite—pharmacokinetics in humans

The clinical pharmacology of 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (I) in patients with advanced cancer was reported previously (1). The data for the time course of the plasma concentration of I following intravenous administration previously were computer fitted to a biexponential equation, and distribution and elimination parameters were calculated (1, 2). These results established that the turnover of unchanged drug in the peripheral compartment was quite rapid, although the elimination phase was moderately prolonged.

However, urinary excretion of metabolized and unmetabolized I accounted for less than 50% of the administered dose of this highly water-insoluble compound (1, 2). Further study of the disposition of unchanged I showed that extensive degradation to tritiated water, retention in the body, and excretion in feces did not account for this discrepancy (1). Furthermore, because the body clearance equaled the plasma clearance for unchanged I, it was previously concluded that the less than quantitative recovery of radioactivity was due to body sequestering of the metabolite and not of unchanged drug (2).



I

To analyze the disposition of I in humans more fully, the kinetics of metabolite formation and elimination were studied and a multicompartment model was developed to describe the disposition of I and its metabolite. Since the chemical identity of the metabolite has not been established, the concentration–time course of the I metabolite in biological fluids was determined by selective extraction of unchanged drug.

To facilitate the derivation of metabolite distribution parameters, the differential equations resulting from the multicompartment model were solved to yield an integrated equation describing the plasma level–time curve of the metabolite after a single intravenous injection of I. These algebraically derived parameters are compared with those obtained by fitting the differential equations.

EXPERIMENTAL

Materials—The reagents were obtained commercially. Compound I[1] was supplied in sealed ampuls ready for intravenous use. Each patient received 250 μCi of 9-^{3}H-I[2] (specific activity of 14.112 mCi/mmole).

Analytical Procedures—The concentration of 9-^{3}H-I and the metabolite in biological fluids was obtained by selective extraction of unchanged drug with chloroform and radioisotope counting as described previously (1). The procedure quantitatively removed unchanged drug without removing the metabolite, as determined by TLC. Further chromatographic analysis of the aqueous phase in several systems confirmed the presence of one major metabolite of I.

Pharmacokinetic Studies—The plasma decay and urinary excretion of I and its metabolite were studied in seven patients who received I, 130–290 mg/m^2 body surface area, during the initial clinical trials of weekly intravenous administration (3). Infusion of the solubilized drug, sample collection, and handling of plasma and urine were described previously (1).

RESULTS

Solution of the differential equations describing the multicompartment model (Scheme I), by the method of Benet (4), yields the following equation, which describes the time course of plasma metabolite con-

[1] VP16-213 (NSC 141540), 100 mg/5 ml, Drug Research and Development, National Cancer Institute, Bethesda, MD 20014.

[2] Supplied by Dr. H. Stähelin, Sandoz Ltd., Basel, Switzerland, through Drug Research and Development, National Cancer Institute, Bethesda, MD 20014.

0022-3549/78/0800-1106$01.00/0

Table I—Parameters [a] for the Plasma Concentration of I Metabolite According to Eq. 1

Patient (mg/m²)	Dose, mg	A_1, μg/ml	α, hr^{-1}	A_2, μg/ml	β, hr^{-1}	A_3, μg/ml	γ, hr^{-1}	Area[b], μg hr/ml
NG (130)	205	1.59 (0.16)	2.21 (0.46)	1.31 (13.37)	0.05 (0.12)	0.27 (13.27)	0.08 (1.23)	29.10
KR (170)	255	2.47 (1.25)	0.92 (0.36)	0.63 (4.18)	0.05 (0.12)	1.80 (3.00)	0.15 (0.37)	22.22
TS (170)	230	4.57 (10.06)	0.64 (0.47)	2.60 (0.40	0.04 (0.004)	1.94 (9.81)	0.33 (0.72)	64.31
JB (220)	415	2.02 (0.73)	0.81 (0.47)	0.29 (1.18)	0.02 (0.10)	1.62 (0.64)	0.11 (0.17)	27.35
RJ (220)	363	4.36 (1.72)	0.71 (0.21)	0.46 (0.24)	0.02 (0.01)	3.83 (1.60)	0.20 (0.07)	35.99
HR (290)	400	3.77 (0.36)	1.21 (0.15)	0.47 (0.15)	0.02 (0.007)	3.29 (0.30)	0.16 (0.02)	39.04
KR (290)	425	5.50 (9.18)	0.70 (0.48)	1.47 (1.26)	0.04 (0.03)	3.99 (8.17)	0.30 (0.44)	40.82

[a] Parameters are quoted ± *SE*. [b] $\text{Area}^{0\to\infty} = \int_0^T C_4\,dt + (A_2e^{-\beta T}/\beta)$ (Ref. 8).

Table II—Calculated I Metabolite Distribution and Clearance Parameters

Patient (Body Weight, kg)	V_4[a], liters	Cl_{rm}[b], ml/min	Cl_b[c], ml/min	Urine Excretion (48 hr), % (Found)
NG (62)	15.84	21.83	96.30	15.57 (15.34)
KR (56)	24.43	32.51	109.02	14.58 (14.87)
TS (48)	16.82	26.46	34.57	35.37 (34.92)
JB (75)	54.01	30.01	171.96	7.98 (7.84)
RJ (62)	40.15	41.68	134.48	16.20 (14.94)
HR (48)	19.55	41.01	124.66	15.49 (—)
KR (56)	21.75	26.17	111.06	11.33 (10.82)
Mean ± *SD*[d]	27.51 ± 14.25	31.27 ± 7.67	111.72 ± 41.86	

[a] V_4 calculated according to Eq. 5. [b] $Cl_{rm} = (\Sigma M_u/\int_0^T C_4\,dt)$ (Ref. 12). [c] $Cl_b = (F_m \text{ dose}/\text{area}^{0\to\infty})$, where F_m is the difference between the dose and the fraction recovered unchanged in the urine (2) (Ref. 10). [d] The means of the central, V_1, and peripheral, V_2, compartment volumes and renal clearance of unchanged drug for the seven patients as determined previously (2) are 8.09 ± 2.30 liters, 9.15 ± 3.70 liters, and 11.80 ± 3.90 ml/min, respectively.

centration, C_4, following the single intravenous administration of I:

$$C_4 = -A_1e^{-\alpha t} + A_2e^{-\beta t} + A_3e^{-\gamma t} \qquad \text{(Eq. 1)}$$

where A_2 and A_3 are the zero-time intercepts given by:

$$A_2 = \frac{k_m \text{dose}}{V_4}\,\frac{(k_{21}-\beta)}{(\alpha-\beta)(\gamma-\beta)} \qquad \text{(Eq. 2)}$$

$$A_3 = \frac{k_m \text{dose}}{V_4}\,\frac{(k_{21}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)} \qquad \text{(Eq. 3)}$$

and α, β, and γ are hybrid transfer constants for disposition processes of unchanged drug and metabolite (4). The disposition rate constant, k_{21}, and the rate constant of biotransformation, k_m, were determined previously (2). The parameter A_1 is determined as a function of the rate constants given by:

$$A_1 = \frac{k_m \text{dose}}{V_4}\,\frac{(k_{21}-\alpha)}{(\beta-\alpha)(\gamma-\alpha)} \qquad \text{(Eq. 4)}$$

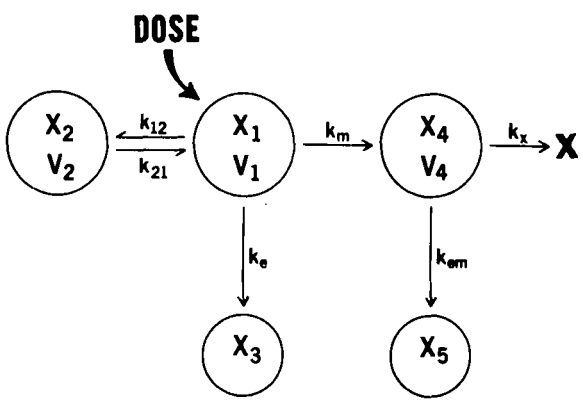
Graphical estimates of the parameters of Eq. 1 were refined by nonlinear least-squares regression analysis, using the Marquardt–Levenburg method (5) in the computer program package[3] MLAB (6, 7). The computed parameter values and the area under the plasma decay curve are listed in Table I.

The volume of the metabolite compartment, V_4, may be calculated as a fraction of the extrapolated volume (9, 10) using Eq. 5 (see proof in *Appendix*):

$$V_4 = \frac{k_m \text{dose}}{A_2(\alpha-\beta) + A_3(\alpha-\gamma)} \qquad \text{(Eq. 5)}$$

The renal clearance of the metabolite, Cl_{rm}, was evaluated by linear least-squares regression analysis (11) of the cumulative amount excreted on the area under the plasma decay curve at the midpoint of the collection interval (12). The renal excretion rate constant, k_{em}, was calculated by algebraic manipulation of Eq. 6:

$$Cl_{rm} = k_{em}V_4 \qquad \text{(Eq. 6)}$$



Scheme I—Multicompartment pharmacokinetic model. The parameters k_{12} *and* k_{21} *are the distribution rate constants between the central compartment,* X_1*, with volume* V_1 *and the peripheral compartment,* X_2 *with volume* V_2*;* k_m *is the first-order rate constant of biotransformation;* k_e *and* k_{em} *are urine* (X_3 *and* X_5) *excretion rate constants; and* k_x *is the transfer rate constant for irreversible loss from the metabolite compartment,* X_4*, with volume* V_4.

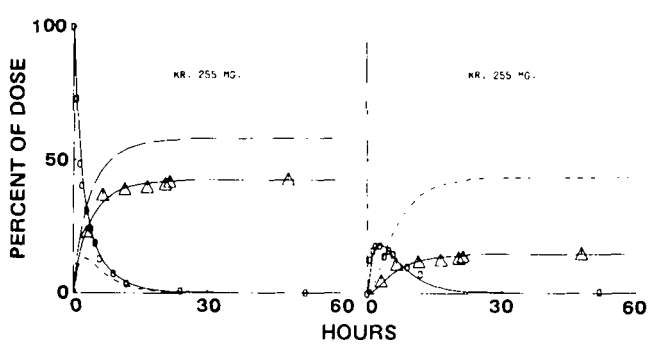


Figure 1—*Left: plot of the distribution, excretion, and metabolism of the percent dose of I. Data points were determined experimentally for serum (○) and urine (△) I concentrations for Patient KR. The theoretical amount of drug in the peripheral compartment,* X_2*, is represented by line ---. The line – – represents the cumulative amount of I metabolized. Right: plot of the formation, disposition, and excretion of the metabolite of I. The symbols ○ and △ represent experimentally determined data points for serum and urine concentrations, respectively. The line --- represents the theoretical amount of depot of I metabolite in body tissues,* X.

[3] Available on a DEC system-10 digital computer, Computer Center, Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD 20014.

Table III—Disposition Rate Constants for the Multicompartment Model [a]

Patient	k_{12}, hr^{-1}	k_{21}, hr^{-1}	k_e, hr^{-1}	k_m, hr^{-1}	k_{em}, hr^{-1}	k_x, hr^{-1}
NG	0.51 (0.02)	0.28 (0.02)	0.05 (0.002)	0.22 (0.009)	0.07 (0.004)	0.31 (0.02)
KR	0.15 (0.01)	0.43 (0.07)	0.14 (0.002)	0.20 (0.004)	0.09 (0.003)	0.26 (0.01)
TS	0.37 (0.02)	0.27 (0.02)	0.10 (0.002)	0.15 (0.004)	0.10 (0.004)	0.06 (0.005)
JB	0.12 (0.01)	0.18 (0.05)	0.06 (0.002)	0.14 (0.008)	0.04 (0.005)	0.28 (0.03)
RJ	0.18 (0.01)	0.16 (0.03)	0.06 (0.002)	0.24 (0.01)	0.07 (0.003)	0.26 (0.02)
HR	0.10 (0.02)	0.02 (0.01)	0.06 (0.003)	0.12 (0.02)	0.14 (0.01)	0.33 (0.09)
KR	0.07 (0.01)	0.14 (0.05)	0.06 (0.001)	0.13 (0.009)	0.09 (0.007)	0.47 (0.05)
Mean ± SD [b]	0.21 ± 0.16	0.21 ± 0.13	0.08 ± 0.03	0.17 ± 0.05	0.08 ± 0.03	0.28 ± 0.12

[a] Results are quoted ± SE of the parameter estimate. [b] The means of the transfer constants for the seven patients as determined previously from the integrated equation (2) are 0.34 ± 0.30, 0.33 ± 0.35, 0.09 ± 0.04, and 0.21 ± 0.05, respectively; those for k_{em} and k_x (see text) are 0.08 ± 0.03 and 0.19 ± 0.09, respectively.

which describes the relationship between the compartment volume and the renal clearance. The difference between the body clearance (renal plus nonrenal) of the metabolite, Cl_b, and the renal clearance was used to determine the rate constant for loss of the metabolite, k_x, to body tissue. Substitution and rearrangement yield:

$$k_x = \frac{Cl_b - Cl_{rm}}{V_4} \qquad \text{(Eq. 7)}$$

The volume and clearance parameters are listed in Table II. The average value and standard deviation of the excretion rate constants as derived here for the metabolite, using Eqs. 6 and 7, are 0.08 ± 0.03 hr^{-1} for k_{em} and 0.19 ± 0.09 hr^{-1} for k_x.

The multicompartment model of I pharmacokinetics, as depicted in Scheme I, was developed by addition of the model for metabolite formation and disposition developed here to the two-compartment open model of unchanged drug distribution and elimination previously described (2). Initial estimates of the rate constants were refined by fitting the parameters of the differential equations to the data calculated as the percent of dose administered. A weighting factor of 1 was used in the weighted least-squares analysis (6, 7). Typical computer fits are shown in Figs. 1 and 2. Table III shows the results of the computer-fitted model parameters for the seven patients as well as the average value of those calculated transfer constants previously published for unchanged drug (2) and obtained here for the metabolite.

DISCUSSION

Compound I is a semisynthetic podophyllotoxin derivative for which the pharmacokinetics of unchanged drug in humans were reported (2). However, because the cumulative excretion of drug is substantially less than the amount of drug administered, of which two-thirds can be accounted for as unchanged drug (1), a multicompartment pharmacokinetic model was developed to ascertain if I or its metabolite was sequestered in the body.

The excellent agreement (Table III) between the calculated values of the transfer constants for unchanged drug previously reported (2) and those obtained here for the metabolite with the disposition constants obtained from fitting the differential equations directly to the data indicates that the drug and its metabolite behave according to the model (Scheme I). According to the model, biotransformation of I occurs only in the central compartment, V_1, in agreement with the previous conclusion obtained from the comparison of body and plasma clearances of unchanged drug (2). The metabolite is distributed into a threefold larger compartment, V_4, which approximates the volume of total body water. From this compartment, the metabolite is eliminated.

Since the renal excretion mechanism is not saturation limited, as indicated by the plot of the urinary excretion rate *versus* the plasma metabolite concentration (13), a nonrenal mechanism for loss of the metabolite must be sought to account for the difference between the amount of the metabolite recovered in the urine (Table II) and the theoretical fraction of I metabolized (2). Furthermore, the larger body clearance of the metabolite as compared to renal clearance (Table II) indicates that the theoretical rate of loss of the metabolite to body tissues is both rapid (Table III) and extensive (Figs. 1 and 2).

In conclusion, the pharmacokinetic analysis of I and its metabolite has identified the metabolite as being retained in the body.

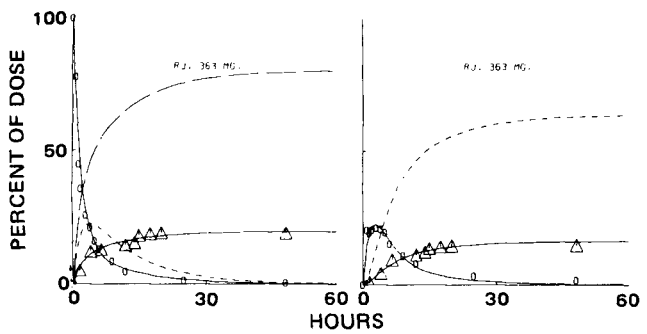


Figure 2—*Same as Fig. 1 but for Patient RJ.*

APPENDIX

Substitutions in Eq. 5 were made from Eqs. 2 and 3, in a manner similar to that reported by Wagner (14), to yield:

$$V_4 = \frac{k_m \text{dose}}{\frac{k_m \text{dose}}{V_4}\frac{(k_{21}-\beta)(\alpha-\beta)}{(\alpha-\beta)(\gamma-\beta)} + \frac{k_m \text{dose}}{V_4}\frac{(k_{21}-\gamma)(\alpha-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}} \qquad \text{(Eq. A1)}$$

$$V_4 = \frac{k_m \text{dose}}{\frac{k_m \text{dose}}{V_4}\left(\frac{(k_{21}-\beta)}{(\gamma-\beta)} - \frac{(k_{21}-\gamma)}{(\gamma-\beta)}\right)} \qquad \text{(Eq. A2)}$$

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ACKNOWLEDGMENTS

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Atlanta meeting, November 1975.