Instantaneous Input Hypothesis in Pharmacokinetic Studies

WIN L. CHIOU ˣ, GILBERT LAM, MEI-LING CHEN, and MYUNG G. LEE

Received December 1, 1980, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication February 20, 1981.

Abstract □ Observed venous plasma concentrations of furosemide, propranolol, griseofulvin, and theophylline at 0.33 and 0.66 min after intravenous bolus injections to unanesthetized dogs were compared with those extrapolated using the instantaneous input hypothesis. At 0.33 min, extrapolated/observed plasma level ratios as high as 20.5, 65.5, 226, and 1.17 were found for these four drugs, respectively. Venous plasma levels peaked at 1 min postinjection in all studies. Total plasma areas ($AUC_{0\to\infty}$) estimated using the instantaneous input principle were higher by as much as 6.0, 6.8, and 19.6% for propranolol, griseofulvin, and furosemide, respectively, when compared with experimental data. The effect on theophylline was negligible. These results suggest the need for cautious interpretation of some venous pharmacokinetic data. More studies in animals and humans are required to assess the magnitude of deviation from the instantaneous input hypothesis for drugs in general.

Keyphrases □ Pharmacokinetics—instantaneous input hypothesis, comparison with conventional extrapolation method, dogs □ Instantaneous input hypothesis—comparison with conventional extrapolation method, dogs □ Venous pharmacokinetics—instantaneous input hypothesis and conventional extrapolation method compared, dogs

In pharmacokinetic studies, venous plasma (blood or serum) level data, usually obtained 1–30 min (1–3) after a rapid intravenous bolus injection, often are employed to fit a polyexponential equation, $\sum_{i=1}^{n} A_i e^{-\lambda_i t}$. The obtained equation, also called the disposition function, then is used for pharmacokinetic analysis (1–10). This method assumes that, after injection, the drug is instantaneously distributed into an initial apparent volume of distribution or the volume of the central compartment as defined in the multicompartmental mammillary modeling analysis. The initial volume of distribution or the volume of the central compartment is calculated by dividing the dose by the extrapolated plasma concentration at time zero, C_p^0, which, in turn, is calculated by the summation of the polyexponential coefficients, $\sum_{i=1}^{n} A_i$. The total area under the plasma concentration–time curve from zero to infinite time, $AUC_{0\to\infty}^{ext}$, usually is obtained by:

$$AUC_{0\to\infty}^{ext} = \sum_{i=1}^{n} \frac{A_i}{\lambda_i} \qquad \text{(Eq. 1)}$$

In Eq. 1, the plasma area from time zero to the first plasma concentration point is, in essence, estimated by the extrapolation based on experimental data obtained later.

The present report compares experimentally obtained plasma concentrations and plasma areas between time zero and 1 min with those estimated by the conventional extrapolation method.

EXPERIMENTAL

Intravenous Bolus Studies—Propranolol[1], furosemide[2], theophylline[3], and griseofulvin[4] were studied in mongrel male conditioned dogs, 18.7–23.7 kg. The drug in solution was injected through an indwelling catheter placed in the cephalic vein. Blood samples were withdrawn from a permanent cannula placed in the femoral vein *via* the saphenous vein. Bolus injection was completed in 10 sec for propranolol and furosemide and in 20 sec for theophylline and griseofulvin. Normal saline, 3–5 ml, was used immediately to flush the catheter to ensure complete drug delivery.

Blood samples were collected before injection and at 0.33, 0.66, 1, 2, 3, 6, 9, 15, 30, 45, 90, 120, 150, 180, 210, 240, 300, and 360 min postinjection. They were centrifuged immediately and stored frozen prior to analysis. The midpoint of injection was set as time zero. Concentrations of drugs in plasma were quantitated by the specific and sensitive high-performance liquid chromotagraphic methods developed previously in this laboratory (11–14).

Two dogs were studied for each drug; five dogs were used in the entire study. At least 3–5 days elapsed between studies for each dog. The doses and body weights of the dogs are summarized in Table I.

Data Analysis—Plasma data from 1 min were fitted to either a two- or three-exponential equation by using a nonlinear least-squares regression program, NONLIN (15), on a digital computer. Plasma data were weighted by the reciprocal concentration squared.

RESULTS AND DISCUSSION

The coefficients and exponents of the polyexponential disposition function based on the NONLIN computer analysis are summarized in Table I. Goodness of fit is supported by the r^2 values, which were all very close to 1.000 (Table I). There is good agreement between the experimental data and the computer-generated curves (Figs. 1–4). However, the plasma concentrations found at 0.33 and 0.66 min were lower than those estimated by the extrapolation method from the polyexponential decay equations in all studies. The differences between the observed and extrapolated levels at 0.33 min are summarized in Table II. Ratios of the extrapolated to observed concentrations up to 20.5, 65.5, and 226 were found for furosemide, propranolol, and griseofulvin, respectively (Table

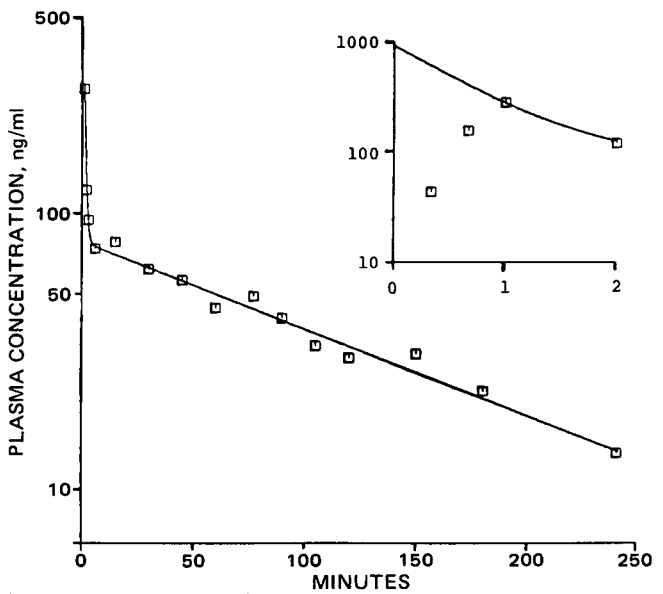

Figure 1—*Venous plasma concentration profile of propranolol following an intravenous bolus injection of 10 mg of propranolol hydrochloride to Dog 2. The solid line was generated by the NONLIN computer program. The insert shows the profile in the first 2 min.*

[1] Propranolol hydrochloride (10 mg/ml), Ayerst Laboratories, New York, N.Y.
[2] Lasix injection (10 mg/ml), Hoechst-Roussel Co., Somerville, N.J.
[3] Aminophylline injection (500 mg/5 ml), Elkins Sinn Co., Cherry Hill, N.J.
[4] Powder (Ayerst Laboratories, New York, N.Y.) dissolved in polyethylene glycol 400 (60 mg/1.5 ml).

0022-3549/81/0900-1037$01.00/0

Table I—Polyexponential Disposition Function Using Nonlinear Least-Squares Regression Analysis of Plasma Concentration Data from 1 Min

Drug	Dose, mg	Dog[a]	A_1, μg/ml	A_2, μg/ml	A_3, μg/ml	λ_1, min^{-1}	λ_2, min^{-1}	λ_3, min^{-1}	r^2
Propranolol	8.8	1	0.842	0.0785	—	1.43	0.00726	—	0.998
	8.8	2	0.887	0.0922	0.0571	0.737	0.0321	0.0103	1.000
Furosemide	40	2	28.5	9.04	1.12	0.955	0.153	0.0261	1.000
	40	3	28.4	5.49	0.764	1.12	0.125	0.0230	1.000
Theophylline	400	3	95.6	21.8	—	0.613	0.0028	—	0.996
	400	4	7.37	20.3	—	0.319	0.0026	—	0.998
Griseofulvin	60	3	9.71	3.35	1.47	1.79	0.187	0.0188	1.000
	60	5	5.92	1.31	0.691	0.515	0.0357	0.0091	1.000

[a] Body weights for Dogs 1–5 were 18.7, 20.3, 19.2, 23.7, and 21.3 kg, respectively.

Table II—Comparisons of Observed and Extrapolated Plasma Concentrations at 0.33 min and Actual and Extrapolated Total Plasma Areas after Intravenous Bolus Injection to Dogs

Drug	Dog	Observed Plasma Level at 0.33 min, μg/ml	Extrapolated Plasma Level at 0.33 min, μg/ml	Extrapolated/ Observed Plasma Level Ratio at 0.33 min	$AUC^{ext}_{0\to\infty}$, min μg/ml	$AUC^{act}_{0\to\infty}$, min μg/ml	Percent Overestimation in Plasma Area
Propranolol	1	0.0430	0.600	14.0	11.41	11.00	3.75 (4.86)[a]
	2	0.0129	0.842	65.5	9.62	9.08	5.97
Furosemide	2	3.22	30.5	9.48	131.9	113.5	16.2 (17.9)[a]
	3	1.25	25.6	20.5	102.5	85.7	19.6
Theophylline	3	88.0	99.7	1.13	7933	7920	0.17 (0.13)[a]
	4	22.9	26.9	1.17	7835	7829	0.08
Griseofulvin	3	0.989	9.96	10.1	101.5	95.0	6.81 (5.38)[a]
	5	0.0308	6.97	226	124.2	119.4	4.72

[a] Mean of the two dog studies.

II). For theophylline, the difference at 0.33 min was relatively much smaller (13 and 17%). The inserts in Figs. 1–4 show the experimental and extrapolated plasma level profiles of the individual dog study for the first 2 min postdosing.

In dogs, the peak plasma levels occurred at 1 min after intravenous injection; but in preliminary studies using anesthetized rabbits, peak plasma (from femoral vein) levels for procainamide (16) and griseofulvin occurred at 2 min after a rapid intravenous bolus injection.

The overestimations of the initial plasma concentrations from the extrapolation of the polyexponential equation probably could be attributed to several factors, such as the lag time due to drug transport from the injection site to the sampling site (17), mixing of the drug in the blood circulation, and drug extraction by the sampling tissue through diffusion across capillary walls. The last factor was supported by the fact that drug concentrations in plasma of blood collected simultaneously from the femoral artery of the same leg were higher than those in venous plasma (16). It has been stated (17) that the zero-time plasma concentration at the normal sampling site immediately after intravenous injection theoretically should be zero. The present data (Figs. 1–4) appear to support this statement.

The areas under the plasma curve between time zero and 1 min after injection were calculated by the "extrapolated" integration method (Eq. 2) and also by the linear trapezoidal rule method using experimentally observed plasma concentrations and assuming zero drug concentration at time zero:

$$AUC^{ext}_{0\text{–}1\ min} = \sum_{i=1}^{n} \frac{A_i}{\lambda_i}(1 - e^{-\lambda_i t}) \qquad \text{(Eq. 2)}$$

where t was set to be 1 min. The overestimation of the plasma area in the first 1 min (ΔAUC) by the extrapolation method is assumed to be equal

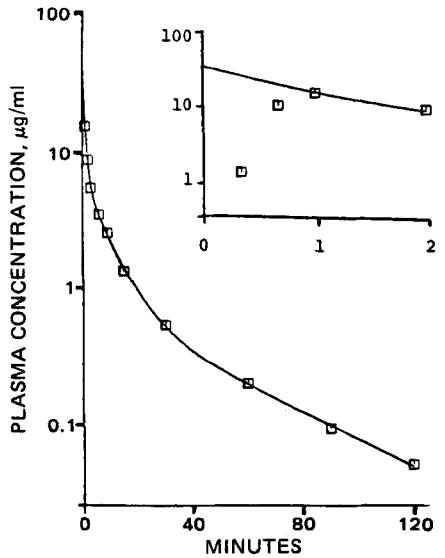

Figure 2—*Venous plasma concentration profile of furosemide following an intravenous bolus injection of 40 mg to Dog 3.*

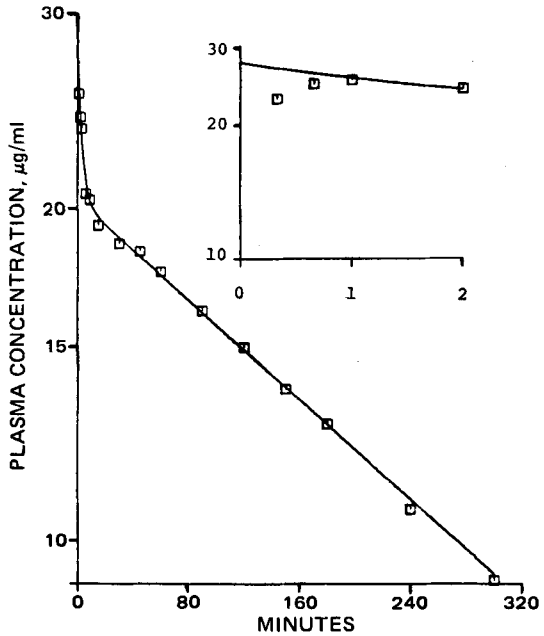

Figure 3—*Venous plasma concentration profile of theophylline following an intravenous bolus injection of 400 mg to Dog 4.*

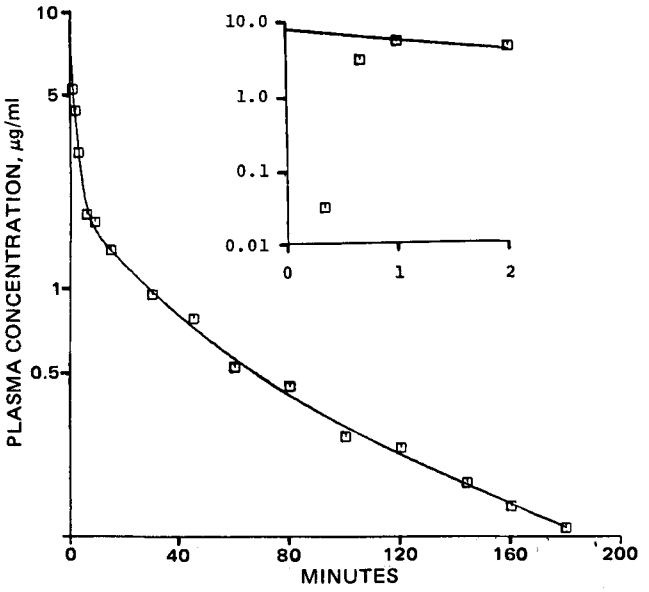

Figure 4—*Venous plasma concentration profile of griseofulvin following an intravenous bolus injection of 60 mg to Dog 5.*

to the difference between the areas estimated by the described two methods. The "correct" (actual) total plasma area, $AUC^{act}_{0\to\infty}$, may be calculated by:

$$AUC^{act}_{0\to\infty} = AUC^{ext}_{0\to\infty} - \Delta AUC \qquad \text{(Eq. 3)}$$

Therefore, the percentage of overestimation in plasma area for the first 1 min by the extrapolation method as compared with the actual total plasma area can be calculated by:

$$\%\ \text{overestimation} = \frac{\Delta AUC}{AUC^{act}_{0\to\infty}} \times 100 \qquad \text{(Eq. 4)}$$

The results of the analyses for the four drugs are summarized in Table II. The mean overestimations for theophylline, propranolol, griseofulvin, and furosemide were 0.13, 4.86, 5.38, and 17.9%, respectively.

The plasma concentration and area data within the 1st min after intravenous injection are different from the prediction using the conventional polyexponential disposition function or multicompartmental mammillary modeling analysis. These results appear to indicate that a very intensive early blood sampling schedule may be required to characterize accurately the plasma level profile during the early period. Although the four drugs studied have markedly different physicochemical properties (two weak acids, one weak base, and one neutral compound), more studies in animals and humans are needed to assess the general magnitude of deviation from the instantaneous input hypothesis.

These findings may have some significant implications in pharmacokinetic studies. For example, the difference between the actual and the extrapolated total plasma area for furosemide in Dog 3 was 19.6% (Table II). This value would result in a difference of 19.7% in the calculated total body clearance (467 *versus* 390 ml/min based on dose/$AUC_{0\to\infty}$). By using the extrapolated total plasma area as a reference, the absolute bioavailability from a completely bioavailable dosage form (assuming correct plasma area measurement) in this dog can be calculated as only ~80%. Furosemide in solution or tablet was reported to be incompletely absorbed (usually <73%) in humans (18–22), although the total diuretic response (relationship with plasma level or dose appears to be complicated, Ref. 22) was often the same following oral or intravenous administration of the same dose (18, 19, 21). If the phenomenon observed in the two dogs in the present study happened similarly in humans, then the absolute bioavailability of furosemide might be greater than reported. Further studies are required to prove this hypothesis.

The results of the present study support the contention (23, 24) that the constant blood withdrawal method may be useful to determine accurately the $AUC_{0\to\infty}$ after an intravenous bolus injection, especially in humans. Furthermore, the extensive blood sampling procedure can be avoided (23–25).

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