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Arterial and Venous Blood Sampling in Pharmacokinetic Studies: Griseofulvin

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Abstract □ The pharmacokinetics of griseofulvin were evaluated simultaneously using both arterial and venous plasma in three dogs and one rabbit after a rapid bolus intravenous dosing. Initial arterial–venous ratios 20 sec after injection were the highest and ranged from 15- to 752-fold for dogs; the ratio was 3240-fold for the rabbit. Both curves decayed paralleling each other at the terminal phase with the venous levels higher than arterial levels by 14–43 and 8.4% for the dogs and the rabbit, respectively. The use of the instantaneous input principle was found to overestimate the total area under the plasma level–time curve by as much as 166%. An exponential term with a negative coefficient was used to account for the short and steep rising phase of plasma levels after injection. Detailed analyses showed significant differences in various calculated pharmacokinetic parameters based on arterial or venous data. The present study exemplifies the need for careful assessment and interpretation of classical pharmacokinetic parameters. It appeared that short intravenous infusion rather than the instantaneous or rapid bolus intravenous injection should be preferred for routine pharmacokinetic studies.

Keyphrases □ Griseofulvin—pharmacokinetics, arterial and venous blood sampling □ Pharmacokinetics—arterial and venous blood sampling, griseofulvin □ Blood sampling—arterial and venous pharmacokinetics of griseofulvin

Preliminary results showing marked and persistent arterial–venous (A–V) plasma concentration differences of six drugs (propranolol, lidocaine, procainamide, furosemide, theophylline, and griseofulvin), following intravenous administration to dogs or rabbits, were recently reported from this laboratory (1). The pharmacokinetic consequences of data analysis by using arterial or venous data on propranolol also has been described (2).

The present report describes in detail arterial and venous plasma level profiles of griseofulvin in three dogs and one rabbit and discusses the resulting effects on pharmacokinetic analysis.

EXPERIMENTAL

Bolus Injection Studies—Griseofulvin[1] (40 mg/ml) in polyethylene glycol 400[2] (1, 3) was injected as a bolus over 20 sec to the cephalic vein in three male mongrel dogs and to the ear vein in one male New Zealand white rabbit. Dogs 1 and 2 were conscious while dog 3 and rabbit 1 were anesthetized with nitrous oxide during the study. The doses administered to each animal are summarized in Table I. The midpoint of the injection was timed zero. Femoral arterial and venous blood samples were withdrawn simultaneously from permanent cannulas in the dogs and a specially designed T-loop in the rabbit. The preparation of the cannulas and the surgical procedure were described elsewhere (2). Heparinized normal saline (10 U/ml) was used for flushing of the cannula during the study. The sampling times, also midpoints of collection, were usually at 0.33, 0.66, 1, 1.33, 1.66, 2, 3, 6, 9, 15, 30, 60, 80, 100, 120, 140, 160, and 180 min

[1] Sigma Chemical Co., St. Louis, Mo.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.

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Table I—Comparison of Arterial–Venous Plasma Difference of Griseofulvin after Intravenous Bolus Administration to Dogs and Rabbit

	Dog 1	Dog 2	Dog 3	Rabbit
Body weight, kg	21.3	19.2	20	3.7
Dose, mg	60	60	60	14
A–V ratio at 0.33 min	176	15	752	3240
Time of intersection, min	4	2	15	45
Percent difference during terminal phase	30.0	43.0	14.7	8.4
Terminal half-life, min	64.8	37.5	73.7	277

in the dog studies, while the collection schedule extended to 300 min in the rabbit. The blood samples were centrifuged immediately to avoid the potential storage effect in the plasma concentration determination (4, 5), and 0.1 ml of plasma was collected in duplicate and frozen until assayed by a fluorometric HPLC method developed earlier in this laboratory (6).

Pharmacokinetic Analysis—The total areas under the plasma level–time curve from time zero to infinity (*AUC*) were estimated by the following three methods:

Method I—The linear trapezoidal rule was used from time zero to the last sampling point followed by the extrapolation method (*i.e.*, the last point concentration divided by the terminal rate constant).

Method II—The logarithmic trapezoidal rule (7) was used from the first to the last sampling point followed by the extrapolation method. The linear trapezoidal rule was used between time zero to the first sampling point. The method was previously employed in a propranolol study (2).

The plasma concentration at time zero in Methods I and II was assumed to be zero (8).

Method III—A NONLIN (9) computer program was employed for the determination of conventional (8) polyexponential equations ($\Sigma A_i e^{-\lambda_i t}$) based on the postpeak plasma data. For the prepeak plasma data, a linear least-squares feathering method was used to obtain another exponential rate constant, m, using a programmable calculator[3].

Since the plasma concentration at time zero was assumed to be zero, the coefficient of this exponential term, M, was set to equal the sum of the positive exponential coefficients, *i.e.*, ΣA_i. The following integration equation was used:

$$AUC = \Sigma\frac{A_i}{\lambda_i} - \frac{M}{m} \quad \text{(Eq. 1)}$$

Standard methods were used to determine the total body clearance, Cl; initial volume of distribution, V_1; apparent volume of distribution at the pseudodistribution equilibrium (10), V; steady-state volume of distribution (2, 11), V_{ss}; mean residence time (12), MRT; and the fraction of dose remaining in the body after bolus injection (2, 13):

$$Cl = \text{dose}/AUC \quad \text{(Eq. 2)}$$

$$V_1 = \text{dose}/C_p{}^0 \quad \text{(Eq. 3)}$$

$$V = \text{dose}/(AUC \times \lambda_n) \quad \text{(Eq. 4)}$$

$$V_{ss} = (\text{dose} \times \int_0^\infty AUC_{t\to\infty}dt)/AUC^2 \quad \text{(Eq. 5)}$$

$$MRT = AUMC/AUC \quad \text{(Eq.6)}$$

$$\text{Fraction remaining in the body} = AUC_{t\to\infty}/AUC \quad \text{(Eq. 7)}$$

where $C_p{}^0$ is the extrapolated plasma concentration at time zero based on the conventional method (8); λ_n is the rate constant of the terminal phase; $AUMC$ is the area under the first moment of the plasma curve, and $AUC_{t\to\infty}$ is the total plasma area from time t to infinity. The AUC values for the calculation of these pharmacokinetic parameters were obtained from Method II.

RESULTS AND DISCUSSION

The arterial and venous plasma concentration profiles of griseofulvin are shown in Figs. 1 and 2. The arterial levels shortly after injection were all much higher than the venous levels indicating an extensive and rapid uptake of griseofulvin by the sampling tissues during a single passage in the leg (1, 2, 11, 13). The maximum A–V ratio for each study occurred at 0.33 min; this ranged from 15 for dog 2 to 3240 for the rabbit (Table I). These ratios decreased to unity at ~2 min for dog 2 and 45 min for the rabbit (Table I) when the net uptake by the sampling tissue was zero (*i.e.*, when the sampling tissue concentration was the maximum). Thereafter, the venous levels were higher than the arterial levels due to the release of griseofulvin from the leg tissues to the venous blood (1, 2, 11, 13). During the apparently parallel arterial and venous terminal phases (Figs. 1 and 2), the venous plasma levels were higher than arterial levels by ~30, 43, 14.7, and 8.4% for dogs 1, 2, 3, and the rabbit, respectively. The terminal half-life in the three dogs studied ranged from 37.5 to 73.7 min, and that for the rabbit was estimated to be 277 min (Table I). A longer sampling time in the rabbit study might be preferred in view of its long terminal half-life.

Various factors affecting the A–V difference during the terminal phase were discussed previously from a theoretical point of view (14). The smaller A–V difference observed in the rabbit study as compared with the dog studies was consistent with the hypothesis (14) that the longer the half-life the smaller the A–V difference in the terminal phase. This is because the rate of release of drug from the sampling tissue to the venous blood would be slower if the half-life were longer (assuming other factors were the same). It should be noted that the terminal arterial or venous plasma half-lives should be theoretically identical (14) as shown in five other drugs in animals (1, 2) and insulin in humans (15).

Unlike the conventional concept of instantaneous input to the central or plasma compartment (1, 2, 14, 16), the venous griseofulvin concentrations after bolus dosing were found to increase from the first 0.33 min and to peak at 1 min in the three dogs and at 2 min in the rabbit (Figs. 1 and 2). For dogs 1 and 2, the arterial plasma levels both peaked at 0.66 min (Fig. 1). These data resembled a rapid absorption plasma level profile

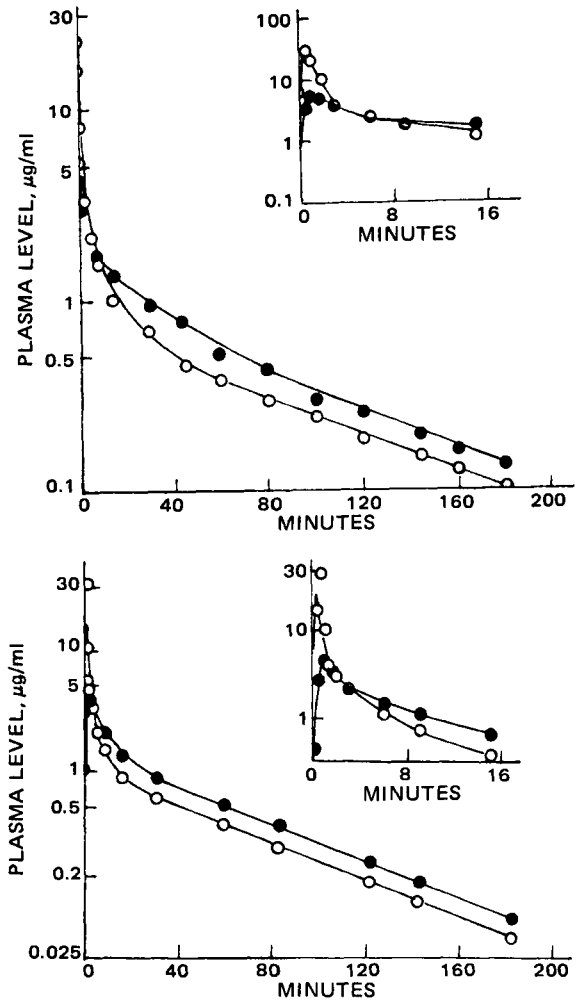

Figure 1—*The arterial (○) and venous (●) plasma level profiles after bolus injection of 60 mg of griseofulvin to dog 1 (upper curve) and dog 2 (lower curve). Solid lines are computer-generated, best-fitted curves. Inserts show the plasma level profiles in the first 16 min.*

[3] Hewlett-Packard, Corvallis, Oreg.

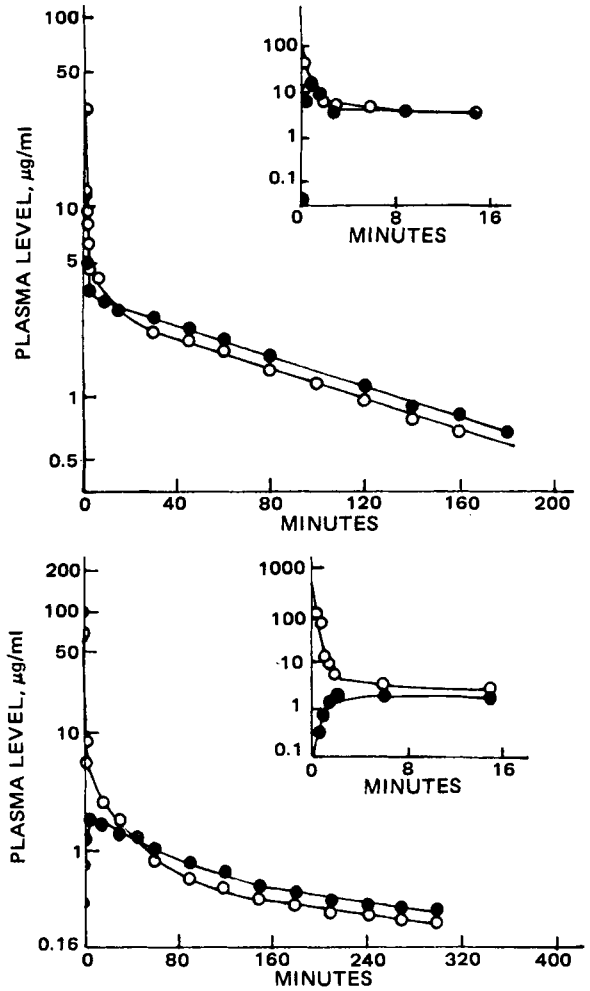

Figure 2—*The arterial (○) and venous (●) plasma level profiles after bolus injection of 60 mg of griseofulvin to dog 3 (upper curve) and 14 mg to the rabbit (lower curve).*

(2, 16). To account for this rising phase, a negative exponential term (Me^{-mt}) was used in curve fittings. The goodness of fit is judged by the closeness between the actual and computer-generated values as shown in Figs. 1 and 2, together with similar *AUC* measurements by the three methods (Table II). Furthermore, the r^2 value for all the fittings was >0.998. In the dog 3 and rabbit studies, the arterial plasma levels were found to peak at 0.33 min, which was the first blood sample; hence, their negative exponential term could not be ascertained.

The importance of recognizing that the plasma level should theoretically be zero at time zero following a bolus injection (8) is best illustrated in dogs 1 and 2. The total arterial plasma areas under the negative exponential curve could be calculated to account for 35 and 166% of the *AUC* for dogs 1 and 2, respectively (100 M/*m*/*AUC* where *AUC* was estimated based on Method II). Failure to consider this factor would result in a large overestimation of the arterial *AUC* in these two dogs if the conventional concept of the instantaneous mixing or polyexponential decay was applied. This effect apparently was minimal for dog 3 as the arterial *AUC* calculated by the conventional method was close to those calculated by the other two methods, assuming zero concentration at time zero (Table II). The effect on the venous *AUC* calculation in most of the studies was also relatively insignificant (Table II). This was in contrast with the furosemide studies in the dogs where the true venous *AUC* values could be overestimated by up to 20% if factors of initial transport lag time (8) and uptake of sampling tissues were not considered (16). The above analysis, together with findings from previous studies (1, 2, 8, 16) indicate the complexity and difficulty in accurately describing the unit-impulse disposition function of drugs (8, 17) in the body. It should be emphasized that an accurate determination of the *AUC* from the instantaneous dosing is important, since the value obtained is often used to calculate other pharmacokinetic parameters. In this regard, it appears that a short-term, intravenous infusion rather than a bolus or instantaneous injection might be preferred. Furthermore, the *AUC* from the infusion study can be more simply estimated by the linear-logarithmic trapezoidal rule-extrapolation method (7) as employed in Method II of the present study.

Theoretically, the arterial *AUC* should equal the venous *AUC* if a drug is not metabolized in the sampling tissues (2, 9, 14, 18). This was confirmed in the dog studies (Table II). In the rabbit study, however, the arterial *AUC* was higher than the venous *AUC* by ~16%. Similar results were found in other drug studies. The reason for such inequality remains to be explored.

Except for the rabbit, the *Cl* and *V* values estimated from arterial and venous plasma data in the three dogs were, as expected, essentially the same (Table III). However, the physiological significance of these values may be different (2, 19, 20). The calculated values for V_1 were found to

Table II—Polyexponential Disposition Function and the Area under Plasma Level-Time Curve of Griseofulvin in Dog and Rabbit Studies

Parameter	Dog 1		Dog 2		Dog 3		Rabbit	
	A	V	A	V	A	V	A	V
M, μg/ml	48.33	8.0	698.27	11.33	—	38.14	—	2.19
A_1, μg/ml	45.48	5.92	692.47	6.85	49.21	34.73	291.20	1.58
A_2, μg/ml	2.15	1.18	4.81	3.07	3.11	3.41	3.59	0.61
A_3, μg/ml	0.70	0.90	0.99	1.41	2.98	—	0.56	—
m, min^{-1}	4.52	1.76	5.04	2.62	—	3.47	—	0.50
λ_1, min^{-1}	1.16	0.53	4.81	1.26	1.90	1.48	3.12	0.02
λ_2, min^{-1}	0.09	0.04	0.25	0.17	0.16	0.01	0.04	0.003
λ_3, min^{-1}	0.01	0.01	0.02	0.02	0.01	—	0.003	—
AUC^a, min μg/ml	114.12	118.78	89.13	97.37	353.76	374.83	388.30	334.82
AUC^b, min μg/ml	111.94	117.64	86.53	95.95	357.94	371.14	383.35	328.46
AUC^c, min μg/ml	116.70	117.37	78.35	95.62	362.60	375.73	418.35	325.49
Percent contribution of terminal phase plasma area to *AUC*	59.98	76.68	63.18	73.73	82.18	90.76	44.62	62.47

[a] Calculated by Method I in *Experimental.* [b] Calculated by Method II in *Experimental.* [c] Calculated by Method III in *Experimental.*

Table III—Summary of Pharmacokinetic Parameters Calculated Based on Arterial or Venous Plasma Data

	Dog 1		Dog 2		Dog 3		Rabbit	
	A	V	A	V	A	V	A	V
Cl, ml/min/kg	25.16	23.94	36.11	32.57	8.38	8.09	9.86	11.51
V_1, liter/kg	0.058	0.352 (6.07)[a]	0.005	0.276 (55.2)[a]	0.053	0.079 (1.49)[a]	0.013	1.73 (133)[a]
V, liter/kg	2.35	2.23	1.95	1.76	0.89	0.86	3.95	4.61
V_{ss}, liter/kg	1.42	1.72	1.24	1.45	0.79	0.84	2.36	3.61
AUMC, min^2 μg/ml	6323.6	8457.2	2969.4	4270.6	33731.8	38730.8	91816.5	102901.3
MRT, min	56.5	71.9	34.3	44.5	94.2	104.4	239.5	313.3

[a] Values in parentheses are V-A ratios.

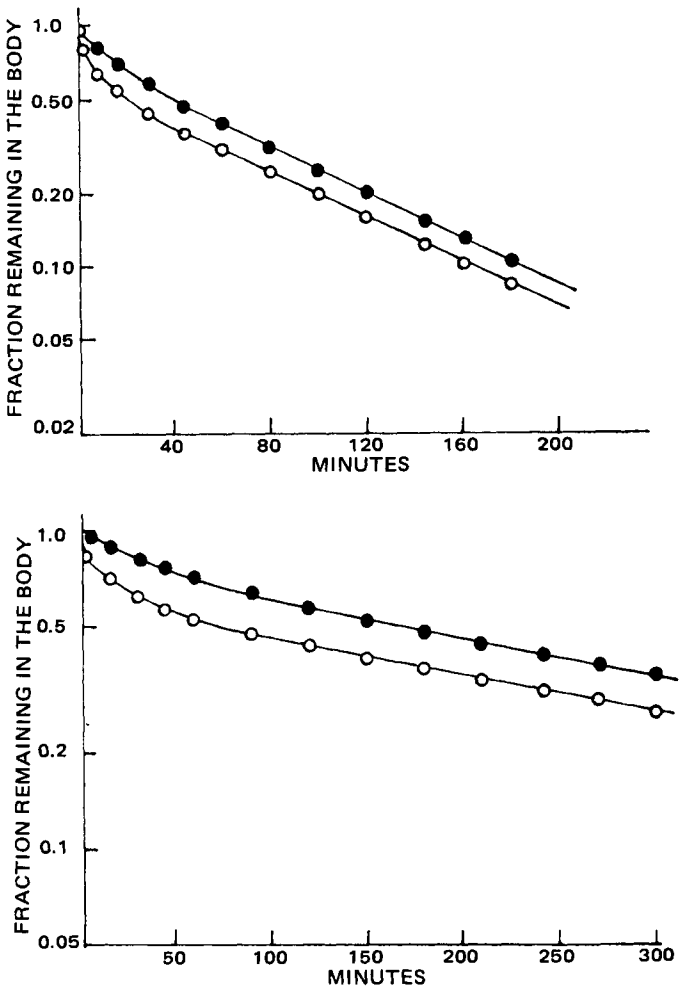

Figure 3—*Calculated fractions of griseofulvin dose remaining in the body after bolus injection of 60 mg of griseofulvin to dog 1 (upper curve) and 14 mg to the rabbit (lower curve). Key: arterial (○) and venous (●) plasma levels.*

vary considerably in the same animal, depending solely on the source of plasma data used (Table III). The venous V_1 values were 6.1, 55.2, 1.5, and 133 times greater than the arterial V_1 values for dogs 1, 2, 3, and the rabbit, respectively. The marked differences primarily were attributed to the initial uptake of griseofulvin by the sampling tissues. Thus, the A–V difference is another factor affecting the calculated V_1 (2, 21). Its implication in the dosing regimen calculation or pharmacodynamic evaluation may have to be considered.

The results of *AUMC* and *MRT* calculations are summarized in Table III. For *AUMC*, those values calculated from the venous data were 33.7, 43.8, 15.8, and 12.1% higher than the arterial data for dogs 1, 2, 3, and the rabbit, respectively. For *MRT*, the corresponding venous data were 27.3, 29.7, 10.8, and 30.8% higher. The effect of the source of blood sampling on the calculated fraction of dose remaining in the body as a function of time in dog 1 and the rabbit is depicted in Fig. 3; the difference was pronounced. For example, at 1 hr the fractions remaining were 53 and 73%, respectively, based on the arterial and venous data for the rabbit. A more marked effect was found for propranolol in dogs and rabbits (2).

Significant and persistent A–V differences were also observed for griseofulvin following intravenous infusion to one unanesthetized dog (1). The A–V plasma levels were found to differ by 20% during the terminal phase. This preliminary study also showed that a similar trend of A–V difference existed when the whole blood was analyzed. The above analyses and discussion suggest that it may be important to investigate the possible A–V difference and its consequences (1, 2, 11, 14, 18, 19) in pharmacokinetic or pharmacodynamic studies of drugs in general.

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