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Potential Effect of Early Blood Sampling Schedule on Calculated Pharmacokinetic Parameters of Drugs after Intravenous Administration

Keyphrases □ Pharmacokinetics—effect of blood sampling time on calculated parameters after intravenous administration □ Volume of distribution—effect of blood sampling time on values calculated after intravenous administration □ Blood sampling time—effect on calculated pharmacokinetic parameters after intravenous administration

To the Editor:

The disposition of a drug in the body often can be studied best by intravenous administration (1–5). Differences in experimental methodology are known to affect the results of a pharmacokinetic study. For example, an insufficient sampling period or the lack of a sensitive assay may result in the underestimation of terminal biological half-lives of drugs (4–9). A nonspecific assay can cause overestimation of the plasma area under the curve and underestimation of the total plasma clearance (10). Sampling devices (11) and blood containers (12) might also affect assay results and, hence, the calculated pharmacokinetic parameters. Marked differences in protein binding (possibly also the concentration) of several drugs and bilirubin in serum and heparinized plasma were demonstrated (13). Differences in the curve-fitting technique also might affect the characterization of pharmacokinetic properties (4, 5, 14, 15).

In conventional pharmacokinetic studies, plasma concentration profiles between the beginning of intravenous injection or infusion (*i.e.*, time zero) and the time of the first blood sample collected usually are extrapolated or predicted based on plasma level data obtained at later times. The accuracy of such an extrapolation method has been questioned (16, 17). Marked underestimations of extrapolated plasma concentrations at time zero and probably of extrapolated plasma areas under the curve between time zero and the first sampling time (15 min) after rapid intravenous injection of sulfamethizole to five dogs were found when a constant blood withdrawal device and the conventional multiple blood sampling techniques were employed simultaneously (16). Disposition kinetics of a drug within the first few minutes after intravenous injection are often more complicated than is commonly recognized (17). For example, the true peak plasma concentration might be several times higher than the extrapolated concentration (17). Furthermore, the peak concentration in humans might occur 0.5–2 min after the end of dosing; at time zero, the true plasma concentration at the normal sampling site should always be zero and not the extrapolated zero-time value.

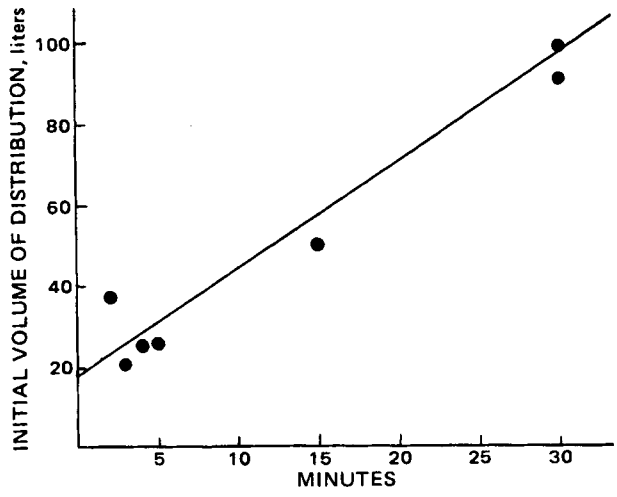

Figure 1—*Correlation between the average initial volume of distribution, V_c, and the time of the first blood sampling after an intravenous dose of digoxin in seven studies on humans with normal renal function; $V_c = 17.4 + 2.53t$ ($r^2 = 0.9452$).*

This review indicates that the early blood sampling schedule used in an intravenous study sometimes might significantly affect the obtained disposition function and, hence, the resultant multicompartmental modeling. In a recent study on quinidine, it was stated that the sampling pattern selected in a study also influences the choice of a model (15). Although it was not elaborated further, this statement is consistent with the contention of the possible early sampling schedule effect.

The literature often shows marked variability in the mean initial volume of distribution or mean volume of the central compartment reported in different intravenous studies on the same drug in similar subjects or patients. Although many factors might account for some of the differences, there often is a general pattern that the mean initial volume of distribution is smaller if the first blood sample is collected earlier in a study. The purpose of this communication is to point out this apparent trend using digoxin, gentamicin, and thiopental as examples.

Mean apparent initial volumes of distribution of digoxin reported or analyzed from six studies (18–23) on healthy adults with normal renal function are used for comparison. Subject characteristics, the duration of intravenous administration, the time of the first blood sampling, and the calculated mean volume value for each study are summarized in Table I. An apparent linear relationship between the mean initial volume of distribution and the time of the first blood sample was found (Fig. 1). The lowest volume, 20 liters, and the highest volume, 99 liters, were obtained from studies with the first blood sample collected at 3 and 30 min, respectively.

Although it is more meaningful to compare the volume of distribution in terms of volume per unit of body weight, this comparison usually is not possible since information on body weight was reported only in one study (21). For that study, the value (24.5 liters) reported in Table I was corrected for a 70-kg body weight. Since these studies involved normal adult subjects with a relatively narrow age range (no age information in Ref. 19), it is unlikely that body weight is a major factor for the marked variation in

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Table I—Subject Characteristics, Duration of Intravenous Digoxin Dosing, Time of First Blood Sampling, and Calculated Initial Volume of Distribution of Digoxin from Six Studies in Normal Adult Subjects and One Study in Neonates and Infants

Study (Ref.)	Number of Subjects	Sex	Age Range, years	Duration of Intravenous Dosing	Time of First Blood Sampling after Dosing, min	Estimated Average V_c[a], liters
1 (18)	4	M	27–35	Bolus	2	37.4[b]
2 (19)	4	—	—	1 min	3	20.0[b]
3 (20)	4	2 M; 2 F	19–40	Bolus	4	25.0[b]
4 (21)	12	M	23–34	60-min infusion	5[c]	24.5[d]
5 (22)	4	3 M; 1 F	22–29	Bolus	15	50.0[b]
6 (23)	6	—	24–26	Bolus	30	99.0[e]
7 (25)	7	—	Neonates and infants	2–3 min	30	91.0[d]

[a] Initial volume of distribution. [b] Estimated by this investigator based on the graphical data from the previous study. [c] Four blood samples also were collected at 0.25, 0.5, 0.75, and 1 hr during infusion. [d] Corrected for 70-kg body weight for comparison. [e] Calculated by Reuning *et al.* (24).

the mean initial volume of distribution. The results (Table I) from a study in neonates and infants (25) also are consistent with the data from the adult studies. The mean initial volume of distribution in these neonates and infants after correction to 70 kg is 91 liters, and the first blood sample in that study (25) was collected at 30 min.

For gentamicin, the mean initial volume of distribution in normal subjects could be estimated as 0.045 liter/kg in one study (26) with the first sample collected 1.0 min after a bolus dose (1.5 mg and 2.0 mg/kg), and as 0.099 liter/kg from another bolus study (27), with the first sample collected at 15 min. In a multiple 1-hr infusion study in patients with normal and moderately impaired renal function, the mean initial volumes of distribution of gentamicin were 0.21 and 0.20 liter/kg, respectively.

The initial volume of distribution of thiopental in one normal subject could be estimated as 6.4 liters from a bolus intravenous study (28) with the first blood sample taken probably at 2.4 min. However, the initial volume of distribution of thiopental in another normal subject in a study conducted in the same laboratory could be estimated as 60.5 liters (29). In the latter study, the first blood sample was collected at ~45 min. In both studies, the data were analyzed based on the two-compartment open model. The plasma thiopental levels at 45 and 120 min in both studies, after correction for the different doses used, were approximately the same.

The results of this study on three drugs indicate that the determination of the apparent initial volume of distribution might be affected by the time at which the first (and probably also the second) blood sample is collected. Although the marked variation in the volume of distribution in these examples might be attributed to the real difference, this factor probably is not major. In general, the most important factor affecting the volume of distribution of the same drug in normal subjects is the body weight. The wide range of the mean initial volume of digoxin from several studies and of thiopental from two (typical) subjects is not accounted for by the presumably relatively much smaller differences in body weight used in each study. Differences in assay accuracy also can be ruled out as a major factor since most assays should be more accurate for plasma samples with higher drug concentrations, which usually were more important in the determination of the initial volume of distribution.

The intersubject variation in the initial volume of distribution from the same study often was much less than the interstudy variation (18, 21, 22, 25), indicating the possible influence of study design on the calculated pharmacokinetic parameter.

The method used in these comparisons of the three drugs is not perfect. A more scientifically designed experiment is being planned to eliminate or minimize most of the other potential contributing factors and to identify the exact mechanisms for the observed phenomena. The results of this preliminary report indicate that one should be prudent in interpreting the markedly different pharmacokinetic parameters, especially the initial volume of distribution, reported in the literature. Some guidelines or standardization of studying and reporting the initial volume of distribution might be useful.

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Mesophase Formation during Cholesterol Dissolution in Ursodeoxycholate–Lecithin Solutions: New Mechanism for Gallstone Dissolution in Humans

Keyphrases □ Cholesterol—mesophase formation during dissolution in ursodeoxycholate–lecithin solutions, mechanism for gallstone dissolution in humans □ Gallstones—mechanism for dissolution in humans, mesophase formation during cholesterol dissolution in ursodeoxycholate–lecithin solutions

To the Editor:

It is well established that oral administration of chenodeoxycholic acid (I) induces bile desaturation in cholesterol and gradual dissolution of cholesterol gallstones in humans (1). Makino *et al.* (2) reported that ursodeoxycholic acid (II), the 7β-hydroxy epimer of I, also can induce bile desaturation in humans, an observation confirmed by other investigators (3).

The cholesterol saturation of bile (percent saturation) is defined as $(C_{sample}/C_s) \times 100$, where C_s is the concentration in the sample if it is saturated fully with cholesterol, *i.e.*, the solubility at equilibrium. In model systems, C_s is determined almost entirely by the molar proportions of bile acids and lecithin (4–6). The C_s value usually is assumed to be that value determined experimentally for the model system having an identical proportion of bile acids and lecithin, and the actual value for percentage saturation is determined graphically (7) or analytically (8). This approach has been assumed to be correct since C_s determined in bile samples from gallstone patients did not differ significantly from that of the model system (6). Accordingly, there has been rather satisfactory agreement between predicted and measured percent saturation in bile samples obtained from gallstone patients (9). The C_s value was considered to be uninfluenced by biliary bile acid composition since changing the relative proportion of the common bile acids (cholic, deoxycholic, and I) in model systems had little influence on C_s (4, 5).

Igimi *et al.* (10) reported that the ability of II to solubilize cholesterol both in the presence and absence of lecithin was strikingly inferior to that of I and concluded that II might well be less efficacious than I for gallstone dissolution. Carey and coworkers (11) confirmed these observations and also reported (12) that the maximum capacity of model bile systems to solubilize cholesterol was depressed in proportion to the percent of II conjugates in bile salt mixtures. Thus, the micellar zone in the phase diagram simulating bile appears to be reduced by the presence of II. Carey and Ko (12) also proposed that a new C_s value must be used to calculate the percent saturation in gallstone patients receiving II and presented an "urso-correction factor" to facilitate such calculations. Nevertheless, initial clinical studies (3) suggested that gallstone dissolution in patients receiving II did not occur more slowly than in those receiving I. Indeed, several instances of rather rapid dissolution were observed. To rationalize these discrepancies, we undertook a systematic study of cholesterol dissolution with II–lecithin solutions.

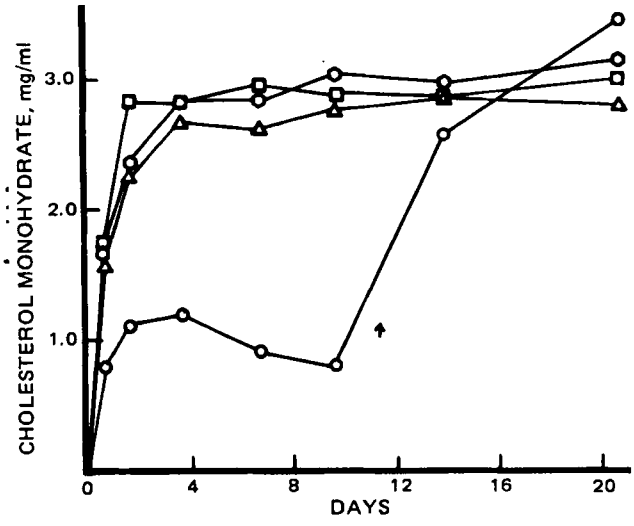

Figure 1—*Apparent solubility profiles of cholesterol monohydrate in 116 mM bile salt, 32 mM lecithin, 0.1 M NaCl, and 0.01 M phosphate buffer media at 37°. Key: ○, glycochenodeoxycholate; □, taurochenodeoxycholate; △, taurocholate; and ○, glycoursodeoxycholate; the arrow indicates the time of occurrence of a nonfilterable cloudiness due to mesophase formation in the glycoursodeoxycholate system.*

In recent dissolution studies[1] under sink conditions using methodology outlined previously (13), we found that over a wide range of bile acid, lecithin, and electrolyte concentrations, the initial dissolution rate of cholesterol monohydrate in II conjugate–lecithin media was two to 70 times lower than in corresponding I media. The dissolution of cholesterol and of gallstones *in vitro* was shown previously (9, 14) to be controlled interfacially, with the interfacial resistance $R = (h/D + 1/P)$ being a function of the dissolution medium composition. The evaluation of R and hence P (the effective permeability coefficient of the interfacial barrier) under sink conditions requires an estimate of C_s, the solute saturation concentration, since $R = AC_s/J$, where A is the area of the dissolving surface and J is the initial dissolution rate (15).

However, in contrast to media containing I and lecithin or I conjugates and lecithin, attempts to measure C_s with

[1] O. I. Corrigan, C. C. Su, W. I. Higuchi, and A. F. Hofmann, manuscript in preparation.

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