Organ Perfusion Studies: A Rebuttal

Keyphrases □ Organ perfusion—pharmacokinetics, sampling □ Pharmacokinetics—organ perfusion, effect of sampling

To the Editor:

Colburn *et al.* (1) discussed the influence of sampling in organ perfusion studies. In this respect we want to make the following critical remarks. These authors defined the elimination rate constant K as Q/V_R, in which Q represents the perfusion flow rate and V_R represents the reservoir volume. According to this equation, however, K is *not* the elimination rate constant.

It is explicitly stated by Rowland *et al.* in their Table I (2), that the rate constant k_{12} of the compartmental model corresponds to $\dot{V}_B/V_R$ of the perfusion model. In Colburn's terminology $\dot{V}_B = Q$. Since Colburn states that $K = Q/V_R$, it is erroneous to call K an elimination rate constant; it simply is the transport rate constant from the reservoir to the eliminating perfused organ in terms of the perfusion model. Similarly, the rate constant k_{12} of the compartmental model represents the transport rate constant from the central to the peripheral compartment in the compartmental model. The elimination rate constant itself, k_e, contrary to the opinion of Colburn *et al.*, is independent of the perfusion flow rate, since it reflects the intrinsic ability of the organ to eliminate drug. As we have pointed out (3), the drug decrease in the reservoir will be more rapid under the influence of sampling than without sampling. Consequently, a pharmacokinetic analysis based on the uncorrected time course of drug concentration in the reservoir will result in overestimation of the parameter k_e.

Colburn *et al.* stated that clearance will be unaffected by sampling from the reservoir. We do not agree with their statement. They define clearance as:

$$CL_o = Q\left(\frac{C_{in} - C_o}{C_{in}}\right) \qquad \text{(Eq. 1)}$$

where C_{in} and C_o represent the inflow and outflow concentrations of the eliminating organ. This expression, however, defines *instantaneous* clearance (2), which is time- and concentration-dependent. A more relevant measure of clearance is the *mean* clearance, which essentially is a steady-state concept. The mean clearance equals:

$$CL = \frac{Q \cdot k_e}{Q/(K_p \cdot V_o) + k_e} \qquad \text{(Eq. 2)}$$

where V_o is the physical organ volume and K_p is the apparent partition coefficient of drug between the eliminating organ and the emergent perfusion fluid (2). This leaves K_p and k_e as two independent parameters to be estimated from the concentration *versus* time curve as measured in the reservoir.

As discussed above, the parameter k_e will be overestimated due to sampling. Similarly, the estimate of the parameter K_p is biased, in a complicated way, by sampling (3). It follows that sampling from the reservoir definitely influences the estimate of clearance. The extent to which clearance is biased by neglecting corrections for sampling is dependent on the numerical values of Q, K_p, k_e, and of course the sample volumes.

In conclusion it can be stated that the instantaneous clearance is the wrong parameter to look at and that the mean clearance estimated from concentration *versus* time curves in the reservoir will certainly depend on sampling from this reservoir.

(1) W. A. Colburn, R. K. Brazzell, and I. Bekersky, *J. Pharm. Sci.*, **72**, 970 (1983).

(2) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).

(3) C. J. Timmer and H. P. Wijnand, *J. Pharmacokinet. Biopharm.*, **5**, 335 (1977).

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Received October 31, 1983.
Accepted for publication February 17, 1984.

Estimation of Mean Residence Time from Data Obtained when Multiple-Dosing Steady State Has Been Reached

Keyphrases □ Pharmacokinetics—multiple dosing, mean residence time

To the Editor:

When the plasma concentration (C) *versus* time (t) profile of a drug, on single dosing, can be described, irrespective of dosing route, as an exponential series:

$$C = \sum_{i=1}^{n} A_i \cdot \exp(-k_i t) \qquad \text{(Eq. 1)}$$

then the concentration *versus* time profile on multiple dosing to steady state, at a constant interval, Υ, can be described (1) as:

$$C_{ss} = \sum_{i=1}^{n} \frac{A_i}{1 - \exp(-k_i \Upsilon)} \cdot \exp(-k_i t) \qquad \text{(Eq. 2)}$$

where C_{ss} represents the plasma concentration at multiple-dosing steady state and, in this case, t is time after the last dose administered. Equation 2 is valid on the assumptions that the dose remains constant, the dosing interval is constant, and clearance is constant. It has been demonstrated (see Ref. 1) that under these conditions:

$$\int_0^\infty C dt = \int_0^\Upsilon C_{ss} dt \qquad \text{(Eq. 3)}$$

When the time course of drug concentration is regarded as a statistical distribution curve (2) the mean residence time (MRT) of the drug, on single dosing, can be defined (3) as:

$$\text{MRT} = \int_0^\infty tCdt \Big/ \int_0^\infty Cdt = \left(\sum_{i=1}^{n} A_i/k_i^2\right) \Big/ \left(\sum_{i=1}^{n} A_i/k_i\right) \qquad \text{(Eq. 4)}$$

In addition to using the analytical integrals of Eq. 1, as shown in Eq. 4, the MRT has been calculated using integrals estimated by the trapezoidal rule (4).

On the basis of Eq. 2, the first moment curve at steady state would be:

0022-3549/84/0600-0854$01.00/0

$$tC_{ss} = \sum_{i=1}^{n} \frac{tA_i}{1 - \exp(-k_i\Upsilon)} \cdot \exp(-k_i t) \quad \text{(Eq. 5)}$$

The integral of Eq. 5 is:

$$\int_0^t tC_{ss}dt = \sum_{i=1}^{n} \frac{A_i}{1 - \exp(-k_i\Upsilon)} \left[\frac{1}{k_i^2}(1 - \exp(-k_i t)) - \frac{t}{k_i} \cdot \exp(-k_i t)\right] \quad \text{(Eq. 6)}$$

$\int_0^\Upsilon tC_{ss}dt$ is, therefore:

$$\int_0^\Upsilon tC_{ss}dt = \sum_{i=1}^{n} \frac{A_i}{k_i^2} - \sum_{i=1}^{n} \frac{A_i\Upsilon \cdot \exp(-k_i\Upsilon)}{k_i[1 - \exp(-k_i\Upsilon)]} = \int_0^\infty tCdt - \Upsilon \sum_{i=1}^{n} \frac{A_i \exp(-k_i\Upsilon)}{k_i[1 - \exp(-k_i\Upsilon)]} \quad \text{(Eq. 7)}$$

Rearranging Eq. 7:

$$\int_0^\infty tCdt = \int_0^\Upsilon tC_{ss}dt + \Upsilon \sum_{i=1}^{n} \frac{A_i \cdot \exp(-k_i\Upsilon)}{A_i[1 - \exp(-k_i\Upsilon)]} \quad \text{(Eq. 8)}$$

Equation 8 indicates that MRT cannot be estimated by using the area under the multiple-dosing first moment curve from time zero to time Υ as a numerator in an equation analogous to Eq. 4. However, since $\int_0^\Upsilon C_{ss}dt = \Sigma_{i=1}^{n} A_i/k_i$ and:

$$\int_0^\infty C_{ss}dt = \sum_{i=1}^{n} \frac{A_i}{k_i[1 - \exp(-k_i\Upsilon)]} \quad \text{(Eq. 9)}$$

then:

$$\sum_{i=1}^{n} \frac{A_i \cdot \exp(-k_i\Upsilon)}{k_i[1 - \exp(-k_i\Upsilon)]} = \int_0^\infty C_{ss}dt - \int_0^\Upsilon C_{ss}dt = \int_\Upsilon^\infty C_{ss}dt \quad \text{(Eq. 10)}$$

Equation 8 is equivalent to:

$$\int_0^\infty tCdt = \int_0^\Upsilon tC_{ss}dt + \Upsilon \int_\Upsilon^\infty C_{ss}dt \quad \text{(Eq. 11)}$$

Table I—MRT Calculation [a]

Time, h	C_{ss}, μg/mL	tC_{ss}, μg·h/mL	$\int_0^t C_{ss}dt$, μg·h/mL	$\int_0^t tC_{ss}dt$, μg·h²/mL
0	124.68	0	0[a]	0[a]
0.0833	103.51	8.62	9.5	0.4
0.1667	89.53	14.93	17.6	1.3
0.25	80.31	20.08	24.6	2.8
0.5	67.34	33.67	43.1	9.5
0.75	63.23	47.43	59.4	19.6
1.0	61.65	61.65	75.0	33.3
1.5	60.09	90.14	105.4	71.2
2.0	58.89	117.78	135.2	123.2
3.0	56.60	169.82	192.9	267.0
5.0	52.30	261.52	301.8	698.3
7.0	48.33	338.30	402.5	1298.1
9.0	44.66	401.90	495.5	2038.4
12.0	39.66	475.96	621.9	3355.2
18.0	31.29	563.22	834.8	6472.6
24.0	24.68	592.43	1002.7	9399.2
36.0	15.36	553.06	1242.9	16810.9
48.0	9.56	458.93	1392.5	22881.9
72.0	3.70	266.64	1551.6	31585.3
96.0	1.43	154.44	1613.1	36429.4
120.0	0.56	66.67	1637.0	38883.2
∞	0	0	1651.2	40942.2

[a] All integrals approximated by using the linear trapezoidal rule.

MRT may be estimated from multiple dosing data by:

$$\text{MRT} = \frac{\int_0^\Upsilon tC_{ss}dt + \Upsilon \int_\Upsilon^\infty C_{ss}dt}{\int_0^\Upsilon C_{ss}dt} \quad \text{(Eq. 12)}$$

The data necessary to compute MRT, using the trapezoidal rule, on multiple dosing to steady state may be obtained by continued sampling of the declining plasma concentrations until, perhaps, 4–7 drug half-lives have elapsed or the limit of detection of the assay is reached, rather than redosing at time Υ. The purpose is to ensure that the postabsorption and postdistribution phase has been reached and the area under the curve from the last sampling time can be properly extrapolated by $(C_m)_{ss}/k_n$ where $(C_m)_{ss}$ is the last sampled concentration and k_n the terminal rate constant. If the dosing interval has been such that the terminal data point, at Υ, were known to be well into the postabsorption and postdistribution phase, then Eq. 12 could be simplified to:

$$\text{MRT} = \frac{\int_0^\Upsilon tC_{ss}dt + \Upsilon(C_m)_{ss}/k_n}{\int_0^\Upsilon C_{ss}dt} \quad \text{(Eq. 13)}$$

In such a case it would be unnecessary to interrupt the dosing schedule.

Wagner (5) and Benet and Galeazzi (6) have used the equation:

$$C = 60.9545 \cdot \exp(-5.060t) + 39.0459 \cdot \exp(-0.03952t) \quad \text{(Eq. 14)}$$

to demonstrate methods of calculating steady-state volume of distribution. Substituting values from Eq. 14 into Eq. 4 to obtain analytical integrals yields MRT = 25.00 h. Values calculated for $\int_0^\infty tCdt$ and $\int_0^\infty Cdt$ by Benet and Galeazzi (6) using the linear trapezoidal rule yield MRT = 24.88 h.

If multiple dosing with Υ = 24 h is assumed:

$$C_{ss} = 60.9545 \cdot \exp(-5.0605t) + 63.7306 \cdot \exp(-0.03952t) \quad \text{(Eq. 15)}$$

Using Eq. 15, plasma concentrations were calculated over a 120-h period (Table I). The first moment curve and cumulative values of $\int_0^t tC_{ss}dt$ and $\int_0^t c_{ss}dt$, calculated using the linear trapezoidal rule, are also given. Using these trapezoidal rule values, according to Eq. 12:

$$\text{MRT} = \frac{9399.2 + 24(648.5)}{1002.7} = 24.90 \quad \text{(Eq. 16)}$$

The analytical integrals, derived from Eq. 15 and substituted into Eq. 12, yield MRT = 25.00 h.

Using analytical integrals, Eq. 4, for single dose data, and Eq. 12, for multiple dosing steady-state data, yield equivalent values for MRT. Using the trapezoidal rule approximations gave MRT 0.4% less than the actual MRT value. The difference is inherent in rounding the calculated plasma concentrations and in the trapezoidal rule approximations. It is, therefore, possible to obtain at least as accurate an estimate of MRT from multiple-dosing steady-state data as from single-dose data providing the plasma sampling schedule is adequate. It might, however, be necessary to interrupt the multi-

ple-dosing regime which might limit the application of the method in patient studies.

Bauer and Gibaldi (7) have proposed an alternative method for performing moment-analysis calculations on multiple-dosing data. It should be noted that their Eq. 7 is equivalent to Eq. 6 in this presentation. The Bauer and Gibaldi method requires the use of calculated concentrations to calculate area under the curve, while this presently proposed method employs only the data observed at multiple-dosing steady state. This raises the possibility of being able to determine whether pharmacokinetic changes, as observed in mean retention times or, perhaps, steady-state volumes of distribution, could have been induced during multiple dosing.

(1) M. Gibaldi and D. Perrier, "Pharmacokinetics," 2nd ed, Dekker, New York, N.Y., 1982, chap. 3.
(2) D. M. Himmelbau and K. B. Bischoff, "Deterministic Systems," Wiley, New York, N.Y., 1968.
(3) W. Perl and P. Samuels, *Circ. Res.*, **25**, 191 (1969).
(4) S. Riegelman and P. Collier, *J. Pharmacokinet. Biopharm.*, **8**, 509 (1980).
(5) J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **4**, 443 (1976).
(6) L. Z. Benet and R. L. Galeazzi, *J. Pharm. Sci.*, **68**, 1071 (1979).
(7) L. A. Bauer and M. Gibaldi, *J. Pharm. Sci.*, **72**, 978 (1983).

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Received October 4, 1983.
Accepted for publication March 30, 1984.

Alterations of α-Lactose During Differential Scanning Calorimetry

Keyphrases □ α-Lactose—alterations, differential scanning calorimetry

To the Editor:

Lactose is a natural disaccharide widely used as a diluent in tablet formations. As a solid it is known to occur either in one of four crystalline forms or in an amorphous state. α-Lactose monohydrate is obtained by crystallization from a super-saturated solution at temperatures <93.5°C, whereas β-lactose crystals are obtained at temperatures >93.5°C (1). During crystallization of β-lactose, no water is incorporated in the crystal lattice. The crystals of β-lactose exist in a nonhygroscopic anhydrous form only, in contrast with α-lactose, which occurs both as monohydrate and as anhydrous α-lactose. Thermal dehydration, or desiccation of the hydrate crystals with suitable liquids, converts α-lactose monohydrate into its anhydrous form. A very hygroscopic product, generally called unstable anhydrous α-lactose, is formed when α-lactose hydrate crystals are heated, mostly *in vacuo*, at temperatures of 100–130°C (2–4). Thermal treatment in a moist atmosphere at temperatures over ~110°C, or desiccation with suitable liquids, such as dry methanol, may result in a nonhygroscopic product, generally called stable anhydrous α-lactose (2, 4).

The different types of lactose are increasingly studied by thermal analysis. Berlin *et al.* (5) determined the heat of desorption of water from α-lactose monohydrate by differential scanning calorimetry. Itoh *et al.* (3, 6) used differential thermal analysis in studying the characteristics of α-lactose hydrate and of β-lactose, next to IR absorption and X-ray powder diffraction techniques. Differential scanning calorimetry has been applied by Ross (7) for the direct measurement of the amount of α- and β-lactose in whey powders.

This communication reports the occurrence of changes in the solid state during differential scanning calorimetry of α-lactoses. Figure 1 illustrates the DSC-curves of α-lactose monohydrate and of stable and unstable anhydrous α-lactose, respectively, as recorded by means of a thermal analyzer[1]. The DSC-curve of α-lactose monohydrate shows an endothermic

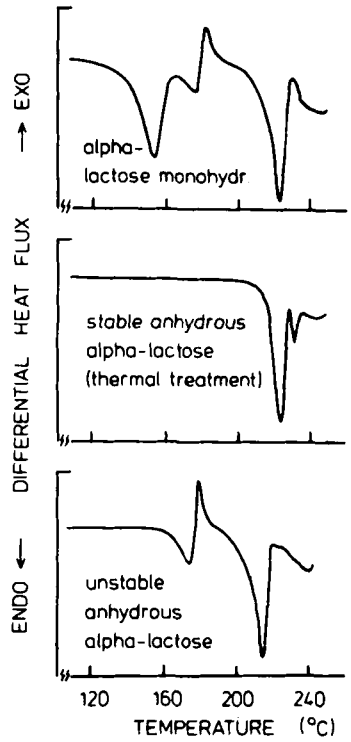

Figure 1—*DSC-curves of α-lactose monohydrate, stable anhydrous α-lactose, and unstable anhydrous α-lactose, respectively, recorded at a heating rate of 10°C/min.*

Table I—Change in β-Content (GLC Determination) and the Solid State (X-ray Diffraction Analysis) of α-Lactose During Thermal Treatment (DSC-cell 910; Heating Rate 10°C/min)[a]

	Temperature, °C						
	20	120	160	180	200	210	220
β-content (%)							
α-Lactose monohydrate	4	5	19	21	23	—	44
Stable anhydrous α-lactose	20	20	—	20	22	34	56
Unstable anhydrous α-lactose	18	—	18	42	—	54	—
Solid State							
α-Lactose monohydrate	AM	SA+(UA)		—	SA+(BA)		
Stable anhydrous α-lactose	SA			SA			
Unstable anhydrous α-lactose	UA				BA		

[a] AM = α-lactose monohydrate; SA = stable anhydrous α-lactose; UA = unstable anhydrous α-lactose; BA = β/α-lactose compound crystal; (UA) and (BA) refer to a heating rate of 2°C/min.

[1] Dupont Model 990 with DSC-cell 910.

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