Rapid Methods for Bioavailability Determination Utilizing Urinary Excretion Data

PAUL J. NIEBERGALL ˣ, EDWIN T. SUGITA, and ROGER L. SCHNAARE

Abstract □ Two equations were developed which enable urinary excretion data to be utilized for estimating drug bioavailability within 12 hr starting between one and two half-lives of the drug, depending upon the relative rates of absorption, distribution, and elimination. Both equations were examined using simulated data for both the one- and two-compartment open models. One equation was tested using literature data with excellent results.

Keyphrases □ Urinary excretion data—equations for rapid estimation of bioavailability □ Bioavailability—equations for rapid estimation using urinary excretion data

The most widely used methods for estimating bioavailability for drugs following linear compartmental pharmacokinetics are based upon the measurement of unchanged drug in blood or urine after the administration of a single dose of drug. While the former readily yields estimates of peak blood level and time to reach peak blood level, as well as the extent of bioavailability, it does contain a number of rather serious pitfalls (1). Among these are:

1. The total area under the curve must be estimated, involving a model-dependent correction for the period between the cessation of sampling and time infinity.
2. The correction term requires a knowledge of the rate constant for the elimination phase.
3. Depending upon the model, parameters such as renal clearance, various rate constants, and volumes of distribution must be assumed to be constant either inter- or intrasubject.
4. The assay procedure must be sensitive enough to determine drug levels approximately one-tenth to one-twentieth that of the peak level.

Measurement of intact drug in the urine has certain advantages. The method is model independent as long as the drug does not exhibit nonlinear pharmacokinetics; samples are easier and less costly to obtain; urinary excretion data are more meaningful in instances where drugs are used to treat urinary tract infections or where there is no correlation between blood levels and pharmacological activity; and the only major assumption is that the average fraction of drug reaching the circulation that is excreted in the urine for a given panel is the same for two or more treatments.

The most serious disadvantage of using urinary excretion data for estimating the extent of bioavailability is that urine samples should be collected for at least seven to 10 half-lives of the drug (1). This requirement can be quite a problem with drugs having relatively long half-lives. This report presents two methods for analyzing urinary excretion data which demonstrate that, provided care is taken during the sampling procedure, the extent of bioavailability can be estimated with urine samples taken over 12 hr starting at one or two half-lives of a drug.

THEORY

When the absorption phase for drugs conforming to the one-compartment open model and both the absorptive and distributive phases for the two-compartment open model have been completed, the equations (2) describing the cumulative amount of drug excreted in the urine as a function of time for the one-compartment open model become:

$$U = U_\infty - \frac{U_\infty k_a}{k_a - k_e} e^{-k_e t} = U_\infty - P_1 e^{-k_e t} \qquad \text{(Eq. 1)}$$

and for the two-compartment open model:

$$U = U_\infty - \frac{U_\infty(k_{tc} - \beta)}{\beta(k_a - \beta)(\alpha - \beta)} e^{-\beta t} \doteq U_\infty - P_2 e^{-\beta t} \qquad \text{(Eq. 2)}$$

in which U is the cumulative amount of drug excreted unchanged in the urine up to time t, U_∞ is the total amount of drug excreted unchanged in the urine, k_a is the absorption rate constant, k_e is the overall elimination rate constant, k_{tc} is the rate constant for the transfer of drug from the tissue to the central compartment, and both α and β are the usual collections of constants representing the distributive and elimination phases, respectively.

In the interest of brevity, since both Eqs. 1 and 2 have the same general form, all further developments will be given using Eq. 1 as the example. If urine samples are collected at uniform time intervals, Δ, the cumulative amount, U', of drug excreted up to time $t + \Delta$ is:

$$U' = U_\infty - P_1 e^{-k_e(t+\Delta)} \qquad \text{(Eq. 3)}$$

Equations 1 and 3 can be rearranged to:

$$U - U_\infty = -P_1 e^{-k_e t} \qquad \text{(Eq. 4)}$$

and:

$$U' - U_\infty = -P_1 e^{-k_e t} e^{-k_e \Delta} \qquad \text{(Eq. 5)}$$

Dividing Eq. 4 by Eq. 5 and rearranging give (3):

$$U' = U(e^{-k_e \Delta}) + U_\infty(1 - e^{-k_e \Delta}) \qquad \text{(Eq. 6)}$$

Thus, a plot of U' *versus* U should give a straight line with a slope equal to $e^{-k_e\Delta}$ and an intercept equal to $U_\infty(1 - e^{-k_e\Delta})$. The intercept divided by 1 minus the slope yields U_∞, and the natural logarithm of the slope divided by the collection time interval (Δ) yields $-k_e$. The final equation for the two-compartment open model is the same as Eq. 6, with the exception that β replaces k_e.

RESULTS

To determine whether the equations describing the cumulative amount of drug excreted in the urine as a function of time would simplify to the forms given by Eqs. 1 and 2 within a reasonable period of time, data were simulated for both pharmacokinetic models. For the one-compartment open model, the fraction of the dose (400 mg) absorbed was assumed to be 0.80, the fraction excreted in the urine unchanged was 0.65, and the volume of distribution was 10 liters.

Data were generated for drugs having half-lives ($T_{1/2}$) of 12, 18, 24, and 48 hr. In each instance, the absorption rate constant was varied, being equal to 2, 5, 10, and 20 times the elimination rate constant, respectively. Constants for the two-compartment open

Table I—Percent of Actual Bioavailability from Data Evaluated over a 12-hr Period Starting at n Half-Lives Using Eq. 6

	R^a			
n	20	10	5	2
1	100.0	100.1	102.7	141.0
2	100.0	100.0	100.1	104.4
3	100.0	100.0	100.0	100.8

[a] R is the ratio of the absorption rate constant to the elimination rate constant.

model were taken from the literature for nortriptyline (4), having a $T_{1/2}$ of approximately 34 hr, assuming that 80% of a given dose would be absorbed and 65% of the dose would be excreted in the urine unchanged.

The data for all simulations were evaluated over 12 or 16 hr starting at one, two, and three half-lives. Thus, when $T_{1/2}$ was equal to 12 hr, the cumulative amount of drug excreted up to 14 hr was plotted *versus* the cumulative amount excreted up to 12 hr, the amount excreted up to 16 hr was plotted *versus* the amount excreted up to 14 hr, and the amount excreted up to 18 hr was plotted *versus* the amount excreted up to 16 hr. This procedure was continued for a 12-hr interval if the collection interval (Δ) was equal to 2 hr; it was continued for a 16-hr interval if the collection interval was equal to 4 hr. The procedure was repeated starting at 24 hr and again at 36 hr.

Except for round-off error in the calculations (percent relative bioavailability was calculated to three significant figures), all data for the one-compartment open model yielded the same results regardless of the $T_{1/2}$ or the collection interval. Therefore, the results obtained for the drug with $T_{1/2}$ equal to 12 hr and a collection interval of 2 hr are representative of the results obtained in the entire study (Table I). It can be seen from Table I that acceptable estimates of bioavailability should be obtainable from urine collections starting at approximately one half-life if the absorption rate constant is equal to at least five times the elimination rate constant. Acceptable values can be obtained after two half-lives if the absorption rate constant is at least equal to twice the elimination rate constant. The data for the two-compartment open model ($T_{1/2}$ = 34 hr) were evaluated over the period of 68–80 hr with a constant time interval of 2 hr. The bioavailability was estimated to within 99.9% of the correct value.

Unfortunately, studies involving the urinary excretion of drugs are not necessarily designed for adequate testing of Eq. 6. In one study (5), urine specimens were collected at constant time intervals starting at approximately two half-lives. The drug, phosphonomycin, has a $T_{1/2}$ of approximately 2.3 hr. The data were evaluated (5) for the pharmacokinetic parameters of a two-compartment open model, and predicted as well as observed cumulative urinary excretion values of the drug were given at various times up to, and including, 36 hr (about 16 half-lives). The data for the 4–8- and the 8–12-hr collection periods were analyzed according to Eq. 6 (Table II). The agreement between U_∞ predicted from Eq. 6 and the actual value is remarkable, considering the fact that only two collection periods could be utilized.

Table II—Evaluation of Literature[a] Data for Phosphonomycin Using Eq. 6

		U_∞, mg	
Subject Number	Eq. 7	Literature Predicted	Literature Actual
9	479.5 (0.99)[b]	479.5 (0.99)	484.3
10	376.1 (1.70)	375.4 (1.51)	369.8
11	460.4 (0.09)	461.4 (0.30)	460.0
12	597.4 (0.20)	598.0 (0.10)	598.6
13	438.4 (0.90)	438.0 (0.99)	442.4
14	407.6 (1.32)	408.2 (1.47)	402.3

[a] Reference 5. [b] The relative error for each prediction is given in parenthesis ($T_{1/2}$ = 2.3 hr).

Since times corresponding to one or two half-lives might not be convenient, a reasonable method would be to give the drug in the morning and start timed urine collections at 24, 48, 72, *etc.*, hr, whichever is equal to at least one half-life. The drug could also be given in the evening and timed urine collections started at 12, 36, 60, *etc.*, hr.

In some instances, it might not be convenient to collect urine specimens at constant time intervals. In such situations, urinary excretion rates can be utilized to predict U_∞. Differentiating Eq. 1 with respect to time gives:

$$dU/dt = \dot{U} = P_1 k_e e^{-k_e t} \qquad \text{(Eq. 7)}$$

Substituting the value for $P_1 e^{-k_e t}$ from Eq. 1 into Eq. 7 and rearranging give:

$$U = U_\infty - (\dot{U}/k_e) \qquad \text{(Eq. 8)}$$

A plot of U *versus* $\dot{U}$ should be linear with the intercept equal to U_∞. Equation 2 can be developed in a similar manner to yield an equation resembling Eq. 8, with the exception that β would replace k_e. All data were analyzed according to Eq. 8 with results identical to those already shown, within round-off error.

SUMMARY

Although technical problems do exist, urine specimens can be collected at short time intervals during the initial phase of a bioavailability study and the data can be analyzed (6) to yield an estimate of the absorption rate constant. Specimens can then be collected at times convenient to each test subject. At 24, 48, *etc.*, hr, whichever is closest to one or two half-lives (depending upon the estimated ratio of the absorption rate constant to the elimination rate constant), all initial samples should be pooled. Samples should then be collected at exactly 2-hr intervals for 12 hr. The data for the 12-hr period should then be analyzed utilizing Eq. 6. If the collection intervals cannot be kept constant during the 12 hr, the data should be analyzed according to Eq. 8.

The data in Tables I and II indicate that the methods presented are valid. Whether useful estimates of bioavailability are obtained in practice will depend upon the care with which the data are collected. The methods presented probably are more sensitive to incomplete bladder emptying than the method in which specimens are collected over seven to 10 half-lives of a drug. However, forcing fluids should dilute the urine enough that the magnitude of this problem can be reduced. Patient variability in bladder emptying can be further reduced through the use of a crossover study. Finally, shortening the collection period from seven to 10 half-lives to approximately one or two half-lives should help with subject compliance with the study protocol.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 25, 1974, from the *Department of Pharmacy (Pharmaceutics), Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104*

Accepted for publication February 19, 1975.

Presented at the APhA Academy of Pharmaceutical Sciences, New Orleans meeting, 1974.

[x] To whom inquiries should be directed. Present address: Marion Laboratories, Inc., Kansas City, MO 64137