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Bioavailability Assessment: Methods to Estimate Total Area (AUC 0–∞) and Total Amount Excreted (A_e^∞) and Importance of Blood and Urine Sampling Scheme with Application to Digoxin

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Five methods are compared to estimate the total area under the digoxin plasma or serum concentration–time curve (AUC 0–∞) after a single dose of drug. To obtain accurate estimates of AUC 0–∞, data required are concentrations at a sufficient number of sampling times to define adequately the concentration–time curve prior to the log-linear phase, and at least three, but preferably four or more equally spaced points in the terminal log-linear phase. One method (designated Method I) requires a digital computer; another (Method III) is the classical method (these two methods do not require equally spaced points in the log-linear phase). Method IIA is the accelerated convergence method of Amidon et al.; *Methods IIB and IIC are modifications of this method, but incorporate usual and orthogonal least squares, respectively, which make them more accurate with real (noisy) data. Methods I and IIC gave very comparable estimates of AUC 0–∞. Results indicate that digoxin administered orally in aqueous solution was completely (100%) absorbed when bioavailability estimates were based on oral and intravenous AUC 0–∞ estimates and the actual doses, whereas formerly only about 80% absorption was reported, based on areas, under plasma concentration curves which were truncated at 96 hr. It is shown that the sampling scheme of blood can produce biased apparent bioavailability estimates when areas under truncated curves are employed, but an appropriate sampling scheme and application of method IIC yield accurate bioavailability estimates. This is important particularly in those bioavailability studies where one is attempting to determine the appropriate label dose for a new "fast-release" digoxin preparation relative to the label dose and bioavailability of currently marketed tablets. It is shown that the magnitudes and variability of apparent elimination rate constants and half-lives of digoxin, estimated from urinary excretion data by the σ^- method, depend on which value of A_e^∞ is used. The formerly reported greater interindividual variability of AUC data compared to A_e data for digoxin is explained in that the AUCs, but not the A_e's, involve the renal clearance, which exhibits considerable inter- and intraindividual variation.*

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KEY WORDS: accelerated convergence method to estimate AUC 0–∞ and A_e^∞; bioavailability; estimation of total areas; estimation of total amounts excreted; blood sampling schemes for digoxin; elimination half-life of digoxin; intra- and interindividual variation of renal clearance of digoxin.

INTRODUCTION

Pharmacokinetic equations appropriate to estimation of absolute or relative bioavailability (used here with the connotation of absorption efficiency only, without the other component of rate of absorption) involve ratios of dose-corrected total areas under plasma or serum concentration–time curves (AUC 0–∞), or total amounts of unchanged drug excreted in the urine in infinite time (A_e^∞) after a single dose of drug (1*a*,2). The word "total" herein refers to AUC 0–∞ or A_e^∞ and not, as often erroneously used in the literature, to indicate AUC 0–T or A_e^T, where T is the investigator's last sampling time; hence AUC 0–T is a *partial* area and A_e^T is a *partial* amount excreted in the urine. In this article, such partial areas and amounts excreted are simply designated by AUC and A_e, respectively.

It is common practice in the digoxin (7–21), as well as the literature for many other drugs, to substitute the particular author's AUC or A_e figures for AUC 0–∞ or A_e^∞ in estimating bioavailability. Such estimates are herein called *apparent bioavailabilities*. For digoxin there have been almost as many blood sampling schemes as investigators. Apparent bioavailabilities depend on the sampling scheme employed, and they may be considerable underestimates of the true bioavailability. This will be very important in establishing the correct dose ratio of new "fast-release" digoxin formulations compared with currently marketed "slow-release" tablets, since an error of the order of 20% could have noticeable effects in the clinical use of digoxin. The shortcomings of reporting such apparent bioavailabilities have been pointed out before by other authors (4,6,16,24). We could find only one article (16) where estimates of A_e^∞ for digoxin had been made, but the method was not given. No article could be found where AUC 0–∞ had been estimated for digoxin after oral administration. Several authors (3,7,9,10,12,14–16,19–21) have collected either 0–6 or 0–10 day digoxin urinary excretion data in those cases when they were employing a radioimmunoassay method for digoxin in plasma or serum which had a sensitivity level of 0.2–0.5 ng/ml and which allowed them to follow digoxin in blood only for about 8 hr. However, as assay has been in the literature (22) since 1972 which allows measurement of digoxin down to 0.08 ng/ml of plasma or serum, and for 96 hr after a single 0.5-mg dose of digoxin (5). This assay has been improved (23) and has a sensitivity limit of 0.05 ng digoxin/ml plasma when a 0.5-ml plasma sample is utilized. The authors have used this improved assay in

several digoxin bioavailability studies where blood was sampled over a 96-hr period.

The articles of Wagner *et al.* (2,5) and Lovering *et al.* (17) suggested that apparent bioavailabilities estimated from partial areas may be close to the true bioavailabilities under certain conditions. In a recent review on digoxin (18) it was stated: "However, if blood sampling is continued for an interval (T) after the dose which is sufficient to allow serum concentrations to become quite small, then AUC 0–T is a good approximation of AUC 0–∞." It was (18) further stated: "In our studies of comparative bioavailability, 4 hr of serum sampling gave results as reliable as 8 or 24 hr of sampling." The same authors (15) also stated: "Extending urine collections beyond 1 day or serum sampling beyond 4 or 8 hr does not necessarily reduce between subject variability or enhance the usefulness of the data." However, Beveridge *et al.* (16) stated: "Therefore, statements on bioavailability [of digoxin] based on areas under plasma curves up to 6 hr may differ from those based on cumulative urinary excretion data, in this case by a factor of about 2 [i.e., a 100% error] and could suggest that bioavailability was much worse than it actually was." This dichotomy of opinion prompted us to examine, in general, the assessment of bioavailability and, in particular, to study digoxin bioavailability. In the process, several new simple methods for estimating AUC 0–∞ and A_e^∞ were devised and applied.

THEORETICAL

Methods for Estimating AUC 0–∞ and A_e^∞

All known methods and the new methods to be presented for estimation of AUC 0–∞ and A_e^∞ depend on accurate estimates of AUC or A_e at various times after administration of a single dose of drug. For AUC the trapezoidal rule (1*b*) is usually employed, and with sufficient sampling times (see rows 6, 7, and 8 of Table III) is accurate for digoxin. An even more accurate method would be that resulting from interpolation by the method of Fried and Zeitz (25), which has been computerized (1*c*), coupled with the trapezoidal rule using both observed and interpolated values. All methods (I, IIA, IIB, IIC, and III) discussed below are applicable *only* to AUC or A_e data in the terminal, log-linear phase. Methods IIA, B, and C below also depend on having three or more blood or urine collections at *equally spaced time intervals* in the terminal log-linear phase of drug elimination; for accurate results, it is later shown that *four* such collections is the minimum necessary. The classical method (Method III) for estimating AUC 0–∞ also depends on an accurate estimate of the apparent elimination rate constant,

which also requires at least three and preferably four or more plasma concentration–time points (i.e., C_p, t pairs).

Method I

Method I depends on nonlinear least-squares fitting to equation 1 of log linear AUC, t or A_e, t data using a suitable program and a digital computer:

$$y = P(1) - P(3)\, e^{-P(2)t} \tag{1}$$

For AUC data, y represents AUC at time t, $P(1)$ represents AUC 0–∞, $P(2)$ represents λ_1, and $P(3)$ represents B_1/λ_1, such that the plasma concentration, C_p, in the log-linear phase is described by equation 2. The B_1 in equation 2 (and later equation 13) is a complex function of model parameters:

$$C_p = B_1\, e^{-\lambda_1 t} \tag{2}$$

For A_e data, y represents A_e at time t, $P(1)$ represents A_e^∞, $P(2)$ represents λ_1, and $P(3)$ is equivalent to $\mathrm{Cl}_R B_1$, where Cl_R is the renal clearance.

To apply the method, we used the program NONLIN (26) and the Amdahl 470V/6 digital computer. $P(1)$, $P(2)$, and $P(3)$ are the parameters estimated in the fittings. The method has two advantages: (a) the points may be equally or nonequally spaced and (b) one obtains the standard deviations of the estimated AUC 0–∞ or A_e^∞ as well as measures of fit of predicted AUC or A_e to observed AUC or A_e values.

Method IIA

Method IIA is the accelerated convergence method of Amidon *et al.* (27). For a series of points, (t_i, Y_i), approaching an asymptote, Y_∞, and obeying first-order kinetics from time t', the general equation 3 applies, where λ_1 is the first-order rate constant:

$$Y_i = Y_\infty[1 - e^{-\lambda_1(t_i - t')}] \tag{3}$$

For three equally spaced points, at intervals, Δt, particular cases of equation 3 may be written as

$$Y_1 = Y_\infty[1 - e^{-\lambda_1 \Delta t}] \tag{4}$$

$$Y_2 = Y_\infty[1 - e^{-2\lambda_1 \Delta t}] \tag{5}$$

$$Y_3 = Y_\infty[1 - e^{-3\lambda_1 \Delta t}] \tag{6}$$

Amidon *et al.* (27) used different symbolism such that Y_1 = their X_n, Y_2 = their X_{n+1}, Y_3 = their X_{n+2}, and Y_∞ = their X'_n. They also plotted the differences on the ordinate but ended up deriving the expression corres-

ponding to these differences being plotted on the abscissa. Hence we prefer to plot the differences on the abscissa in order to apply Methods IIB and IIC discussed later.

Consider a rectalinear plot of $Y = Y_i$ (ordinate) vs. $X = Y_{i+1} - Y_i$ (abscissa) with the equation of the straight line being $Y = a + bX$; two points on the line are $(Y_2 - Y_1)$, Y_2 and $(Y_3 - Y_2)$, Y_3; the line extrapolates back to give an ordinate intercept, a, equal to Y_∞; the slope of the line, b, is given by equation 7, and the slope is *negative* since $Y_1 < Y_2 < Y_3$ and $(Y_2 - Y_1) > (Y_3 - Y_2)$.

$$b = \frac{(Y_2 - Y_3)}{(Y_2 - Y_1) - (Y_3 - Y_2)} = \frac{Y_3 - Y_2}{Y_3 - 2Y_2 + Y_1} \tag{7}$$

When $Y = Y_3$, the equation of the line is given by

$$Y_3 = Y_\infty + \left[\frac{Y_3 - Y_2}{Y_3 - 2Y_2 + Y_1}\right](Y_3 - Y_2) = Y_\infty + \frac{(Y_3 - Y_2)^2}{Y_3 - 2Y_2 + Y_1} \tag{8}$$

Rearrangement of equation 8 gives equation 9, which is equivalent to the equation given by Amidon *et al.* (27):

$$Y_\infty = Y_3 - \frac{(Y_3 - Y_2)^2}{Y_3 - 2Y_2 + Y_1} \tag{9}$$

The validity of equation 9 with respect to first-order kinetics is readily checked by substituting for Y_1, Y_2, and Y_3 in equation 9 from equations 4 through 6 and showing that the right-hand side is equal to the left-hand side.

As a simulation for AUC data after bolus intravenous administration of digoxin, let the values of the parameters of equation 1 be $P(1) = 44.67$, $P(2) = 0.0122$, and $P(3) = 37.44$. Substitution of these values and $t = 24$, 48, and 72 hr into equation 1 yielded the values below.

t	Y_i
24	$16.73 = Y_1$
48	$23.82 = Y_2$
72	$29.12 = Y_3$

Substitution of the above values into equation 9 and simplification gave $Y_\infty = 44.81$. The actual value of $P(1) = Y_\infty = \text{AUC } 0\text{–}\infty = 44.67$, while the estimated value by this method is 44.81. Hence with these error-free data the method gave an answer with an error of +0.45%.

Method IIB

Method IIB is a modification of Method IIA, but three or more points may be utilized (see Fig. 3 as an example). Equation 10 is a generalization of

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