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Further Considerations on Model-Independent Bioavailability Estimation

S. HWANG ˣ and K. C. KWAN

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Abstract □ Assumptions attendant to model-independent bioavailability estimation were reexamined. Particular attention was given to the situation where an intravenous reference is not available and nonrenal clearance is assumed to be constant between treatments. Under these circumstances, the previously proposed approximation was compared with other bioavailability estimators. On the basis of error analysis, a procedure was devised to yield optimal relative bioavailability estimates.

Keyphrases □ Bioavailability—model-independent estimation □ Drug availability—model-independent estimation □ Models—bioavailability estimation, equations

In a previous report (1), a model-independent method to assess bioavailability was suggested. The procedure calls for an initial determination of plasma clearance from an intravenous reference and assumptions concerning changes therein following the test dose(s). The proposed solutions are exact except when an intravenous reference is not available and nonrenal clearance is assumed to be unchanged between treatments. For this latter situation, an approximate solution was suggested initially with the support of a simulated example (1) and verified subsequently with experimental results (2).

This report provides a rigorous analysis of this approximation and the means to optimize its solutions. Its merits are examined relative to those of the dose-adjusted ratio of urinary recoveries of unchanged drug and of the area under the plasma concentration–time curve. It will be shown that where the nonrenal clearances are unchanged, the proposed approximation (1) is always superior to area ratios and often is better than urine ratios. Conditions under which relative bioavailability estimates should be optimal are discussed.

THEORETICAL

Bioavailability following a nonintravascular treatment, x, can be estimated by:

$$F^x = \frac{\dot{V}^x_{cl,p} AUC^x_\infty}{D^x} = \frac{\dot{V}^x_{cl,p} U^x_\infty}{D^x \dot{V}^x_{cl,r}} \qquad \text{(Eq. 1)}$$

where F is the fraction of the dose, D, absorbed; AUC_∞ is the total area under the plasma concentration–time curve; U_∞ is the total amount of unchanged drug excreted in the urine; and $\dot{V}_{cl,p}$ and $\dot{V}_{cl,r}$ are the plasma and plasma renal clearances, respectively. Except for $\dot{V}^x_{cl,p}$, the terms on the right side of Eq. 1 are known or can be calculated from plasma and/or urinary excretion data following treatment x.

On the other hand, plasma clearance must be determined by an independent experiment. Ideally, an intravenous tracer dose is administered concurrently with x such that the plasma clearance of the labeled drug becomes the estimate of $\dot{V}^x_{cl,p}$. An alternative solution was proposed (3, 4) whereby plasma clearance is estimated from separate treatments in which the renal drug clearance is perturbed in a controlled manner. The assumptions are that the perturbing influence on the kidney remains constant with time and that the same dose fraction is absorbed between

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treatments. More typically, however, the intravenous reference is administered to the test subject separately so that some assumption must be made concerning the constancy of plasma clearance between treatments. For example, if plasma clearance remains unchanged, *i.e.*, $\dot{V}^x_{cl,p} = \dot{V}^{iv}_{cl,p}$, the bioavailability of x is given by the dose-adjusted ratio of the areas under the plasma concentration curve:

$$F^x = \frac{D^{iv}(AUC^x_\infty)}{D^x(AUC^{iv}_\infty)} \qquad \text{(Eq. 2)}$$

However, if plasma clearance changes in proportion to the observed renal clearance change[1], the dose-adjusted ratio of urinary recoveries is the more accurate estimator, *i.e.*:

$$F^x = \frac{D^{iv}U^x_\infty}{D^xU^{iv}_\infty} \qquad \text{(Eq. 3)}$$

Finally, if nonrenal clearance is constant between treatments:

$$F^x = \frac{AUC^x_\infty}{D^x}(\dot{V}^{iv}_{cl,p} - \dot{V}^{iv}_{cl,r} + \dot{V}^x_{cl,r}) \qquad \text{(Eq. 4}a\text{)}$$

$$F^x = \frac{U^x_\infty}{D^x\dot{V}^x_{cl,r}}(\dot{V}^{iv}_{cl,p} - \dot{V}^{iv}_{cl,r} + \dot{V}^x_{cl,r}) \qquad \text{(Eq. 4}b\text{)}$$

In the absence of an intravenous reference and when $\dot{V}_{cl,p}$ is assumed constant, the bioavailability of treatment x relative to another nonintravascular treatment, s, is given by:

$$\frac{F^x}{F^s} = \frac{D^sAUC^x_\infty}{D^xAUC^s_\infty} \qquad \text{(Eq. 5)}$$

and when the ratio of $\dot{V}_{cl,r}/\dot{V}_{cl,p}$ remains constant between treatments:

$$\frac{F^x}{F^s} = \frac{D^sU^x_\infty}{D^xU^s_\infty} \qquad \text{(Eq. 6)}$$

By analogy to Eqs. 4*a* and 4*b*, the corresponding expression for relative bioavailability when nonrenal clearance remains constant is:

$$\frac{F^x}{F^s} = \frac{U^x_\infty}{D^x\dot{V}^x_{cl,r}}\left[\frac{\dot{V}^s_{cl,p}}{F^s} - \frac{(\dot{V}^s_{cl,r} - \dot{V}^x_{cl,r})}{F^s}\right] \qquad \text{(Eq. 7)}$$

Equations 2–7 are exact insofar as their respective assumptions hold. While the application of Eqs. 2–6 is straightforward, Eq. 7 cannot be evaluated because of the presence of unknowns $\dot{V}^s_{cl,p}$ and F^s on the right side. As an approximation to Eq. 7, it was suggested by Kwan and Till (*K–T*) that (1):

$$\left(\frac{F^x}{F^s}\right)_{K-T} = \frac{1}{[\dot{V}^x_{cl,p}]_{ex}}([\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r} + \dot{V}^x_{cl,r}) \qquad \text{(Eq. 8)}$$

where $[\dot{V}_{cl,p}]_{ex}$ is defined as an experimentally derivable clearance such that:

$$[\dot{V}^{niv}_{cl,p}]_{ex} = \frac{\dot{V}^{niv}_{cl,p}}{F^{niv}} = \frac{D^{niv}(\dot{V}^{niv}_{cl,r})}{U^{niv}_\infty} \qquad \text{(Eq. 9)}$$

and niv refers to a nonintravascular treatment such as x or s. The error incurred by the use of Eq. 8 instead of Eq. 7 therefore is:

$$|(F^x/F^s)_{K-T} - (F^x/F^s)_{Eq.\ 7}| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}}\left(\frac{1}{F^s} - 1\right) \qquad \text{(Eq. 10)}$$

where:

$$\delta = \dot{V}^s_{cl,r} - \dot{V}^x_{cl,r} \qquad \text{(Eq. 11)}$$

Since treatments x and s are nonintravascular, one should be able to estimate the bioavailability of s relative to that of x by the same approximate method:

$$\left(\frac{F^s}{F^x}\right)_{K-T} = \frac{1}{[\dot{V}^s_{cl,p}]_{ex}}([\dot{V}^x_{cl,p}] - \dot{V}^x_{cl,r} + \dot{V}^s_{cl,r}) \qquad \text{(Eq. 12)}$$

in which treatment x is designated as the reference for calculation purposes. Then, the bioavailability of x relative to s is simply the reciprocal of $(F^s/F^x)_{K-T}$:

$$\frac{1}{(F^s/F^x)_{K-T}} = \frac{[\dot{V}^s_{cl,p}]_{ex}}{[\dot{V}^x_{cl,p}] - \dot{V}^x_{cl,r} + \dot{V}^s_{cl,r}} \qquad \text{(Eq. 13)}$$

The error resulting from the use of Eq. 13 as an approximation to Eq. 7 is:

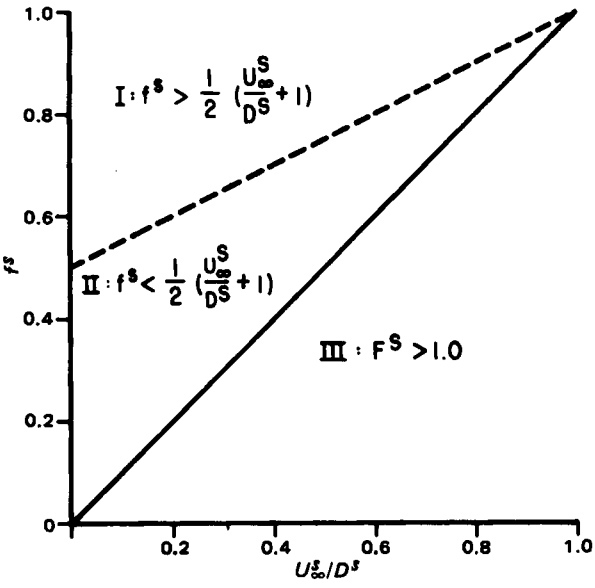

Figure 1—*Plot of* f^s versus U^s_∞/D^s. *On the dashed line,* $f^s = 0.5 + 0.5(U^s_\infty/D^s)$, *the errors associated with the uses of* $(U^x_\infty D^s/U^s_\infty D^x)$ *and* $(F^x/F^s)_{K-T}$ *are equal. In Region I,* $(U^x_\infty D^s)/(U^s D^x)$ *is preferred over* $(F^x/F^s)_{K-T}$, *and vice versa in Region II.*

$$\left|\frac{1}{(F^s/F^x)_{K-T}} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}}\frac{[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,p}}{F^s([\dot{V}^x_{cl,p}]_{ex} + \delta)} \qquad \text{(Eq. 14)}$$

In addition to the trivial case where $\delta = 0$, it is evident from Eqs. 10 and 14 that the error term vanishes as F^s approaches 1.0 and as $[\dot{V}^x_{cl,p}]_{ex}$ approaches $\dot{V}^x_{cl,p}$, respectively. Equation 15 shows that estimates of bioavailability from Eq. 8 generally will differ from those from Eq. 13. The choice between the two can be made by considering the difference between their respective errors, Eqs. 14 and 10:

$$\left|\frac{1}{(F^s/F^x)_{K-T}} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| - \left|\left(\frac{F^x}{F^s}\right)_{K-T} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}([\dot{V}^x_{cl,p}]_{ex} + \delta)}|([\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r}) - ([\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r})| \qquad \text{(Eq. 15)}$$

All terms on the right side of Eq. 15 are experimentally derivable. Since the quotient preceding the braces, { }, always is positive, the sign of Eq. 15 is determined by the relative magnitude of $[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$ for the two treatments. If $[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r}$ is greater than $[\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r}$, $(F^x/F^s)_{K-T}$ is closer to the true value and is preferred. If the converse holds, $1/(F^s/F^x)_{K-T}$ should be chosen. In the unlikely event that the two quantities within braces are identical, the same relative bioavailability estimate would result. The same criteria apply in studies involving three or more nonintravascular dosages. In other words, the treatment yielding the smallest value for $[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$ should be the reference for calculation.

According to Eq. 15, the first step in the estimation of relative bioavailability under the assumption of constant nonrenal clearance is to calculate $[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$ for all treatments in all subjects. For each subject, the treatment with the smallest $[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$ then is selected as the reference to which the bioavailability of all other treatments is compared. The resulting bioavailability ratios are rearranged or combined so that the desired reference, s, appears in the denominator.

In view of the approximate nature of Eqs. 8 and 13, their merits relative to other estimators should be examined. Conceivably, either the ratio of the dose fraction recovered in the urine or the ratio of the area under the plasma curve may be similarly adequate as an approximation even though nonrenal clearance is unchanged between treatments. Therefore, if nonrenal clearance truly is constant between treatments, the error associated with the use of the dose-adjusted area ratio (*i.e.*, Eq. 5) as a relative bioavailability estimate would be:

$$\left|\frac{AUC^x_\infty/D^x}{AUC^s_\infty/D^s} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{F^s[\dot{V}^x_{cl,p}]_{ex}} \qquad \text{(Eq. 16)}$$

Comparison of the uncommon terms on the right side of Eq. 10 with those of Eq. 16 shows that $(1/F^s) > (1/F^s) - 1$. Thus, under the prescribed

[1] This assumption is synonymous with saying that the fractional urinary recovery, $f = \dot{V}_{cl,r}/\dot{V}_{cl,p}$, remains constant between treatments.

Table I—Simulated Data for Relative Bioavailability Calculation

Oral Treatment	Dose, mg	Dose Fraction Absorbed, F^a	Dose Fraction Excreted in Urine, U_∞/D	Renal Clearance $\dot{V}_{cl,r}$, ml/min	$[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$
1	500	0.536	0.265	223.1	618.8
2	500	0.680	0.306	186.6	423.2

[a] As estimated in Table II of Ref. 1.

conditions, Eq. 8 estimates the true bioavailability better than does the ratio of dose-adjusted areas under the plasma curve. In light of the previous discussion on optimization, estimates by Eq. 13, when applicable, should be even better than those by Eq. 8.

Similarly, the absolute difference between the dose-adjusted ratio of urinary recoveries (*i.e.*, Eq. 6) and the true value is:

$$\left|\frac{U^x_\infty/D^x}{U^s_\infty/D^s} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}}\left(\frac{D^s}{U^s_\infty} - \frac{1}{F^s}\right) \quad \text{(Eq. 17)}$$

Comparison of the uncommon terms on the right side of Eqs. 10 and 17 shows that $(D^s/U^s_\infty) - (1/F^s)$ may be greater than, equal to, or less than $(1/F^s) - 1$. Thus, even when the nonrenal clearance is constant between treatments, the urinary excretion ratio approximates relative bioavailability better than does Eq. 8 in some situations. Intuitively, such situations prevail when renal excretion is the dominant drug elimination route. A quantitative assessment of situations in which one method is superior to the other is as follows.

The difference between Eqs. 10 and 17 can be expressed as:

$$\left|\left(\frac{F^x}{F^s}\right)_{K\text{-}T} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| - \left|\frac{U^x_\infty/D^x}{U^s_\infty/D^s} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}}\frac{D^s}{U^s_\infty}\left(2f^s - 1 - \frac{U^s_\infty}{D^s}\right) \quad \text{(Eq. 18)}$$

where f^s is the ratio of the renal clearance to plasma clearance. When the right side of Eq. 18 is positive, the urinary recovery ratio is better; when it is negative, $(F^x/F^s)_{K\text{-}T}$ is preferred. Whether Eq. 18 is greater or less than zero depends on the relative magnitude of f^s and U^s_∞/D^s. When Eq. 18 is zero:

$$f^s = \tfrac{1}{2}\left(1 + \frac{U^s_\infty}{D^s}\right) \quad \text{(Eq. 19)}$$

A plot of f^s *versus* U^s_∞/D^s is shown in Fig. 1, which is clearly divided into three regions. Equation 19 separates Region I, representing situations where $f^s > \frac{1}{2}[1 + (U^s_\infty/D^s)]$ when urinary excretion ratios should be used,

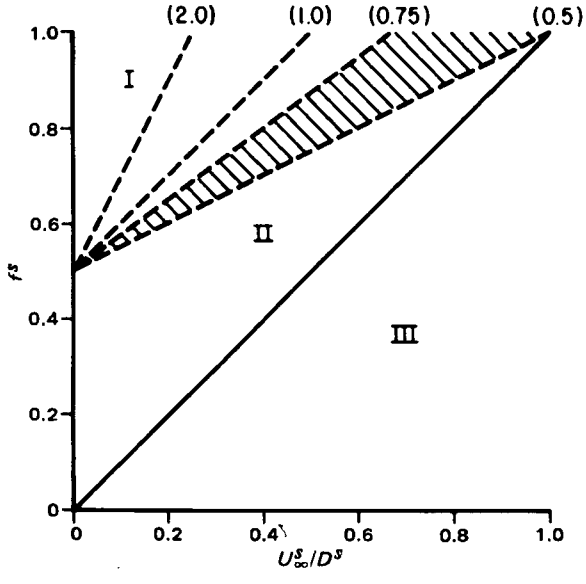

Figure 2—*Plot of* $f^s = 0.5 + \alpha(U^s_\infty D^s)$ *(Eq. 20). The* α *value for each line is specified in parentheses. The shaded area indicates the expansion of Region II by using* $1/(F^s/F^x)_{K\text{-}T}$ *instead of* $(F^x/F^s)_{K\text{-}T}$ *as* α *increases from 0.5 to 0.75.*

Table II—Sample Calculation of Relative Bioavailability of Oral Treatment 1 to Oral Treatment 2

True Value F^1/F^2	$\frac{AUC^1_\infty}{AUC^2_\infty}$[a]	$\frac{U^1_\infty}{U^2_\infty}$	$\left(\frac{F^1}{F^2}\right)_{K\text{-}T}$	$\frac{1}{(F^2/F^1)_{K\text{-}T}}$
0.788	0.724	0.866	0.768	0.757

[a] The *AUC* values were estimated by dividing total urinary recovery by renal clearance.

from Region II, where $f^s < \frac{1}{2}[1 + (U^s_\infty/D^s)]$ when $(F^x/F^s)_{K\text{-}T}$ should be favored. Region III, representing $f^s < (U^s_\infty/D^s)$ (or $\dot{F}^s > 1$), is not experimentally relevant.

In accord with the preceding discussion on the optimal choice of a reference in relative bioavailability calculations, there will be times when $1/(F^s/F^x)_{K\text{-}T}$ is a better estimator than $(F^x/F^s)_{K\text{-}T}$. Under these circumstances, the demarcation between Regions I and II must change so as to enlarge Region II. Therefore, the difference between Eqs. 14 and 17 is:

$$\left|\frac{1}{(F^s/F^x)_{K\text{-}T}} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| - \left|\frac{U^x_\infty/D^x}{U^s_\infty/D^s} - \left(\frac{F^x}{F^s}\right)_{Eq.\ 7}\right| = \frac{|\delta|}{[\dot{V}^x_{cl,p}]_{ex}}\frac{D^s}{U^s_\infty}\left\{2f^s - 1 - \frac{U^s_\infty}{D^s} - \frac{U^s_\infty}{D^s}\frac{([\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r}) - ([\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r})}{[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r} + \dot{V}^s_{cl,r}}\right\} \quad \text{(Eq. 20)}$$

Evidently, the critical value at which Eq. 20 becomes zero is when:

$$f^s = \frac{1}{2} + \left\{\frac{([\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r}) - ([\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r})}{[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r} + \dot{V}^s_{cl,r}} + \frac{1}{2}\right\}\frac{U^s_\infty}{D^s} \quad \text{(Eq. 21)}$$

Like Eq. 19, Eq. 21 is a straight line with an intercept of 0.5. Unlike Eq. 19, the slope of Eq. 21 varies with each specific pair of treatments x and s. However, since $1/(F^s/F^x)_{K\text{-}T}$ is used only when $[\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r}$ is greater than $[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r}$, a positive contribution to the slope is assured. Thus, Eq. 20 represents a family of lines with slopes equal to or greater than 0.5 and a common intercept at $x = 0, y = 0.5$. Figure 2 illustrates the moving boundary between Regions I and II for a range of slopes. The shaded area in Fig. 2 represents the expansion of Region II at the expense of Region I if the slope increases from 0.5 to 0.75.

Application of Eqs. 19 and 21 requires some knowledge of the excretion characteristics of the drug. In the absence of an intravenous reference, f^s is an unknown, while all terms on the right side of Eqs. 19 and 21 are experimental observations. If it is known from previous studies that f generally is less than 0.5, Eq. 8 or 13 clearly is preferable to urinary excretion ratios. For given f values greater than 0.5, the superior estimator can be deduced from observed U_∞/D values with the aid of Eqs. 15, 19, and 21 and Fig. 2. That is to say, the choice between $(F^x/F^s)_{K\text{-}T}$ and $1/(F^s/F^x)_{K\text{-}T}$ depends on the criterion set by Eq. 15. Whether either of these terms is preferable to the urinary recovery ratio then depends on f for the drug and the actual observations, *e.g.*, U_∞ and $\dot{V}_{cl,r}$, for a given subject.

For comparisons involving $(F^x/F^s)_{K\text{-}T}$, Eq. 19 or the line having a slope equal to 0.5 in Fig. 2 applies. When $1/(F^s/F^x)_{K\text{-}T}$ is more appropriate, a slope first must be calculated in accordance with Eq. 21, and the proper line must be constructed in Fig. 2. For example, when $U^s_\infty/D^s = 0.4$ and the slope by Eq. 21 is 1.0, Fig. 2 shows that the urinary excretion ratio would be a better estimator of relative bioavailability than $(F^x/F^s)_{K\text{-}T}$ if f^s is >0.7 and superior to $1/(F^s/F^x)_{K\text{-}T}$ if f^s is >0.9.

EXAMPLE

To test the conclusions from the forgoing error analyses, it is necessary to use an example for which the correct answer is known. The simulated data from the previous report (1) are suitable for this purpose because all aspects of drug disposition except renal clearance were kept constant between treatments and the bioavailability of the two oral treatments was fixed *a priori* (and confirmed by comparison to the intravenous reference). Table I summarizes the information necessary to the present discussion.

Various bioavailability estimates of Treatment 2 relative to Treatment 1 are shown in Table II. The accuracy of each approximation can be ranked according to its proximity to the true value, which is the ratio of their absolute bioavailabilities. Since $[\dot{V}_{cl,p}]_{ex} - \dot{V}_{cl,r}$ is smaller after Treatment 2, $(F^1/F^2)_{K\text{-}T}$ should be closer to the true value than $1/(F^2/F^1)_{K\text{-}T}$. This is the case. Also, as predicted, the result by the *AUC*

ratio is worse than that by $(F^1/F^2)_{K\text{-}T}$. Finally, the urinary recovery ratio is the worst approximation of all, which is not unexpected since renal excretion is only a minor elimination route ($f < 0.50$).

DISCUSSION

An approximate solution for relative bioavailability estimation between nonintravascular doses was suggested previously (1). Since then, numerous inquiries have been received concerning the nature and source of its inexactitude, particularly under the assumption of constant nonrenal clearance between treatments. Simulation studies were not revealing; reasonably accurate estimates usually were obtained except when unrealistically large perturbations were considered. On the other hand, the present theoretical analysis appears to offer new insights.

Whereas the ratio of areas under the plasma curve and of urinary excretions are exact relative bioavailability determinants when their respective assumptions prevail, Eqs. 8 and 13 are only approximations when nonrenal clearance remains constant between treatments. In addition, Eqs. 8 and 13 give different answers for a given data set. However, criteria were established so that the more accurate of the two estimates always can be identified. The choice of reference for calculations should be made for each comparison within a study to ensure the best possible estimates.

Despite their approximate nature, Eqs. 8 and 13 always are preferable to area ratios and often are superior to urinary excretion ratios when nonrenal clearance is constant. The only exception is when the drug is eliminated predominantly by renal excretion. Predominance now has been defined as when $\dot{V}_{cl,r}/\dot{V}_{cl,p}$ is greater than either $\frac{1}{2}(1 + U^s_\infty/D^s)$ or:

$$\frac{1}{2} + \left\{\frac{1}{2} + \frac{([\dot{V}^s_{cl,p}]_{ex} - \dot{V}^s_{cl,r}) - ([\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r})}{[\dot{V}^x_{cl,p}]_{ex} - \dot{V}^x_{cl,r} + \dot{V}^s_{cl,r}}\right\} \frac{U^s_\infty}{D^s}$$

depending on whether Eq. 8 or 13 is to be used, respectively. Thus, given some idea of the usual fraction excreted unchanged following an intravenous dose, a decision can be made whether the urinary excretion ratio, Eq. 8, or Eq. 13 should be chosen.

The constant nonrenal clearance assumption ordinarily is favored for another reason. Given that observed renal clearances differ between treatments, the likelihood that compensatory nonrenal clearance changes will occur to maintain a constant plasma clearance or a constant ratio of renal to plasma clearance seems remote. On the other hand, adjustments in plasma clearance based only on observed changes in renal clearance simply corroborate experimental evidence.

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Mass Spectrometry of Chlorambucil, Its Degradation Products, and Its Metabolite in Biological Samples

S. Y. CHANG ˣ, B. J. LARCOM, D. S. ALBERTS, B. LARSEN, P. D. WALSON, and I. G. SIPES

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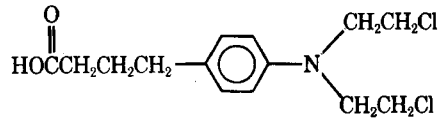
Abstract □ A sensitive and specific method for the determination of chlorambucil and its metabolite in biological fluids is reported. The method is based on selected-ion monitoring detection following simple extraction of the parent compound, its metabolite, and an internal standard (chlorambucil-d_8) from plasma and urine samples. The precision (reproducibility) of the method was 94.3 ± 1.3% with 200 ng of chlorambucil added to 1 ml of plasma. Chlorambucil degradation or alkylation of plasma proteins was minimal with plasma incubated at 24° for 4 hr. However, chloroambucil recovery decreased to 56% after plasma incubation at 37° for 4 hr. Three chlorambucil degradation products in ethyl acetate solution were found, and their structures were studied by mass spectrometry.

Keyphrases □ Chlorambucil—analysis, mass spectrometry, degradation products and metabolite, human plasma and urine □ Antineoplastic agents—chlorambucil, degradation products and metabolite, mass spectrometry, human plasma and urine □ Mass spectrometry, selected-ion monitoring—chlorambucil, degradation products and metabolite, human plasma and urine

The anticancer drug chlorambucil (I) is useful in the treatment of chronic lymphocytic leukemia, ovarian carcinoma, nodular lymphocytic lymphoma, and myelocytic leukemia (1, 2). Various analytical methods were reported for the quantitation of I, including the colorimetric determination of 4-(*p*-nitrobenzyl)pyridine derivatives (3, 4) and UV spectrophotometric (5) and chlorine titrimetric (6) methods. None of these methods provides the sensitivity and accuracy needed to study I pharmacokinetics and metabolism in humans. The mass spectrometric determination of I in plasma was reported recently (7). This



I

method requires several extraction steps, including a back-extraction of I from the aqueous solution at alkaline pH.

The quantitative method presented here is based on a one-step extraction followed by a determination of I and a metabolite in plasma and urine by mass spectrometry using a deuterated I internal standard. This method was used to study the *in vitro* I stability in plasma as well as to characterize the *in vitro* degradation products. The method was applied to the quantitation of I and a metabolite in human plasma and urine samples.

EXPERIMENTAL

Chlorambucil-d_8 Synthesis—The synthesis of chlorambucil-d_8 (labeled at *N*-chloroethyl) was adapted from a literature method (8). 4-(*p*-Nitrophenyl)butyric acid[1], 500 mg, was reacted with ethereal diazomethane[1]. The resulting methylnitrophenylbutyrate was dissolved in ethyl acetate–methanol (9:1 v/v) through which hydrogen gas was bubbled continuously for 8 hr in the presence of palladium[2] with the solvent

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Ventron Corp., Danver, Mass.

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