An Improved Intercept Method for the Assessment of Absorption Rate in Bioequivalence Studies

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INTRODUCTION

In contrast to the general consensus that the use of the area under the curve (AUC) of a plasma concentration versus time profile is an efficient indicator of the extent of absorption in bioavailability/bioequivalence studies, the currently used measures for the assessment of the rate of absorption have been considered to be problematic: C_{max} (maximum plasma concentration) is confounded by the extent of drug absorption and it is insensitive to changes in rate of absorption, T_{max} (time at which maximum concentration occurs) suffers from a lack of well established statistical tests applicable to such a discrete variable whereas both C_{max} and T_{max} depend upon sampling schedule. These shortcomings have been addressed in a plethora of publications where several alternative rate indicators have been proposed (1–8).

Recently, a sensitive and specific method for the determination of the equivalence of absorption rates was proposed by Endrenyi and Al-Shaikh (9). The method is based on Eq. 1 which was derived assuming a linear one-compartment model after a first-order Taylor expansion around $t = 0$:

$$\frac{k_{aT}}{k_{aR}} \approx \frac{C_T/AUC_T}{C_R/AUC_R} \tag{1}$$

where k_{aT} and k_{aR} are the absorption rate constants, C_T and C_R are the plasma concentrations before T_{max} while the symbols T and R denote the test and reference formulation, respectively. The method requires the calculation of the y-intercept, by linear regression of the $(C_T/AUC_T)/(C_R/AUC_R)$ values or their logarithms versus time. According to Eq. 1, this intercept is an estimate of the ratio of absorption rate constants or its logarithm of them assuming identical elimination characteristics for the test and the reference formulation. This new metric exhibits very promising kinetic and statistical properties when a linear one-compartment model kinetics is considered (9).

However, the estimation of a single intercept does not allow for separate indirect measures of the absorption rate for the test and reference formulation. Therefore, a different procedure to that traditionally used for construction of confidence intervals was proposed (9). Currently, the statistical evaluation of C_{max} data is based on an analysis of variance which necessitates separate figures for the test and the reference formulation. Further, the application of the proposed method (9) presupposes that samples for both formulations have been collected at identical time points. There are situations (e.g. when deviations from sampling schedule are encountered or comparisons between extended and immediate release formulations are made) where this presupposition does not hold. Finally, in the presence of lag time the method requires appropriate corrections not only in time values but in concentration values, too. Interpolation techniques should be employed for these kinds of corrections.

In this note we propose a modified intercept approach which maintains the favorable characteristics of the proposed method (9) and lacks the drawbacks delineated above.

THEORY

In order to develop a methodology for the assessment of absorption rate in bioequivalence studies, instead of the conventional pharmacokinetic functions $C = f(t)$, functions of the general form $(C/t) = g(t)$ were considered. By doing so, the change of the quotient [concentration/time], which can be considered as a "typical" rate quantity, can be studied as a function of time.

Assuming one-compartment model disposition with first-order absorption, the plasma concentration changes with time according to:

$$C = (FD/V)\frac{k_a}{k_a - k_e}(e^{-k_e t} - e^{-k_a t}) \tag{2}$$

where F is the fraction of dose D absorbed, V is the volume of distribution and k_e is the elimination rate constant. Dividing both sides of Eq. 2 by t and taking the limit for $t \to 0$ of the resulting equation we have:

$$\mathrm{Lim}_{t\to 0}\left(\frac{C}{t}\right) = (FD/V)\frac{k_a}{k_a - k_e}\mathrm{Lim}_{t\to 0}\frac{(e^{-k_e t} - e^{-k_a t})}{t} \tag{3}$$

By applying L' Hospital's rule one gets:

$$\mathrm{Lim}_{t\to 0}\left(\frac{C}{t}\right) = (FD/V)\frac{k_a}{k_a - k_e}\mathrm{Lim}_{t\to 0}\frac{(e^{-k_e t} - e^{-k_a t})'}{t'}$$

$$= (FD/V)\frac{k_a}{k_a - k_e}(k_a - k_e) = (FD/V)k_a \tag{4}$$

Based on Eq. 4 and applying an identical syllogism to that used for Eq. 1, one can infer that the extrapolated y-intercept obtained by linear regression analysis of the plot of the ratios (C/t) versus time, $\mathrm{intercept}_{C/t}$, provides an estimate for $(FD/V)k_a$:

$$\mathrm{Intercept}_{C/t} \approx (FD/V)k_a \tag{5}$$

Assuming one-compartment model disposition with zero-order absorption, the plasma concentration changes with time (during the absorption phase) according to the following equation:

$$C = \frac{k_0}{Vk_e}(1 - e^{-k_e t}) \tag{6}$$

where k_0 is the zero-order absorption rate constant. Applying the same methodology used for obtaining Eq. 4, one gets:

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$$\text{Lim}_{t\to 0}\left(\frac{C}{t}\right) = \frac{k_0}{Vk_e}\,\text{Lim}_{t\to 0}\frac{(1-e^{-k_e t})'}{t'} = \frac{k_0}{V} = (FD/V)\frac{1}{\tau} \tag{7}$$

where τ is the duration of the zero-order absorption phase. It can be seen that equations 4 and 7 are essentially equivalent.

In a similar manner one can also prove that the same limits are derived assuming either the linear two-compartment model or the two-compartment model with zero-order absorption.

These findings suggest that, regardless of the disposition kinetics, $\text{intercept}_{C/t}$ depends exclusively upon FD/V and the absorption rate constant (or the equivalent $1/\tau$). However, the coefficient FD/V can be eliminated by dividing $\text{intercept}_{C/t}$ with the corresponding AUC value. This treatment is in full analogy with the recently proposed method (9). By doing so, the ratio $\text{intercept}_{C/t}$/AUC can be used as an indicator of the absorption rate constant if one assumes identical elimination characteristics for the test and the reference formulation.

RESULTS AND DISCUSSION

Due to the exponential character of the $(C/t) = g(t)$ function, the (C/t) versus time curves are nonlinear with (C/t) continuously decreasing with time. However, the corresponding semi-logarithmic plots are almost linear during the absorption phase. An example is shown in Figure 1, assuming a linear one compartment model and values of k_a/k_e ranging from 1.25 to 8.00. Inspection of Figure 1, reveals that the nonlinear character of the plot during the absorption phase becomes less pronounced as the ratio k_a/k_e decreases. In fact, for the specific case where $k_a = k_e = k$, the entire plot of $\ln(C/t)$ versus time has been shown to be linear with an intercept equal to $\ln[(FD/V)k]$ (10):

$$\ln(C/t) = \ln[(FD/V)k] - kt \tag{8}$$

The corresponding plots for the linear two-compartment model (not shown) were found to be of similar shape. In addition, (C/t) versus time, direct and semi-logarithmic plots were constructed (not shown) for the case of a one-compartment model with zero-order absorption. In all cases, the initial limbs of the semi-logarithmic plots are almost linear. Therefore, in practice, an estimate of the $\text{intercept}_{C/t}$ can be obtained by linear regression analysis of the $[\ln(C/t), t]$ data from time $t = 0$ to time $t = T_{max}$.

In order to evaluate the performance of the method in the assessment of the absorption rate constant, errorless simulated data and realistic sampling schedules were used. Thus, assuming one compartment model disposition with first order kinetics and various values of k_a/k_e, the $\text{intercept}_{C/t}$ values were estimated by linear regression analysis of $[\ln(C/t), t]$ data up to T_{max}, Figure 2. The regression lines shown in this Figure substantiate the valid estimation of $\text{intercept}_{C/t}$. The effect of changing the ratio of the absorption rate constant for test and reference formulations on corresponding ratios for the $\text{intercept}_{C/t}$ is shown in Figure 3. The T/R ratios were calculated considering a formulation with $k_a/k_e = 1.25$ as the reference. For comparative purposes, in Figure 3 the T/R ratios for C_{max} are also shown. It can be seen that in contrast to C_{max} which (as expected) has a poor sensitivity in reflecting changes of k_a values, the $\text{intercept}_{C/t}$ performs almost ideally and this behavior is maintained over a wide range of values of the ratio k_a/k_e, i.e. from 0.02 to 50.0

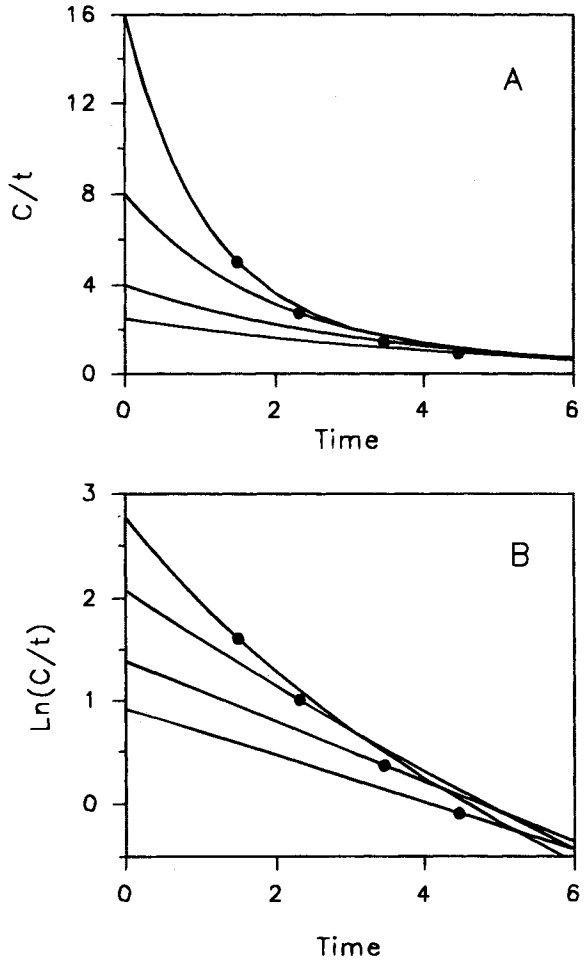

Fig. 1. (C/t) vs time plots (A) and $\ln(C/t)$ vs time plots (B) assuming linear one-compartment model kinetics with $k_e = 0.20$, $FD/V = 10$, and k_a values equal to (bottom to top) 0.25, 0.40, 0.80, and 1.60. The dot on each curve indicates the (C_{max}, T_{max}) point.

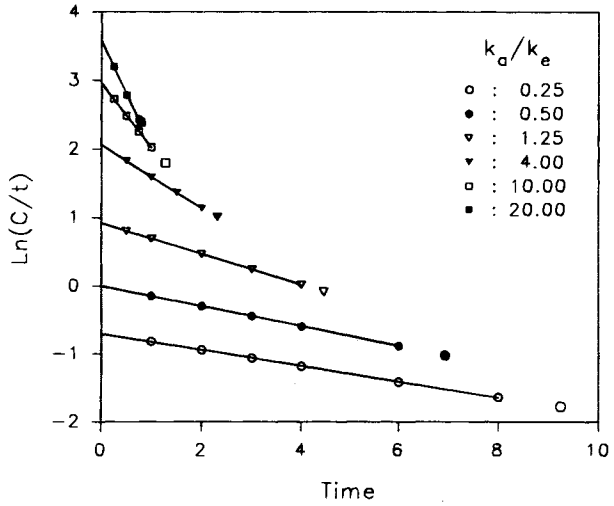

Fig. 2. $\ln(C/t)$ vs time simulated data assuming linear one-compartment model kinetics with $k_e = 0.20$, $FD/V = 10$, and k_a/k_e values ranging from 0.25 to 20. Each line is the regression line using all data points up to T_{max} excluding the (C_{max}, T_{max}) datum. For presentation purposes this datum is shown on the right end of each line.

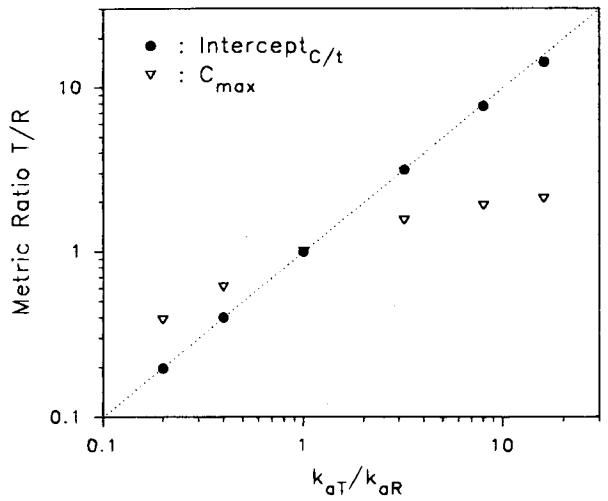

Fig. 3. The effect of changing the ratio of the absorption rate constant for test (k_{aT}) and reference (k_{aR}) formulations on corresponding ratios for intercept$_{C/t}$ and C_{max}. Intercept$_{C/t}$ estimates were obtained from the data of Figure 2; theoretical values for C_{max} were used. The formulation with k_a/k_e = 1.25 was used as reference formulation. The dotted line indicates complete concordance.

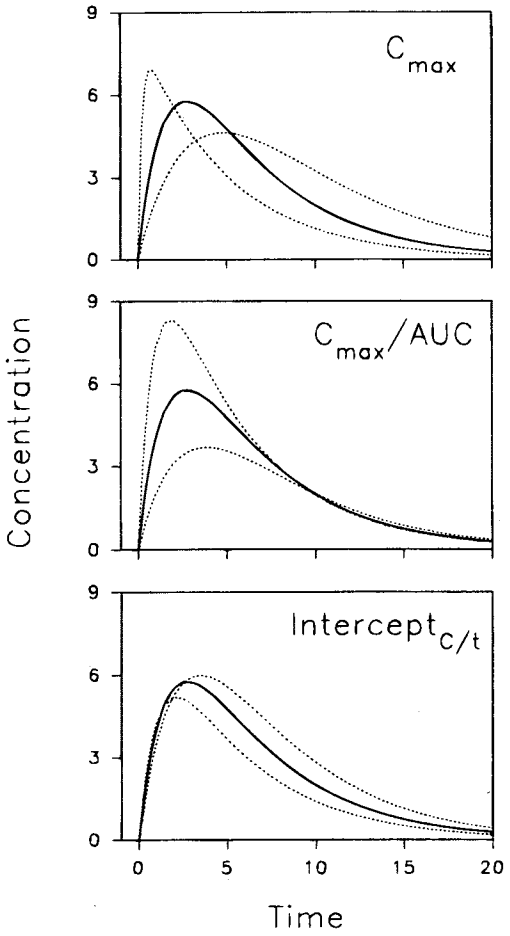

Fig. 4. Concentration vs time profiles for the reference and the test formulation. Continuous lines correspond to the reference formulation assuming linear one compartment model kinetics with k_e = 0.20, FD/V = 10, and k_a = 0.60. Dotted lines correspond to «accepted» extreme test profiles assuming ±20% difference on AUC values and ±20% difference on C_{max}, C_{max}/AUC and intercept$_{C/t}$ values.

(not shown). This is a major advantage since in most of the proposed metrics (including C_{max}) kinetic sensitivity varies remarkably with the value of the ratio k_a/k_e (5).

The focal point of the debate on the assessment of the absorption rate in bioequivalence studies is the definition of the objectives (5): Is the clinical safety and efficacy of the product, and therefore C_{max}, the only objective or the pharmaceutical quality (actual release rate) is also of importance? In contrast to previously proposed metrics which are not sensitive in reflecting changes of the absorption rate constant (5), intercept$_{C/t}$ enables for addressing both objectives. Assuming one-compartment model kinetics, values of the pharmacokinetic parameters were assigned for the reference formulation, and the corresponding AUC and rate metrics were calculated. Considering ±20% difference for both the AUC and the rate metrics, the pharmacokinetic parameters for the test products were estimated with an iterative method. The corresponding (C, t) extreme profiles for the test formulation were generated (Figure 4). Relying on these profiles it can be concluded that:

- The "accepted" extreme test profiles based on AUC and C_{max} differed dramatically in k_a values (k_{aT}/k_{aR}: 0.37 and 7.57). The well known insensitivity of C_{max} in reflecting changes of k_a values is intensified when a simultaneous variation in AUC occurs. For example, it can be shown that when $k_{aR}/k_e \geq 5$ and $F_T \approx 0.8F_R$ then C_{maxT} is always lower than $1.2C_{maxR}$ irrespective of the value of k_{aT}. An extremely high value of the absorption rate constant may be of clinical importance in certain situations.
- Based on AUC and C_{max}/AUC, the k_a values of the "accepted" extreme test profiles varied less (k_{aT}/k_{aR}: 0.55 and 1.93) but C_{max} values were outside the ±20% range. The high variation of C_{max} values may also be of clinical importance in certain situations.
- Based on AUC and intercept$_{C/t}$, the k_a values of the "accepted" extreme test profiles differed minimally (k_{aT}/k_{aR}: 0.67 and 1.50) whereas both C_{max} and C_{max}/AUC were within the ±20% interval.

A further conclusion derived from the above observations is that intercept$_{C/t}$ better reflects changes in absorption rate than the currently used C_{max}. Due to this high kinetic sensitivity of intercept$_{C/t}$, relaxing of the ±20% boundaries would be advisable. Such a proposal has been also pointed out in the context of the use of Eq. 1 (9).

It should be noted that the above conclusions were drawn using a moderate value for the ratio of the rate constants, k_{aR}/k_e = 3. However, these conclusions were found to be valid for a wide range of k_{aR}/k_e values. For C_{max} or C_{max}/AUC differences between the test and the reference absorption rate constants become more pronounced as k_{aR}/k_e increases whereas for intercept$_{C/t}$ these differences are independent of the value of k_{aR}/k_e.

In conclusion, intercept$_{C/t}$ is linearly related to the absorption rate constant. Compared with other metrics the intercept$_{C/t}$, estimated from linear regression of ln(C/t) versus time ($0 < t < T_{max}$) data, reflects the absorption rate constant with higher sensitivity. In parallel, this approach also improves the previous method (9) considerably. First, separate estimates for intercept$_{C/t}$

can be obtained for the test and the reference formulation and, therefore, traditional statistical comparisons using analysis of variance can be applied. Second, intercept$_{Cl_t}$ can be estimated in situations where the sampling schedules for the test and the reference formulation are not identical.

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