The Duration of Measuring Partial AUCs for the Assessment of Bioequivalence

Laszlo Endrenyi,[1,3] Ferenc Csizmadia,[1] Laszlo Tothfalusi,[1] Alfred H. Balch,[2] and Mei-Ling Chen[2]

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Purpose. To determine favourable sampling conditions for assessing bioequivalence by the comparison of partial AUCs in the early phase of concentration-time profiles.

Methods. Two-period crossover trials were simulated. They assumed a wide range of the ratios of absorption rate constants of the test (T) and reference (R) formulations (k_{aT}/k_{aR}). Averages and standard deviations of the corresponding ratios of simulated partial AUCs (AUC_{pT}/AUC_{pR}) were determined together with the statistical power of assessing bioequivalence, i.e., the percentage of simulated trials in which bioequivalence was declared.

Results. The power for stating bioequivalence was high when AUC_p was recorded until the earlier rather than the later of two peaks in each subject. Similarly, power was comparatively high when AUC_p was measured until the time of the reference peak instead of multiples of this time. Power was high also when AUC_p was determined until the fixed true, population mean time of the reference formulation instead of multiples of this time. The pattern for the kinetic sensitivity paralleled that found for the power, while the standard deviations changed generally in the opposite direction.

Conclusions. The effectiveness (power) of evaluating bioequivalence in the early phase of concentration-time profiles by partial AUCs generally decreases when the duration for measuring the metric is extended. Among the investigated designs, determination of partial AUCs until the earlier of two peaks in each subject is the most powerful.

KEY WORDS: bioequivalence; partial AUC; absorption rate; experimental design; crossover trials.

INTRODUCTION

Ratios of areas under the curves (AUCs) contrasting plasma concentrations (C) with time (t) have been widely used to determine the equivalence of the extent of absorption. However, Chen (1) suggested that ratios of partial AUCs (AUC_p) measured until the peak of the reference product or the earlier peak of whichever formulation was tested, could usefully evaluate the equivalence of absorption rates. In a refined interpretation, the measure would compare the *early phase* of concentration-time profiles, or the early "exposures" (2). This application of partial AUCs should be distinguished from the truncation of AUCs which is performed in the later segments of the profiles. Truncations are used for comparisons of extents of absorption.

While the suggestion for the use of partial AUCs is promising, the indicated condition for their evaluation may not be optimal. Moreover, analyses of observed and simulated trials indicate that the variation of partial AUCs, and their ratios, can be reduced if the duration of their evaluation is extended (3,4). Therefore, an investigation is presented which evaluates ratios of partial AUCs under various conditions. It is intended to find circumstances which are favourable for the effective evaluation of bioequivalence in the early phase of concentration-time profiles.

METHODS

Simulation of Bioequivalence Trials

Computer-simulated bioequivalence trials were performed. Two-period randomized crossover trials were conducted in which the test (T) and reference (R) formulations were assumed to be administered in random sequences to the subjects. 24 individuals were included in each trial.

The conditions of the simulations were essentially the same as in the basic scenario of Bois et al. (5,6) as well as Tothfalusi and Endrenyi (7). First-order absorption and single-exponential disposition were assumed. The distributions of the kinetic parameters, including their population means and inter- and intraindividual coefficients of variation are summarized in Table 1. The main deviation from the assumptions of Bois et al. (5,6) was that the kinetic parameters were considered to follow lognormal and not truncated normal distributions.

The assumed *experimental design* placed greater emphasis on observations in the early phase of studies than the scheme of Bois et al. (5,6). The design points were assumed to be 0,.15,.30,.45,.6,.8,1.0,1.25,1.50,1.75,2.0,2.5,3.0,3.5,4,5,6 and 8 hr. These points took into account that the true, population average peak time of the reference formulation was $T^0_{max,R}$ = 1.33 hr and that the corresponding peak time of the test product $T^0_{max,T}$ varied between approximately 0.6 and 2.7 hr with a wider variation expected for individual values of $T_{max,T}$. (The superscript refers to true values calculated from kinetic model parameters.) The sampling points replaced those of Bois et al. (5,6), in the same region, of 0,0.25,0.5,1.0,1.5,2,4,6 and 8 hr.

Figure 1 illustrates the sampling points on the concentration-time profile of the reference formulation (R). Profiles of test product are included at the extremes of the studied conditions with k^o_{aT}/k^o_{aR} = 1/3.5 and 3.5 (T_1 and T_2). The figure demonstrates that the sampling points are generously sufficient for the evaluation of the reference formulation and the determination of bioequivalence especially when the test formulation is absorbed more slowly than the reference product ($k^o_{aT} < k^o_{aR}$). However, when the opposite condition prevails and the absorption of the test formulation is faster than that of the reference product ($k^o_{aT} > k^o_{aR}$) then the suggested design points are barely sufficient for analysis.

Again as in Bois et al. (5,6), normally distributed observations were assumed with means equalling the concentrations predicted by the model in each subject, with both formulations. The model for the variances had two components. In addition to a coefficient of variation (CV) of 10% of the true concentrations, a constant term was assumed which equalled 0.1LQ. LQ, the limit of quantitation, was assumed to be $0.01C^0_{max}$.

[1] University of Toronto, Department of Pharmacology, Toronto, Ontario M5S 1A8, Canada.

[2] Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, Maryland.

[3] To whom correspondence should be addressed. (e-mail: l.endrenyi @utoronto.ca)

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Table 1. Distributions, Means, Inter- and Intraindividual Coefficients of Variation (C.V.), and Truncations of Parameters

Parameter	Distribution	Mean[a]	Inter-/Intra-subject C.V.		Truncation
Volume of distribution, V (L kg^{-1})	Lognormal	1.0	10	10	—
Clearance, CL (L hr^{-1} kg^{-1})	Lognormal	0.347	20	20	—
Absorption rate constant, k_a (hr^{-1})	Lognormal	1.39	20	20	—
Bioavailability, F	Uniform	0.5	—	—	0.4-0.6[b]

[a] Population means for interindividual variations. The intraindividual variations had means at the sample parameter value for a given subject.
[b] Range for interindividual variation. The range for intraindividual variation was -0.1 to 0.1 around the parameter value sampled for a given subject.

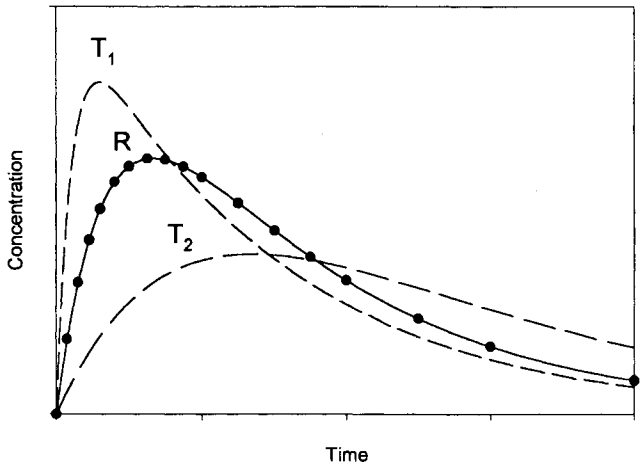

Fig. 1. Concentration-time profiles of the reference (R) and test (T) formulations. Profiles for the test formulation are illustrated for conditions when its absorption is faster (T_1) or slower (T_2) than that of the reference product. The sampling points considered in this study are shown on the reference curve.

1,000 crossover trials were simulated under each condition. The simulation software was written in Pascal object language (Delphi version 2, Borland).

Evaluation of Bioequivalence Trials

Partial AUCs of the test and reference formulations ($AUC_{p,T}$ and $AUC_{p,R}$) were calculated by the trapezoidal procedure until various durations, for each subject in every simulated trial. Properties of the ratios of partial AUCs ($AUC_{p,T}/AUC_{p,R}$) were evaluated at various true ratios of the assumed absorption rate constants ($k^o_{a,T}/k^o_{a,R}$). The properties included the averages and standard deviations of the logarithmic AUC_p-ratios, and the power curves characterizing the assessment of bioequivalence.

The relationship between the logarithmic AUC_p- and k_a-ratios characterizes curves of *kinetic sensitivities* (8). Slopes approximating 1.0 suggest satisfactory, high sensitivity while small values indicate low sensitivities and give rise to weak analyses. Kinetic sensitivity is a component also of the observed variation of a metric. Consequently, a small recorded variation does not necessarily represent a favourable condition.

Power curves contrast the probability of declaring bioequivalence with increasing deviations between characteristics of the two formulations. In the ideal case, without parameter and observational errors, the acceptance of bioequivalence is stated in all cases as long as the contrast between the characteristics does not reach preset regulatory limits. In contrast, the absence of bioequivalence is declared consistently when the difference between the characteristics exceeds the regulatory limits. In the presence of parameter and observational variations, the relationship between probability and the difference of characteristics is shallower and less clear-cut. A condition or metric should be favoured in comparison with another if the observed power curve shows a smaller deviation from the ideal, square shape, i.e., if it exhibits smaller statistical responsiveness to deviations and variations of observations and extraneous parameters (8). Thus, the effectiveness of a metric for the determination of bioequivalence can be characterized mainly by the behaviour of its power curves.

The power curves illustrate that the error of declaring bioequivalence for actually bioinequivalent products (the "consumer risk") is limited; the risk is 5% when the bioequivalence limits are reached. Conversely, the error of stating bioinequivalence for truly bioequivalent formulations (the "producer risk") can also be read off the curves as the percentage of rejected (i.e., not accepted) trials at the condition of bioequivalence.

The power curves evaluated under the various design conditions would coincide in the absence of parameter and observation variabilities. Figure 2 presents, for the sake of illustration, power curves for C_{max} with increasing variations of the measurement errors. In the absence of both observational and parameter variations, bioequivalence is always rejected outside the regula-

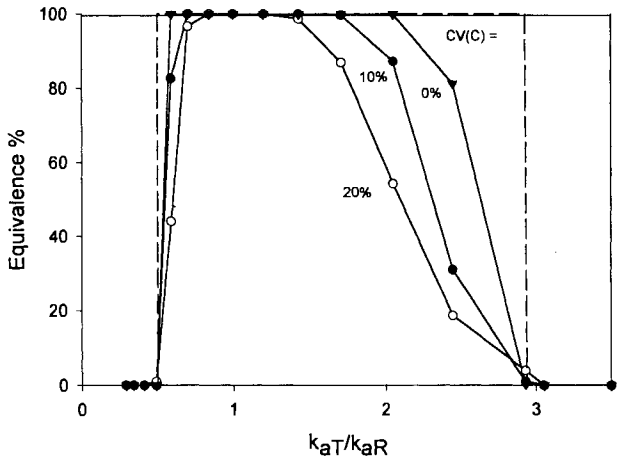

Fig. 2. Effect of increasing variability on power curves characterising the assessment of bioequivalence. For the sake of illustration, the coefficient of variation of the observational errors was set at various levels, and the % acceptance of bioequivalence by C_{max}-ratios was recorded. In the absence of both parameter and observational variabilities, bioequivalence is accepted within the regulatory limits and rejected outside them; this condition is illustrated by dashed lines.

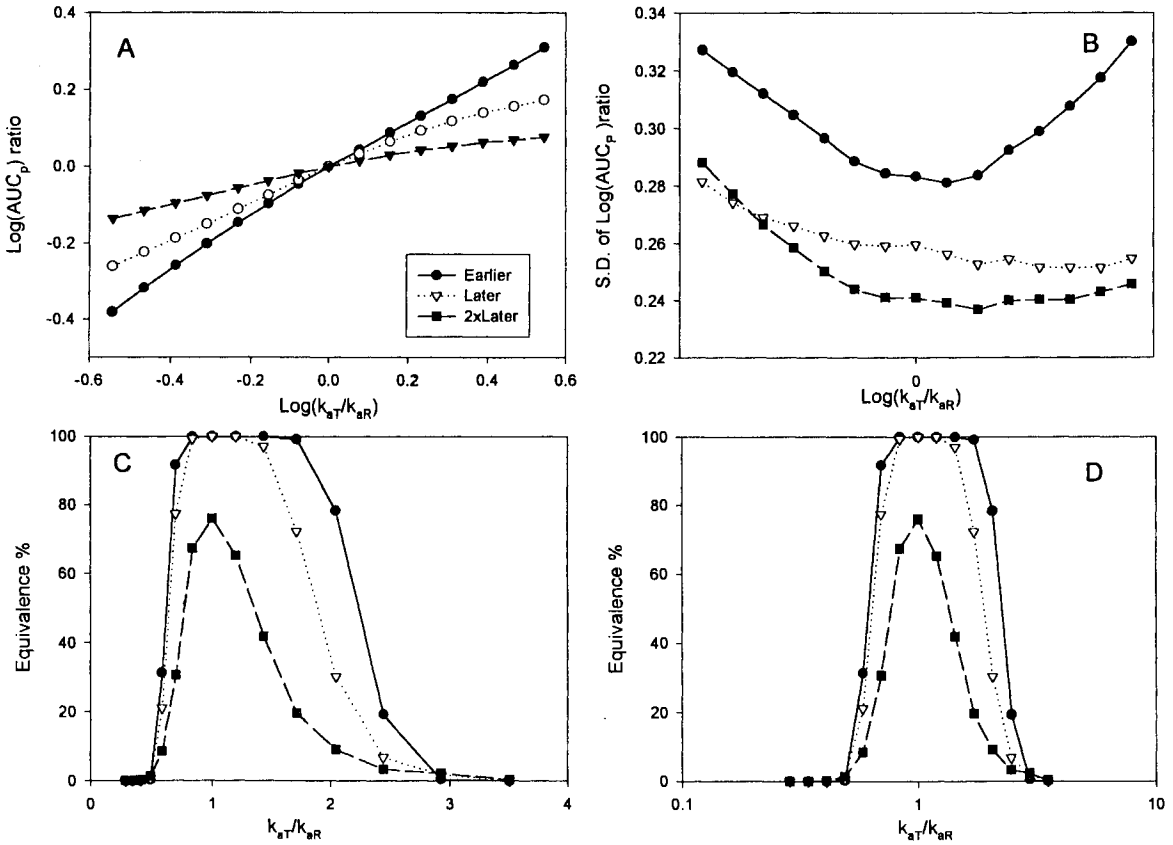

Fig. 3. Properties of partial AUCs evaluated until the earlier concentration peak (filled circles), or the later peak (open triangles), or till twice the time of the later peak (filled squares). (A) Calculated kinetic sensitivity curves contrasting the logarithmic ratio of partial AUCs of two formulations with the corresponding ratio of absorption rate constants. (B) Standard deviations of the logarithmic ratios of partial AUCs simulated at various logarithmic k_{aT}/k_{aR} ratios. (C) and (D) Power curves for the acceptance of bioequivalence, obtained by simulation at various k_{aT}/k_{aR} ratios. The k_{aT}/k_{aR} axis is (C) linear, or (D) logarithmic.

tory limits and always accepted within them. The decision is less and less clear with increasing errors. The curves somewhat parallel those presented by Endrenyi et al. (9).

Power was evaluated by observing, at a given ratio $k^o_{a,T}/k^o_{a,R}$ the proportion of simulated trials in which the acceptance of bioequivalence was declared. The estimation of power was repeated at different ratios of $k^o_{a,T}/k^o_{a,R}$. For the determination of the power curves in the simulations, it was necessary to set bioequivalence limits. The limits were assumed to correspond to a range of 0.80–1.25 for the ratio of true, population average maximum concentrations ($C^0_{max,T}/C^0_{max,R}$). The limits in terms of the $k^o_{a,T}/k^o_{a,R}$ ratios were then calculated from the kinetic sensitivities. Under the chosen kinetic conditions, the limits of the $k^o_{a,T}/k^o_{a,R}$ ratio were 0.501 and 2.767.

The partial AUCs were determined until various time points. They included either the earlier or later peak recorded in each subject, the time of the reference peak observed in each subject, various multiples of these times, and a selection of fixed time points. The characteristics of ratios of partial AUCs were compared for these design conditions.

RESULTS

Presentation of the Results

Each set of Figures 3–6 contains 4 diagrams. The first of these, Figure A, shows observed kinetic sensitivities which contrast ratios of the metric with ratios of the absorption rate constants of the two formulations. It is useful to display a double-logarithmic representation of the contrast. For one, the logarithmic ratio of the metrics is applied for the assessment of bioequivalence. Figure B illustrates, in each set, the standard deviation of the logarithmic ratio of the metric. Figures C and D show power curves; Figures C present them against a linear scale of k_a-ratios, and Figures D against a logarithmic one.

Figure 3 presents results obtained until (a) the earlier, and (b) the later of the two peaks in each individual, and (c) until (just before) twice the T_{max} of the later peak.

Figure 4 shows results recorded until (a) the reference peak observed in each subject (at $T_{max,R}$), and (b,c,d) until just before 1.5, 2.0 and 2.5 times $T_{max,R}$, respectively.

Figure 5 illustrates results obtained until fixed time periods. Accordingly, the simulated experiments were performed until (a) just before the true, population average reference peak (at $T^o_{max,R}$), and (b,c,d) until just before 1.5, 2.0 and 2.5 times $T^o_{max,R}$, respectively.

Figure 6 compares results calculated at the shortest duration by the 3 design strategies: until the earlier peak observed in each subject, until $T_{max,R}$ and just before $T^o_{max,R}$.

Summary of the Results

The sense of conclusions is identical in the first 3 sets of diagrams (Figures 3–5).

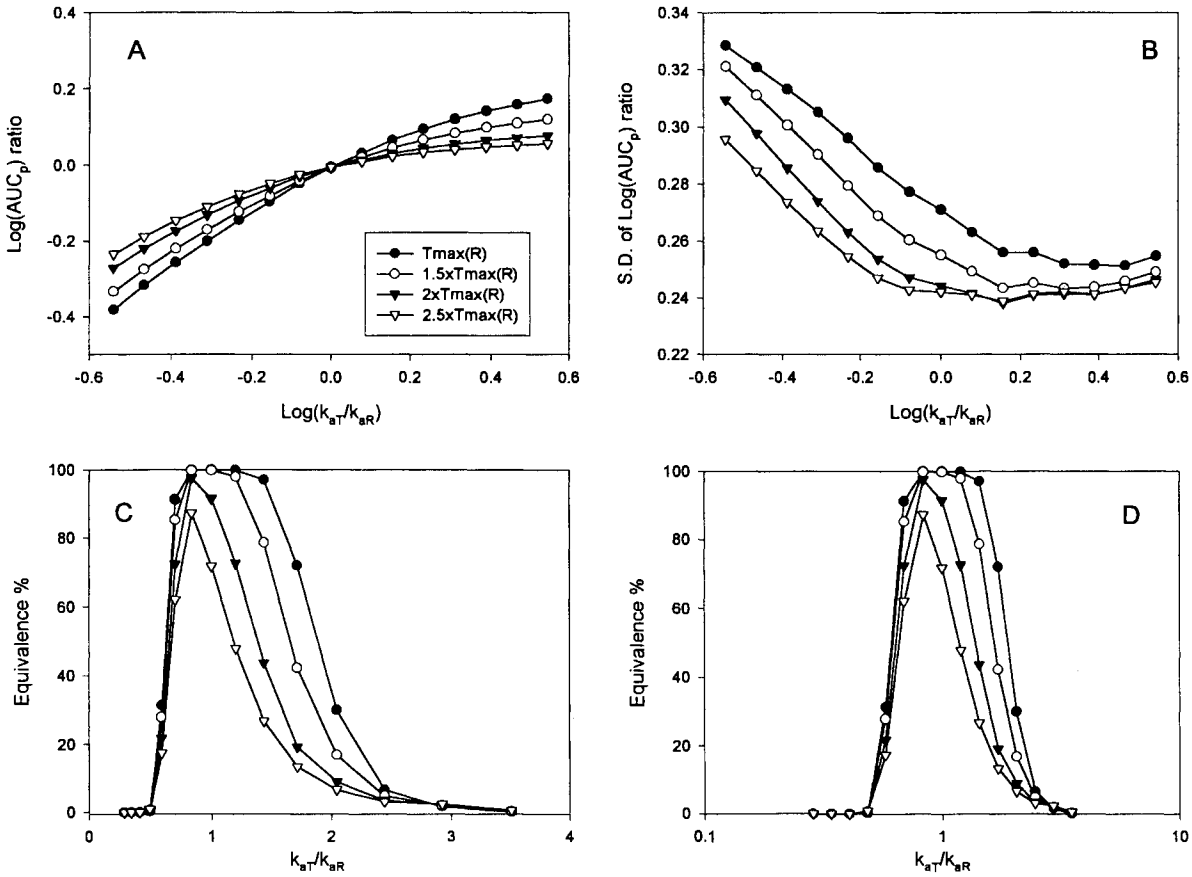

Fig. 4. Properties of partial AUCs evaluated until the concentration peak of the reference formulation (filled circles), or till 1.5, 2 or 2.5 times the time of the peak for the reference product (open circles, filled triangles, and open triangles, respectively). The structure of the figure is similar to that of Fig. 3.

The kinetic sensitivity of the AUC_p-ratio deteriorates as the duration of the study is extended (Figures 3A–5A).

The variation of the AUC_p-ratio improves as the duration of the investigation is lengthened (Figures 3B–5B). The improvement slows down beyond $2T_{max,R}$.

The effectiveness (power) of evaluating bioequivalence with AUC_p-ratios deteriorates as the duration of analyses is extended (Figures 3C–5C, 3D–5D).

In the comparison of the three basic design strategies, evaluation of the partial AUC until the earlier peak in each individual yields the highest kinetic sensitivity but also the highest variation (Figures 6A, 6B). The net effect of the two components is that this strategy yields, overall, somewhat higher power than the other two approaches (Figures 6C, 6D). There are only slight differences between the results of determining partial AUCs either until $T_{max,R}$ or till $T^o_{max,R}$.

DISCUSSION

The principal conclusion of this study is that the effectiveness (power) of evaluating bioequivalence by partial AUCs is reduced when the duration applied for the determination is extended. The reduction is demonstrated by the contrasts of power curves. The conclusion is reached from the following considerations. The power of evaluating bioequivalence was favourable when AUC_p was determined until the earlier rather than the later of the two peaks in each subject (Figs. 3C and 3D). The power was higher when AUC_p was evaluated until the peak of the reference formulation than until a later time (Figs. 4C and 4D). Also, when measurements of AUC_p until a fixed time point were considered, evaluation of the partial AUC to the true, population average T^o_{max} of the reference formulation yielded higher power than determinations until later times (Figs. 5C and 5D). Finally, in the comparison of the three favourable strategies chosen above (Figs. 3–5), determination of partial AUC until the earlier peak resulted in the highest power (Figs. 6C and 6D).

The conclusion of favouring the measurement of partial AUCs until the earlier peak in bioequivalence studies parallels the suggestion of Macheras et al. (10). These authors based their recommendation on calculations analogous to the evaluation of kinetic sensitivities.

The diminishing effectiveness of partial AUCs with extended duration of their determination arises from the contributions of two sources. On the one hand, the variation of logarithmic AUC_p-ratios decreases (down to a limit) as the duration of determining AUC_p rises. This is in agreement with earlier experience (3,4). On the other hand, extension of the duration results also in the deterioration of the kinetic sensitivity of the AUC_p-ratio. The net effect of the two contributions appears to be detrimental to the effectiveness of assessing bioequivalence.

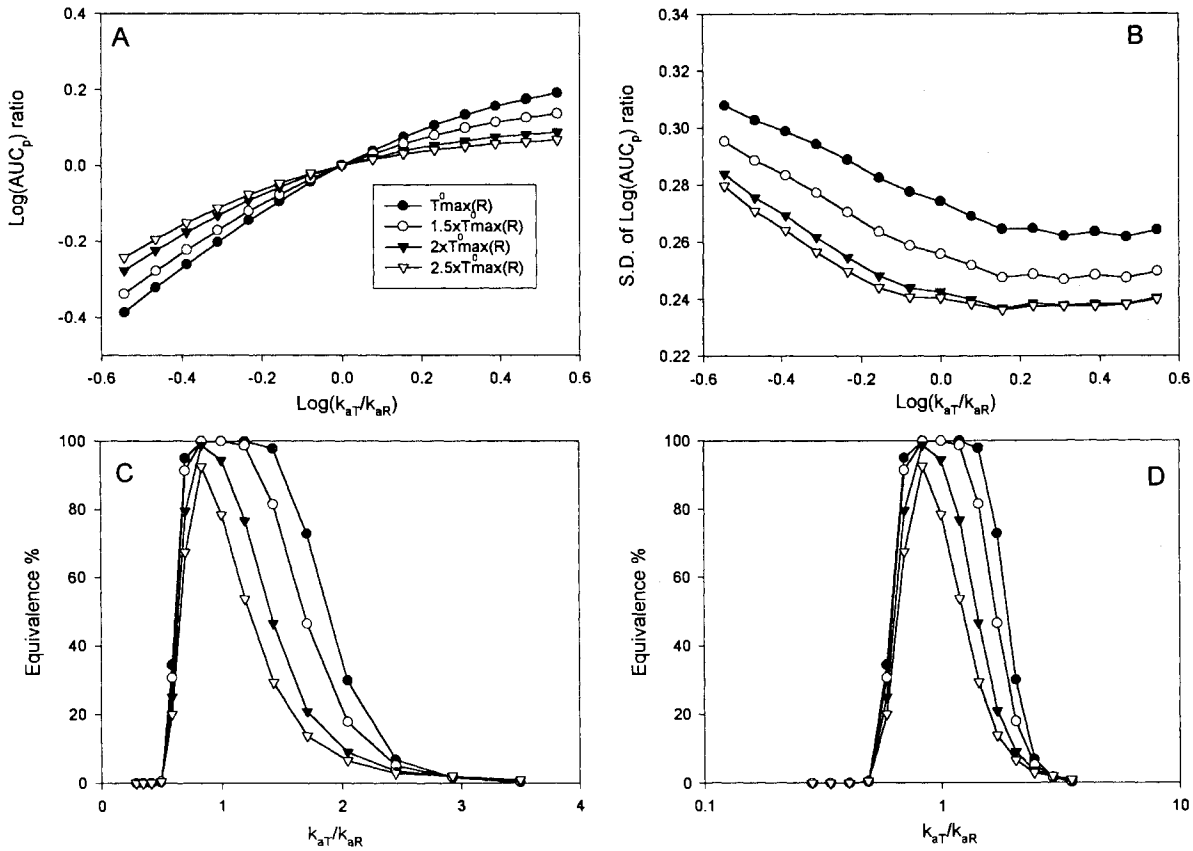

Fig. 5. Properties of partial AUCs evaluated until fixed time points. The fixed final points equal either the time of the true, population average reference peak, or 1.5, 2, 2.5 times this value (filled circles, open circles, filled triangles, and open triangles, respectively). The structure of the figure is similar to that of Fig. 3.

Partial AUCs of two formulations are compared in order to determine bioequivalence in the early phase of the concentration-time profiles. Such assessments can be relevant when the similarity of absorption rates is important as in the case of some cardiovascular drugs.

When the determination of bioequivalence in the early phase of concentration-time profiles (i.e., early exposure) is important then more than the usual number of observations should be allocated to this segment. Simulation studies (to be published) indicate that bioequivalence in the early phase can be effectively evaluated from an average of only three measurements on the ascending arm of the concentration-time profile. Nevertheless, in some investigations it may not be possible to obtain a sufficient number of observations until the earlier of two peaks. On these occasions, the partial AUCs could be determined either until the later peak or till T_{max} of the reference formulation.

The properties of partial AUCs evaluated under additional kinetic conditions (flip-flop, zero-order, two-compartmental kinetics) will be presented in a separate publication.

The kinetic sensitivities are much higher when the test product is absorbed more slowly than the reference formulation ($k_{aT} < k_{aR}$) than under the reverse condition, i.e., when the test formulation is absorbed more rapidly than the reference product ($k_{aT} > k_{aR}$) (Figures 3A–6A). This is the main reason that, especially under unfavourable design conditions, the bioequivalence limits are substantially asymmetric in the logarithmic scale. This can give rise to asymmetric power curves (Figures 4D, 5D).

Simulations not shown here demonstrated that the effectiveness of evaluating bioequivalence by the intercept method (11) and its Macheras variant (12) also deteriorated when the duration of their analysis was extended. This behaviour is actually expected for the intercept metric.

The results indicate that, among the investigated design strategies, determination of partial AUCs until the earlier peak is the most effective for the assessment of bioequivalence.

CONCLUSIONS

The effectiveness (power) of determining bioequivalence in the early phase of concentration-time profiles by partial AUCs generally diminishes when the duration for determining the metric is extended. Among the investigated design strategies, calculation of the partial AUC until the earlier of the two contrasted peaks is the most effective.

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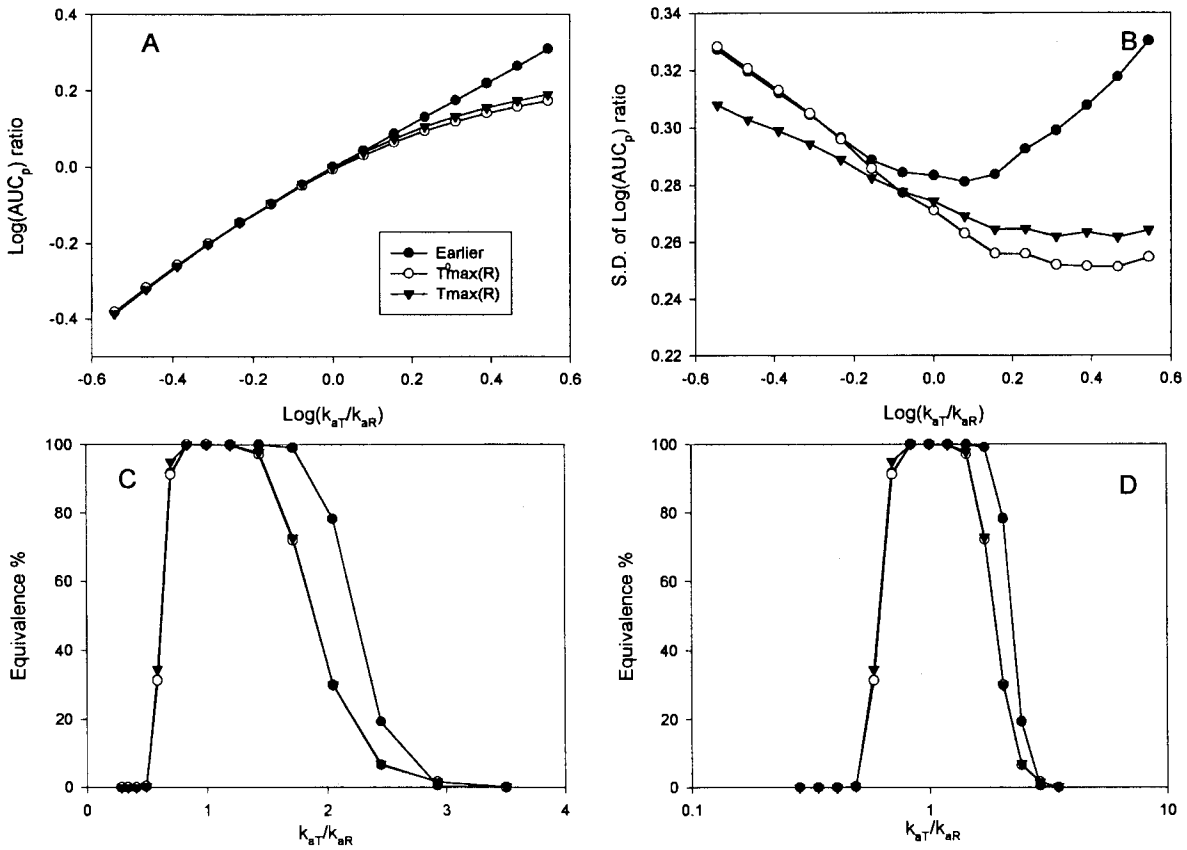

Fig. 6. Comparison of properties for partial AUCs among three design strategies. The partial AUCs are evaluated either until the earlier peak (filled circles), or till the maximum concentration for the reference formulation (filled triangles), or until the fixed time of the population average peak concentration of the reference formulation (open circles). The structure of the figure is similar to that of Fig. 3.

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