Use of Truncated Areas to Measure Extent of Drug Absorption in Bioequivalence Studies: Effects of Drug Absorption Rate and Elimination Rate Variability on this Metric

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Purpose. To compare the applicability and accuracy of truncated area (AUCt; where t represents truncated time) versus area to the last quantifiable time point [AUC(0-T)] for assessing bioequivalence. Drugs with either very low or very high intra-subject variability in clearance (CL) were selected for study. Clearance variability was defined by the number of subjects with a quantifiable plasma value (Cp) at each collection time from 24 hrs to last collection time (T).

Methods. Data for amiodarone and danazol, drugs with different distributions of subject CL were examined. For amiodarone, the number of subject samples observed (test + reference) at the time of the last quantifiable concentrations was 60 at 240 hrs(T), 16 at 144 hrs and 4 at 96 hrs; while danazol had 4 at 96 hr(T), 3 at 72 hrs, 16 at 60 hrs, 7 at 48 hrs, 14 at 36 hrs, 11 at 24 hrs, 13 and 2 at 16 and 12 hrs, respectively. Simulations (Scenarios A and B) were performed to obtain populations (N = 24) with CL patterns similar to those of amiodarone and danazol. For scenario A (CL pattern similar to amiodarone), log-normally distributed CL values (28.8 L/HR) with intra-subject coefficient of variation (CV) of 25%, 40% and 60% gave the desired CL pattern. Scenario B (CL pattern similar to danazol) required that a subpopulation with an increase in CL of 40% from baseline (i.e., 40.32 L/HR) in 5%, 10% and 20% of the population represent the desired distribution. Power was evaluated by the percentage of times the simulated trials were declared bioequivalent (i.e., the number of times the test vs. reference 90% CI was within 80–125%), while accuracy was determined when the true difference in fraction absorbed (i.e., 1.25) was within the CI. Each simulation was repeated 300 times.

Results. The simulation results for Scenario A indicated that the statistical results using truncated area (AUCt) had power and accuracy equivalent to that obtained using the AUC(0-T) metric. However, results for Scenario B indicated that AUCt had less power and accuracy than that obtained using AUC(0-T). The confidence interval (CI) for amiodarone was the same whether AUC (0-T) or AUCt was used as the metric for extent, while for danazol, the AUC(0-T) and AUCt differed in the lower limit by 7%.

Conclusions. The truncated area, AUCt, has the greatest power and accuracy when the population clearance is such that most subjects have measurable plasma concentrations at last collection time(T), resulting in a proportional loss of data from each subject.

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INTRODUCTION

A survey of current pharmacokinetic literature reveals that there has been growing interest in evaluating different metrics to measure the rate and extent of drug absorption (1–4). Areas computed to time of maximum concentration, Tmax (5–7), have been investigated as a metric for rate of absorption. Truncated areas (areas under the curves computed to various times between zero and the last quantifiable plasma concentration) have been proposed as a replacement of AUC∞ or AUC(0-T), especially for drugs with long half-lives (8). If applied to drug with inappropriate kinetics (i.e., population has subjects with an increased clearance) then truncated areas can increase the risk of declaring drugs bioequivalent when they are not, a Type II statistical error.

The influence of rate of absorption (Ka) on the estimation of truncated areas has been discussed elsewhere as has the influence of the rate of elimination (ke) under certain circumstances (9). Although it has been previously observed that Ka does not influence the magnitude of the area under the curve, it does greatly influence curve shape. This may be pivotal when partial areas are used to evaluate extent of absorption, especially for early portions of the curve. The fact that ke (e.g., clearance) within subjects affects the amount of area eliminated from each subject's plasma curve following truncation has not been addressed.

A recent simulated pharmacokinetic (PK) study reported that as the ratio of available fraction of test drug to available fraction of reference drug [Fa(test)/Fa(reference)] approached 1.0, the use of AUCt to measure extent of drug absorption increased the probability of meeting the 90% confidence interval criterion for bioequivalence compared to use of AUC(0-T) to measure extent (8). It was also shown that if a drug has a clearance with a CV of at least 15%, or a high limit of quantitation, or exhibits 2-exponential disposition calculation of partial area from time zero to the time for one half-life provides a better estimate of extent of absorption than does AUC(0-T). In the same study, three-fold differences in Ka between simulated formulations was shown to increase the bias of the AUCt estimate by approximately 5% compared to the estimate obtained for AUC∞ via extrapolation. Investigators have shown that Ka influences the areas computed to time of Tmax and that as the ratio of Ka(test):Ka(reference) increases, the probability of declaring bioequivalence decreases(1).

The use of truncated areas for the estimation of extent of absorption is potentially very useful. However, the use of this metric may increase the risk of accepting drugs as being equivalent when in fact they are not, or Type II statistical error. The influence of Ka on truncated AUC is of special concern whenever the ratio of available fraction of test drug to available fraction of reference drug [Fa(test)/Fa(reference)] is ≥ 1.25. In these situations, regulatory agencies attempt to manage the risk of type II statistical error/consumer risk by establishing a nominal acceptance level of 5%. The current investigation attempted to determine how the power and accuracy of truncated areas were affected by Ka and CL.

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METHODS

Bioequivalence Studies

Evaluation of single-dose, two-treatment, two-period, randomized crossover bioequivalence studies performed *in vivo* on generic amiodarone tablets and danazol capsules revealed striking differences in CL pattern. For amiodarone, the majority of subjects had their last quantifiable drug plasma concentrations at 240 hrs, at 144 hrs or at 96 hrs. On the other hand, for danazol, this pattern was more variable. The subjects' last quantifiable drug plasma concentrations occurred at seven different collection times (96 hrs, 72 hrs, 60 hrs, 48 hrs, 36 hrs, 24 hrs and 12 hrs).

Amiodarone

The subjects in the study were healthy males (N = 41) ages 18–35 and within 10% of ideal weight. At the beginning of each study period, subjects received a 400 mg oral dose of amiodarone tablets. Subject blood samples for plasma drug assay were collected at 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 72, 96, 144, 240, 336, 504, and 672 hrs after dosing. The washout period between test versus reference product doses was 9 weeks. Although samples were taken until 672 hrs, levels were quantifiable to 240 hrs.

Danazol

The subjects in this study were healthy, non-pregnant females (N = 35), ages 29–48. At the beginning of each study period, subjects received a 200 mg oral dose of danazol capsules. Samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hrs after dosing. The washout period between doses was 2 weeks.

Analytical Methods

Amiodarone and danazol plasma concentrations were analyzed by high performance liquid chromatography methods. For amiodarone, the assay was linear over the range of 5.01 to 1001 ng/mL with a CV ranging from 1.5 to 10%. The accuracy of the assay was ranged from 96 to 106%. Standard curves of Danazol were linear over the concentration range of 2 to 100 ng/mL. The precision of assay was ranged from 2.2 to 13.4%, while the accuracy was 98.1 to 98.2%.

Monte Carlo Simulations and Pharmacostatistical Models

Monte Carlo simulations patterned after the amiodarone and danazol *in vivo* studies were performed using a one-compartment model with first-order absorption and elimination. The conditions of the simulations were similar to those presented in a recent publication (8). The pattern of the number of subject last quantifiable concentrations versus the scheduled sampling times, as observed for the drugs in the in vivo studies, was simulated by adding specified levels of stochastic variation to the Ka, CL, and volume of distribution (Vd) parameters for each subject at each trial period through use of a random-number generator. The level of variability was assumed to remain constant throughout the study periods and a log-normal distribution was assumed. All simulated studies were done using a ratio of Fa(test):Fa(reference) equal to 1.25. A uniform distribution was assumed for Fa with averages of 0.99 (test) and 0.79 (reference) and a range of ± 0.01.

Mean pharmacokinetic parameters for Simulation Study A, which patterned the CL distribution of amiodarone, were Vd = 1000 L, CL = 28.8 L/hr, T1/2 = 24 hrs, and Ka = 1.154 hr-1. Intra-subject %CV of 25%, 40%, and 60% was added to the subjects' CL; 25% CV was added to Ka; and 10% CV to Vd. Study period and sequence were assigned in a randomized, balanced manner to mimic the usual two-period crossover bioequivalence study design.

For Simulation B, the pharmacokinetic parameters were the same as those in Simulation A with the exception of the subpopulation which has an increase in CL (10). Simulation Set B patterned the CL distribution observed for danazol in the *in vivo* studies. In 5%, 10%, and 20% of the study subjects a 40% increase in the baseline CL of 28.8 L/hr (that is, to 40.32 L/hr) was made with ratio of Ka (test): Ka(reference) set equal to 4 and ratio of Ka(reference):ke is approximately 7.

Conditions for Simulation Set C were the same as those for Simulation Set B, above, except that Ka(test):Ka(reference) ratios were set equal to 1, 2 and 3, (rather than 4) and Ka(reference):ke values of 40, 30, and 20, were used, in addition to the value of 10 used in Simulation B. All simulations were done with a 40% increase in baseline CL added to 5% of the population. The Ka(test):Ka(reference) values are given in Table 2.

Under each condition, 300 crossover trials were simulated using SAS on a Compac 133MH DeskPro PC.

Generation of Concentration Values and Evaluation of Data

Simulations A, B, and C were performed using a one-compartment model with plasma sampling times set at 0, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, 48, 60, 72, 84 and 96 hrs following drug administration. For Simulation Study A, random assay error was added to the concentrations generated, assuming zero means and variances (v), as defined by the following model(3): $V = (0.01 \times Cmax)^2 + (0.1 \times \text{true concentration})^2$. For Simulation Studies B and C, random assay error was added to the generated concentrations assuming the following formula for standard deviation (11): $\sigma_C = 0.2C + 0.1$. The lower limit of quantitation (LLOQ) for the simulations was set equal to one-tenth of the Cmax value, with values below this set equal to zero. The area under the curve computed from time zero to the last quantifiable plasma concentration, AUC(0-T), was calculated using the trapezoidal rule.

The area under the curves (contrasted with time) values were truncated up to 24 hrs (from a total collection period of 96 hrs) to simulate an abbreviated sampling interval for each trial. The AUC∞ was calculated by dividing the value of the last measurable plasma drug concentration by the elimination rate constant and adding the result to the AUC(0-T).

The 90% confidence interval for each simulated trial was computed and recorded as equivalent when it was in the range 0.8–1.25. The power of the test was defined as the probability of declaring equivalence. Accuracy of the simulations was assessed by determining if the true difference in the Fa(test)/Fa(reference), set equal to 1.25 in the simulations, was within the calculated confidence interval.

RESULTS

In Vivo Bioequivalence Study Data

For the amiodarone tablet *in vivo* study, the Test/Reference(T/R) ratios for AUC(0-T) and AUC_{96} were essentially identical, as were the corresponding 90% CI's (Table 1).

For the danazol capsule study, the T/R ratios for AUC (0-T) and AUC_{36} were essentially identical; however, a decrease in the ANOVA RMSE from 27.3% to 25.2% for the AUC_{36} compared to the AUC (0-T) raised the lower limit of the 90% CI for the AUC_{36} by 5%, thus narrowing the CI range (Table 1).

A further difference between amiodarone and danazol is shown by the distribution of the times at which the last quantifiable test and reference concentrations for these drugs in the subjects were observed, as presented in Fig. 1 (top graph). In Simulation Study A, Fig. 1–bottom panel, the number of times recorded as the last quantifiable drug concentration (using 60% intra-subject CV in CL) occurred at 4 time points, which compares closely with the number of last quantifiable time points (3) observed in the clinical amiodarone study. In Simulation Study B, the number of times recorded as the last quantifiable drug concentration (using 40% increase in baseline CL for 20% of the population) occurred at 7 time points, which compares closely with the number of last quantifiable time points (8) observed in the clinical danazol study.

Simulation Studies

For Simulation Study A (CL pattern similar to amiodarone), Table 2 presents the power and accuracy of the AUC_{24} and AUC(0-T) CI's when Fa(T):Fa(R) is set at 1.25, and intra-subject CV are set at 25%, 40%, and 60%. These data indicate that the power is at the established level of 5% irrespective of error level and that the accuracy is approximately 90%.

For simulation Set B (CL patterned after danazol), Table 3 shows that the inclusion of subpopulations in the data set affects the power and accuracy of AUCt when used to measure the extent of drug absorption. The data show that if the CL level in 5, 10 and 20% of the study subjects is increased 40% above baseline, the AUC(0-T) CI has less power but more accuracy in measuring extent of drug absorption than does the corresponding AUC_{24} CI.

In Simulation C, the Ka(test):Ka(reference) ratio affects the power and accuracy of the AUC(0-T) and AUC_{24} CI These CI results are presented for AUC(0-T) and AUC_{24} in the top and lower panels, respectively, of Fig. 2. The graphs show that as the ratio [Ka(reference):ke] decreases, and the ratio [Ka(test):Ka(reference)] increases, the probability of meeting the confidence interval criterion for equivalence is greater for AUC_{24} compared to AUC(0-T). However, in the presence of the included subpopulations, the accuracy of the CI estimate decreases to well below 90% as the Ka(reference):ke ratio falls below 30, with lower accuracy observed for the AUC_{24} CI than for the AUC(0-T) CI.

DISCUSSION

Another report has discussed many potential problems in using truncated area as a measure of extent of drug absorption in bioequivalence studies (9). Some of these problems may lead to a determination of equivalence for extent of drug absorption even when the test product PK parameters are quite different from those of the reference. Among the concerns listed were:

1. The ke is important when test product bioavailability is more than 120% that of the reference product and the test product absorption is less than 80% that of the reference. As ke is increased, the absorption phase contributes progressively more to the area measured, which can lead to a false determination of bioequivalence.
2. Absorption and elimination rates may influence T/R ratios for AUCt more than for AUC(0-T), resulting in an overestimation of relative bioavailability for those drugs with low absorption rates (that is, low Ka/ke ratios) and underestimation of this parameter for those drugs with high absorption rates (that is, high Ka/ke ratios).

Table 1. Arithmetic Mean Values, Root Mean Square Error from ANOVA, and 90% CI for AUC∞, AUC(0-t), and AUCt

Drug	Area under the curve	Mean AUC (ng/ml*hr) Test (T)	Ref (R)	Ratio T/R	%RMSE	CI low Upp
Danazol	AUC∞	629	645	0.98	27.3	81–107
	AUC(0-t)	438	481	0.91	28.2	77–98[a]
	AUC_{96}	438	481	0.91	27.6	78–98[a]
	AUC_{72}	431	474	0.91	27.5	79–99[a]
	AUC_{60}	426	467	0.91	27.3	80–99
	AUC_{48}	413	452	0.91	26.5	80–98
	AUC_{36}	394	395	1.00	25.2	81–98
	AUC_{24}	361	395	0.91	23.1	81–98
Amiodarone	AUC∞	8652	8702	0.99	20.6	94–109
	AUC(0-t)	8251	8321	0.99	20.8	94–109
	AUC_{504}	8251	8321	0.99	20.8	94–109
	AUC_{336}	8251	8321	0.99	20.8	94–109
	AUC_{240}	8251	8321	0.99	20.8	94–109
	AUC_{144}	7523	7592	0.99	19.6	94–108
	AUC_{96}	6920	6982	0.99	19.2	94–108

[a] CI-Outside the acceptable limit of 80–125.

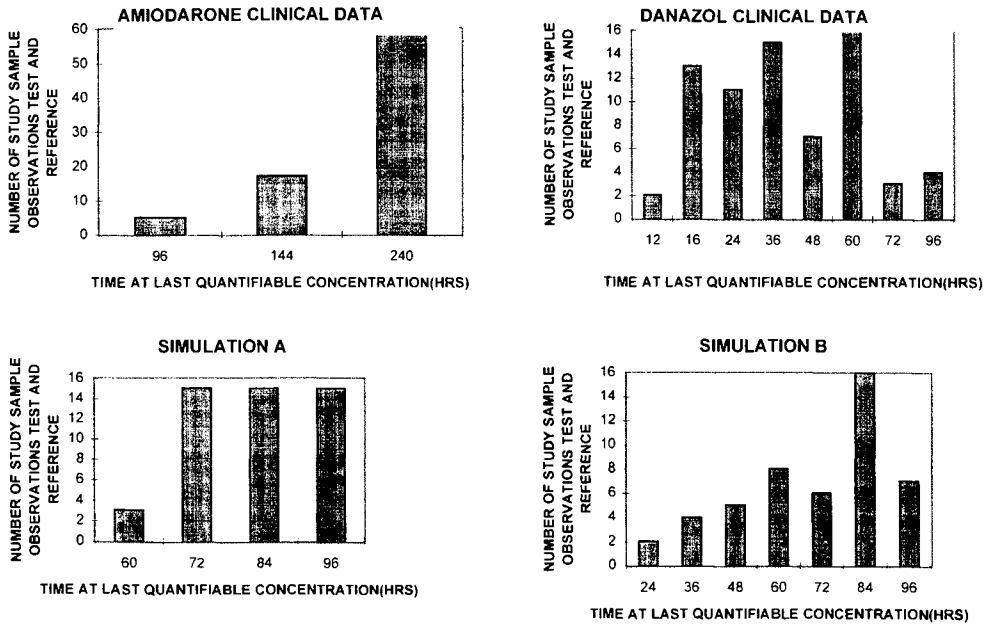

Fig. 1. Comparison of clinical data from amiodarone and danazol (upper graphs) with simulated data from Simulation A and Simulation B respectively, showing the comparability of the experimental and simulated distribution patterns of the times for the last quantifiable concentration in the study subjects.

The current study adds a concern over variability of CL to those others listed above.

Two distinct levels of CL variability were observed, as exemplified by amiodarone and danazol. For amiodarone, all last quantifiable subject plasma drug concentrations were observed at only three different time points: at 96, 144, and 240 hrs, indicating relatively low variability. For danazol, the last measurable concentrations occurred at seven different sampling times from 12 to 96 hrs, indicating relatively high variability. Based upon data from the simulated studies, the danazol pattern was best represented by including subpopulations having clearances substantially increased over the baseline levels established for amiodarone.

When a shortened post-dosing plasma sampling interval resulted in a proportional loss of area per subject, as in the amiodarone studies, the probability of meeting the bioequivalence and accuracy criteria was independent of the length of sampling time used to calculate the AUC 90% CI (see Table 1, and Simulation Set A in Table 2). This is supported by the results of another simulation study recently published (8).

However, when the pattern of last quantifiable concentrations becomes similar to that of danazol (as in Simulation Studies B and C), a non-proportional amount of area is lost from each subject with the use of AUCt and one may obtain differing results depending on the length of sampling time used to calculate the CI (Table 1, Table 3, and Fig. 2). The Simulation Set C data in Table 3 and Fig. 2 clearly show that even though the probability of declaring bioequivalence may be higher when AUC_{24} is used in place of AUC(0-T) to compute the 90% CI, the estimate is less accurate, especially as the Ka/ke ratio decreases and the Ka(test)/Ka(reference) ratio increases.

Truncated areas under the curve are potentially very useful in the determination of bioequivalence, but more than just increased power (probability of declaring bioequivalence) must be demonstrated; the results must also be accurate. Otherwise, one would risk increasing the Type II statistical error and consumer risk whenever truncated areas were used. A serious concern arises for drugs such as danazol, which exhibit variable CL patterns. In that case, AUCt's computed to different sampling times may yield different 90% CI's and provide unreliable

Table 2. Impact of Intrasubject Variability in CL on the Ability of AUC(0-T) and Truncated Areas up to 24 hrs (AUC_{24}) to Determine Extent of Absorption as Measured by the Probability of Concluding Equivalence (i.e., Power) and Accuracy of Each Metric When the F(T)/F(R) Ratio is 1.25

Subpopulation with increased clearance	AUC_{24} Power	AUC_{24} accuracy	AUC(0-T) Power	AUC(0-T) accuracy
25%	6%	90%	6%	92%
40%	7%	90%	6%	91%
60%	5%	90%	6%	90%

Note: Simulation Set A was based on baseline values of Ka(t)/Ka(r) = 3 and Ka(r)/ke = 40.

Table 3. Effect of Subpopulation on the Power and Accuracy of AUC_{24} and AUC(0-T) to Determine Extent of Absorption for Simulated Bioequivalence Studies in Simulation Set B

Percent of subpopulation with increased clearance	AUC_{24} Power	AUC_{24} accuracy	AUC(0-T)[a] Power	AUC(0-T)[a] accuracy
5%	56%	44%	44%	55%
10%	53%	46%	43%	56%
20%	53%	46%	40%	60%

Note. The Simulation was based on baseline values of Ka(t)/Ka(r) = 4 and Ka(r)/ke = 10. The increase in CL in the subpopulation was 40%.

[a] For Simulation Set B, AUC(0-T) = AUC(0-96).

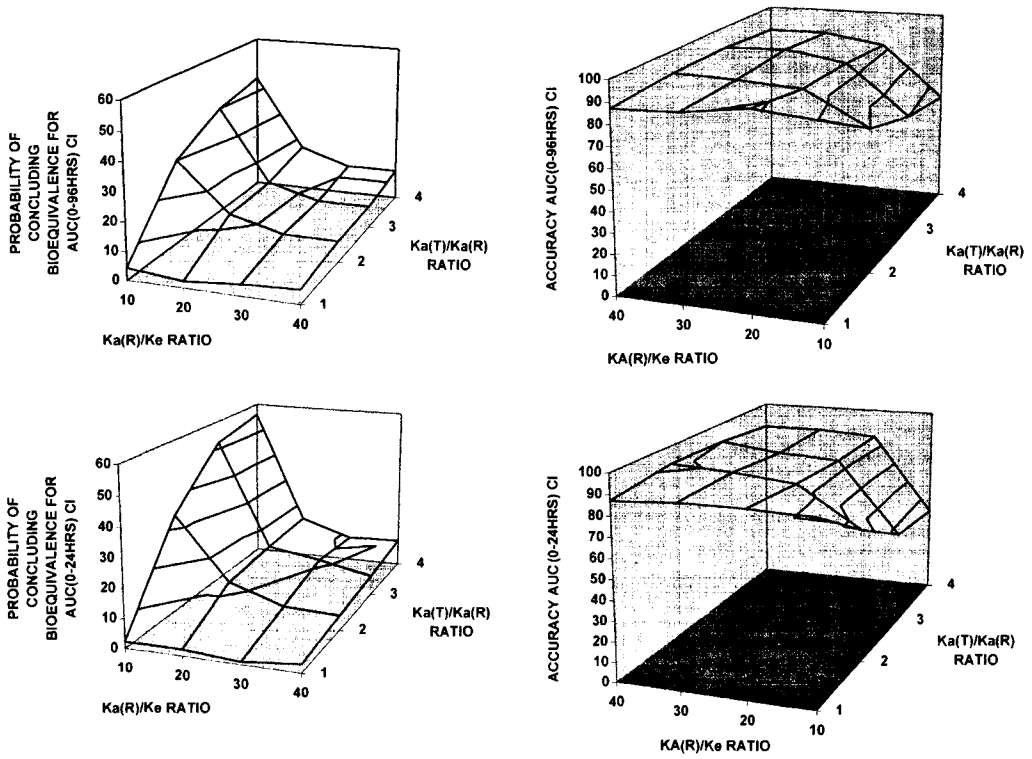

Fig. 2. Power and accuracy of the AUC(0-96) (top panel) and TAUC(0-24) (bottom panel) as metrics to determine extent of absorption as a function of test/reference Ka values and the ratio of Ka(reference)/Ke.

answers to the question of bioequivalence. Our data do demonstrate, however, that AUCt is a useful and appropriate metric for those drugs with relatively low variability in CL, such as amiodarone.

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