DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 16(1), 157-163 (1990)

AREA UNDER THE CURVE ESTIMATION IN BIOEQUIVALENCE STUDIES

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The determination of bioequivalence is a matter of considerable practical importance to the pharmaceutical industry, consumers and the Federal Food and Drug Administration. It is generally accepted that the parameters of primary importance in most decisions of bioequivalence are area under the curve to the time of the last measurable concentration (AUC), area to infinite time (AUCINF), peak concentration (Cmax), and time of peak concentration (Tmax). The last of these, Tmax, is influenced entirely by the rate of drug absorption, while the first, AUCINF, is entirely a measure of extent of drug absorption. Cmax is influenced by both the rate and extent of drug absorption. AUC is an intermediate value in the calculation of AUCINF. To establish that a generic drug formulation is equivalent to its branded counterpart, one must conduct a bioequivalence study to demonstrate that the two products have comparable rates and extents of drug absorption.

In many instances, the Food and Drug Administration provides useful, standard guidances for the conduct of the bioequivalence study. In other instances, the design of the study must be determined from the available scientific literature. The typical bioequivalence study consists of a two-way crossover comparison of a generic product (test) to an innovator's product (reference) in a group of healthy, normal subjects. Each product is administered, on separate occasions, to each subject. The order of administration is randomized and balanced and the time between separate administrations is sufficiently long as to eliminate carry-over effects. Serial blood samples obtained after each administration are used to characterize the individual blood level curves. Individual Cmax and Tmax values are generally determined directly from the blood level profiles. Area to the time of the last measurable concentration (AUC) is calculated by the standard trapezoidal method. AUCINF is calculated by appending to AUC the quantity obtained by dividing the last measurable concentration by the estimated terminal rate of monoexponential decay of the blood level curve. Statistical analyses are conducted and confidence intervals are constructed on the differences between test and reference means. As Tmax is extremely sensitive to the time of sampling as well as to the rate of drug absorption, the confidence intervals for this parameter are generally too wide to be useful in determining bioequivalence. Usually, if the difference between the test mean and reference mean is less than 20% of the reference mean for AUC, AUCINF, Cmax and Tmax and the 90% nonsymmetrical confidence intervals on the difference for AUC, AUCINF and Cmax are within +/- 20% of the reference mean, the test product is considered to be bioequivalent to the reference product.

TABLE 1

Compartmental Models and Reference Product Parameters

One-Compartment:

$$C_t = [(Ka \cdot F \cdot X) / (V \cdot (Ka-Ke)] (e^{-Ke \cdot t} - e^{-Ka \cdot t})$$

$F = 1.0, \quad X = 250, \quad V = 10, \quad Ke = 0.12, \quad Ka = 2.0$

Two-Compartments:

$$C_t = L\, e^{-a \cdot t} + M\, e^{-b \cdot t} + N\, e^{-Ka \cdot t}$$

$$L = [Ka \cdot F \cdot X \cdot (K_{21}-a)] / [V \cdot (Ka-a)(b-a)]$$
$$M = [Ka \cdot F \cdot X \cdot (K_{21}-b)] / [V \cdot (Ka-b)(a-b)]$$
$$N = [Ka \cdot F \cdot X \cdot (K_{21}-Ka)] / [V \cdot (a-Ka)(b-Ka)]$$

$F = 1.0, \quad X = 1000, \quad V = 10, \quad a = 0.141, \quad b = 0.033$
$Ka = 0.40, \quad K_{21} = 0.05$

Our examination of the published literature has not revealed any rigorous considerations of the relative importance of AUC and AUCINF in judging the bioequivalence of two products. The extrapolation process in calculating AUCINF involves blood concentrations in the most variable analytical region, causing AUCINF to be inherently less precise and, possibly, less accurate, than AUC. For this reason, we feel that emphasis should be placed on AUC in the determination of bioequivalence, with AUCINF providing only secondary support for the conclusions drawn from AUC. An obvious question arises as to what proportion of the total AUCINF should be characterized before AUC is a reliable measure of bioequivalence. We explored this question by simulating typical blood level profiles for two products, test and reference, under one- and two-compartment behavior (Table 1).

Our simulations dealt with conditions of inequivalent rates of absorption (+/- 10%, 20%), inequivalent extents of absorption (+/- 10%, 20%) and combinations of inequivalent rates and extents of absorption. The sampling times used in the simulations were typical of those used in bioequivalence studies and were adequate for characterizing at least 95% of the theoretical reference AUCINF. Our examinations were based on incremental AUC calculations from time zero to the time of each sample.

The case of a test product which was inequivalent to the reference product only in extent of absorption was trivial. AUC representing any proportion (5%, 10%, 90%, etc.) of the theoretical AUCINF demonstrated the inequivalence. This was true for both compartmental models we examined and is exactly what one expects, as blood concentrations at all sampling times are directly proportional to the fraction of drug absorbed from the pharmaceutical product.

When the rate of absorption for the test product was the only difference between it and the reference product, the ratio of test AUC to reference AUC was essentially 1.0 (0.97 to 1.03) by the time at which 40% of the area was characterized in the one-compartment model and 60% was characterized in the two-compartment model. AUC to a time at which less than 40% of AUCINF was characterized for the one-compartment model or less than 60% for the two-compartment model was actually a better indicator of the rate of absorption difference than were either Cmax or Tmax.

When both rate and extent of absorption for the test were inequivalent to those of the reference, AUC accurately reflected the fraction of drug absorbed for the test product after 45% of the AUCINF was

TABLE 2

Selected Examples From The One-Compartment Model

	Test-to-Reference Ratios:				
Area Measured	F	Ka	Area	Cmax	Tmax
5 %	1.00	0.80	0.89	***	***
36%	1.00	0.80	0.98	0.97	1.33
5 %	1.00	1.20	1.07	***	***
36%	1.00	1.20	1.02	1.02	1.00
5 %	0.90	0.80	0.80	***	***
36%	0.90	0.80	0.88	0.87	1.33
5 %	1.20	0.80	1.06	***	***
36%	1.20	0.80	1.17	1.17	1.33
5 %	1.20	1.20	1.31	***	***
36%	1.20	1.20	1.22	1.22	1.00

*** Reference Cmax was observed only after 10% of area was measured.

characterized for the one-compartment model and 60% for the two-compartment model. AUC to a time at which a lesser proportion of AUCINF was characterized was found to be a more sensitive indicator of the rate of absorption difference than were either Cmax or Tmax.

The results of our simulations (Tables 2 and 3) were not unexpected. We had hypothesized that AUC, rather than AUCINF, would provide a reliable measure of extent

TABLE 3

Selected Examples From The Two-Compartment Model

Area Measured	Test-to-Reference Ratios: F	Ka	Area	Cmax	Tmax
10%	1.00	0.80	0.86	***	***
60%	1.00	0.80	0.97	0.93	1.25
10%	1.00	1.20	1.12	***	***
60%	1.00	1.20	1.02	1.06	1.00
10%	0.90	0.80	0.77	***	***
60%	0.90	0.80	0.87	0.84	1.25
10%	1.20	0.80	1.03	***	***
60%	1.20	0.80	1.16	1.12	1.25
10%	1.20	1.20	1.35	***	***
60%	1.20	1.20	1.23	1.27	1.00

*** Reference Cmax was observed only after 16% of area was measured.

of absorption. What wasn't anticipated was that the AUC could be calculated to a time at which significantly less than 80% of the AUCINF was characterized and still provide this reliability. Of particular interest was the sensitivity of incremental AUC (representing 10%, 20%, 30% of AUCINF) to differences in rates of absorption. This finding is understandable when one considers that the absorptive phase of the blood level profile, during

which the rate of absorption has maximal influence on concentration, occurs in the pre- and early post-peak sampling times for both compartmental models.

Based on our work and upon the consideration that AUCINF is an imprecise parameter in most bioequivalence work, we suggest that AUC be the prime parameter of interest. We also feel that bioequivalence study designs which result in an average of at least 60% of the AUCINF being measured are acceptable. The inclusion of subjects with less than 60% of the area characterized would simply have the effect of making AUC sensitive to rate of absorption differences, as well as extent of absorption differences. We would, however, out of practical considerations of having sufficient concentration data, advocate excluding from analyses those subjects in which some reasonable proportion (eg. 55%) of the reference AUCINF has not been characterized. A determination of bioequivalence based upon data from a properly conducted study with these restrictions would provide justification for accepting the test product as being bioequivalent to the reference product.

We are continuing our studies on this topic with further compartmental simulations and hope to publish additional pertinent material in the near future.