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An Area Correction Method To Reduce Intrasubject Variability In Bioequivalence Studies

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ABSTRACT Purpose. This paper investigates the use of a corrected area (*AUC•K)* to compensate for intrasubject variability in bioequivalence studies. **Methods.** Using computer simulation, this technique was applied to bioequivalence studies for two *drugs*. Both drugs exhibit first-order absorption and linear one-compartment disposition kinetics and total elimination by the liver. Drug I has a low intrinsic clearance (Cl_{int}) and is not bound to blood components, while Drug II has a high Cl_{int} and is highly bound. Two-way crossover trials, each including 24 subjects, were simulated using a spread-sheet program, which also performs ANOVA and provides 90% confidence intervals for C_{max}, *AUC* and *AUC•K*. The intrasubject CV for the parameter of interest was 30%. For all other pharmacokinetic parameters, the intrasubject CVs were 10%. **Results.** *Drug I:* With high variability in Cl_{int}, *AUC's* were concluded to be bioequivalent in 335, 303, 222, 102 and 32 of 500 trials for mean difference in % absorbed ($\Delta A = [A_{test} - A_{ref}]x100/A_{ref}$), -5%, -10%, -15% and -20% respectively. The corresponding numbers of trials that passed for *AUC•K* were 500, 500, 500, 382 and 23. *Drug II:* With high variability in Cl_{int}, 273, 281, 190, 106 and 29 of 500 trials passed for *AUC* at ΔA of 0%, -5%, -10%, -15% and -20% respectively. The corresponding numbers that passed for *AUC•K* were 378, 351, 239, 113 and 38 trials. For both drugs, when high variability was assigned to *V*, area correction reduced the number of trials passing for *AUC*. When the same intrasubject %CV was assigned to both *Cl* and *V*, area correction resulted in no change (Drug I) or a decrease (Drug II) in the number of passing trials. Assigning high intrasubject %CV to ΔA did not appear to alter the outcome of the simulation. **Conclusion.** Area correction appears to be helpful only when high intrasubject variability exists in clearance and not in the other parameters. It may be more helpful for drugs with low, compared to high Cl_{int} since in the latter case variability in Cl_{int} is reflected in both systemic clearance and bioavailability. It is recommended that area correction be attempted in bioequivalence studies of drugs where high intrasubject variability in clearance is known or suspected. It should be avoided where there appears to be a difference in K between treatments. The value of this approach in regulatory decision making remains to be determined.

INTRODUCTION

In studies designed to assess bioavailability and bioequivalence, it is often assumed that clearance, *Cl*, remains constant in the same individual during all treatment periods; therefore, the relative bioavailability, F_R is calculated as:

$$F_R = \frac{AUC_{test}}{AUC_{ref}} \bullet \frac{Dose_{ref}}{Dose_{test}} \qquad \textbf{(1)}$$

It is reasonable, nevertheless, to believe that *Cl* may not be constant, but rather changes from period to period with some degree of intrasubject variability. In randomized, crossover bioavailability/bioequivalence studies, however, the influence of such variability on the outcome of the study, particularly on the

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estimated mean value F_R is minimized. It is the distribution around this mean value that is largely affected by intrasubject variability. The larger the intrasubject variability in parameters influencing *AUC*, namely absolute bioavailability and clearance, the larger the variance of the estimated F_R. Consequently, it becomes less likely that the confidence interval calculated around this parameter would fall within the limits accepted by regulatory authorities for establishing bioequivalence.

Correction of *AUC* for half-life was first suggested by Wagner (1) for across-study comparisons. Koup and Gibaldi (2) presented a theoretical basis for using the same correction as a means of reducing variability around estimated mean F_R in bioequivalence and bioavailability studies. The method is based on the assumption that *Cl* is not necessarily constant in the same subject between treatments, but that the volume of distribution, *V*, is. The latter assumption is reasonable unless drastic physiologic changes are taking place during the course of the study, *e.g.* changes in protein concentration, adipose tissue fraction of body weight, hydration state, *etc.* Therefore,

$$FR = \frac{Cl_{test} \bullet AUC_{test}}{Cl_{ref} \bullet AUC_{ref}} \bullet \frac{Dose_{ref}}{Dose_{test}} \qquad \textbf{(2)}$$

Assuming *V* is constant and substituting *V*•*K* for clearance, where *K* is the terminal disposition rate constant, it follows that,

$$FR = \frac{AUC_{test} \bullet K_{test}}{AUC_{ref} \bullet K_{ref}} \bullet \frac{Dose_{ref}}{Dose_{test}} \qquad \textbf{(3)}$$

Also,

$$FR = \frac{AUC_{test} \bullet t_{1/2,ref}}{AUC_{ref} \bullet t_{1/2,test}} \bullet \frac{Dose_{ref}}{Dose_{test}} \qquad \textbf{(4)}$$

Bioequivalence assessment is carried out using both *AUC* and *AUC*•*K* values for individual subjects.

Area correction has been used in a number of New Drug Applications (NDA's), mainly to show bioequivalence between market and clinical formulations. For example, this method was used to test bioequivalence of a tablet formulation to the clinical capsule formulation of a cardiovascular drug in 18 healthy volunteers. The drug was readily and almost completely absorbed, less than 30% bound to plasma proteins, had a volume of distribution of 50 L, and was mainly metabolized by the liver with a total plasma clearance of 20-25 L/h. Systemic plasma clearance had overall coefficient of variations (CVs) of 20%-40% in various studies. In the bioequivalence study, the mean (CV) F_R was 1.19 (35%) and 1.09 (18%) before and after correction, respectively. The corresponding 90% confidence intervals were 88%-130% and 99%-113%, respectively. The estimated power of the study increased from 39% to approximately 100% and the estimated number of subjects needed to detect a 20% difference with an α of 0.05 decreased from 47 to 8 as a result of the correction. This, and other similar cases, prompted a simulation study the results of which are presented in this paper. The objective of the study was to examine the applicability of the area correction method in bioequivalence studies for two “drugs” having markedly different pharmacokinetic properties.

METHODS

The software used for this study was a modification of a bioequivalence simulation spreadsheet program previously described (3). The modification enables the assessment of bioequivalence based on *AUC*•*K* in addition to C_{max} and *AUC*. The program simulates multiple two-way crossover bioequivalence studies and provides 90% confidence intervals for natural logarithm-transformed parameters. The procedure assumes first-order absorption and linear, one-compartment disposition.

Two *drugs* were used in this study. Drug I has a low intrinsic clearance (Cl_{int}) and is not bound to blood components, while Drug II has a high Cl_{int} and is extensively bound. The population means of the pharmacokinetic parameters used for the simulations are listed in Table 1. The intersubject and intrasubject CVs for the parameter(s) of interest were set at 40%

and 30%, respectively. For all other parameters, the intersubject and intrasubject CVs were 20% and 10%, respectively, except for f_u where no variation was allowed. The CV for the dose and the assay were 2% and 10%, respectively. No assay lower limit of quantification was built into the simulation.

Table 1: Population Mean Pharmacokinetic Parameters for Simulation of Bioequivalence Studies with Drug I (Low-Clearance) and Drug II (High-Clearance)

Parameter	Drug I	Drug II
Dose, mg	10	1000
A[a],%	80	80
k_a, hr^{-1}	0.5	0.5
V, L	50	500
Cl_{int}[b], L/h	7.5	5000
Unbound (blood), %	100	5
Q_H[c], L/h	90	90
Extraction Ratio[d], %	7.7	74
F[e], %	74	21
Cl_s[d], L/hr	6.9	66
K[f], hr^{-1}	0.139	0.132

[a] *Percent of the dose absorbed for the reference products; A for the test products were set at 80%, 76%, 72%, 68% and 64%*
[b] *Hepatic intrinsic clearance*
[c] *Hepatic blood flow*
[d] *From the well-stirred model (Reference 5)*
[e] *A•(1-Extraction ratio)*
[f] *Cl_s/V*

Sampling times were set to 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24 and 30 hours. *AUC(0-t)* and *AUC* were calculated using the linear trapezoidal rule and *K* was determined using the last 6 points on the *lnC vs. t* plot.

The simulation strategy involved the introduction of high intrasubject variability (intrasubject %CV = 30%) in: a) intrinsic clearance (Cl_{int}), b) volume of distribution *(V)*, c) both Cl_{int} and *V* and d) the percent of the dose absorbed *(A)*. Differences in *A* ($\Delta A = [A_{test} - A_{ref}]x100/A_{ref}$) were set to 0%, -5%, -10%, -15% and -20%. Five hundred trials were run at each set of conditions. In each case, the overall %CV of F_R and the number of trials passing the 80-125% bioequivalence criterion for *AUC* and *AUC•K* were examined.

RESULTS

The overall CVs of F_R estimates and the number of trials, out of 500, passing the 80-125% bioequivalence criteria before and after area correction for Drug I and Drug II are listed in Tables 2 and 3, respectively. The tables also list the number of simulated studies in which area correction resulted in a *gain*, defined as a change in outcome of a particular trial from fail to pass or a *loss*, defined as a change in outcome from pass to fail.

Drug I:

With high intrasubject variability in clearance, the overall CVs of F_R decreased from 43% or 44% before correction to 16% after correction. Only 335 of 500 trials (67%) passed bioequivalence for *AUC* when the test product was identical to the reference product (*i.e.* $\Delta A = 0\%$); all 500 trials passed with area correction. The number of trials passing bioequivalence at ΔA of -5%, -10% and -15% increased after area correction from 303, 222 and 102, respectively to 500, 500 and 382, respectively, and the individual trial gains clearly outnumbered the losses. At ΔA of -20%, area correction did not improve the number of trials passing bioequivalence; 32 and 23 trials passed before and after correction, respectively.

With high intrasubject variability in volume, area correction resulted in an increase in the overall CV of F_R and a reduction in the number of trials passing bioequivalence (Table 2). On the other hand, area correction had no effect on the overall CVs of F_R or the number of trials passing bioequivalence when both clearance and volume were highly variable, or when the fraction absorbed was highly variable.

Drug II:

With high intrasubject variability in clearance, the overall CVs of F_R decreased slightly: from 46% before correction to 38% or 39% after correction. Only 273 of 500 trials (55%) passed bioequivalence when the test product was identical to the reference product (*i.e.* $\Delta A = 0\%$); area correction caused this

number to increase to 378 (76%). The number of trials passing bioequivalence at ΔA of -5%, -10%, -15% and -20% changed from 281, 190, 106 and 29, respectively to 351, 239, 113 and 38, respectively. Trial gains outnumbered losses, but the difference between the two numbers became progressively smaller as the magnitude of ΔA increased (Table 3).

Table 2: Coefficients of Variation of Relative Bioavailability and the Number of Trials (out of 500) Passing the Bioequivalence Criteria for AUC before and after Correction

Drug I, Low Clearance

	Before Correction		*After Correction*			
ΔA	**%CV**	**Number Pass**	**%CV**	**Number Pass**	**Gain[a]**	**Loss[b]**
I. Clearance Highly Variable:						
0%	44	335	16	500	165	0
-5%	43	303	16	500	197	0
-10%	43	222	16	500	278	0
-15%	43	102	16	382	293	13
-20%	44	32	16	23	17	26
II. Volume Highly Variable:						
0%	19	500	43	330	0	170
-5%	19	497	43	300	1	198
-10%	19	454	43	184	9	279
-15%	19	278	43	94	29	213
-20%	19	44	43	31	27	40
III. Clearance and Volume Highly Variable:						
0%	43	334	42	342	109	101
-5%	44	260	43	286	130	104
-10%	44	188	43	181	106	113
-15%	44	98	43	102	79	75
-20%	44	17	42	18	16	15
IV. Fraction Absorbed Highly Variable:						
0%	38	408	36	399	29	38
-5%	37	374	36	361	38	51
-10%	40	277	38	272	40	45
-15%	40	164	38	171	40	33
-20%	40	78	38	72	23	26

[a] *Number of trials which failed before, but passed after correction.*

[b] *Number of trials which passed before, but failed after correction.*

Table 3: Coefficients of Variation of Relative Bioavailability and the Number of Trials (out of 500) Passing the Bioequivalence Criteria for AUC before and after Correction.

Drug II, High Clearance

	Before Correction		*After Correction*			
ΔA	**%CV**	**Number Pass**	**%CV**	**Number Pass**	**Gain[a]**	**Loss[b]**
I. Clearance Highly Variable:						
0%	46	273	38	378	121	16
-5%	46	281	39	351	93	23
-10%	46	190	39	239	64	15
-15%	46	106	39	113	33	26
-20%	46	29	39	38	17	8
II. Volume Highly Variable:						
0%	23	500	47	269	0	231
-5%	23	486	47	254	5	237
-10%	23	411	46	184	22	249
-15%	23	203	47	81	28	150
-20%	23	35	47	27	23	31
III. Clearance and Volume Highly Variable:						
0%	46	284	56	157	55	182
-5%	46	257	56	150	50	157
-10%	46	188	54	118	49	119
-15%	46	98	56	56	25	67
-20%	46	28	56	23	16	21
IV. Fraction Absorbed Highly Variable:						
0%	40	376	42	350	30	56
-5%	40	345	42	316	30	59
-10%	40	257	42	240	34	51
-15%	41	174	43	164	28	38
-20%	40	80	43	80	23	23

[a] *Number of trials which failed before, but passed after correction.*
[b] *Number of trials which passed before, but failed after correction.*

When high intrasubject variability was introduced in volume, area correction was accompanied by an increase in overall %CV of F_R and a reduction in the number of trials passing bioequivalence (Table 2). Area correction was also accompanied by an increase in the overall %CV's of F_R and a decrease in the number of trials passing bioequivalence when both clearance and volume were highly variable. When the fraction absorbed was highly variable, area correction did not show any pronounced effect on either parameter.

DISCUSSION

Area correction resulted in reduction of the overall %CV around the estimated mean F_R and an increase in the number of studies fulfilling the bioequivalence criteria when intrasubject variability in clearance was high (Tables 2, 3). This improvement was more pronounced for the low-clearance drug (Drug I) than for the high-clearance drug (Drug II). This preferential improvement should be expected since for Drug II variability in intrinsic clearance is reflected in both systemic clearance and bioavailability. Area correction only compensates for the portion of variability reflected in systemic clearance. On the other hand, variability in the intrinsic clearance of Drug I is almost entirely expressed in systemic clearance. For example, at ΔA of -10%, area correction increased the number of successes for Drug I (Table 2) from 222 (44%) to 500 (100%) and for Drug II (Table 3) from 190 (38%) to 239 (48%). The number of trials in which area correction resulted in trial loss was greater in case of Drug II compared to Drug I, except at ΔA of -20%. Also the ratio of the number of losses to the number of gains was greater for Drug II than for Drug I, except at ΔA of -20% (Tables 2, 3).

Area correction resulted in a decrease in the number of successful trials in the somewhat unrealistic situation where the volume of distribution showed high intrasubject variability. It was not of any help when absorption was highly variable or when both clearance and volume varied to the same extent.

The findings of the present study confirm previous suggestions made by Koup and Gibaldi (2) and by Gibaldi and Perrier (4). In their textbook, Gibaldi and Perrier (4) suggested that "It is probably reasonable to attempt the half-life correction in most bioavailability studies but to accept it only when it results in a substantial decrease in the standard deviation of the mean value of *F or* F_R." It was also noted by Koup and Gibaldi (2) that area correction should be avoided when a systematic difference in *K* appears to exist between the test and reference treatments, as this most likely reflects a difference in absorption rather than variability in clearance. It is recommended that the intrasubject variability in pharmacokinetics be systematically determined during drug development, thus enabling the pharmaceutical scientists to anticipate the utility, or lack thereof, of the proposed correction method in future bioequivalence studies.

The risk of the proposed correction resulting in approval of products that are truly not bioequivalent appears to be minimal. During preliminary work, it was noted that practically no simulated trials (0-2 out of 500) passed the bioequivalence criteria, with or without correction, when the difference in A was 25% or greater.

CONCLUSION

Area correction is likely to help when intrasubject variability in clearance contributes significantly to the total variability in F_R. It will not help if high variability in F_R is largely due to absorption. It is recommended that area correction be attempted in bioequivalence studies of drugs where high intrasubject variability in clearance is known or suspected. It should be avoided, however, where there appears to be a difference in K between treatments. The regulatory acceptability of this analysis needs further evaluation.

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