Title

Evaluation of a rate-adjusted area under the concentration curve method to reduce the impact of variability in bioequivalence testing

Adam J Lucas*, Kayode Ogungbenro**, Shuying Y Yang***, Leon J Aarons**, Chao Chen***

Evotec Ltd*, University of Manchester**, GSK***

Corresponding author: Chao Chen

Clinical Pharmacology Modelling & Simulation, GlaxoSmithKline,

1 Iron Bridge Road, Uxbridge UB11 1BT, UK

email chao.c.chen@gsk.com; phone +44 771 780 1734

ABSTRACT

Aim: To quantify the utility of the rate-adjusted area under the concentration curve method in increasing the probability of a correct and conclusive outcome of a bioequivalence (BE) trial for highly variable drugs when clearance (CL) varies more than volume of distribution (V).

Methods. Data from a large population of subjects were generated with variability in CL and V parameters and used to simulate a two-period, crossover BE trial. The 90% confidence interval for formulation comparison was determined following BE assessment using the area under the concentration curve (AUC) ratio test, and the proposed rate-adjusted AUC ratio method. An outcome of bioequivalent, non-bioequivalent or inconclusive was then assigned in relation to predefined BE limits.

Results: We illustrate the utility of the rate-adjusted AUC method for BE testing when CL varies more than V. The approach is expected to enhance the probability of correctly assigning BE or non-BE and to increase study power to further reduce the risk of an inconclusive trial.

Conclusions: The rate-adjusted AUC method represents a simple and readily applicable approach to enhance the BE assessment of drug products when CL varies more than V.

Keywords

Bioequivalence, rate-adjusted area under the concentration curve, crossover, clearance

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What is already known about this subject

- Current regulatory guidelines for bioequivalence (BE) evaluations remain focused on comparison of area under the concentration curve (AUC). Pharmacokinetic principles dictate that direct comparison of AUC must assume constancy of clearance (CL).
- It has been proposed that, when volume of distribution (V) remains relatively constant between treatments, there is a benefit of adjusting AUC by elimination rate constant as a measurable parameter to account for variation in CL.

What this study adds

- Through simulations, we quantify the utility of a rate-adjusted AUC method for BE testing when CL varies more than V. The approach enhances the probability of correctly assigning BE or non-BE and furthermore increases study power and lowers the risk of an inconclusive trial.

INTRODUCTION

Scientifically rigorous evaluation of formulation bioequivalence (BE) allows correct regulatory decisions on new formulations or major changes of manufacturing process of existing formulations, consequently mitigating the risk of inadequate efficacy or compromised safety of a pharmaceutical product.

Variability presents a considerable challenge in the successful design and delivery of formulation BE testing whilst minimising drug exposure to trial participants. Regulatory guidance recommends a crossover design to diminish the impact of between-subject variability (BSV) through subjects effectively acting as their own control. Yet between-occasion variability (BOV) also occurs due to time dependent physiological variation, in addition to both environmental and experimental influences. BOV is therefore an important factor in crossover trial designs. Alternatively, there are situations where it may be necessary to conduct a parallel group study, requiring a larger sample size, which potentially introduces a further elevated risk of BSV, and therefore the propensity for an unsuccessful trial.

Regulatory guidance remains principally focussed on standard approach of comparing the ratio of area under the concentration curve (AUC) between formulations, and applying a statistical test to determine whether on average the products can be considered equivalent within predefined limits. Even with meticulous efforts to minimise variability through careful study design, BE testing can fail to demonstrate equivalency despite there being no discernible difference attributable to formulation or active moiety, but rather from physiological variation [1–4]. Careful prospective attention to possible sources of variation on a case-by-case basis could allow reduced subject numbers to sufficiently power a conclusive trial.[5]

An earlier report estimated approximately 30% of the products being tested in regulatory BE studies as highly variable drugs (HVD). HVD are defined by variation in exposure metrics such as AUC in excess of 30%. Use of AUC in BE testing of HVD required on average, 55 subjects compared to 32 for lower variability drugs. Furthermore, this analysis estimated that around 60% of HVD owed high variability to disposition of the active moiety – volume of distribution (V) or clearance (CL) - pharmacokinetic (PK) characteristics, rather than formulation properties. [6]

Equivalency of test formulation (T) and reference formulation (R) is typically assessed using relative bioavailability (F_T/F_R) at comparable doses:

$$\frac{F_T}{F_R} = \frac{AUC_T}{AUC_R} \qquad \text{(equation 1)}$$

Yet equation 1 assumes constancy in CL because in reality, bioavailability (F) = AUC · CL, and therefore must be expressed according to equation 2:

$$\frac{F_T}{F_R} = \frac{AUC_T \cdot CL_T}{AUC_R \cdot CL_R} \qquad \text{(equation 2)}$$

High BOV in CL can therefore potentially lead to excessive variation in F_T/F_R not relevant to the assessment of formulation average BE. As a result, a trial could fail to be conclusive, or require greater subject numbers to counteract the variability.

Furthermore, it is conceivable that CL variability may exceed that of V, which could be anticipated to remain more constant between study groups or test periods. On this basis, equation 2 can be expressed as equation 3 ('rate-adjusted AUC' method ($AUC \cdot k_e$)) as an alternative approach to BE assessment given that elimination rate constant (k_e) = CL/V:

$$\frac{F_T}{F_R} = \frac{AUC_T \cdot k_{e,T}}{AUC_R \cdot k_{e,R}} \qquad \text{(equation 3)}$$

Despite the approach first proposed by Wagner based upon fundamental PK principles [7], it has only attracted limited attention in the BE community or by the regulators. Abdallah [8] reported BE trial simulations of two hypothetical drugs with high variability in CL and V and reported an increased achievement of BE when utilising the rate-adjusted AUC method when CL variability exceeded that for V. In addition, this effect was more dramatic when CL was low, owing to the greater influence of changes to intrinsic CL. The reported simulations also concluded a detrimental effect on BE outcome when variability for V exceeded CL.

In a recently reported case, the use of uncorrected AUC data failed to demonstrate BE of an extended-release lamotrigine formulation against a standard strength tablet in a parallel group design [9]. Yet post-hoc analysis revealed bimodal distribution of half-lives between study groups, indicative of CL subpopulations owing to greater variability associated with CL/F compared to V/F. These findings supported the successful re-evaluation of trial data utilising the rate-adjusted AUC method to demonstrate formulation BE.

The objective of this work is to systematically quantify the potential value of the rate-adjusted AUC method on BE trial outcome. A range of formulation F_T/F_R scenarios, trial group sizes, and relative differences in CL and V variability were tested in simulated BE trials.

METHODS

Simulation approach

A population (n=10,000) of potential trial subjects was randomly assigned unique values of CL and V from a multivariate lognormal distribution (CL-V; correlation coefficient r=0.3) with a moderate BSV coefficient of variation (CV) = 40%. Furthermore, BOV scenarios were applied between test periods according to Table 1. Three F_T/F_R conditions were tested to replicate possible BE outcome differences between T and R: no difference (F_T/F_R=1, bioequivalent), a clinically meaningless difference (F_T/F_R=1.15, bioequivalent), and a clinically relevant difference (F_T/F_R=1.35, non-bioequivalent). In addition, F_T and F_R parameters were set with CV=5% to allow for variability related to sources such as drug content or release mechanism.

Two period and two sequence crossover trials (N=1000) were simulated for a range of sample sizes (n=4-300). For each trial, subjects were randomly sampled from the large population without replacement. For overall balance, half of the trial subjects received T in the first period, then R in the second period; and the remaining half of subjects received R first followed by T. Simulations were conducted using R (2017).[10]

BE assessment method

Both the conventional AUC ratio test and rate-adjusted AUC ratio test were determined according to equation 4 and equation 5, respectively, based on the principles set out in equations 1-3 assuming unit doses for all tests.

$$AUC\,ratio = \frac{AUC_T}{AUC_R} = \frac{F_T/CL_T}{F_R/CL_R} \qquad \text{(equation 4)}$$

$$AUC \cdot k_e\,ratio = \frac{AUC_T \cdot k_{e,T}}{AUC_R \cdot k_{e,R}} = \frac{F_T/V_T}{F_R/V_R} \qquad \text{(equation 5)}$$

The mean estimate and 90% confidence interval (CI) of the ratio of AUC and AUC ⋅ke between T and R were then derived for each trial. For the purpose of this analysis, the proportion of trials

with three different outcomes (*bioequivalent*, *non-bioequivalent*, or *inconclusive*) was determined according to Figure 1.Error: Reference source not found following comparison of the lower and upper 90% CI to pre-defined BE limits of 0.8 and 1.25 respectively – boundaries per regulatory requirement and widely regarded as clinically meaningful limits. Categorisation as *inconclusive* was included to highlight the value of sample-size saving and of avoiding a false trial conclusion.

RESULTS

The probability of a trial outcome (classified as bioequivalent, non-bioequivalent, or inconclusive) using the standard AUC ratio test and the rate-adjusted AUC ratio test for the reported simulations are presented graphically in Figure 2. The probability difference of each outcome when using the rate-adjusted AUC method compared to the standard AUC method is presented graphically in Figure 3. Finally, the sensitivity to change in CL BOV on the probability of a trial outcome was assessed for the scenario in which F_T/F_R = 1.15, and is presented graphically in Figure .

In all scenarios and regardless of the BE assessment method, the probability of a correctly assigned trial outcome increased with sample size, and the prospect of inconclusiveness diminished. When F_T/F_R deviates from unity within BE limits, an appreciable risk was evident for falsely claiming non-bioequivalent. Despite increasing sample size raising the likelihood of correctly assigning bioequivalent and decreasing risk of inconclusiveness, the propensity for incorrectly concluding non-bioequivalent rose to a maximum before declining. This observation is attributable to the CI as a function of sample size, and where it sits in relation to BE limits when $F_T/F_R \neq 1.00$.

Scenario: clearance is more variable than volume

When formulations are exactly bioequivalent (F_T/F_R = 1.00), comparable sample sizes allowed the rate-adjusted AUC method to have a higher probability of correctly concluding bioequivalent, a lower probability of incorrectly concluding non-bioequivalent, and a lower risk of inconclusiveness (Figure 2). In other words, to achieve the same study power, the rate-adjusted AUC method required comparatively smaller sample sizes.

When BOV in CL (CV=35%) exceeded BOV for V (CV=10%) in a two-way crossover trial for bioequivalent (F_T/F_R=1) formulations, the rate-adjusted AUC method led to a maximum

increased probability of 0.78 over the standard AUC ratio test (Figure 3). The advantage was most discernible at low subject numbers before the benefit lessened with increasing sample size. The risk of incorrectly concluding non-bioequivalent in this scenario was low using the uncorrected AUC method (probability of <0.18), and reduced further using rate-adjusted AUC, with a negligible probability of <0.042 (Figure 2).

Use of uncorrected AUC data when CL variability is high, indicates that while subject numbers are low there is an appreciable risk of an inconclusive trial (0.46 for n=12 subjects). Increasing sample size steadily decreased this risk, yet greater than n=30 subjects were necessary to make the likelihood negligible. Conversely, use of rate-adjusted AUC data resulted in a minimal risk of inconclusiveness even for small sample sizes (maximum reduction of 0.65 compared to uncorrected AUC data) (Figure 2 and Figure 3).

When a true formulation difference occurs (e.g. F_T/F_R=1.15), deviating from 1.00 but within BE limits, the comparison still holds. However, it is evident that the uncorrected AUC method has a higher probability of incorrectly concluding non-bioequivalent than the correct assignment of bioequivalent when sample sizes are small. In this scenario, despite a notable risk of incorrect assignment of non-bioequivalent at small sample sizes, use of the rate-adjusted AUC method delivered a greater probability of a correct trial outcome regardless of group size compared to the uncorrected AUC method (Figure 2). The maximum probability gain using the rate-adjusted AUC method versus uncorrected AUC was 0.6, and the benefit did not diminish until subject numbers were high (Figure 3). The maximum benefit was therefore lower compared to the scenario in which formulations are exactly equivalent. Yet because this situation presents a risk of wrongly concluding non-bioequivalent, the rate-adjusted AUC method reduced the probability of this occurrence by up to 0.54 compared to using the standard AUC ratio test (Figure 3). The risk posed to an incorrect trial outcome at low subject numbers was present using both methods (probability of 0.32-0.41) but continued to rise to 0.58 (n=30 subjects) for the AUC ratio test before declining with increasing sample size (Figure 2 and Figure 3).

When formulations are truly non-bioequivalent (e.g. F_T/F_R = 1.35), the rate-adjusted AUC method demonstrated a higher probability of correctly concluding non-bioequivalent, and likewise a lower prospect of inconclusiveness (Figure 2).

Scenario: both clearance and volume are similarly variable

When CL and V BOV were comparable (BOV=20%), the use of rate-adjusted AUC made no discernible difference to the trial outcomes compared to using uncorrected AUC data (Figure 2 and Figure 3).

Scenario: clearance is less variable than volume

When the formulations are stringently bioequivalent (F_T/F_R = 1.00), comparable group sizes allowed the standard AUC method to have a higher probability of correctly concluding bioequivalent, a lower probability of falsely determining non-bioequivalent, and a lesser risk of inconclusiveness (Figure 2). In other words, to achieve equivalent study power, the conventional method required smaller sample sizes for the correct trial outcome. When the F_T/F_R = 1.15, deviating from 1.00 but still bioequivalent, the advantages of the conventional method still hold. Finally, when the formulations are truly non-bioequivalent (F_T/F_R = 1.35), the conventional method maintained the higher probability of reaching the correct conclusion.

Impact of variability in clearance

Where formulations are truly bioequivalent but with a moderate difference (F_T/F_R = 1.15), there are considerable risks of inconclusive trials or wrongly claiming non-bioequivalent, even at relatively high sample sizes (Figure 2). Therefore, the BE test outcome by each method – AUC ratio and AUC·ke ratio – for a wide range of BOV in CL (from 10 to 100%) was further investigated for this situation to determine the benefit of the rate-adjusted AUC method as the extent of CL variability increases.

Using the AUC ratio, the more variable CL is, the less likely it is that the correct conclusion of bioequivalent will be reached. Even when CL is only moderately variable, the probability of concluding bioequivalent is very low. This is true regardless of the sample size. Worse, with increasing sample size, the probability of claiming non-bioequivalent becomes higher than the probability of the trial being inconclusive (Figure 4).

Conversely, when considering the rate-adjusted AUC method, the issue of increasing CL variability is eradicated. As expected, small sample sizes increased the probability of inconclusiveness. While slightly more subjects introduces enhanced risk of incorrectly concluding non-bioequivalent, moderate subject numbers become sufficiently high to deliver a correct trial outcome, regardless of variability of CL.

DISCUSSION

The use of a crossover design is common practice in BE testing to assess comparability of formulation bioavailability. Regulatory guidance remains focused on comparison of AUC to judge whether an alternative formulation will, on average, deliver equivalent exposure to an existing product. From first principles, both F and CL govern AUC; therefore, this established approach must assume constancy in CL to detect true formulation differences.

Successful testing of HVD presents a considerable challenge of potentially excessive subject numbers being needed to overcome variability. Despite best efforts to minimise variability through careful study design there is a substantial risk of inconclusiveness and therefore failure in BE testing. Other approaches rely upon higher-order designs that enable assessment of BOV. Determination of such variability derives from repeat testing, and therefore poses further safety risk due to increased drug exposure. [11,12]

In this study, simulation of BE trials with BOV for CL in excess of that for V demonstrates enhanced propensity for a correct outcome with fewer subjects using the rate-adjusted AUC approach. Unsurprisingly, the probability of a conclusive trial increases with expanding sample size and likewise improves as the true formulation ratio approaches unity. When CV(CL)=35% and CV(V)=10% (3.5-fold difference) the rate-adjusted AUC method potentially delivers a comparable outcome using $1/6^{th}$ of the number of subjects compared to the standard AUC approach. It may not be uncommon that F_T/F_R is not 1.0 for two formulations that are bioequivalent. One important finding from the simulation is that in this situation, when CL is markedly more variable than V, the conventional approach of uncorrected AUC ratio is expected to have a detrimental consequence of a high probability of concluding non-bioequivalent even with a large sample size (Figure 4).

When V varies more than CL, the uncorrected method is justified by PK principles. This was confirmed by the herein simulations, which showed that utilising rate-adjusted AUC was detrimental to subject requirements compared to using AUC alone, and as such could be expected to result in an increased likelihood of an inconclusive outcome or the worst-case scenario of falsely concluding BE when it does not truly exist.

An important consideration in planning a BE trial is sample size requirements to provide sufficient statistical power. In practice, one may plan a study on the basis of no formulation difference (i.e. $F_T/F_R = 1$), in anticipation of a small difference (e.g. $F_T/F_R = 1.15$), or apply an

assurance approach by assuming a distribution of potential F_T/F_R values.[13] For the purpose of this simulation, n=30 subjects may be recruited to provide 90% power by the uncorrected AUC method, assuming $F_T/F_R = 1$ when CV(CL)=35% and CV(V)=10%. Yet depending on the level of BOV, this may over-power the study leading to wasted resource and unnecessary drug exposure. Conversely, depending on the actual formulation difference this may result in a substantial risk of an incorrect trial outcome. This simulation indicates the growing significance of study power when a formulation difference occurs ($F_T/F_R \neq 1$) as there is an increased risk of both inconclusiveness and incorrectly concluding non-bioequivalent. Correctly determining BE in this instance requires higher subject numbers to shrink the 90% CI such that it is contained within BE limits. Use of the rate-adjusted AUC method remained equally beneficial when CL varied more than V, as it accounts for a portion of the variability, and therefore contracts the 90% CI.

This simulation indicates that for HVD (BOV(CL)=35%, BOV(V)=10%), subject recruitment to sufficiently power a study using the uncorrected AUC method would be in the order of n=30 to n=60. This requirement further increases as the true formulation difference inflates. These numbers agree with the typical sample size reported in the literature for HVD (n=55). Somewhat fewer subjects were required using the rate-adjusted AUC method when CL is the root cause of variability, broadly in keeping with the average group size also reported previously for lower variability drugs using the standard AUC approach. [6]

Findings from this exercise suggest that for better efficiency and a more reliable conclusion, a BE study should be powered for rate-adjusted AUC, instead of AUC alone, as the endpoint when BOV(CL) is higher than BOV(V). In practice, CL and V are not available for the BOV comparison when intravenous PK data for R are not available; and their BOVs are not estimable when intravenous PK is not studied more than once in the same subjects. In these situations, the BOVs for CL/F and V/F estimated from non-intravenous administrations may serve as the surrogates. When the BOVs for CL/F and V/F are not available, the current practice of using BSVs in the place of BOVs may be adopted with the expectation that higher BSV generally indicates higher BOV. Prospective knowledge of potential variability will add value in determining and justifying an appropriate study design.

The performance of rate-adjusted AUC in the simulated scenarios indicates that the method should be considered by the regulators for its potential utility in testing HVD and reducing

unnecessary human exposure in BE trials. The simulations herein clearly indicate that use of rate-adjusted AUC in BE testing must be carefully considered owing to the possibility of both a beneficial or detrimental impact on a trial outcome dependent upon the source of variability. *A priori* knowledge of variability associated with R would be insightful and could enable identification of the root cause of the variability.

Following oral application, k_e is a readily measurable parameter. Yet, it follows that such measurement must be made with accuracy. Potential for flip-flop kinetics must be negligible to minimise the risk of absorption-limited kinetics. Furthermore, for drugs displaying multi-compartmental behaviour it is important to ensure use of the terminal elimination phase, avoiding influence from distribution processes. Accurate identification of the terminal half-life will depend on many factors: dose and drug exposure in relation to analytical detection limits, sampling times, and PK linearity. Anticipating use of k_e therefore requires careful study planning and may even be inappropriate for some product types such as slow release formulations. Assuming k_e has been determined with sufficient accuracy then an indirect assessment of whether it is appropriate to proceed using rate-adjusted AUC could be obtained from determining the variance associated with corrected versus uncorrected AUC data. If deemed appropriate for use; the rate-adjusted AUC method could be considered as an alternative to study designs involving repeat administration, increased subject recruitment, or the need to use expanded BE limits.

For long half-life products, the regulators recommend alternative strategies such as a parallel group design. Such studies may need to consider greater potential risk of variation from BSV given multi-factorial possibilities of variation amongst the population. Broadly speaking, the greatest propensity for variability within a population will likely be CL; yet potential variability in V should not be underestimated given the influence of physiological parameters such as age, weight, and body composition. Despite careful control of subject assignment through randomisation; trial groups may ultimately represent different PK populations, and therefore lead to failure to control for variability. Therefore, variability in V in some circumstances may limit the utility of rate-adjusted AUC in parallel group designs compared to crossover in which differences/changes in body composition are less likely over the duration of a study.

Despite a justified scientific basis, the BE community and regulatory guidelines do not acknowledge the use of the rate-adjusted AUC approach. Correction of AUC by the readily

measurable parameter k_e could account for variation in CL related to the individual subject rather than formulation. This represents a practical and useful path forward to reduce such variability particularly in the case of HVD, and therefore trial size required to power a conclusive outcome.

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Table 1. Between-occasion variability (BOV) for clearance (CL) and volume (V) used in the simulations

		Formulation relative bioavailability (test: reference)		
BOV Scenario		**1**	**1.15**	**1.35**
BOV(CL) > BOV(V)	BOV(CL)	35%	35%	35%
	BOV(V)	10%	10%	10%
BOV(CL) = BOV(V)	BOV(CL)	20%	20%	20%
	BOV(V)	20%	20%	20%
BOV(CL) < BOV(V)	BOV(CL)	10%	10%	10%
	BOV(V)	35%	35%	35%

Figure 1. Schematic of bioequivalence (BE) limits and outcomes. Arrows represent lower and upper (left and right arrowheads, respectively) limits of the 90% confidence interval in relation to pre-defined limits used to classify a trial outcome as bioequivalent, non-bioequivalent, or inconclusive.

Figure 2. Simulation scenario outcomes representing the overall probability of a trial outcome as a function of the sample size using both area-under the concentration curve (AUC) and rate-adjusted AUC ratio tests. In each scenario, clearance (CL) and volume of distribution (V) between-subject variability (BSV)=40% (CV). Solid lines: tested by AUC ratio; dashed lines: tested by rate-adjusted AUC ratio. Green: claiming bioequivalent; Red: claiming non-

bioequivalent; Orange: inconclusive. F_T/F_R = relative bioavailability, BOV = between-occasion variability.

Figure 3. Probability difference of simulation trial outcomes using rate-adjusted area-under the concentration curve (AUC) relative to uncorrected AUC as a function of the sample size. Green: claiming bioequivalent; Red: claiming non-bioequivalent; Orange: inconclusive. F_T/F_R = relative bioavailability, BOV = between-occasion variability, CL = clearance, V = volume of distribution.

Figure 4. Probability of trial outcome being bioequivalent (top), non-bioequivalent (middle) and inconclusive (bottom) using either area-under the concentration curve (AUC) (left) or rate-adjusted AUC (right) ratio tests for a range of clearance (CL) between-occasion variability (BOV) (10 - 100%) and sample sizes, while volume of distribution (V) BOV is kept at 20%. Colour from red to green indicates low to high probability, respectively. Formulation relative bioavailability (F_T/F_R) for test: reference is 1.15. BSV for CL and V are both kept at 40%.