Biometrical evaluation of bioequivalence trials using a bootstrap individual direct curve comparison method

E. ZINTZARAS¹, P. BOUKA² and A. KOWALD³

¹ National Agricultural Research Foundation, Athens, Greece
² Hellenic Organization for Medicines, Athens, Greece
³ Max Planck Institute, Berlin, Germany

Received for publication: September 3, 2001

Keywords: Bioequivalence, individual bioequivalence, bootstrap, curve comparison

SUMMARY

Bioequivalence of two medicinal, or veterinary, products is established by comparing the mean of bioavailability measures, such as AUC and C_max, following administration of the test (T) and reference (R) products. However, the use of these parameters has several drawbacks, e.g., they do not take into consideration the overall pharmacokinetic profile shape. Therefore, concerns have been raised regarding their appropriateness for assessment of bioequivalence. To overcome the limitations of these bioequivalence parameters, direct curve comparison metrics methods were recently proposed on an average basis. In this paper, an individual based direct curve comparison method for assessing bioequivalence is proposed. The bioequivalence of T and R in each subject is evaluated by a new curve comparison metrics $\delta$. The metrics $\delta$ is the absolute sum of the difference between two curves. The significance of the metrics for each subject is assessed by bootstrapping. An overall bioequivalence of T and R may be considered if less than 25% of the subjects show statistically different profiles.

INTRODUCTION

Bioavailability means the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action (1,2).

In the majority of cases, bioavailability is estimated by the parameters AUC (extrapolated area under the plasma concentration-time curve) and C_max (the maximum plasma concentration) which are derived from plasma/serum concentration-time curves.

Similarly, in most cases bioequivalence is established by comparing the pharmacokinetic parameters AUC and C_max which express the extent and rate of absorption, respectively. Two drug products are bioequivalent if their bioavailabilities after administration in the same molar dose are similar to such a degree that their effects, with respect to both efficacy and safety, will be essentially the same (1,2).

The bioequivalence of the T and R are proven by conducting a bioequivalence trial. The design of a bioequivalence trial is usually a two-period crossover. The statistical evaluation of the trial is currently based on the corresponding ANOVA after log-transforming the data. The assessment of bioequivalence is based on the 90% confidence interval (c.i.) for the ratio (T/R) of the average
bioavailability of the test (T) and the reference (R) products. Bioequivalence of the formulations is declared if the 90% c.i. is within preset regulatory limits (0.80, 1.25) (1,2,3,4).

However, use of these pharmacokinetic parameters has several drawbacks: AUC does not take into consideration the profile shape of the concentration-time curve; \( C_{\text{max}} \) is not a good estimate of the absorption rate, it depends on a single-point and its use alone cannot discern differences in \( t_{\text{max}} \) or \( t_{\text{lag}} \) time (elapsed time until the drug appears in the blood) between formulations (5). An additional criticism of AUC and \( C_{\text{max}} \) as bioequivalence parameters is that they are confounded since each parameter is a measure of extent of drug availability (6,7,8).

In an effort to reduce reliance on rate and extent of absorption Tozer et al. (9) introduced the concept of 'exposure' and pointed out that the goal of bioequivalence trials should be to assure that the shape of the concentration-time curve of the test product is sufficiently similar to that of the reference product.

The limitations of the AUC and \( C_{\text{max}} \) as bioequivalence metrics may be overcome by using direct curve comparison metrics like \( f_1 \) and \( f_2 \) (7,8,9,10,11).

The \( f_1 \) metrics is a function of the average absolute difference between two curves and measures difference:

\[
f_1 = \left[ \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \right] 100,
\]

where \( R_i \) is the reference concentration at the ith time, \( T_i \) is the test concentration at the ith time and \( n \) is the number of time points.

The \( f_2 \) metric is a function of the reciprocal of mean square root of the sum of square distances at all points and measures similarity:

\[
f_2 = \log \left[ 1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right]^{0.5} 100.
\]

These metrics are commonly used for in vitro dissolution profile comparison (10,11). Only recently the \( f_1 \) metrics has been applied to pharmacokinetic profile comparisons. However, the bioequivalence is assessed based on an average basis, i.e. the metric of each subject is calculated and then from the individual subjects the median, or the mean, of the metrics is calculated and its significance is evaluated by ANOVA in a similar way to AUC and \( C_{\text{max}} \) (7,8,12). Although this approach compares the profiles directly, it is actually based on a summary statistic, such as the mean, and does not take into account the differences in the test (T) and reference (R) profiles in each subject directly. These methods eventually pool the data variability ignoring the proportional trend of this variability in each subject.

Anderson and Hauck (13) introduced the concept of 'individual bioequivalence' indicating that the goal of a bioequivalence trial is to ensure that an individual could be switched from the reference product to the test product with unchanged efficacy and safety (switchability) (13,14). Bioequivalence criteria that focus on switchability are termed individual bioequivalence criteria. The individual bioequivalence has some advantages compared with the average bioequivalence: (i) it allows comparison of intra-individual variances, (ii) it detects existing subject-by-formulation interactions and (iii) it promotes inclusion of heterogeneous population of subjects in bioequivalence trials (15,16,17,18). However, individual bioequivalence just takes into account the within-subject variability and not the significance of the bioequivalence in each subject. Furthermore, questions remain on the optimal use of replicate trial designs and the proposed criterion for evaluation of bioequivalence between products (16).

In this paper, an individual based direct curve comparison method for assessing bioequivalence is proposed. The comparison is based on a new metrics, \( \delta \), which is the absolute sum of the difference between two curves. This approach utilizes all data points, compares profiles at the same time points and achieves assessment in a single evaluation.

The significance of the metrics for each subject is assessed by using bootstrapping: a resampling method for constructing the distribution of the metrics (19). In this way bioequivalence is assessed in each subject. When a certain proportion of subjects (e.g. less than 25%) does not have statistically similar test (T) and reference (R) pharmacokinetic profiles then the two products are considered non-bioequivalent.

Metrics like \( f_1 \) and \( f_2 \) are not performing properly when the bootstrapping is used because they cancel out the swift of the curves.

The results of the proposed method are compared to the results when the average bioequivalence is applied since this method is recommended by the regulatory authorities of the leading industrialized countries.

METHODS AND RESULTS

Assessment of bioequivalence of the test (T) and reference (R) products is based on comparison of the plasma concentration-time profiles of the test and reference