Bioequivalence and Bioequivalency Testing

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PROLOGUE
This lecture material is covered in one and one-half fifty-minute lecture periods. The primary objectives of the lecture are to: (i) review interpatient and intrapatient pharmacokinetic variability; (ii) introduce the concepts of therapeutic equivalence and bioequivalence; (iii) introduce the current FDA standards on bioequivalence; (iv) introduce the basic approach of bioequivalency testing; (v) provide an example of a clinical study assessing bioequivalence, showing calculations and conclusion regarding bioequivalency; and (vi) discuss the current FDA standards of bioequivalence as they relate to the approval of generic formulations of highly variable drugs and to the interchangeability of formulations of drugs with low therapeutic indices. The overall goal of the lecture is to introduce pharmacy students to bioequivalence and to bioequivalency testing, such that they may appropriately address the concerns of patients regarding generic substitution and appropriately advise physicians regarding the interchangeability of approved drug formulations.

INTRODUCTION
Generic drug formulations are often substituted for ‘brand name’ (i.e., innovator) formulations by pharmacists in an effort to reduce the cost of prescription-drug therapy. In most states, generic substitution is allowed and encouraged, provided that the generic formulation is deemed to be therapeutically equivalent to the innovator formulation by the FDA. The FDA publishes a list of drug products and equivalents, which is entitled, Approved Drug Products with Therapeutic Equivalence Evaluations, and is commonly known as the Orange Book. The FDA’s designation of ‘therapeutic equivalence’ indicates that the generic formulation is (among other things) bioequivalent to the innovator.
formulation and signifies the FDA’s expectation that the formulations are likely “to have equivalent clinical effect and no difference in their potential for adverse effects.”(1)

The 1984 Amendments to the Drug Price Competition and Patent Term Restoration Act require that manufacturers seeking approval of generic formulations submit to the FDA data demonstrating bioequivalence to the innovator drug product (1). Currently, bioequivalence is determined by assessing the equivalence of the rate and extent of drug absorption. Typically, this investigation is made through a 12-36 subject, two-treatment crossover study, conducted with healthy normal adult subjects. Under current FDA standards, bioequivalence is concluded in cases where the innovator and test product differ in terms of their rate and extent of absorption by -20/+25 percent or less. The FDA guidance, *Statistical Procedures for Bioequivalence Studies Using a Standard Two-treatment Crossover Design*, outlines a statistical method for assessing data and provides a decision rule for concluding bioequivalence(2).

In spite of the efforts of the FDA, controversy and disagreement exist regarding the therapeutic equivalence of drug products listed in the Orange Book. Additionally, there is a seemingly continual debate in the pharmaceutical literature over the appropriateness of the FDA’s recommended approach of testing the rate and extent of drug absorption(3-6). Perhaps in part as a consequence of these debates, and perhaps in part due to the publicity of the recent generic drug scandals, pharmacists are often queried by patients, health care professionals, and HMO representatives regarding the therapeutic equivalence of drug products. This lecture is designed to strengthen the students’ understanding of bioequivalence and bioequivalence testing, in an attempt to increase students’ confidence in addressing these questions.

**LECTURE FORMAT AND CONTENT**

**Interpatient and Intrapatient Pharmacokinetic Variability**

Interpatient variability in pharmacokinetics is recognized by pharmacists and other health care professionals as an important factor to consider in evaluating and planning drug therapy.

In fact, much of the work in the field of clinical pharmacokinetics is devoted to attempts to understand and overcome this form of pharmacokinetic variability. Interpatient variability is often best demonstrated by examining the concentration vs. time profiles of a drug following the administration of a fixed dose to several patients. Between-patient differences in pharmacokinetics (i.e., the processes of drug absorption, distribution, metabolism and excretion) are expressed as differences in resultant concentration vs. time profiles (Figure 1)(7).

Intrapatient pharmacokinetic variability refers to variabilities in pharmacokinetics that occur within a patient from dose to dose during the course of drug therapy (Figure 2)(8). In contrast to the mindfulness of practitioners regarding interpatient variability, intrapatient variability is virtually ignored in discussions of pharmacokinetics, outside of those in the bioequivalence literature. The relative lack of attention paid to intrapatient pharmacokinetic variability may be partially explained by the argument that drugs exhibiting high intrapatient pharmacokinetic variability rarely are drugs with low therapeutic indices, as this combination would not be likely found in an approved drug(9). This argument, which likely has merit, implies that intrapatient pharmacokinetic variability is rarely a factor in maintaining the safety and efficacy of drug therapy. However, intrapatient variability appears as a key factor in designing and assessing bioequivalence studies.
**Cosmetic Act:** Bioequivalence

The main focus of this code signifies that the formulation has been submitted with a demonstration of bioequivalence. The ‘AB’ Therapeutic Equivalence Codes are employed by the FDA to indicate the level of bioequivalence of pharmaceutical formulations. The FDA classes as therapeutically equivalent those products that meet the following general criteria: (i) they are approved as safe and effective; (ii) they are pharmaceutical equivalents in that they, (a) contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, and (b) meet compendial or other applicable standards of strength, quality, purity, and identity; (iii) they are bioequivalent in that, (a) they do not present a known or potential bioequivalence problem, and they meet an acceptable in vitro standard, or (b) if they do present such a known or potential problem, they are shown to meet an appropriate bioequivalence standard; (iv) they are adequately labeled; and (v) they are manufactured in compliance with Current Good Manufacturing Practice regulations.

It is important to note that the FDA designation of therapeutic equivalence does not consider several formulation characteristics, including packaging, scoring configuration, shape, or pharmaceutical excipients (e.g., colors, preservatives, flavors). These formulation characteristics may be important to the care of particular patients (e.g., patients allergic to certain pharmaceutical excipients). With this limitation, however, the FDA has the expectation that therapeutically equivalent products will produce the same clinical effect and toxicity profile of the prescribed product.

**Therapeutic Equivalents.** Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling.

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**Therapeutic Equivalence Codes**

The FDA provides a code to indicate the level of bioequivalence demonstrated for therapeutic equivalents. The ‘AB’ code signifies that the formulation has been submitted with a study demonstrating bioequivalence. The main focus of this lecture is to instruct students in process and requirements needed to attain the ‘AB’ rating.

**Bioequivalence**

Bioequivalence is defined to the class using the terms provided in section 505(j)(7)(B) of the Federal Food, Drug, and Cosmetic Act:

“The rate and extent of absorption of the test drug do not show a significant difference from the rate and extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses;” or “the extent of absorption of the test drug does not show a significant difference from the extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses and the difference from the reference drug in the rate of absorption of the drug is intentional, is reflected in its proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.”

**Metrics of the Rate and Extent of Drug Absorption**

Bioequivalence studies attempt to gain insight on formulation “switchability” (i.e., the ability to substitute one formulation for another without concern of the potential for reduced effectiveness or increased probability of adverse effects). A key assumption is that switchability may be inferred from plasma concentration vs. time data and metrics reflecting the rate and extent of drug absorption. The area under the plasma concentration vs. time curve (AUC) is commonly employed as the metric describing the extent of drug absorption, while the maximal concentration observed following drug administration (Cmax) is the metric recommended by the FDA to evaluate the rate of drug absorption.

The AUC resultant from a single dose of a drug formulation is commonly assessed with the linear trapezoidal method:

\[
AUC = \int_0^\infty Cdt \approx \sum_{i=1}^{n} \left( \frac{C_i + C_{i+1}}{2} \right) \times (t_i - t_{i-1}) + \frac{C_n}{\lambda} \quad \text{Eq. 1.}
\]

Where \( C \) refers to the concentration of drug in plasma, \( t \) refers to the time of sample collection, and \( z \) refers to the magnitude of the terminal In-linear slope of the declining concentration vs. time data. Cmax is determined by observation from the collected concentration vs. time data.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test AUC ng/ml x h</th>
<th>Reference AUC ng/ml x h</th>
<th>Test Cmax ng/ml</th>
<th>Reference Cmax ng/ml</th>
</tr>
</thead>
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<td>4020</td>
<td>140</td>
<td>226</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>211</td>
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<td>20.1</td>
</tr>
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<td>3</td>
<td>905</td>
<td>572</td>
<td>78.8</td>
<td>51.8</td>
</tr>
<tr>
<td>4</td>
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<td>815</td>
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</tr>
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<td>715</td>
<td>54.7</td>
<td>40.6</td>
</tr>
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<td>6</td>
<td>2460</td>
<td>1940</td>
<td>76.4</td>
<td>52.6</td>
</tr>
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<td>1930</td>
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</tr>
<tr>
<td>11</td>
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Fig. 3. Schematic representation of the standard two-treatment crossover study design commonly employed in bioequivalence trials.
Table II. Data transformations performed in analysis of the simulated cyclosporine bioequivalence study

<table>
<thead>
<tr>
<th>Subject</th>
<th>lnAUC test</th>
<th>lnAUC reference</th>
<th>Difference test-reference</th>
<th>lnCmax test</th>
<th>lnCmax reference</th>
<th>Difference test-reference</th>
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</thead>
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<td>-0.45</td>
<td>4.94</td>
<td>5.42</td>
<td>-0.48</td>
</tr>
<tr>
<td>2</td>
<td>4.77</td>
<td>5.35</td>
<td>-0.58</td>
<td>2.61</td>
<td>3.00</td>
<td>-0.39</td>
</tr>
<tr>
<td>3</td>
<td>6.81</td>
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<td>0.46</td>
<td>4.37</td>
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<td>0.42</td>
</tr>
<tr>
<td>4</td>
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<td>4.65</td>
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<tr>
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<tr>
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<tr>
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<td>6.91</td>
<td>0.04</td>
<td>4.71</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SD</td>
<td>0.368</td>
<td></td>
<td></td>
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<td></td>
<td>0.405</td>
</tr>
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</table>

Two-Treatment Crossover Study

The typical study design employed in bioequivalence studies is the two-treatment, two-period, crossover design (Figure 3). In this study design, subjects are randomly separated into two groups of equal number. The innovator formulation is administered to group 'A' in the first study period, and the test formulation is administered to group 'B' in the first period. During the second study period, group 'A' receives the test formulation and group 'B' receives the innovator formulation. The first and second study periods are separated by a washout period, which is designed to be of sufficient duration to allow elimination of the drug administered in the first period. Subjects are separated into two groups to allow identification of 'period' or 'sequence' effects in the study results.

Bioequivalence Study Example

Data (AUC and Cmax) are presented from a simulated cyclosporine bioequivalence study with 12 subjects. Briefly, data were simulated with ADAPT II software(11) using a one compartment model and assuming the following values for pharmacokinetic parameters (mean ± interpatient SD): bioavailability 0.2±0.4, absorption rate constant 0.28h-1±0.13, clearance 53.8L/h±21.4, volume of distribution 301L±217. Intrapatient variances in model parameters were assumed as follows: bioavailability (0.2± mean value)2, absorption rate constant (0.23± mean value)2, volume of distribution and clearance (0.1± mean values)2. Data were simulated for a two-treatment, crossover study of 300mg of cyclosporine administered orally. These data were simulated assuming the same parameter values and variances for both study periods; consequently, there was no ‘true’ difference between the formulations in terms of their rate and extent of absorption. However, these parameter values lead to moderately high values of intrapatient variability in AUC (percent coefficient of variation: 19.3 percent) and in Cmax (percent coefficient of variation: 23 percent). These intrapatient variabilities agree well with the experimental results of Kovarik et al. in their investigation of intrapatient variability in cyclosporine pharmacokinetics following administration of the Sandimmune oral formulation(12). Simulated AUC and Cmax data are presented in Table I.

Analysis of Data for Bioequivalence Determination

A step-by-step approach is used to evaluate data for the determination of bioequivalence.

1. Calculation of the natural log of the AUC and Cmax data

The FDA advocates logarithmically transforming AUC and Cmax data prior to analysis(2). Either log10 or the natural logarithms of the data may be used. Natural logarithms are used in this example (Table II).

2. Calculation of the difference between the transformed data for each metric for each subject

The transformed value of the reference AUC is subtracted from the transformed value of the test AUC for each patient. This procedure is repeated for the transformed Cmax values (Table II).

3. Calculation of the mean difference between the transformed data

The mean difference exhibited in the transformed data are determined by summing the individual differences and dividing by the number of subjects in the study (e.g., (lnAUCtest-lnAUCreference)/number of subjects). The mean differences for the example data set are provided in Table II.

4. Calculation of the standard deviation of the difference between the transformed data

Standard deviation calculation is performed through the use of a hand-held calculator by students in class. Results are shown in Table II.

5. Selection of the appropriate t-value

T-values appropriate for the construction of confidence interval limits are dependent on the a-value of the statistical test and on the on the degrees of freedom of the test. For bioequivalence studies, a is always 0.05 and the degrees of freedom are determined as n-1, where n is the number of patients in the study. Common subject sample sizes and t-values for bioequivalence studies are as follows: 12-subjects: 1.7959, 18-subjects: 1.7396, 24-subjects: 1.7139.

6. Calculation of the high and low bounds of confidence intervals about the mean difference of the transformed data

High and low confidence bounds are calculated as:

$$HB = e^{(MD + [t-value \times SD / \sqrt{n}])}$$

$$LB = e^{(MD - [t-value \times SD / \sqrt{n}])}$$

Where HB and LB refer to the high and low bound of the confidence interval; MD and SD refer to the mean difference and standard deviation of the transformed metric. For example, for the AUC data, the HB of the confidence interval may be calculated as:

$$HB = e^{(MD + [t-value \times SD / \sqrt{n}])} = e^{(-0.0427[1.7959 \times 0.368/\sqrt{12}]) = 1.16}$$

The confidence intervals for AUC and Cmax are 0.79-1.16 and 0.76-1.15, respectively.
6. Decision rule
Bioequivalence is concluded if the confidence intervals about the ratio of AUC and Cmax both fall within the range of 0.8-1.25. That is the lower bounds of the confidence intervals must be greater than or equal to 0.8 and the upper bounds of the confidence intervals must be less than or equal to 1.25. In the present example, bioequivalence is not concluded as the lower bound of each confidence interval is less than 0.8.

Limitations of the Present Approach of Determining Bioequivalence

Formulations of ‘Highly Variable’ Drugs. Highly variable drugs have been defined as those demonstrating intrapatient variabilities greater than 30 percent in metrics of the extent and rate of absorption (e.g., AUC and Cmax) (9). Unfortunately, present FDA bioequivalence standards tend to penalize against generic formulations of drugs exhibiting high intrapatient variability, as this high variability often translates to wide confidence intervals about the mean difference in the bioavailability metrics (as demonstrated in the example above). For example, Tsang et al. have shown a failed bioequivalence determination from a small study assessing verapamil absorption from the same lot of sustained release tablets, administered on two occasions in a crossover study (8). In practical terms, large numbers of subjects are often required to produce confidence interval limits within the current guidelines. It has been argued that the current standards present an unfair obstacle to companies developing formulations of these drugs; however, the FDA is currently considering a draft guidance to address this concern (13).

Additional Concerns. The intent of the FDA designation of therapeutic equivalence is to ensure the switchability of drug formulations. Unfortunately, the typical approach of assessing bioequivalence is through small, tightly controlled studies with normal healthy subjects. Commonly, subjects are not permitted to take other medications during the study period; additionally, several factors are often controlled. For example, subjects are often selected based on age, weight, and smoker-status. Moreover, variables such as the timing and content of meals, liquid intake, and the amount of physical activity allowed during the study period are often regulated. These controlled factors may differentially influence the rate and extent of absorption of compared formulations; consequently, the results of these studies may not be indicative of results obtained in less controlled situations (e.g., typical outpatient therapy). These concerns are particularly significant when one considers the switchability of formulations of drugs with low therapeutic indices.

HOMEWORK ASSIGNMENT

Ten 12-patient bioequivalence trials are simulated for five different levels of intrapatient variability (i.e., 10 trials with 0, 10, 20, 30, and 40 percent intrapatient variability in bioavailability and absorption rate) with a one-compartment population model, using ADAPT II software (11). The simulations are performed such that no ‘true difference’ exists between the test and innovator formulation in the extent or rate of absorption. Each student is provided with data from a unique clinical trial and is required to determine if the results are indicative of bioequivalence. Results of the homework are reviewed in the next class. Students report the results of their study, which allows determination of the fraction of studies yielding the conclusion of bioequivalence under each level of intrapatient variability. The results (invariably) demonstrate that the fraction of studies showing bioequivalence decreases with increasing intrapatient variability; however, equally impressive is the intrapatient variability in AUC and Cmax shown in studies yielding the conclusion of bioequivalence. Overall, the homework exercise strengthens the students’ understanding of bioequivalence, and particularly the students’ understanding of the influence of intrapatient variability in bioequivalence studies.

AUTHOR’S NOTES

It is the author’s belief that the above homework assignment greatly enhances student understanding of the process and limitations of bioequivalency testing. In turn, the educational value of the homework assignment is believed to be enhanced through the distribution of individual data sets with specific values of intrapatient variability (and potentially specific ‘true differences’ in the rate and extent of drug absorption). Data of this nature are unavailable in the literature, but may be generated with clinical trial simulation software (as described above). Please contact the author if you wish to obtain copies of recently used data sets or copies of executable files capable of performing the clinical trial simulations described.


**References**