CHAPTER 9

Biopharmaceutical and Pharmacokinetic Characterization of Nutraceuticals

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INTRODUCTION

Some very important components of the drug approval process along with demonstrating safety and efficacy are biopharmaceutical and pharmacokinetic characterization. This is simply documenting the degree of absorption of the active ingredient(s) from the dosage form, for example, tablet absorption into the systemic circulation in which the active ingredient is assumed to be in equilibrium with the biophase, i.e., site of action. The following discussion will focus on guidelines for the pharmaceutical industry for biopharmaceutical and pharmacokinetic characterization of drugs. This brief summary will provide some insights into biopharmaceutical and pharmacokinetic characterization of nutraceuticals if it is eventually mandated by the FDA.
BIOPHARMACEUTICS: BIOAVAILABILITY AND BIOEQUIVALENCE

Oral Drug Dosage Forms

A commonly used dosage form for the delivery of medicines is the oral dosage form, such as tablets, capsules, suspensions, and solutions. Two important parameters that are related to the degree of absorption of the active ingredient of the dosage form are bioavailability and bioequivalence. Bioavailability is the measurement of the rate and extent of the active ingredient that reaches the systemic circulation and bioequivalence is when two different drug products with the same active ingredient have similar bioavailability [Shargel 1993]. For example, a generic company wants to market its own version of a brand name drug because the patent has expired.

Bioavailability and bioequivalence studies are important components of the drug approval process. Bioavailability for oral dosage forms can be documented via systemic exposure profile. This can be determined by measuring the active ingredient and, in some cases, its metabolite(s) from the system circulation, i.e., blood. An important regulatory concern is that the data from the dosage form used in clinical trials provide documentation of efficacy and safety. The bioavailability data can be used as a gold standard for subsequent bioequivalence studies [Food and Drug Administration 2003].

Four important types of studies to document bioavailability and bioequivalence in descending order of preference are as follows:

1. Pharmacokinetic
   a. Plasma
      i. \( t_{\text{max}} \)
      ii. \( C_{\text{max}} \)
      iii. AUC
   b. Urine
      i. \( D_{\infty} \)
      ii. \( dD/dt \)
      iii. \( t^{\infty} \)
2. Pharmacodynamic
3. Clinical
4. Dissolution

The active ingredient can be accurately quantitated pharmacokinetically in the plasma and urine, which gives the most objective data on bioavailability. Plasma data can determine three important parameters: \( t_{\text{max}} \), \( C_{\text{max}} \), and AUC. \( t_{\text{max}} \) is the time to reach maximum drug concentration in the plasma after administration. \( C_{\text{max}} \) is the maximum drug concentration in the plasma after administration. \( C_{\text{max}} \) is often a good marker for safety and efficacy for the drug because drug concentration in the plasma often corresponds to drug action and drug toxicity. AUC is the area under the drug concentration-time curve, and this is a measure of the degree of bioavailability. The AUC is the amount of drug that reaches the systemic circulation. Often,
the AUC is proportional to drug dose, unless there is saturation of drug metabolizing enzymes [Shargel 1993; Food and Drug Administration 2003].

The drug excreted in the urine can be used to estimate bioavailability. However, for this to be valid, the drug must be excreted significantly. Also, from a clinical trial participant perspective, peeing in a cup is less traumatic than a venopuncture. $D_u$ is the total amount of drug excreted in the urine and this is related to the amount of drug absorbed. $dD_u/dt$ is the rate of drug excreted in the urine, and it is graphically similar to the drug plasma concentrations. $t^*$ is the time for maximum urinary excretion, and this can be a useful parameter for bioequivalence studies [Shargel 1993; Food and Drug Administration 2003].

Pharmacodynamic studies or acute pharmacological effect studies measure drug action, for example, a reduction in blood pressure. Pharmacokinetic studies are preferred over pharmacodynamic studies. However, in some instances, a pharmacokinetic study is not possible, for example, no available bioassay or a bioassay with unacceptable accuracy or reproducibility. Thus, pharmacodynamic studies can be used to estimate the bioavailability. When conducting a pharmacodynamic study, measurements of drug action should be evenly divided over at least three half-lives of the drug to be within the estimated AUC [Shargel 1993; Food and Drug Administration 2003].

When a pharmacokinetic or pharmacodynamical study is not possible, then a clinical study can be used. The measurement of a clinical study is simply that the therapeutic treatment of the drug is a success, for example, complete cure from cancer. The assumption is that a therapeutic success occurred because there was enough bioavailability when the drug was administered. However, there can be a lot of pharmacodynamic and/or pharmacokinetic variability, such as diet, disease, or genetics, which can make it difficult to determine the cause of patient differences to drug action, such as therapeutic success or failure [Shargel 1993; Food and Drug Administration 2003].

In certain cases, dissolution studies, i.e., in vitro studies, can be used instead of the previously mentioned studies. This is especially true for drugs that are very soluble, permeable, and quickly dissolving. In addition, food-effect studies should be conducted because food can affect the bioavailability and bioequivalence of a drug dosage form, and this important topic will be discussed in more detail. Also, certain drug dosage forms contain multiple active ingredients that may be natural or synthetic, and it is unreasonable to quantify each active ingredient. Therefore, it is acceptable to conduct bioavailability studies with nutraceuticals because some, such as botanicals, potentially contain many active ingredients [Shargel 1993; Food and Drug Administration 2003].

Food Effect

Food-effect bioavailability and fed bioequivalence studies for oral drug dosage forms are part of IND applications and abbreviated new drug applications, respectively.
This includes both immediate-release and modified-release drug dosage forms. These studies are important because food can affect the rate and extent of absorption, which can impact the safety and efficacy of drugs and the focus of this chapter, namely nutraceuticals. Food can alter the bioavailability by various mechanisms, such as delay gastric emptying, alter gastrointestinal (GI) pH, and alter GI metabolism. High-calorie and high-fat meals, such as bacon, eggs, toast, hash browns, and a glass of milk, are used for food-effect bioavailability and fed bioequivalence studies because these are more likely to affect GI physiology, which can cause the largest effect when compared against fasting controls. It is important to keep in mind that food can affect the pharmacokinetics of the active ingredient and/or excipients, which can lead to the alteration of the bioavailability, and this is most likely to occur when the drug dosage form is taken right after the meal. The following are study considerations:

1. General design
2. Subject selection
3. Dosage strength
4. Test meal
5. Administration
6. Sample collection

The general design of the food-effect bioavailability study is fed versus fasting, single-dose, randomized, crossover design with an appropriate washout period. The fed bioequivalence study would include a comparison of the test and reference drug dosage forms. At least 12 healthy subjects should be included in bioavailability and bioequivalence studies unless safety concerns warrant the inclusion of patients with the disease state that is being studied, for example, antineoplastics for cancer treatment. The dosage strength to be studied is usually the highest strength that will be marketed. A high-fat, high-calorie meal is used as a test meal for food-effect bioavailability and bioequivalence studies. This type of meal is most likely to affect the GI tract, leading to the maximal affect on systemic availability. Subjects of fasted treatments should have fasted overnight for at least 10 h, and the drug dosage form, such as tablet or capsule, should be swallowed with 240 ml of water. No food should be eaten for at least 4 h after the dose was given. Subjects of fed treatments should have fasted for at least 10 h overnight, and they should have had 30 min to consume the high-calorie, high-fat meal, after which the oral dosage form with 240 ml of water is swallowed. No food should be eaten for at least 4 h after the dose was given. For both treatment arms, subjects receive standardized meals for lunch and dinner at the same time each day. Sample collection is usually from the plasma, and the types of pharmacokinetic parameters that are examined are AUC, t_{max}, C_{max}, and t_{lag} (for modified release dosage forms) [Food and Drug Administration 2002].

**Clozapine Tablets: Dissolution Testing**

An example of in vivo bioequivalence and in vitro dissolution testing guidance is with the clozapine tablet dosage form, which is required for abbreviated new
drug applications. Clozapine (Clozaril®) is used for the treatment of schizophrenia. Some important side effects of clozapine are agranulocytosis, seizures, and syncope. Because of the side effects, it is recommended that healthy subjects not be used for bioequivalence studies. The goal of the bioequivalence and dissolution studies is to compare the generic clozapine with the brand Clozaril® with regard to the rate and extent of absorption at equal doses. This is important because the generic form must demonstrate safety and efficacy just like the brand drug dosage form. If company X has marketed a popular nutraceutical in a tablet form and now company Y wants to market its tablet form of the identical nutraceutical, future regulations might require bioequivalence studies. An equal number of patients in a randomized schedule will receive either the generic or brand tablet dosage form at the same dose with 240 ml of water every 12 h for 10 days. Then the patients should be switched to the other tablet for a second period of 10 days. The highest tablet strength is used, which is 100 mg. The study must be first approved by an IRB and the names, titles, and curriculum vitae of medical and scientific directors should be documented. Patients who have been stable while taking clozapine for three months are eligible for the study. The patients white blood cell counts, blood pressure, heart rate, and body temperature need to be carefully monitored. Food does not appear to affect the bioavailability of clozapine. Although on day 10, patients should fast for 8 h before and 4 h after the administration of the morning dose because 14 venous blood samples will be collected from times 0.25–12 h. The following pharmacokinetic data should be collected and evaluated for bioequivalence:

1. Patient and mean drug concentrations
2. Patient and mean trough concentrations (C_{min,SS})
3. Patient and mean peak concentrations (C_{max,SS})
4. Patient and mean steady-state AUC_{interdose}
5. Patient and mean percentage drug concentration fluctuation
6. Patient and mean t_{max}

Two additional factors to consider are batch size and potency. The test batch should be no less than 10% of the largest batch planned for full production or a minimum of 100,000 units. The assayed potency of the generic should not differ from that of the brand by more than 5%. Dissolution testing should be conducted for all strengths of both generic and brand, with an n = 12 for each. Note that the same batch as the bioequivalence study is used [Food and Drug Administration 2005].

**PHARMACOKINETICS**

Before drugs can be approved for marketing, their pharmacokinetic profile must be studied. In particular, the investigational drug’s biotransformation or metabolism must be studied because the metabolism can affect the safety and efficacy of a drug. Inhibition of drug metabolism can lead to high concentrations, which can lead to toxicity, and induction of drug metabolism can lead to low concentrations, which can lead to lack of efficacy. Most drug dosage forms contain one active ingredient and
many inactive ingredients or excipients. However, nutraceuticals may contain many active and inactive ingredients, especially with botanical or herbal nutraceuticals, which could potentially affect its safety and efficacy and therefore potentially complicate active ingredient studies.

**Drug Metabolism**

The following are studies of *in vitro* drug metabolism that can lead to a better understanding of potential drug interactions [Food and Drug Administration 1997]:

1. Hepatic cytochrome P450
2. Additional hepatic enzymes
3. GI tract enzymes
4. Animal studies

Cytochrome P450 is a large family of enzymes that metabolize the majority of drugs. Some clinically important examples are CYP3A4, CYP2D6, and CYP2C9. The liver is an important site for drug metabolism, and the human liver microsomes are a common *in vitro* technique to study drug metabolism. Isolated hepatocytes and precision cut liver slices can give a more complete understanding of drug metabolism. Unfortunately, these two techniques have limited enzyme stability. The investigational drug is incubated with human liver microsomes, and different inducers and inhibitors of cytochrome P450 can be tested. A positive result might require additional *in vivo* drug metabolism and drug interaction studies [Food and Drug Administration 1997].

There are many additional hepatic enzymes that can influence drug metabolism. Some clinically important examples are glucuronidation, sulfation, and acetylation. The *in vitro* tests to evaluate their drug metabolism are not as well developed as with the cytochrome P450s.

The GI tract enzymes are especially important for oral drug dosage forms because of gut metabolism and subsequent absorption into the system circulation. Two clinically important enzymes that can affect drug absorption are CYP3A4 and P-glycoprotein. Therefore, compounds that inhibit CYP3A4 and P-glycoprotein can lead to increased concentrations of the investigational drug, and, conversely, compounds that induce CYP3A4 and P-glycoprotein can lead to decreased concentrations of the investigational drug [Food and Drug Administration 1997].

*In vitro* or *in vivo* animal studies during preclinical drug development can help elucidate the safety and efficacy of potential drugs, including nutraceuticals. Of special interest are potentially active metabolites found during human *in vitro* studies. These potentially active metabolites can be studied in animals to determine their safety and efficacy, which can help determine future *in vitro* or *in vivo* human studies. Furthermore, animal studies can be used to examine drug interactions with regard to parent drug and its metabolites. A difficulty with animal studies is how they relate to humans. For example, a metabolite that is toxic to the selected experimental animal species might not be toxic to humans [Food and Drug Administration 1997].

*In vitro* tests can help elucidate the metabolic pathway of the investigational compound, i.e., nutraceutical and this can determine the type of human *in vivo* tests that
need to be conducted. For example, if the in vitro tests indicate that the primary route of metabolism for the nutraceutical is via CYP3A4, then one would want to follow up with human in vivo tests on CYP3A4 inducers and inhibitors. However, if the in vitro tests concluded that the nutraceutical is not a substrate for CYP2C9, then it is not necessary to conduct human in vivo studies with CYP2C9 inhibitors and inducers [Food and Drug Administration 1997].

An important consideration is the timing of the in vitro metabolic studies. There are two important goals for the in vitro metabolic studies:

1. Identify important routes of metabolism and metabolites
2. Identify potential drug interactions

The earlier this information can be determined the better, but this must be balanced with capital expenditure. A company does not want to invest its precious capital if the nutraceutical encounters early problems and it is dropped from advancing. However, regulatory agencies are not going to allow a pharmacologically active compound to the market until there are in vitro and in vivo drug metabolism studies. Therefore, it is suggested that in vitro metabolic studies be conducted during Phase II preclinical drug development [Food and Drug Administration 1997].

If during in vitro drug metabolism studies the nutraceutical of interest is found to have activity as either an inhibitor or inducer, then human in vivo drug interaction studies should be conducted.

Drug Interactions

The following study design is adapted from drug-drug interaction studies as recommended by the FDA with its key components [Food and Drug Administration 1999]:

1. Design
2. Subjects
3. Interacting drug selection
4. Route of administration
5. Dose
6. Pharmacokinetic endpoints
7. Statistics

There are many different study designs that can be conducted, such as one-sequence crossover or randomized crossover. The selection of the study design can really depend on several factors, such as length of drug use or toxicity of the drug. These studies in general examine the concentration of the compound of interest, i.e., the nutraceutical, with the interacting compound, i.e., the inducer or inhibitor. An important consideration with the interacting compound is that inhibitors usually exert their activity much sooner than inducers, because inducers usually work by increasing the protein synthesis of the drug-metabolizing enzyme [Food and Drug Administration 1999].
Subjects for drug-drug interaction studies or nutraceutical-drug interaction studies are usually conducted in healthy volunteers unless safety concerns warrant the use of volunteers with the disease state of interest. In addition, pharmacogenomic analysis of the subject’s drug-metabolizing enzymes is encouraged because polymorphisms of the enzymes can dramatically affect safety and efficacy. This is especially true for CYP2D6, UGT1A1, etc. [Food and Drug Administration 1999].

The selection of the interacting drug with the nutraceutical is a very important consideration. Two general types of experiments should be considered:

1. Nutraceutical as the interacting compound to the drug
2. Drug as the interacting compound to the nutraceutical

The nutraceutical could cause either inhibition or induction of drug-metabolizing enzymes, which could affect drug concentrations. This information is important because drugs such as cyclosporine with a narrow therapeutic window, i.e., a specific concentration range, could lead to toxicity or lack of efficacy if the CYP3A4 enzyme is inhibited or induced. Table 9.1 lists several nutraceuticals that inhibit or induce a cytochrome P450 and the affected drug. Another possibility to explore is that a drug could affect the concentrations of the nutraceutical via inhibition or induction of drug-metabolizing enzymes. These enzymes can also biotransform endobiotics and xenobiotics, such as testosterone and benzo[a]pyrene. Therefore, these enzymes are not restricted to biotransform drugs only. Table 9.2 lists several drugs that can induce or inhibit drug-metabolizing enzymes that could be used to design the appropriate in vitro tests with the follow up in vivo tests if deemed necessary. Potentially, the FDA would like to know if a drug could affect the concentration of the nutraceutical if taken together [Food and Drug Administration 1999].

The route of administration for the in vivo drug-nutraceutical interaction study would depend on the dosage form that will be marketed. For example, if the nutraceutical will be formulated via a capsule only, then studies with an intravenous solution or oral suspension would not be necessary [Food and Drug Administration 1999].

The dose selection for the drug-nutraceutical interaction should use the maximum approved dose for the nutraceutical and drug in the shortest dosing interval to

<table>
<thead>
<tr>
<th>Nutraceutical</th>
<th>Enzyme</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grapefruit</td>
<td>CYP3A4</td>
<td>Felodipine [Baily et al. 1991]</td>
</tr>
<tr>
<td>Seville Orange</td>
<td>CYP3A4</td>
<td>Felodipine [Malhotra et al. 2001]</td>
</tr>
<tr>
<td>St. John’s Wort</td>
<td>CYP3A4</td>
<td>Cyclosporine [Bauer et al. 2003]</td>
</tr>
<tr>
<td>Watercress</td>
<td>CYP2E1</td>
<td>Chlorzoxazone [Leclercq, Desager, and Horsmans 1998]</td>
</tr>
<tr>
<td>Pomelo</td>
<td>CYP3A4</td>
<td>Tacrolimus [Egashira et al. 2004]</td>
</tr>
<tr>
<td>Lime</td>
<td>CYP3A4</td>
<td>Felodipine [Bailey et al. 2003]</td>
</tr>
</tbody>
</table>
detect the interaction. The dose of both might need to be further reduced because of safety concerns [Food and Drug Administration 1999].

The following pharmacokinetic endpoints could be recommended to be measured for drug-nutraceutical interactions: AUC, C$_{max}$, t$_{max}$ and sometimes CL, V$_{d}$, and T$_{1/2}$. For example, if nutraceutical A induces the metabolism of drug B, then the pharmacokinetic endpoints to be measured in a drug-nutraceutical interaction study would be AUC, C$_{max}$, and t$_{max}$ [Food and Drug Administration 1999].

An important statistical consideration for drug-nutraceutical interaction studies would be to capture clinically significant interactions, and this can be determined as 90% confidence intervals about the geometric mean ratio of the pharmacokinetic endpoints with and without the interacting compound [Food and Drug Administration 1999].

### REFERENCES


