Chapter 9
Design and Data Analysis in Drug Interaction Studies

David E. Nix and Keith Gallicano

9.1 Study Rationale

Drug interaction studies should be considered for drugs that are likely to be administered concomitantly to large numbers of patients. The drugs may be indicated for the same disease process, and their use in combination is considered therapeutically rational. Alternatively, the drugs may have different indications, but the two disease processes occur frequently in the same population. Drugs involved in interactions are divided into precipitant drugs (drugs that cause a change in the pharmacokinetics and/or pharmacodynamics of another drug) and object drugs (drugs affected by the precipitant drug). A drug can act as a precipitant drug and an object drug at the same time when two drugs affect each other during concomitant administration.

To study large numbers of potential interactions routinely for all drugs is not feasible or desirable. Consequently, screening methods are required to identify drugs that are likely to interact. A chemist who is knowledgeable about drug interactions affecting gastrointestinal absorption may be able to identify potential interactions involving chelation, physical binding, or other incompatibility. Metabolism of object drugs may be studied using in vitro cytochrome P450 (CYP) enzyme preparations to identify enzymes involved in the metabolism [1, 2]. Databases are available that list drugs that inhibit or induce various CYP subtypes. Once metabolism is determined to be a major elimination pathway and the responsible enzyme subtypes are known, these databases can be used to identify potential precipitant drugs [3]. Preliminary interaction studies of substrates with metabolic inhibitors and inducers can be performed using the same in vitro enzyme preparations as those used to

D.E. Nix (✉)
The University of Arizona College of Pharmacy, Tucson, AZ, USA
e-mail: nix@pharmacy.arizona.edu

K. Gallicano
Novum Pharmaceutical Research Services, Murrieta, CA, USA

M.P. Pai et al. (eds.), *Drug Interactions in Infectious Diseases: Mechanisms and Models of Drug Interactions*, Infectious Disease,
https://doi.org/10.1007/978-3-319-72422-5_9

determine metabolic pathways of substrates [2, 4]. Similar methods have been adapted to investigate drug interactions involving intestinal metabolism and drug transport [5–7].

Interactions involving protein binding displacement are not usually clinically significant. However, protein binding interactions should be examined for drugs that [1] exhibit high binding to plasma proteins (>90%), [2] have a narrow therapeutic index, [3] occupy most of the available plasma protein binding sites at clinically relevant concentrations, and [4] have a small volume of distribution (<10 L/70 kg). Drugs that are the most important candidates for drug interaction studies are those that are restrictively cleared by an elimination organ; a concern is also apparent for drugs that are nonrestrictively cleared, have a narrow therapeutic index and a small volume of distribution, and are administered intravenously. In the former case, a transient increase in unbound concentration could produce harmful adverse effects [8, 9]. Preliminary protein binding studies can be carried out in vitro, recognizing that metabolites may contribute to protein binding displacement interactions. Interactions involving renal clearance changes may be expected for drugs that are mainly eliminated by renal excretion. For these drugs, the presence of significant tubular secretion or reabsorption suggests possible interactions. Pharmacodynamic interactions should be suspected for drugs that have similar pharmacologic or toxicologic effects.

9.2 Study Design: General Issues

Current regulatory guidances provide some insight into designs for in vivo drug interaction studies [10, 11]. These guidances recommend three designs: (1) randomized crossover, (2) one-sequence crossover, or (3) parallel. A position paper by Pharmaceutical Research and Manufacturers of America (PhRMA) Drug Metabolism and Clinical Pharmacology Technical Working Groups has defined a minimal best practice for in vitro and in vivo pharmacokinetic drug-drug interaction studies targeted to drug development, with the goal of harmonizing approaches by regulatory agencies and industry sponsors [12]. The US Food and Drug Administration maintains a web page that covers many issues regarding drug-drug interactions in drug development from lists of prototype inhibitors and substrates to decision trees, labeling, and dose adjustment (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm; Accessed 3/1/2017).

Drug interaction studies involve the measurement of pharmacokinetics or a specific pharmacodynamic effect in the presence and absence of an interacting drug. Such studies typically employ a within-subject design in which individuals receive both treatments in either fixed or random order. A fixed-order design (single sequence) denotes a longitudinal study in which the treatments are administered sequentially over two or more time periods. Longitudinal studies are often conducted in patients who are receiving long-term therapy of the object drug or those

undergoing treatment with drugs possessing long elimination half-lives (>24 h). A two-period, longitudinal study involves the administration of the object drug alone followed by measurement of the pharmacokinetics or effect parameter(s) in period 1. A washout period may or may not be necessary. Then, the object and suspected precipitant drugs are concomitantly administered in period 2. Measurements of the pharmacokinetics or effect parameters are then repeated following administration of the combination treatment. In the longitudinal design, potential period effects are confounded with the treatment effects. If a change in the clearance (Cl) of the object drug is observed, the change may have been caused by the precipitant drug or by some other intercurrent event. Perhaps the food intake differed between the two periods (treatment phases), or a portion of the subjects acquired a mild viral infection between the two periods. If females are included as subjects, the number of subjects in the luteal phase of the menstrual cycle may differ between the two periods.

The study must be designed with full knowledge of the pharmacokinetics of both drugs. If the study involves single doses of the object drug, then adequate washout of the first dose must be allowed before starting the second treatment phase. For the control treatment, measuring serum concentrations or effect for at least four to five half-lives is important. If reduced clearance and increased half-life are expected, the sampling time may need to be extended following concomitant treatment compared to the control period. If the study involves multiple-dose administration of the object drug, then the serum concentrations should reach steady state during both periods. Steady state may take longer during the interaction phase if the half-life is prolonged.

The major advantage of a two-period, longitudinal design is that the potential for carryover effect from prior administration of the precipitant drug is avoided. A switchback design in which the object drug is replicated at least once after the precipitant drug is discontinued is useful to determine the effects of starting and stopping a metabolic inhibitor or inducer on the baseline characteristics of the object drug. Such a design was used to establish the rebound to baseline pharmacokinetic parameters of steady-state zidovudine at 14 d after rifampin was discontinued in period 2 [13].

9.2.1 Crossover Designs

A crossover study evaluates treatments administered in two or more planned sequences with subjects randomly allocated to the different sequences. The design is characterized by T, P, and S in which T is the number of treatments, P is the number of periods, and S is the number of sequences. All of these numbers must be ≥ 2 [14]. Designs that have a single (fixed) sequence are sometimes referred to as "crossover-like," but should be considered as a longitudinal study.

There are two main types of crossover designs: nonreplicated and replicated. Nonreplicated designs have the same number of treatments as periods, and the

number of possible sequences increases as the factorial of T (i.e., when $T = 3$, $S = 6$). Replicate designs have more periods than treatments, such that at least one treatment is replicated within a subject. Optimum designs are those that are balanced with equal numbers in each sequence and balanced for carryover effects and variance for the given number of treatments. A design that has each treatment followed by a different treatment the same number of times is balanced for carryover. The presence of a carryover effect is important to assess in drug interaction studies, and enough subjects in each sequence are needed to allow testing of this effect. In a variance-balanced design, each treatment appears the same number of times in each period.

The simplest nonreplicated crossover design is the 2, 2, 2 design. Suppose treatment A involves giving the object drug alone and treatment B involves giving the object drug with the precipitant drug. Subjects would receive the two treatments in one of two sequences, AB or BA, in which treatment A or B would be given during the first period and then switched to the other treatment during the second period. Carryover effects may be introduced for subjects receiving treatment B (sequence BA) in the first period if drug exposures of the object drug are increased by the precipitant drug. An adequate washout period must be planned between the two periods to prevent differential carryover in the two sequences. This may sometimes be difficult if the duration of an "adequate" washout period is not known a priori. Carryover and sequence effects, however, are confounded in the 2, 2, 2 design, and studies in which the two treatments are replicated must be conducted for optimal evaluation of carryover effects.

When nonreplicated studies involve more than two periods, the number of sequences should be carefully planned rather than testing all possible sequences. Usually a subset of sequences is chosen that defines a variance-balanced design. In a three-period, crossover pharmacokinetic study with treatments A, B, and C, six possible sequences ABC, ACB, BAC, BCA, CAB, and CBA must be included to maintain a carryover-balanced design. If carryover is a concern when the object and precipitant drugs are given together in treatments B and C, then a large sample size may be required to ensure an adequate number of subjects per sequence to test the carryover effect. A three-period crossover study may also be used to study potential bidirectional interactions. Here, treatments including drug A, drug B, and drugs A + B are required. A four-period, crossover study would have 4 or 24 possible sequences. The goal is to select four sequences from a 4×4 Latin square in which each treatment is administered once during each of four periods, each subject receives all four treatments, and each treatment follows the other three treatments once (balanced for carryover). An example of a "Williams design" involves the four sequences (ABCD, BDAC, CADB, and DCBA). The total number of subjects is selected as a multiple of 4 and subjects are randomized in blocks of 4 to undergo treatments in the sequence assigned [15].

There is considerable interest in replicate crossover designs for bioequivalence studies in which the test and reference treatments are administered each on two separate occasions. This allows for assessment of intraindividual variability in systemic exposure and estimation of carryover effects. The analysis of replicate designs

considers that some individuals may differ from the mean response and allows for the determination of "individual bioequivalence." Optimal designs for carryover estimation of the two treatments are AA, BB, AB, and BA for two-period designs, ABB and BAA for three-period designs, and AABB, BBAA, ABBA, and BAAB for four-period designs [14, 16–18]. Switchback designs, either ABA and BAB or ABAB and BABA, are preferred to estimate the intraindividual variability [14]. Similar designs may be employed for drug interaction studies because they increase the confidence that a drug interaction detected is a true interaction.

Replicate measurements may also be obtained in more traditional study designs. As an example, the object drug may be administered as a multiple-dose regimen, and measurements can be made during more than 1 day or dosing interval before changeover to the next treatment. This was done in a randomized crossover study to investigate the interaction between cimetidine and theophylline [19]. Theophylline was administered at a subject-specific dose (concentration controlled) for 23 days. Subjects received treatment 1 (cimetidine or placebo) on days 5–11, washout on days 12–16, and treatment 2 (cimetidine or placebo) on days 17–23. The order of cimetidine and placebo treatments was randomly assigned. The pharmacokinetics of theophylline were assessed on the first, fourth, and seventh days of each treatment period. In the analysis, the data from the fourth and seventh days were treated as replicate measurements of the effect at steady state. Because theophylline exhibits large interindividual variability in clearance, doses were adjusted in a run-in phase to provide similar mean steady-state concentrations before evaluating the interaction. This example also shows how concentration control can be incorporated into the design of a drug interaction study.

9.2.2 Parallel Designs

A parallel design may be used for evaluating drug interactions. However, such designs are less desirable, because the drug variability is usually greater between individuals than within individuals. A simple parallel design study consists of two groups of subjects/patients, one group that is receiving the object drug and one that is receiving the object drug concomitantly with the suspected precipitant drug. Most studies of this type are performed in patient populations that are receiving the drug or drugs therapeutically. There may be problems with comparability of the two patient groups in terms of pharmacokinetics of the object drug regardless of the precipitant drug. The two groups may or may not be randomly selected. If random assignment is not used, additional issues of bias must be considered. When studies of this type are necessary, the use of population modeling may be used for evaluating the presence or absence of the interaction. An example of using population modeling to evaluate a drug interaction involved imipramine and alprazolam [20]. The parallel design may be advantageous for drugs with long elimination half-lives in studies where a long washout period is impractical for a crossover or longitudinal design. When there are safety concerns, randomized studies may not be ethical.

If a population can be identified that requires one or both of the drugs of interest for therapeutic reasons, a convenience sample (sparse or rich sampling) can be used with population PK modeling to study their potential interaction.

A placebo-controlled, parallel-group study can be conducted when possible inherent group differences in a parallel design or time-dependent effects in a single-sequence longitudinal design are a concern. Subjects in each group receive treatment on more than one occasion, and treatment effects are adjusted for baseline values in the first period (placebo) of each treatment group. Alternatively, the mean treatment differences are estimated within each group, and then these differences are compared between treatment groups. A placebo-controlled, parallel-group design was used to show no clinically significant effect of indinavir on the pharmacokinetics of voriconazole [21] and to demonstrate that ritonavir inhibited the metabolism of rifabutin [22].

9.2.3 Mechanistic Aspects

Drug interactions may be very complex. The mechanism of potential interaction is important to hypothesize from in vitro studies, previous clinical and preclinical studies, and experience with other related drugs. Such knowledge is essential to planning a good drug interaction study. Most studies are designed to evaluate the effect of a precipitant drug on an object drug. The precipitant drug may cause some physical or physiologic effect that alters the pharmacokinetics or pharmacodynamics of the object drug. Several questions need to be posed about the precipitant drug in relation to developing the study methods. What are the doses and administration schedules that are relevant to clinical practice? Is the interaction concentration dependent within the range of clinically achievable concentrations? Does the interaction take time to develop (e.g., P450 induction)? What is the primary goal of the study (e.g., to find the maximum potential interaction)? In some circumstances, one may be interested in whether the pharmacokinetics and/or pharmacodynamics of both drugs are affected by concomitant administration.

Multiple dosing of the precipitant drug is often desirable. The object drug may be administered as a single dose or in a multiple-dose regimen designed to achieve steady state. A single dose of the object drug may be appropriate when inhibition of elimination is suspected and safety concerns are substantial. In such cases, unpredictable accumulation would be avoided. One exception occurs when an object drug undergoes extensive first-pass metabolism and the precipitant drug inhibits this metabolism. Much greater systemic bioavailability may result even with single-dose administration.

Concerns about multiple-dose studies are exemplified by a study of voriconazole effects on cyclosporine pharmacokinetics. This study included renal transplant patients receiving treatment with cyclosporine that was continued throughout the study. Subjects received voriconazole or placebo for 7.5 days (period 1), underwent a washout period of at least 4 days, and then received the alternate treatment

(voriconazole or placebo) for 7.5 days. Although 14 subjects were entered, only 7 completed the study and 7 were withdrawn during the voriconazole treatment. Voriconazole resulted in a mean 1.7-fold increase in cyclosporine exposure [23]. Although a multiple-dose regimen of the object drug may simulate clinical use and provide greater applicability, safety would favor a single-dose study in healthy subjects first. The addition of procedures to limit exposure to high concentrations during the interaction phase for a follow-up multiple-dose study needs to be considered. For example, the study could employ a dose reduction during the combination treatment. More extensive knowledge of the potential study outcomes, frequent and careful clinical monitoring, and perhaps real-time drug concentration monitoring may be necessary when the object drug is administered in a multiple-dose regimen.

9.2.4 Study Population

Drug interaction studies are most commonly performed in healthy volunteers. Healthy subjects are easier to recruit, the investigators can better control concomitant medications and activities, and study participation may be safer compared to patients with target illnesses. There is no compelling reason why performing a pharmacokinetic interaction study in healthy volunteers is less desirable than performing the study in a target population likely to receive both drugs, unless disease in the target population influences the magnitude of interaction or safety considerations prevent the use of healthy volunteers. The elderly are often cited as a group more susceptible to drug interactions. This is true because elderly patients receive more drugs and interactions only occur when two or more drugs are given concurrently [24]. In addition, geriatric patients and patients with organ failure may eliminate drugs more slowly and achieve higher concentrations than healthy/young counterparts. Administering reduced doses in these special populations designed to achieve exposure that is similar to that observed in healthy volunteers may reduce potential differences in exposure.

Interaction studies that involve pharmacodynamic assessments may or may not be best performed in the target population, depending on the nature of the pharmacodynamic effect. Suppose an object drug reduces wheezing and acute bronchospasm and increases forced expiry volume in 1 s (FEV-1) in patients with asthma. Administration of a precipitant drug in combination with the object drug leads to worsening of symptoms and lowering the FEV-1 in asthma patients. However, these effects are not seen in patients without asthma. Such an interaction would need to be studied in the target population.

One report of an interaction between a laxative polymer and digoxin found a pharmacokinetic interaction consistent with a 30% decrease in digoxin absorption. The concluding statement was “there was no consequence of this interaction on heart rate and atrial ventricular conduction.” The study was conducted in healthy volunteers, and digoxin administration was not associated with changes in atrial

ventricular conduction with or without the laxative administration. Although a small decrease in heart rate was noted following digoxin dosing, the laxative did not alter the observed change [25]. This study demonstrates the importance of using relevant pharmacodynamic parameters and a relevant study population. The pharmacodynamic parameter should be a validated surrogate marker and be sensitive to changes in response. Had the study been conducted in patients with atrial fibrillation and used a therapeutic dose of digoxin, changes may have been apparent. Discussions on specific issues relating to pharmacodynamic drug interactions are beyond the scope of this chapter because the endpoint parameters depend on the pharmacology of the specific drug class and the characteristics of the parameter itself.

9.3 Pharmacokinetic Interaction Studies

9.3.1 Interactions Affecting Drug Absorption

Drug interactions may involve absorption or other aspects of drug delivery. This chapter does not address pharmaceutical or physicochemical interactions that occur in vitro or ex vivo such as incompatibility involving intravenous admixtures or mixing within intravenous administration tubes. Drug interactions commonly occur with drugs that are administered orally. Most of these interactions involve the effect of a precipitant drug on gastric pH or physical interactions between the two drugs. If an acidic environment in the stomach is required for optimal dissolution, reduced absorption in the presence of drugs that increase gastric pH may occur. The interaction between acid suppressants (e.g., cimetidine or omeprazole) and ketoconazole or itraconazole is a classic example of this type of interaction [26, 27]. Interaction studies should be performed for drugs that have greatly reduced solubility at neutral pH compared to pH < 3. One must be careful to provide sufficient doses of the acid suppressant to increase gastric pH to >6 during the absorption period [28]. Continuous monitoring of gastric pH is recommended to ensure that the target pH is attained.

Many drugs bind or complex with other drugs, thereby preventing gastrointestinal absorption. Examples of this type of interaction include tetracycline and calcium carbonate, ciprofloxacin and aluminum antacids or iron products, and norfloxacin and sucralfate [29–31]. These interactions occur when both drugs are present in the stomach and upper gastrointestinal tract at the same time. Maximum interaction usually occurs when the precipitant drug is administered slightly before or at the same time as the object drug [30]. Although not well studied, differences in gastric pH, gastric emptying time, and transintestinal elimination of drug may influence the extent of these interactions.

In the infancy of pharmacokinetics, drug absorption after oral administration was regarded as a passive diffusion process affected by pH (portion unionized) and lipophilicity. We now know that the process is extremely complex and involves many

transporters located in the basolateral and apical (lumen) sides of the gastrointestinal epithelium. There are numerous transporters on the apical membrane, some of which serve to facilitate absorption of drugs (e.g., peptide transporter 1 (pPEPT1) and organic cation transporters (OCT1/3)), and others serve to limit intestinal absorption (e.g., p-glycoprotein (pGP), multidrug resistance protein (MRP2)) [32]. Beta-lactam antibiotics are very hydrophilic drugs and would be expected to poorly diffuse across lipid membranes. However, some beta-lactams exhibit high oral bioavailability through vectorial apical to basal transport utilizing PEPT1 on the apical side and MRP3 on the basolateral side [32]. In contrast, fluoroquinolone antibiotics may inhibit PEPT1; however, this interaction is based on in vitro assessment of potential rather than clinical studies [33]. The best-known efflux transporter is probably pGP, which has a substrate specificity similar to CYP3A4. Substrate drugs are absorbed through the intestinal mucosa into the enterocytes and then transported back out into the intestinal lumen by pGP. There is an abundance of CYP3A4 present to metabolize the same substrate, resulting in a cycle of entry, efflux, and metabolism, which substantially limits bioavailability of some drugs. Strong inhibitors of pGP such as itraconazole or HIV protease inhibitors inhibit both pGP and CYP3A4 and result in very large increases in bioavailability of drugs like nadolol [34, 35]. In 2010 an International Transporter Consortium was formed and identified seven transporters of particular importance including pGP, BCRP, organic anion-transporting polypeptides (OATP1B1 and OATP1B3), organic cation transporter (OCT2), and organic anion transporters (OAT1 and OAT2). The list was updated to include multidrug and toxin extrusion proteins (MATE1 and MATE2K), multidrug resistance protein (MRP2, MRP3, and MRP4), and bile salt export pump (BSEP). Candidate probe substrates and inhibitors were proposed, although the substances often lack specificity for a given transporter [36].

The liver also operates with involvement of transporters. Drugs entering the portal circulation or in systemic circulation can be transported into hepatocytes by organic anion transport proteins (OATP1B1, OATP1B3, OATP2B1, OATP1A2, OAT1, OAT2) and sodium-taurocholate cotransporting polypeptide (NTCP). The drug can be extruded from the hepatocyte into blood by MRP3, MRP4, or MRP5. Finally, a drug can be transported into bile using several transporters including pGP, MDR3, MRP2, BCRP, and BSEP [34].

The most recent FDA guidance for drug interactions provides a decision tree for evaluating transporter drug interactions [10]. The guidance recommends that a cell-based assay be used to evaluate whether the drug is a substrate for pGP or BCRP, particularly if the drug is intended for oral administration. If the drug undergoes hepatic or biliary secretion to a significant extent (Cl $\geq$25% of total clearance), it is important to investigate whether the drug is a substrate for OATP1B1 and/or OATP1B3 using an in vitro system. If any of the screening results show that the drug is a substrate for these transporters, selected in vivo drug interaction studies are recommended. A list of known inhibitors and inducers for common transporters is provided in the FDA guidance. There should also be screening to determine if the drug induces or inhibits selected transporters. Assessment of effects on pGP is recommended, for example, if the drug inhibits or induces CYP3A4 in vitro [10].

9.3.2 Interactions Affecting Drug Distribution

Drug distribution may be affected by drug interactions. However, many studies conclude differences in volume of distribution that represent artifact rather than true differences. Changes in volume of distribution should be examined using intravenous dosing whenever possible. When oral administration is used, apparent changes in volume of distribution may represent changes in bioavailability. Comparisons should be made using steady-state volume of distribution (V_{ss}) only. Frequently V_{area} (also designated as V_z) is used for comparisons. However, this parameter is greatly affected by changes in the terminal elimination rate constant.

Steady-state volume of distribution may also be affected by experimental problems. Suppose a drug is well described using a three-compartment model when administered alone. The same drug is given after 10 d of rifampin treatment and the clearance is greatly enhanced. Drug concentrations are substantially lower following rifampin treatment, and the profile is best described using a two-compartment model. Presumably, the third exponential phase would remain present, but the concentrations may be undetectable with the assay used. V_{ss} is equal to mean residence time (AUMC/AUC) multiplied by systemic clearance (Cl) for an intravenous bolus dose, where AUMC is the area under the first moment of the plasma concentration-time curve. Although AUC would be decreased and Cl increased as a result of the interaction, these parameters would be affected minimally by missing the third exponential phase. However, the third exponential phase contributes a large portion of the total AUMC for the control treatment. Excluding this phase following rifampin treatment will cause an apparent decrease in the V_{ss}. Thus, problems fitting the control and interaction phases to the same model with equal reliability could result in apparent changes in V_{ss} when no true change occurred. Similar problems would occur with non-compartmental analysis, but the problem would not be as apparent.

Examples of drug interactions affecting distribution include the interaction between ceftriaxone and drugs that increase free fatty acid concentrations (e.g., heparin). Free fatty acids displace ceftriaxone from protein binding. In this example, there were profound physiologic changes due to cardiopulmonary bypass, administration of high-dose heparin and methylprednisolone, and intravenous fluids. Along with this there were profound changes in the free ceftriaxone concentrations and renal clearance [37]. On a positive note, the free (active) ceftriaxone concentrations would be highest during the operation and could boost efficacy as a prophylactic antibiotic; however, persistence with longer operations may be reduced. Such an interaction is generally not clinically significant because the increased free fraction (microbiologically active drug) results in no change in average steady-state unbound concentrations in plasma even though renal clearance is increased. In general, for drugs that are highly protein bound, protein displacement interactions may be clinically relevant when the object drug has a narrow therapeutic range and a small volume of distribution (<10 L/70 kg) [8, 9].

A potentially significant situation involves parenterally administered drugs that exhibit a high extraction ratio. Here nearly all of the drug that passes through the organ is removed or metabolized including both bound and unbound drugs. Displacement from protein binding will have no effect on the total clearance of the drug. However, the increased free fraction of drug may result in greater pharmacodynamic activity while the precipitant drug is present. For the interaction to be significant, the object drug must have a narrow therapeutic index so that the increase in free drug concentration will have toxicologic significance. Overall, protein binding displacement interactions are rarely clinically significant.

9.3.3 Interactions Affecting Renal Excretion

Changes in renal excretion of drugs can be subdivided into effects on filtration, secretion, and reabsorption. Glomerular filtration of drugs is limited by protein binding and only unbound drug is filtered. Drug interactions involving displacement of an object drug from serum protein will result in transiently higher unbound serum concentrations and lead to increased renal clearance for object drugs that have a low renal extraction ratio. The clinical significance of protein binding displacement is limited by the compensatory increase in renal clearance as lower total serum concentrations from increased clearance compensate for the increased free fraction.

Tubular secretion involves active transport of drugs from the serum to the tubular lumen mediated by a number of drug transporters. Separate transport systems are present for cationic and anionic compounds, but these transport systems have a very low degree of specificity. Various transport proteins are located on the basolateral side of the proximal tubular cells including OAT1/3, OAT2, OATP4C1, and OCT2, which are in the solute carrier family (SLC22A). These transporters mediate facilitated transport across an electrochemical gradient often exchanging for an ion (e.g., Na^+ and H^+) or another solute (e.g., dicarboxylate). The substrate is delivered from blood to the cytoplasm of proximal tubule cells [38]. OAT1/3 transport represents important pathways for secretion of many beta-lactam antibiotics, tetracycline, ciprofloxacin, acyclovir, adefovir, cidofovir, entecavir, stavudine, tenofovir disoproxil fumarate, and zidovudine. OCT2 is an important transporter for lamivudine and zalcitabine, although other anti-infective drugs are also substrates [39]. As with CYP450 enzymes, in vitro systems have been developed using probe drugs including furosemide for OAT1/3 and metformin for OCT2. These cell-based systems can be used to screen for potential inhibitor drugs [40]. In another system, adefovir and benzylpenicillin were used as probes for OAT1 and OAT3. Probenecid inhibited the uptake of both adefovir and benzylpenicillin, whereas para-aminohippurate (PAH) selectively inhibited adefovir uptake. These in vitro results were predictive of the interaction observed in humans, although a 47% increase in benzylpenicillin renal clearance induced by PAH was not expected. Penicillins, cephalosporins, and carbapenems are transported in the kidney by OAT1 and to a greater extent by OAT3 promoting the accumulation of the drugs in the cytoplasm of renal tubular cells.

Other transport proteins may be involved in extruding the drugs from the cell into the tubular lumen. A few members of these beta-lactam family have produced nephrotoxicity, and the possibility exists that this is related to transport and accumulation of too much drug in the proximal tubular cells. Antiviral drugs including adefovir, cidofovir, and tenofovir undergo transport by OAT3 and cause nephrotoxicity [41]. In the case of cidofovir, probenecid is used to reduce nephrotoxicity by inhibiting basolateral transport and intracellular accumulation in renal tubular cells [42].

Transporters are also integrated on the apical membrane of tubular cells and are involved in getting drug from the cytoplasm to the tubule lumen. Cation transport proteins include pGP, organic cation transporters (OCTN1/2), and MATE1/2. The organic anion transport proteins include MRP2/3, OAT4, and urate transporter (URAT1). OCTN, OAT4, and MATE1/2 are in the SLC family (SLC22A or SLC47A), whereas pGP and MRP are ATP-dependent active transporters [38]. Much less is known about the role of the apical efflux proteins in the context of drug interactions. Digoxin is a well-known substrate for pGP for which clarithromycin serves as an inhibitor. As pGP is found in many tissues, components of the interaction are difficult to dissect. However, clarithromycin coadministration with intravenously administered digoxin results in about 20% increased digoxin exposure (AUC) in part because of a 40% reduction in non-glomerular clearance [43]. From a toxicology perspective, accumulation of drug in the proximal tubule is a major determinate of kidney toxicity. Such accumulation is related to the balance of uptake across the basolateral membrane and trafficking across the apical membrane (extrusion and passive re-update). Minimizing update by inhibiting basolateral transport and being careful not to inhibit apical extrusion may be important to modulate nephrotoxicity risk.

Precipitant drugs may inhibit tubular secretion resulting in reduced renal clearance. Drugs that are extensively eliminated in the urine and have significant tubular secretion (renal clearance of free drug greater than 150% of glomerular filtration) are good candidates for studying this interaction mechanism. The normal glomerular filtration rate is about 120 mL/min, and the renal blood flow is approximately 1100 mL/min for a 70 kg adult. A drug can have a renal clearance approaching renal blood flow rate, as is observed with PAH, owing to its extensive tubular secretion. However, drugs that exhibit restricted to intermediate renal clearance are more susceptible to drug interactions involving inhibition of tubular secretion. The partitioning of a drug into red blood cells and the ability to diffuse out of red blood cells may also influence tubular secretion.

Probenecid may be administered with certain beta-lactam drugs to prolong their elimination rate. The beta-lactam agents most affected by this interaction have a high ratio of renal clearance to glomerular filtration rate and rely on the kidney as their major clearance organ. Before penicillin resistance was prevalent, a combination of probenecid and high-dose amoxicillin was used to provide single-dose treatment for uncomplicated gonorrhea [44].

To assess drug interactions involving renal excretion, collection of both urine and plasma (or serum) is required. A measure of the glomerular filtration rate before or

during the study is helpful to explore the mechanism of interaction. Glomerular filtration rate (GFR) can be determined by radiolabeled ^{99m}Tc-diethylenetriamine pentaacetic acid clearance, ^{125}I–iothalamate clearance, inulin clearance, or creatinine clearance (with concurrent cimetidine treatment) [45–47]. Measurement of creatinine clearance also serves as a rough measure of GFR. However, overestimation of GFR is expected owing to a small component of tubular secretion. Although unusual, the tubular secretion of creatinine may be large. As cimetidine inhibits the tubular secretion of creatinine, concurrent treatment during urine collection can improve the estimate of GFR [47].

Competitive inhibition of tubular secretion is typically concentration dependent and is influenced by the concentration of the precipitant and object drugs. Concentration-dependent renal clearance of the object drug is established by collecting urine in intervals less than or equal to one half-life duration. Blood samples collected at the beginning and end of each urine collection interval are a minimum requirement, but more blood samples taken during the collection interval will provide a better estimate of plasma AUC. The renal clearance is calculated for each interval and would be expected to increase as drug concentrations (plasma AUC) decline. A precipitant drug may have only minor effect on the renal clearance when concentrations of the object drug are high, because saturation may already be present. However, a drug that potently inhibits tubular secretion should prevent the increase in renal clearance seen at low concentrations of the object drug. The precipitant drug must be present in sufficient concentrations throughout the observation period to observe inhibition. Thus, continuous infusion or frequent dosing of the precipitant drug may be required unless the half-life of the precipitant drug is long. An interaction study also may be planned using dosing regimens likely to be used in clinical practice. However, information about the mechanism of interaction may be lost. An assumption usually made in pharmacokinetics is that clearance of the object drug is stable during each assessment period. If there are large differences in peak and trough drug concentrations of the precipitant drug over the period in which the pharmacokinetics of the object drug is assessed, this assumption may be violated because the degree of inhibition depends on inhibitor concentration. Information about the mechanism of interaction may also be lost if urine is collected in only one interval to obtain the average renal clearance.

Tubular reabsorption is usually a passive process whereby drug present in the tubular lumen (high concentration) diffuses back into the capillary lumen and returns to circulation. The drug must be unionized to diffuse across the tubular membrane. Interactions occur from altered pH in the tubular lumen or from physical interaction between the precipitant and object drug within the tubular lumen. An independent measure of tubular secretion, filtration, and reabsorption is not possible in the clinical setting. Instead, only the overall renal clearance is measured, and the intrinsic clearance is compared to GFR to classify the elimination as net tubular reabsorption, filtration, or net tubular secretion.

9.3.4 Interactions Affecting Drug Metabolism

CYP enzymes metabolize many anti-infective drugs whose pharmacokinetics are affected by drugs that inhibit or induce these enzymes. Several anti-infective agents act as inhibitors (ritonavir, ciprofloxacin, etc.) or inducers (rifampin, rifabutin, etc.) of CYP enzymes. Goals for a metabolism interaction study are important to establish. The goal may be to determine if a clinically significant interaction is likely between two drugs or to determine more broadly if a drug serves as a precipitant drug involving a particular enzyme system. The precipitant drug should be administered in a clinically relevant, multiple-dose regimen with sufficient duration to achieve steady-state pharmacokinetic conditions. Longer durations of treatment may be required for time-dependent interactions. For example, the maximum induction with rifampin takes 10–13 days [48]. When no prior knowledge is available, multiple dosing for at least 1 week is usually sufficient. A longitudinal design in which the object drug is studied alone and then following treatment with the precipitant drug is preferred in the absence of prior knowledge about the interaction offset time. If the offset time is of interest, the object drug may be studied again one or more times after the precipitant drug is stopped.

More than 50% of drugs that undergo metabolism are metabolized primarily by CYP3A enzymes. These enzymes are induced by rifampin, rifabutin, phenytoin, carbamazepine, and barbiturates and are present in the gastrointestinal tract, liver, and other organs. CYP3A4 enzymes are responsible for first-pass metabolism of many drugs, and their inhibition may lead to pronounced increases in systemic bioavailability of orally administered object drugs that undergo first-pass metabolism. Precipitant drugs may induce or inhibit CYP3A4. Candidate object drugs are those that rely on metabolism by CYP3A4 enzymes for a substantial portion of their clearance. Midazolam is an excellent marker of CYP3A4 activity because its elimination depends almost entirely on hydroxylation by CYP3A subfamily of enzymes to form 1-hydroxy midazolam [49, 50]. Drugs that affect CYP3A activity in the gastrointestinal tract or liver may affect the apparent clearance of oral midazolam. *N*-demethylation of erythromycin is also catabolized by CYP3A and this metabolism occurs mostly in the liver. The intravenous administration of [[14]C-*N*-methyl]-erythromycin and measurement of $[14]CO_2$ in breath provide a convenient marker of CYP3A4 activity in the liver (not gastrointestinal tract) [51–53] even though potential limitations of the test have been identified [54]. Cortisol is metabolized to 6β-hydroxycortisol by CYP3A4 isozymes. The measurement of urinary 6β-hydroxycortisol/cortisol ratio remains fairly stable without circadian differences. Agents that affect CYP3A4 enzyme activity usually cause changes in the 6β-hydroxycortisol/cortisol ratio [52, 53]. These markers are useful tools to identify induction or inhibition of CYP3A4, although changes in clearance may not correlate quantitatively among the different markers.

Other common metabolic enzyme pathways involve CYP1A2 and the polymorphic CYP2D6 and CYP2C19 isozymes. Probe drugs are caffeine and theophylline for CYP1A2 [55, 56], debrisoquin and dextromethorphan for CYP2D6 [57], and

omeprazole and mephenytoin for CYP2C19 activity [58]. For caffeine and theophylline, changes in systemic clearance are usually evaluated. The measurement of paraxanthine/caffeine ratio in saliva at 6 h after caffeine intake also correlates with CYP1A2 activity [59]. CYP2D6 activity can be assessed by measuring changes in the dextromethorphan/dextrorphan ratio in urine [57]. CYP2C19 activity can be evaluated from the urinary *S*-mephenytoin/*R*-mephenytoin ratio after administration of racemic mephenytoin [60].

Markers of CYP isozyme activity are useful to evaluate whether a potential precipitant drug affects metabolism. There is also a need to evaluate whether a drug serves as an object drug resulting in toxicity, loss of therapeutic activity, or reduced effectiveness. Agents that are known to inhibit CYP1A2 (cimetidine, enoxacin), CYP3A4 (itraconazole, ketoconazole), CYP2D6 (quinidine, cimetidine), and CYP2C19 (omeprazole, fluconazole) are well known [61–65]. However, not all of these drugs have specific effects on only one isozyme. Rifampin, rifabutin, carbamazepine, and phenytoin are inducers of CYP3A4 and other enzymes [62, 63]. Lists of enzyme inhibitors and enzyme substrates can be found in recent publications [62, 64, 65].

If feasible, active or toxic metabolites in plasma and urine should be measured because the magnitude and direction of metabolite pharmacokinetic changes are often unpredictable. Multiple metabolic enzymes and pathways can confound predictions. The AUC of metabolite may be altered even if the metabolite is not a product of the affected pathway. Detectable changes in AUC of the parent drug may not be apparent if a minor metabolic pathway is affected or if compensatory changes in hepatic and renal clearance occur. Thus, there is a danger in concluding "no interaction" from data involving only the parent drug. Metabolic parameters such as the metabolic AUC ratio and the urinary recovery ratio of metabolite to parent drug can give useful information on mechanisms of interaction, particularly if the metabolite is eliminated exclusively by renal excretion.

9.3.4.1 Impact of Pharmacogenomics

Metabolic interactions are sometimes complicated by the existence of polymorphic enzyme expression. A recent trend in metabolic interaction studies is to characterize subjects by genotype and/or phenotype into extensive, intermediate, or poor metabolizers. In several of the studies reviewed, subjects were recruited without considering genotype or phenotype, leading to a very low number of subjects in less common metabolic groups [66–74]. Although more difficult and more expensive, the design would be improved by recruiting subjects based on genotype or phenotype with a target minimum number of subjects in each category. Larger clinical trial units should consider developing a subject database that includes genotype results for enzymes such as CYP3A5, CYP2C19, CYP2C9, and CYP2D6. Subject recruitment could be planned using a predictor panel concept similar to that used in microbiology to examine susceptibility against a panel of bacteria with categorized resistance mechanisms [66].

The impact of metabolic polymorphisms may vary substantially as demonstrated in the following examples. The effect of ritonavir on voriconazole exposure was studied in 20 subjects, which included 8 homozygous extensive metabolizers (EMs), 8 heterozygous EMs, and 4 poor metabolizers (PMs) based on CYP2C19 genotype. Total exposure ($AUC_{0-\infty}$) was increased 54% in homozygous EMs, 94% in heterozygous EMs, and 907% in PMs. Voriconazole Cl/F varied about eightfold during the placebo phase and part of this variation was from metabolizer status. Adding ritonavir resulted in about 70-fold variation from the highest Cl/F in a homozygous EM subject at baseline to the lowest Cl/F in a PM subject receiving ritonavir [67]. Findings have been mixed with CYP2C19. Moclobemide resulted in a significant increase in omeprazole AUC, an effect that was limited to EMs [70]. However, in the case of tacrolimus with administration of either lansoprazole or rabeprazole, an interaction was noted only in CYP2C19 PMs who also had the CYP3A5*3/*3 genotype. CYP3A activity becomes more important in these subjects as CYP3A4 and CYP3A5 exhibit a similar substrate profile [71]. Clarithromycin inhibited CYP2C19-mediated metabolism of omeprazole in EMs, IMs, and PMs to a similar extent. However, clinical implications become apparent after considering that coadministration of the two drugs in PMs resulted in 30-fold higher exposure (AUC) compared to the AUC in the EM group receiving omeprazole alone [72]. In contrast, oral contraceptives were shown to enhance carisoprodol AUC by 60% overall; however, there was no difference with respect to CYP2C19 genotype (EMs versus IMs) [73].

CYP3A4 is not polymorphic in expression; however, a small portion of the population expresses CYP3A5, which metabolizes essentially the same substrates as CYP3A4. Consequently, subjects expressing CYP3A5 tend to be EMs. Drugs that inhibit CYP3A4 may not have the same magnitude of effect on CYP3A5, which is typically less susceptible to inhibition [68]. Using grapefruit juice as an enzyme inhibitor of both CYP3A4 and CYP3A5, the urinary 6β-hydroxycortisol/cortisol ratio varied depending on CYP3A5 genotype. Likewise, genotype of MDR1 (pGP) was associated with urinary 6β-hydroxycortisol/cortisol ratio in a pattern that suggested that both polymorphisms affect this cortisol endpoint [69].

Some interactions are extremely complex as noted with the mixed inhibitor inducer HIV protease inhibitor combination, tipranavir/ritonavir. The combination produced weak induction of CYP1A1, moderate induction of CYP2C19, potent induction of pGP, and potent inhibition of CYP2D6 and CYP3A after multiple dosing [74].

Given the potential differences in the effects of metabolic interactions based on genotype, either measuring genotype or perhaps planning studies with genotype entry criteria should be considered.

9.3.5 Interactions Affecting Other Elimination Pathways

Some drugs are eliminated by fecal excretion and are excreted in bile or by transintestinal elimination. Enterohepatic recycling occurs when drugs are eliminated in bile as conjugates. Deconjugation may occur in the small intestine, thereby allowing

for reabsorption of the parent drug. A precipitant drug that interferes with deconjugation will prevent enterohepatic recycling (reabsorption) and increase the apparent clearance. Potential examples of this interaction type involve antibacterial drugs and oral contraceptives [75]. Valproic acid (VPA) is a very lipophilic drug which undergoes conjugation to an acylglucuronide metabolite. The VPA-glucuronide can either be excreted by the kidney or hydrolyzed back to the parent drug in the liver. The enzyme responsible for VPA-glucuronide hydrolysis is an acylpeptide hydrolase found in liver cytosol. Thus VPA-VPA-glucuronide bidirectional cycling results in longer persistence of VPA in the body. This interesting pharmacokinetic phenomenon was explained after the interaction between VPA and meropenem was recognized, leading to markedly increased VPA clearance. Subsequently, meropenem and other carbapenem antibiotics were shown to inhibit this acylpeptide hydrolase [76].

Precipitant drugs that physically trap or bind another drug within the gastrointestinal lumen may also enhance the clearance of the object drug. Examples of this interaction include iron salts or aluminum hydroxide with doxycycline [77, 78].

9.4 Pharmacostatistical Techniques

Advances have been made in the past decade to facilitate detection and evaluation of drug interactions. The intent of this section is to focus on the recommended approaches for presenting and analyzing pharmacostatistical drug interaction data. In discussions below, the terms “test” and “reference” treatments refer to the administration of the object and precipitant drugs in combination (test) and administration of the object drug alone.

9.4.1 Statistical Analysis Approach

There are many approaches, both parametric and nonparametric, to analyze comparative pharmacokinetic data from drug interaction studies. The statistical strategy recommended by regulatory agencies in the United States [10] and Europe [11, 79], editors of clinical pharmacology journals [80], and others [81, 82] is to adapt the confidence interval approach used in average bioequivalence studies [14, 83]. A bioequivalence study is a type of comparative bioavailability study conducted to demonstrate that the shape and magnitude of blood or plasma concentration-time profiles produced by the drug formulations under study are sufficiently alike that therapeutic equivalence can be assumed. In drug interaction studies, the aim is to determine whether the interaction is clinically meaningful from differences in concentration-time profiles or other pharmacokinetic characteristics between test and reference treatments. Comparison between profiles is at a minimum based on maximal exposure (C_{max}) and overall exposure (AUC) and presented as a mean ratio (MR: test/reference). The two one-sided t-test is based on the null hypotheses: H_{01}, MR $\leq$ lower bound, and H_{02}, MR $\geq$ upper bound. The lower and upper bounds need

to be specified in advance, and values of 0.8 and 1.25, respectively, are typically used for log-transformed pharmacokinetic parameters in bioequivalence and drug interaction studies. The alternate hypothesis is that the MR falls within the specified lower and upper bounds, H_{alt}: lower bound < MR < upper bound.

In traditional analysis, the null hypothesis stipulates that parameters for the object drug are equivalent for the test and reference treatment. When a significant difference is found, the null hypothesis would be rejected and a difference would be concluded. A small, clinically unimportant difference may be statistically significant at the 5% level of significance ($\alpha = 0.05$). The lack of significance does not necessarily imply "no interaction." In such cases, the statistical power, or probability of detecting a specified difference, must be considered. The specified difference should be a change that would be considered clinically important given the available pharmacodynamic and toxicologic information. A large, clinically important difference between treatments may not be statistically significant if sample size is small and within- and/or between-individual pharmacokinetic variability is large. Therefore, classical statistical approaches that attempt to confirm an interaction by rejecting the null hypothesis of "no difference" are inappropriate because the consumer risk is not controlled.

An equivalence approach is necessary to adequately address the risk to the consumer. Because a drug-drug interaction consists of different drug treatments, one should test the null hypothesis of "nonequivalence" by demonstrating "equivalence" or "lack of pharmacokinetic interaction," as first proposed by Steinijans et al. [84] In this manner the risk to the patient of a clinically relevant interaction can be defined within established limits. Generic drugs are approved on the basis of bioequivalence compared to a reference product. Risk to consumers is considered low for most drugs when substituting a generic drug that is considered bioequivalent. The same principle applies when a potential interacting drug is studied, and despite concomitant administration, the exposure to the object drug remains equivalent to the object drug given alone.

Two important assessment criteria must be defined before invoking the equivalence approach: [1] the range of clinically acceptable variation in pharmacokinetic response of the affected drug and [2] the risk to the consumer of incorrectly concluding a "lack of pharmacokinetic interaction." The range of clinically acceptable variation defines the equivalence range (clinical no-effect boundary). The range can be based on population average dose-related and/or individual concentration-response relationships derived from PK/PD models and other available information about the object drug that relates to the extent of difference in exposure caused by the interaction that is of no clinical consequence [10]. The consumer risk is the type I or α-error in statistics, which is usually set at 5%.

The equivalence method is based on two one-sided *t*-test procedure of rejecting the null hypothesis that the mean test/reference ratio is less than the lower equivalence limit or greater than the upper equivalence limit. At the 5% level of consumer risk, this procedure is operationally identical to the method of declaring equivalence (or lack of interaction) if the 90% confidence interval for the mean test/reference ratio is entirely within the specified equivalence range. More generally, the

$100 \times (1–2\alpha)\%$ confidence limits around the ratio (test/reference) of the means or medians of the test and reference treatments constrain the consumer risk to $100 \times (\alpha)\%$ as well as indicate the precision by the width of the confidence interval. In bioequivalence studies the accepted equivalence range is ±20%, which corresponds to a lower limit of 80% and an upper limit of 120% for original data or 125% for log-transformed data. A range of ±20% seems reasonable to assess product quality, but for drug interactions these limits may be wider or narrower depending on the patient population and the therapeutic index and pharmacokinetic variability of the object drug. For example, a range of clinically acceptable variation of 30% for changes in zidovudine AUC was suggested [85], whereas a range variation of 50% for changes in indinavir AUC was proposed [86]. Equivalence limits of the form (θ, $1/\theta$) have been proposed for data on both the original and logarithmic scales, where θ is the lower limit for the test/reference ratio [87]. The upper limit would be the reciprocal (e.g., limits of 0.8 and 1.25). No dose adjustment is required if the confidence interval falls within the no-effect boundary. Also, there is no requirement that the boundary must be symmetrical around a mean ratio of 1.0 or 100% [88]. For example, the lower bound could be 80% to ensure no loss of efficacy, but the upper bound could be set at 150% for a drug with low risk of toxicity.

Statistical inferences are made on either absolute (test-reference) or relative (test/reference) differences in the arithmetic means, geometric means (from logarithmic transformed data), harmonic means (from reciprocal transformed data), or medians of pharmacokinetic variables. Parametric analysis of variance (ANOVA) models appropriate for the study design are used to test differences in means (C_{max} and AUC), and nonparametric methods such as the Wilcoxon rank sum test or Wilcoxon signed-rank test are used to test differences in medians (t_{max}). If the study design is unbalanced from an unequal number of subjects in each sequence (crossover) or from missing data, assessments are based on least-squares means. Because clinicians prefer to think in terms of relative rather than absolute changes, pharmacokinetic differences are usually expressed as a ratio (test/reference). Confidence limits around these mean ratios for within-subject comparisons in crossover studies and between-group comparisons in parallel studies are constructed from the residual mean-square error (MSE) term in ANOVA. The ANOVA provides exact confidence limits for relative differences of geometric means if the distribution of variables is truly lognormal. Only approximate limits for relative differences of arithmetic means are possible, because ANOVA ignores variability in the reference mean and treats the reference mean as a constant instead of as a variable when dividing by the reference mean to convert a test/reference difference to a test/reference ratio, unless Fieller's theorem is applied [89]. Nonparametric approximate 90% confidence limits can be calculated for two-period, two-sequence crossover studies [90]. One should be cautious in concluding "no interaction" when approximate confidence limits generated from parametric or nonparametric techniques are within but near the equivalence limits. Also, inferences on mean data may not reflect how certain individuals in the study population respond to the interaction. A particular stratum of individuals may show an apparent interaction even though the overall mean data indicate no pharmacokinetic interaction.

9.4.2 Logarithmic Transformation of Pharmacokinetic Variables

All pharmacokinetic variables, except those such as t_{max} that depend on discreet sampling times, are logarithmically transformed before ANOVA [14, 84, 91]. Harmonic means have been proposed for inferences on half-life [92]. Transformation converts a multiplicative model to an additive model, which is the basis of ANOVA [ln(test/reference) = ln(test) – ln(reference)]. Decisions on t_{max} are best handled by nonparametric analysis. Most pharmacokinetic data have positively skewed distributions created by the truncation of these quantities at zero and have variances that depend on the mean. Transformation reduces the skewness and brings the distribution of data closer to normal. However, the main reason for transforming the data is to stabilize or make equal the within-subject (crossover study) or between-group (parallel study) variance and not to normalize the between-subject parameters [91]. Another advantage of transformation is that it is the best way to handle ratios for relative or proportional differences, and calculation of the associated confidence limits is straightforward.

For most studies the outcome will not change regardless of whether the original or log scale is used. There are two instances where conclusions can be opposite in a within-subject design [91]. If certain subjects with larger than average responses show larger than expected absolute differences, variability is increased on the original scale, whereas larger than expected absolute differences for smaller than average responses are expanded on the log scale. If this occurs, for example, when fast and slow metabolizers are studied together, then the within-subject variability and the relative mean changes can be different on the two scales.

9.4.3 Crossover Design and Analysis of Variance

The ANOVA for a crossover design includes the effects of sequence, subject within sequence, treatment, period, and, except for the 2, 2, 2 design, carryover. All effects except the sequence effect are tested by the MSE term. The sequence effect is tested against the subject-within-sequence effect. Any subgroup comparison of fixed effects (e.g., males and females) is tested with the subject mean-square term.

The sequence effect measures the difference between the groups of subjects defined by their sequence. In statistical parlance, a true sequence effect is known as the treatment-by-period interaction, which is a measure of the differential effect of the treatment (test-reference) in each of the periods. In the 2, 2, 2 design, the sequence effect is caused by three confounded sources: [1] a difference between subjects in the two sequences (i.e., group effects), [2] an unequal carryover of one treatment into the next period compared to the other treatment, or [3] a treatment-by-period interaction. In this case a significant sequence effect ($p < 0.1$) requires

further explanation and evaluation of questions including: Was randomization appropriate? Was the washout period sufficient? Were trial conditions, analytical methodology, and clinical settings applied consistently? However, a true sequence effect (i.e., group effect) does not invalidate the determination of bioequivalence [93]. A sequence effect in the 2, 2, 2 design can be due to unequal carryover between treatments, in which case the analysis of period 1 data should be presented separately; carryover should be evaluated by checking the pre-drug plasma assay results.

The period effect measures the difference between study periods or alternatively the differential effect of the treatment in each of the sequences. In a 2, 2, 2 study, the period effect is completely confounded with treatment-by-sequence interaction. Any difference in treatment comparison (test-reference) between the two sequence groups cannot be distinguished from period effects. If there are carryover effects or if more than two periods are included, then the period effect and treatment-by-sequence interaction are not interchangeable. The period effect can be caused by equal carryover in each sequence from period to period, bias in analytical data if samples in each period were analyzed in different batches, differences in the study environment or procedures, and changes with time in stage of disease. As with sequence effect, carryover can be ruled out by checking the pre-dose drug concentrations before period 2.

In a 2, 2, 2 study, the presence of a treatment effect (i.e., period-by-sequence interaction) implies that differences between periods are in opposite directions for the two sequence groups (if P2 – P1 in S1 is negative, then P2 – P1 in S2 may be positive). The estimate of treatment differences will not be biased if a period effect is present.

If the test treatment is determined not to be bioequivalent, then a treatment effect may be expected; however, treatment effects may also be observed when a bioequivalent determination is made for products with low intra-subject variability. A significant treatment effect may be entirely ignored when equivalence criteria are met.

The MSE term is a measure of the intra-subject variability and is usually converted to a coefficient of variation (CV_W) to estimate the consistency of the magnitude of interaction among the subjects [94]. The CV_W is estimated as $100\% \times (e^{MSE} - 1)^{½}$ for logarithmic transformed data and as $100\% \times (MSE)^{½}/Y$ for original data, where Y is either the least-squares mean of the reference treatment or the combined mean of the two least-squares treatment means being compared. The goal of any within-subjects design is too minimize the CV_W. The interaction is considered highly variable for a particular pharmacokinetic parameter if the CV_W is >30%. The CV_W is a very informative parameter but is rarely reported in the literature. Values for a number of drugs orally administered in crossover bioequivalence studies have been tabulated by Steinijans et al. [95] The CV_W is important to know because the width of the confidence interval around the difference of treatment means, the calculation of post hoc power to detect these differences, and an estimation of sample sizes for planning future interaction studies require an estimate of CV_w.

There are a number of sources of variation in CV_W: the true intra-subject pharmacokinetic variation exhibited by a single person, analytical variability (measurement errors), within-batch variation in manufacture of the drug formulation, nonadherence to the medications, and the random subject-by-treatment interaction. This latter source is caused by random variability of treatments within subjects or within identifiable subgroups of the population studied. Each individual may behave differently to the test treatment, or subjects in subgroups may show similar variation within subgroups but different responses to the test treatment among subgroups. An example could be smokers responding differently from nonsmokers to one of the treatments. On the log scale, the random subject-by-treatment interaction is minimized if all subjects show the same relative change in the same direction.

9.4.4 Sample Size and Post Hoc Power Calculations

The sample size of the study needs to be planned with consideration of the purpose of the study. If the purpose of the study is to evaluate a potential drug interaction that is suspected based on preliminary data, the sample size can be somewhat conservative. However, if the goal is to demonstrate the lack of interaction for an individual drug when a member of the same drug class exhibits the interaction (class labeling), then the sample size should be larger. Estimations of sample size for a within-subject drug interaction study require a knowledge of CV_w for the interaction. CV_w values for drug interaction studies may be greater than those reported for drugs in bioequivalence studies [95] because not all subjects will respond to the precipitant drug to the same degree. Tables of sample sizes for 2, 2, 2 crossover designs to attain a power of 80% or 90% at the 5% nominal level for a given CV_w and expected relative difference in treatment medians or means are published for the multiplicative (logarithmic) model with equivalence ranges of 0.7–1.43 [96], 0.8–0.25 [87, 97], and 0.9–1.11 [96]. Similar tables are published for the additive (original) model [98] and for parallel designs [87, 99]. The minor influence of the between-subject coefficient of variability on sample size estimates for the 2, 2, 2 crossover design is demonstrated by Hauschke et al. [87]

Post hoc power calculations have limited utility but can be used for negative studies to estimate differences that could be detected with a certain power (usually 80% at the 5% significance level) or to estimate the power of the study to detect a specified difference (usually 20% difference from reference at the 5% significance level). These calculations require an estimation of the standard error of the difference in mean or medians. General equations for point hypothesis testing for original and logarithmic data using a central *t*-distribution are provided in references [89, 100]. General equations for interval hypothesis testing using a noncentral *t*-distribution for crossover and parallel designs are given in references [87, 89].

9.5 Pharmacokinetic Metrics and Characteristics

The major assumption in bioequivalence is that the Cl of the drug under investigation is constant over the course of the study and that AUC is a pure characteristic of extent of bioavailability (F). In drug interactions both clearance and bioavailability can change after oral administration. Therefore, changes in AUC can result from alterations in either parameter. Schall et al. [101] proposed the terminal elimination half-life ($t_{½,z}$) and the ratio of AUC/$t_{½,z}$ as characteristics for Cl and F, respectively, in drug-drug interaction studies. Interpretation involves looking at the ratio of $t_{½}$ (test/reference) and ratio of AUC/$t_{½,z}$(test/reference ratio). Note that $AUC_{0\text{-}\infty}$ should be used for a single-dose case and $AUC_{0\text{-tau}}$ should be used for steady state. Assuming a constant volume of distribution, if the $t_{½,z}$ ratio is >1, then the interaction results from reduced Cl, and if the $t_{½,z}$ ratio is <1, the interaction results from increased Cl. If AUC/$t_{½,z}$ (test/reference ratio) is >1, then the interaction is at least in part due to increased bioavailability. Finally, if AUC/$t_{½,z}$ (test/reference ratio) is <1, then the interaction is at least in part due to decreased bioavailability. However, interactions may result from a mixture of altered Cl and altered bioavailability.

Because AUC is a composite characteristic of Cl and F, and peak drug levels (C_{max}) reflect both rate and extent of absorption, these metrics can be used to indicate drug exposure [102]. AUC is the ideal metric for total systemic drug exposure and C_{max} is a measure of peak systemic exposure. The term drug exposure conveys more clinical relevance than the term "rate and extent of drug absorption" because drug safety and effectiveness are concerns in drug interaction studies.

9.6 Presentation and Interpretation of Drug Interaction Data

There are generally three ways to present comparative pharmacokinetic data for changes in the test treatment relative to the reference treatment: [1] a test/reference ratio expressed as a percentage; [2] an x-fold change, where x is the test/reference ratio; or [3] a percentage change [(test/reference ratio – 1) × 100%]. For example, an AUC ratio of 200% indicates a twofold increase and a 100% increase in AUC. Often x-fold changes are confused with percentage change, and the reader needs to be aware of which method of calculation was used.

Current thinking favors expressing the results in terms of a test/reference geometric mean ratio and the corresponding 90% confidence limits for AUC and C_{max} parameters. The use of 95% confidence limits should not be confused with 90% confidence limits. The former bounds will be wider and may lead to different conclusions in equivalence testing. Reporting the 95% confidence limits is another way of reporting a test of significance at the 5% level of significance. For example, AUC of bosentan increased 2.1-fold (95% confidence interval 1.5–2.7) after concomitant administration with ketoconazole [103]. The 95% confidence interval

would be examined to determine if it includes the value 1.0, and if not, as in this case, a statistically significant interaction at the 5% level of significance ($p < 0.05$) would be concluded. Use of statistical testing for difference should not be used for the reasons cited in this chapter.

9.6.1 No-Effect Boundary

The "no-effect boundary" or acceptable range needs to be established a priori. If a drug interaction is concluded, the clinical significance of the interaction and recommendations on how to manage the interaction need to be formulated. The FDA guidance for drug interaction studies allows two approaches for developing a no-effect boundary [10]. The first approach is to describe the range of the selected exposure parameters over a range of doses that are normally used. The sponsor should include information on dose and/or concentration-response studies or PK/PD models to support the recommendation. If the exposure parameters remain within this range in the presence of a potential precipitant drug, the sponsor could conclude that "no clinically significant interaction is present." The second approach defaults to bioequivalence criteria where the 90% confidence interval for geometric mean exposure parameter ratio (test/reference) falls within 80–125% [10]. This latter approach is most commonly used.

The use of bioequivalence criteria should eliminate a substantial portion of studies that statistically conclude a drug interaction when only small clinically insignificant differences occur. As an example, digoxin steady-state AUC was 25.5 ng·h/ml after digoxin alone and 23.9 ng·h/ml after digoxin plus zaleplon (a hypnotic agent). From a test of significance (ANOVA, $p = 0.018$), a drug interaction would have been concluded. The geometric mean ratio (test/reference) was 93% with a 90% confidence interval of 89–98%, and this would more appropriately lead to a "no-effect" conclusion [104]. Potential problems with the equivalence approach include too small of a sample size and high variability. If the sample size is too small, confidence intervals tend to be wide, and this could result in a 90% confidence interval that falls outside of the "no-effect boundary" despite a mean ratio near 100%. Too large of a sample size with the bioequivalence approach does not cause adverse consequences other than excessive study costs and ethical issues of imparting risk to numbers of subjects greater than needed. For tests of difference, too small of a sample size will lead to low power and inability to detect an important drug interaction, and too large of a study population may cause detection of small, clinically insignificant changes.

Not only does the no-effect boundary need to be established a priori, use of unconventional ranges needs to be justified. In a study evaluating the effect of montelukast on digoxin, several problems are apparent. The authors used a no-effect boundary of 70–143% without appropriate justification. Digoxin exhibits a narrow therapeutic index and relatively low variability in exposure parameters in a healthy population.

The mean digoxin $AUC_{0-\infty}$ was 43.2 ng·h/ml for digoxin alone and 39.2 ng·h/ml for digoxin plus montelukast. Although the 90% confidence interval for $AUC_{0-\infty}$ was 70–118%, the authors concluded that montelukast has no effect on the pharmacokinetics of digoxin [105]. The use of this expanded no-effect boundary for a drug with a narrow therapeutic index is concerning. Moreover, the 90% confidence interval is too wide to fit within the range of 80–125%. The study involved a small sample size ($n = 10$) and did not address power.

In another study, which evaluated the effects of proton pump inhibitors on theophylline, the no-effect boundary was expanded to 70–143% for steady-state C_{max}, but not for steady-state AUC [106]. There is no pharmacokinetic basis to suspect a change in rate of absorption of theophylline from acid suppression, and the reason for the expanded boundary was not addressed. Because the observed 90% confidence limit for steady-state C_{max} fell within the range of 80–125%, conclusions remain appropriate. In some cases involving drugs (e.g., ethionamide) with moderate to high variability in exposure parameters, it may be difficult to obtain 90% confidence intervals that fall within the usual no-effect boundaries, requiring the use of large sample sizes or expanded boundaries [107].

An example of a study that used an expanded no-effect boundary and provided justification involved interactions between didanosine, indinavir, ketoconazole, and ciprofloxacin [108]. A no-effect boundary of 75–133% was used. The authors cited a study where the AUC of indinavir was increased 29% with clarithromycin administration, and the interaction was concluded to be not clinically significant. For ciprofloxacin, the authors cited the package insert and a publication and considered that a 48% increase in ciprofloxacin AUC in elderly subjects did not result in a recommendation for reducing the dose. For ketoconazole, the authors cited a study that reported a 59% increase in ketoconazole AUC when administered with food compared to fasting and considered that the labeling did not contain a recommendation for administering ketoconazole with food [108]. In another study, in which ketoconazole significantly increased the exposure of desloratadine, the interaction was concluded to be not clinically relevant as no changes in ECG parameters were observed [109]. Although such observation does not totally rule out clinical significance in special populations, the value of concomitant pharmacodynamic assessment is apparent.

9.6.2 Studies to Confirm Clinical Strategy

Another potential area of misinterpretation is when the doses and/or dosing intervals of the drug under investigation are different in the test and reference arms of the study. This may occur if the purpose is to obtain equivalent drug exposures over a specified time period in the absence and presence of an interacting drug. The magnitude of pharmacokinetic effect can appear smaller or larger if the control dose is larger or smaller. For example, 800 mg of indinavir every 8 h was estimated to give

about the same AUC over 24 h as 400 mg indinavir every 12 h in the presence of 400 mg ritonavir every 12 h; from single-dose indinavir data, the magnitude of the interaction was actually about a fivefold increase in AUC if 400 mg of indinavir was used as the reference [110]. Depending on the purpose of the study, the analysis may compare the exposures between the two treatments; however, to avoid confusion, analysis should still be done with Cl or dose normalized AUC to characterize the extent of the interaction.

9.7 Summary

Since publication of the first edition of this chapter in 2001, issues still remain to be resolved concerning optimal design of drug interaction studies. Traditional issues, such as defining the research hypothesis (question of interest), determining the appropriate study population (healthy volunteers or patients), determining the study design (crossover, longitudinal or parallel, washout requirements, etc.), deciding between single-dose or steady-state, and deciding which pharmacokinetic and/or pharmacodynamic endpoints to evaluate, should depend on knowledge of the drugs involved, preliminary data on the potential interaction, and general knowledge of pharmacokinetics and drug interactions. Defining whether a drug interaction exists is now well accepted by regulatory agencies as an equivalence problem where endpoints are compared between the object drug given with and without the precipitant drug. The acceptable clinical no-effect boundary should be specified a priori, but allowing flexibility depending on the therapeutic index of the object drug and variability of the endpoints.

References

1. Ekins S (1996) Past, present, and future applications of precision-cut liver slices for in vitro xenobiotic metabolism. Drug Metab Rev 28(4):591–623
2. Decker CJ, Laitinen LM, Bridson GW et al (1998) Metabolism of amprenavir in liver microsomes: role of CYP3A4 inhibition for drug interactions. J Pharm Sci 87(7):803–807
3. Bonnabry P, Sievering J, Leemann T et al (2001) Quantitative drug interactions prediction system (Q-DIPS): a dynamic computer-based method to assist in the choice of clinically relevant in vivo studies. Clin Pharmacokinet 40(9):631–640
4. Rodrigues AD, Wong SL (1997) Application of human liver microsomes in metabolism-based drug-drug interactions: in vitro-in vivo correlations and the Abbott Laboratories experience. Adv Pharmacol 43:65–101
5. Koudriakova T, Iatsimirskaia E, Utkin I et al (1998) Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: mechanism-based inactivation of cytochrome P4503A by ritonavir. Drug Metab Dispos 26(6):552–561
6. Hochman JH, Yamazaki M, Ohe T et al (2002) Evaluation of drug interactions with p-glycoprotein in drug discovery: in vitro assessment of the potential for drug-drug interactions with p-glycoprotein. Curr Drug Metab 3(3):257–273

7. Benet LZ, Cummins CL, Wu CY (2003) Transporter-enzyme interactions: implications for predicting drug-drug interactions from in vitro data. Curr Drug Metab 4(5):393–398
8. Rolan PE (1994) Plasma protein binding displacement interactions--why are they still regarded as clinically important? Br J Clin Pharmacol 37(2):125–128
9. Sansom LN, Evans AM (1995) What is the true clinical significance of plasma protein binding displacement interactions? Drug Saf 12(4):227–233
10. FDA US (2012) Guidance for industry: drug interaction studies – study design, data analysis, implications for dosing and labeling recommendations; draft guidance. February 2012, Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 1–79
11. EMA (2012) Guideline on the investigation of drug interactions. Committee for Human Medicinal Products, European Medicines Agency, June 21, 1–59
12. Bjornsson TD, Callaghan JT, Einolf HJ et al (2003) The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PHARMA) perspective. Drug Metab Dispos 31(7):815–832
13. Gallicano KD, Sahai J, Shukla VK et al (1999) Induction of zidovudine glucuronidation and amination pathways by rifampicin in HIV-infected patients. Br J Clin Pharmacol 48(2):168–179
14. Ormsby E (1994) Statistical methods in bioequivalence. CRC Press, Boca Raton
15. Wang BS, Wang XJ, Gong LK (2009) The construction of a williams design and randomization in cross-over clinical trials using SAS. J Stat Softw 29(1):1–10
16. Fleiss JL (1989) A critique of recent research on the two-treatment crossover design. Control Clin Trials 10(3):237–243
17. Vuorinen J (1997) A practical approach for the assessment of bioequivalence under selected higher-order cross-over designs. Stat Med 16(19):2229–2243
18. Chow SC, Liu JP (1992) On assessment of bioequivalence under a higher-order crossover design. J Biopharm Stat 2(2):239–256
19. Nix DE, Di Cicco RA, Miller AK et al (1999) The effect of low-dose cimetidine (200 mg twice daily) on the pharmacokinetics of theophylline. J Clin Pharmacol 39(8):855–865
20. Grasela TH Jr, Antal EJ, Ereshefsky L et al (1987) An evaluation of population pharmacokinetics in therapeutic trials. Part ii. Detection of a drug-drug interaction. Clini Pharmacol Ther 42(4):433–441
21. Purkins L, Wood N, Kleinermans D et al (2003) No clinically significant pharmacokinetic interactions between voriconazole and indinavir in healthy volunteers. Br J Clin Pharmacol 56(Suppl 1):62–68
22. Cato A 3rd, Cavanaugh J, Shi H et al (1998) The effect of multiple doses of ritonavir on the pharmacokinetics of rifabutin. Clini Pharmacol Ther 63(4):414–421
23. Romero AJ, Le Pogamp P, Nilsson LG et al (2002) Effect of voriconazole on the pharmacokinetics of cyclosporine in renal transplant patients. Clini Pharmacol Ther 71(4):226–234
24. Cadieux RJ (1989) Drug interactions in the elderly. How multiple drug use increases risk exponentially. Postgrad Med 86(8):179–186
25. Ragueneau I, Poirier JM, Radembino N et al (1999) Pharmacokinetic and pharmacodynamic drug interactions between digoxin and macrogol 4000, a laxative polymer, in healthy volunteers. Br J Clin Pharmacol 48(3):453–456
26. Piscitelli SC, Goss TF, Wilton JH et al (1991) Effects of ranitidine and sucralfate on ketoconazole bioavailability. Antimicrob Agents Chemother 35(9):1765–1771
27. Blum RA, D'Andrea DT, Florentino BM et al (1991) Increased gastric pH and the bioavailability of fluconazole and ketoconazole. Ann Intern Med 114(9):755–757
28. Lebsack ME, Nix D, Ryerson B et al (1992) Effect of gastric acidity on enoxacin absorption. Clini Pharmacol Ther 52(3):252–256
29. Lehto P, Kivisto KT, Neuvonen PJ (1994) The effect of ferrous sulphate on the absorption of norfloxacin, ciprofloxacin and ofloxacin. Br J Clin Pharmacol 37(1):82–85
30. Nix DE, Watson WA, Lener ME et al (1989) Effects of aluminum and magnesium antacids and ranitidine on the absorption of ciprofloxacin. Clin Pharmacol Ther 46(6):700–705

31. Parpia SH, Nix DE, Hejmanowski LG et al (1989) Sucralfate reduces the gastrointestinal absorption of norfloxacin. Antimicrob Agents Chemother 33(1):99–102
32. Muller J, Keiser M, Drozdzik M et al (2017) Expression, regulation and function of intestinal drug transporters: an update. Biol Chem 398(2):175–192
33. Arakawa H, Kamioka H, Kanagawa M et al (2016) Possible interaction of quinolone antibiotics with peptide transporter 1 in oral absorption of peptide-mimetic drugs. Biopharm Drug Dispos 37(1):39–45
34. Shugarts S, Benet LZ (2009) The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res 26(9):2039–2054
35. Misaka S, Miyazaki N, Yatabe MS et al (2013) Pharmacokinetic and pharmacodynamic interaction of nadolol with itraconazole, rifampicin and grapefruit juice in healthy volunteers. J Clin Pharmacol 53(7):738–745
36. Momper JD, Tsunoda SM, Ma JD (2016) Evaluation of proposed in vivo probe substrates and inhibitors for phenotyping transporter activity in humans. J Clin Pharmacol 56(Suppl 7):S82–S98
37. Jungbluth GL, Pasko MT, Beam TR et al (1989) Ceftriaxone disposition in open-heart surgery patients. Antimicrob Agents Chemother 33(6):850–856
38. Yin J, Wang J (2016) Renal drug transporters and their significance in drug-drug interactions. Acta Pharm Sin B 6(5):363–373
39. Ivanyuk A, Livio F, Biollaz J et al (2017) Renal drug transporters and drug interactions. Clin Pharmacokinet 56(8):825–892
40. Ebner T, Ishiguro N, Taub ME (2015) The use of transporter probe drug cocktails for the assessment of transporter-based drug-drug interactions in a clinical setting-proposal of a four component transporter cocktail. J Pharm Sci 104(9):3220–3228
41. Hagos Y, Wolff NA (2010) Assessment of the role of renal organic anion transporters in drug-induced nephrotoxicity. Toxins 2(8):2055–2082
42. Hsu V, de LT Vieira M, Zhao P et al (2014) Towards quantitation of the effects of renal impairment and probenecid inhibition on kidney uptake and efflux transporters, using physiologically based pharmacokinetic modelling and simulations. Clin Pharmacokinet 53(3):283–293
43. Rengelshausen J, Goggelmann C, Burhenne J et al (2003) Contribution of increased oral bioavailability and reduced nonglomerular renal clearance of digoxin to the digoxin-clarithromycin interaction. Br J Clin Pharmacol 56(1):32–38
44. Megran DW, Lefebvre K, Willetts V et al (1990) Single-dose oral cefixime versus amoxicillin plus probenecid for the treatment of uncomplicated gonorrhea in men. Antimicrob Agents Chemother 34(2):355–357
45. Gaspari F, Perico N, Remuzzi G (1997) Measurement of glomerular filtration rate. Kidney Int Suppl 63:S151–S154
46. Brochner-Mortensen J (1985) Current status on assessment and measurement of glomerular filtration rate. Clin Physiol 5(1):1–17
47. Hellerstein S, Erwin P, Warady BA (2003) The cimetidine protocol: a convenient, accurate, and inexpensive way to measure glomerular filtration rate. Pediatr Nephrol 18(1):71–72
48. Baciewicz AM, Chrisman CR, Finch CK et al (2008) Update on rifampin and rifabutin drug interactions. Am J Med Sci 335(2):126–136
49. Wandel C, Bocker R, Bohrer H et al (1994) Midazolam is metabolized by at least three different cytochrome P450 enzymes. Br J Anaesth 73(5):658–661
50. Thummel KE, Shen DD, Podoll TD et al (1994) Use of midazolam as a human cytochrome P450 3A probe: ii. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. J Pharmacol Exp Ther 271(1):557–566
51. Lown KS, Thummel KE, Benedict PE et al (1995) The erythromycin breath test predicts the clearance of midazolam. Clini Pharmacol Ther 57(1):16–24
52. Watkins PB, Turgeon DK, Saenger P et al (1992) Comparison of urinary 6-beta-cortisol and the erythromycin breath test as measures of hepatic P450iiiA (CYP3A) activity. Clini Pharmacol Ther 52(3):265–273

53. Hunt CM, Watkins PB, Saenger P et al (1992) Heterogeneity of CYP3A isoforms metabolizing erythromycin and cortisol. Clini Pharmacol Ther 51(1):18–23
54. Chiou WL, Jeong HY, Wu TC et al (2001) Use of the erythromycin breath test for in vivo assessments of cytochrome P4503A activity and dosage individualization. Clini Pharmacol Ther 70(4):305–310
55. Sarkar MA, Jackson BJ (1994) Theophylline N-demethylations as probes for P4501A1 and P4501A2. Drug Metab Dispos 22(6):827–834
56. Ziebell J, Shaw-Stiffel T (1995) Update on the use of metabolic probes to quantify liver function: caffeine versus lidocaine. Dig Dis 13(4):239–250
57. Anthony LB, Boeve TJ, Hande KR (1995) Cytochrome P-450iiD6 phenotyping in cancer patients: debrisoquin and dextromethorphan as probes. Cancer Chemother Pharmacol 36(2):125–128
58. Desta Z, Zhao X, Shin JG et al (2002) Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet 41(12):913–958
59. Fuhr U, Rost KL, Engelhardt R et al (1996) Evaluation of caffeine as a test drug for CYP1A2, NAT2 and CYP2E1 phenotyping in man by in vivo versus in vitro correlations. Pharmacogenetics 6(2):159–176
60. Brockmoller J, Rost KL, Gross D et al (1995) Phenotyping of CYP2C19 with enantiospecific hplc-quantification of R- and S-mephenytoin and comparison with the intron4/exon5 g-->a-splice site mutation. Pharmacogenetics 5(2):80–88
61. Guengerich FP (1997) Role of cytochrome p450 enzymes in drug-drug interactions. Adv Pharmacol 43:7–35
62. Tanaka E (1998) Clinically important pharmacokinetic drug-drug interactions: role of cytochrome P450 enzymes. J Clinical Pharmacy Ther 23(6):403–416
63. Lomaestro BM, Piatek MA (1998) Update on drug interactions with azole antifungal agents. Ann Pharmacother 32(9):915–928
64. Caraco Y (1998) Genetic determinants of drug responsiveness and drug interactions. Ther Drug Monit 20(5):517–524
65. Shannon M (1997) Drug-drug interactions and the cytochrome P450 system: an update. Pediatr Emerg Care 13(5):350–353
66. Bradford PA, Sanders CC (1993) Use of a predictor panel to evaluate susceptibility test methods proposed for piperacillin-tazobactam. Antimicrob Agents Chemother 37(12):2578–2583
67. Mikus G, Schowel V, Drzewinska M et al (2006) Potent cytochrome P450 2C19 genotype-related interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir. Clini Pharmacol Ther 80(2):126–135
68. Isoherranen N, Ludington SR, Givens RC et al (2008) The influence of CYP3A5 expression on the extent of hepatic CYP3A inhibition is substrate-dependent: an in vitro-in vivo evaluation. Drug Metab Dispos 36(1):146–154
69. Li D, Abudula A, Abulahake M et al (2010) Influence of CYP3A5 and MDR1 genetic polymorphisms on urinary 6 beta-hydroxycortisol/cortisol ratio after grapefruit juice intake in healthy Chinese. J Clin Pharmacol 50(7):775–784
70. Cho JY, Yu KS, Jang IJ et al (2002) Omeprazole hydroxylation is inhibited by a single dose of moclobemide in homozygotic em genotype for CYP2C19. Br J Clin Pharmacol 53(4):393–397
71. Miura M, Inoue K, Kagaya H et al (2007) Influence of rabeprazole and lansoprazole on the pharmacokinetics of tacrolimus in relation to CYP2C19, CYP3A5 and MDR1 polymorphisms in renal transplant recipients. Biopharm Drug Dispos 28(4):167–175
72. Furuta T, Ohashi K, Kobayashi K et al (1999) Effects of clarithromycin on the metabolism of omeprazole in relation to CYP2C19 genotype status in humans. Clini Pharmacol Ther 66(3):265–274
73. Bramness JG, Skurtveit S, Gulliksen M et al (2005) The CYP2C19 genotype and the use of oral contraceptives influence the pharmacokinetics of carisoprodol in healthy human subjects. Eur J Clin Pharmacol 61(7):499–506

74. Dumond JB, Vourvahis M, Rezk NL et al (2010) A phenotype-genotype approach to predicting CYP450 and p-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. Clinical Pharmacol Ther 87(6):735–742
75. Zachariasen RD (1994) Loss of oral contraceptive efficacy by concurrent antibiotic administration. Women Health 22(1):17–26
76. Suzuki E, Nakai D, Ikenaga H et al (2016) In vivo inhibition of acylpeptide hydrolase by carbapenem antibiotics causes the decrease of plasma concentration of valproic acid in dogs. Xenobiotica 46(2):126–131
77. Nguyen VX, Nix DE, Gillikin S et al (1989) Effect of oral antacid administration on the pharmacokinetics of intravenous doxycycline. Antimicrob Agents Chemother 33(4):434–436
78. Neuvonen PJ, Penttila O (1974) Effect of oral ferrous sulphate on the half-life of doxycycline in man. Eur J Clin Pharmacol 7(5):361–363
79. Muller HJ, Gundert-Remy U (1994) The regulatory view on drug-drug interactions. Int J Clin Pharmacol Ther 32(6):269–273
80. Waller PC, Jackson PR, Tucker GT et al (1994) Clinical pharmacology with confidence. Br J Clin Pharmacol 37(4):309–310
81. Fuhr U, Weiss M, Kroemer HK et al (1996) Systematic screening for pharmacokinetic interactions during drug development. Int J Clin Pharmacol Ther 34(4):139–151
82. Kuhlmann J (1994) Drug interaction studies during drug development: which, when, how? Int J Clin Pharmacol Ther 32(6):305–311
83. Pidgen AW (1992) Statistical aspects of bioequivalence--a review. Xenobiotica 22(7): 881–893
84. Steinijans VW, Hartmann M, Huber R et al (1996) Lack of pharmacokinetic interaction as an equivalence problem. Int J Clin Pharmacol Ther 34(1 Suppl):S25–S30
85. Gallicano K, Sahai J, Swick L et al (1995) Effect of rifabutin on the pharmacokinetics of zidovudine in patients infected with human immunodeficiency virus. Clin Infect Dis 21(4):1008–1011
86. De Wit S, Debier M, De Smet M et al (1998) Effect of fluconazole on indinavir pharmacokinetics in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 42(2):223–227
87. Hauschke D, Kieser M, Diletti E et al (1999) Sample size determination for proving equivalence based on the ratio of two means for normally distributed data. Stat Med 18(1):93–105
88. Huang SM, Lesko LJ, Williams RL (1999) Assessment of the quality and quantity of drug-drug interaction studies in recent NDA submissions: study design and data analysis issues. J Clin Pharmacol 39(10):1006–1014
89. Chow S, Liu JP (2000) Design and analysis of bioavailabilty and bioequivalence studies, second edition, revised and expanded. Marcel Dekker, New York
90. Wijnand H (1996) Some nonparametric confidence intervals are non-informative, notably in bioequivalence studies. Clin Res Reg Affairs 13:65–75
91. Midha KK, Ormsby ED, Hubbard JW et al (1993) Logarithmic transformation in bioequivalence: application with two formulations of perphenazine[erratum appears in j pharm sci 1993 dec;82(12):1300]. J Pharm Sci 82(2):138–144
92. Roe DJ, Karol MD (1997) Averaging pharmacokinetic parameter estimates from experimental studies: statistical theory and application. J Pharm Sci 86(5):621–624
93. Zintzaras E (2000) The existence of sequence effect in cross-over bioequivalence trials. Eur J Drug Metab Pharmacokinet 25(3–4):241–244
94. Hauschke D, Steinijans WV, Diletti E et al (1994) Presentation of the intrasubject coefficient of variation for sample size planning in bioequivalence studies. Int J Clin Pharmacol Ther 32(7):376–378
95. Steinijans VW, Sauter R, Hauschke D et al (1995) Reference tables for the intrasubject coefficient of variation in bioequivalence studies. Int J Clin Pharmacol Ther 33(8):427–430
96. Diletti E, Hauschke D, Steinijans VW (1992) Sample size determination: extended tables for the multiplicative model and bioequivalence ranges of 0.9 to 1.11 and 0.7 to 1.43. Int J Clini Pharmacol, Ther Toxicol 30(Suppl 1):S59–S62

97. Hauschke D, Steinijans VW, Diletti E et al (1992) Sample size determination for bioequivalence assessment using a multiplicative model. J Pharmacokinet Biopharm 20(5):557–561
98. Liu JP, Chow SC (1992) Sample size determination for the two one-sided tests procedure in bioequivalence. J Pharmacokinet Biopharm 20(1):101–104
99. Chow SC, Wang H (2001) On sample size calculation in bioequivalence trials.[erratum appears in j pharmacokinet pharmacodyn. 2002 feb;29(1):101]. J Pharmacokinet Pharmacodyn 28(2):155–169
100. Gallicano K, Sahai J, Zaror-Behrens G et al (1994) Effect of antacids in didanosine tablet on bioavailability of isoniazid. Antimicrob Agents Chemother 38(4):894–897
101. Schall R, Hundt HK, Luus HG (1994) Pharmacokinetic characteristics for extent of absorption and clearance in drug/drug interaction studies. Int J Clin Pharmacol Ther 32(12):633–637
102. Tozer TN, Bois FY, Hauck WW et al (1996) Absorption rate vs. exposure: which is more useful for bioequivalence testing? Pharm Res 13(3):453–456
103. van Giersbergen PL, Halabi A, Dingemanse J (2002) Single- and multiple-dose pharmacokinetics of bosentan and its interaction with ketoconazole. Br J Clin Pharmacol 53(6):589–595
104. Sanchez Garcia P, Paty I, Leister CA et al (2000) Effect of zaleplon on digoxin pharmacokinetics and pharmacodynamics. Am J Health Syst Pharm 57(24):2267–2270
105. Depre M, Van Hecken A, Verbesselt R et al (1999) Effect of multiple doses of montelukast, a cyslt1 receptor antagonist, on digoxin pharmacokinetics in healthy volunteers. J Clin Pharmacol 39(9):941–944
106. Dilger K, Zheng Z, Klotz U (1999) Lack of drug interaction between omeprazole, lansoprazole, pantoprazole and theophylline. Br J Clin Pharmacol 48(3):438–444
107. Auclair B, Nix DE, Adam RD et al (2001) Pharmacokinetics of ethionamide administered under fasting conditions or with orange juice, food, or antacids. Antimicrob Agents Chemother 45(3):810–814
108. Damle BD, Mummaneni V, Kaul S et al (2002) Lack of effect of simultaneously administered didanosine encapsulated enteric bead formulation (videx EC) on oral absorption of indinavir, ketoconazole, or ciprofloxacin. Antimicrob Agents Chemother 46(2):385–391
109. Banfield C, Herron J, Keung A et al (2002) Desloratadine has no clinically relevant electrocardiographic or pharmacodynamic interactions with ketoconazole. Clin Pharmacokinet 41(Suppl 1):37–44
110. Hsu A, Granneman GR, Cao G et al (1998) Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 42(11):2784–2791