Mechanism-based Preclinical Approaches for the Evaluation of Drug-Drug Interactions

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Summary: Pharmacokinetic drug-drug interactions (PDDI), in which the pharmacokinetic clearance of one drug is altered by a co-administered drug, can be divided mechanistically into two general categories: 1. inhibitory PDDI, the inhibition of the metabolic clearance of one drug by a co-administered drug; and 2. inductive PDDI, the enhancement of the metabolic clearance of one drug by a co-administered drug. It is believed that drug-drug interaction potential can be defined using a mechanism-based approach which includes the following sets of studies. 1: Identification of the major drug metabolizing enzymes. 2: Evaluation of the inhibitory potential for drug-metabolizing enzymes. 3. Evaluation of the induction potential for drug-metabolizing enzymes. Human liver-derived experimental systems: hepatocytes, liver microsomes, and cDNA-expressed microsomes, are useful experimental systems for this mechanistic approach to the evaluation of drug-drug interaction potential preclinical.

Key words: Drug-drug interactions, *In vitro* systems, Human liver, Hepatocytes, Liver microsomes, cDNA microsomes, Drug metabolism, Pharmacokinetics

Introduction: Mechanism and Clinical Significance of Pharmacokinetic Drug-drug Interactions

Multiple drug therapy is widely practiced to treat either a medical disorder or to treat several concurrently-existing ailments in the same patient. It is now known that drugs may interact with each other, with serious pharmacological and/or toxicological consequences. Drugs interact mainly by a phenomenon known as pharmacokinetic drug-drug interactions-alteration of the metabolic clearance of a drug by a co-administered drug. Although pharmacokinetic drug-drug interactions can occur during absorption, distribution, and elimination phases following initial drug administration, interference with drug metabolism appears to be the predominant mechanism. The interference can occur via inhibition or induction of the metabolism of the drug by a co-administered drug. Both mechanisms of pharmacokinetic drug-drug interactions can have serious clinical consequences.

Inhibition of drug metabolism results in an increase in plasma/tissue drug concentrations of administered drugs, which can lead to toxicity, especially with drugs that have a narrow therapeutic index. Thus pharmacokinetic drug-drug interactions via the inhibitory mechanism is a safety concern. A well-established example of a drug-drug interaction via an inhibitory mechanism is the occurrence of *torsades de pointes* ventricular arrhythmia

in patients receiving concomitant therapy with the non-sedating antihistamine terfenadine, with azole antifungals such as ketoconazole, itraconazole, clotrimazole, and with macrolide antibiotics such as erythromycin.¹⁻³⁾ Terfenadine is believed to be metabolized by CYP3A4.⁴⁾ It has been suggested that the adverse drug interactions observed with terfenadine are related to inhibition of its metabolism by the co-administered drugs, leading to the accumulation of the parent terfenadine to a cardiotoxic level.⁵⁾

Conversely, induction of drug metabolism can result in a decrease in drug concentration to a level that is no longer efficacious. A significant drug-drug interaction *via* an induction mechanism is the interaction between rifampin and oral contraceptives.⁶⁻⁸⁾ Rifampin is an antimicrobial agent that is a known inducer of CYP3A4 in the human.⁹⁻¹¹⁾ The key active ingredients of oral contraceptives, estrogen and progesterone analogs, and substrates of CYP3A4.¹²⁾ Rifampin administration has been found to lead to uterine bleeding and pregnancies in women taking oral contraceptives; this is believed to be due to the enhanced clearance of the active ingredients.¹³⁾ In addition to its interactions with oral contraceptives, rifampin also has been reported to decrease the therapeutic effects and/or plasma concentration of other drugs, including verapamil,^{14,15)} cyclosporin¹⁶⁾; doxycycline,^{17,18)} itraconazole,¹⁹⁾ prednisolone²⁰⁾ and zidovudine.^{21,22)} While drug-drug interactions due to enzyme inhibition are primarily a safety concern, therapeutic

Table I A comparison of individual *in vitro* human drug-metabolizing systems for their strengths in their applications to drug metabolism and drug-drug interaction studies

System	Strengths
Hepatic microsomes	<ol style="list-style-type: none"> 1. Ease of storage (-80°C) and use 2. Well-characterized experimental system 3. Multiple human comparison in same experiment 4. Pooling of microsomes from multiple donors for a "normalized" human 5. Active phase I oxidation enzymes
Hepatocytes	<ol style="list-style-type: none"> 1. Intact cell with ICP 2. Well-characterized experimental system 3. Active phase I(oxidation), II(conjugation) and III(transmembrane export) pathways 4. Physiological enzyme and cofactor concentrations 5. Active gene expression, allowing the evaluation of enzyme induction 6. Can model bioaccumulation or active excretion 7. Can study sequential metabolism 8. Relatively easy storage (cryopreservation in liquid nitrogen)
cDNA microsomes	<ol style="list-style-type: none"> 1. Only one isoform present, therefore can be used effectively for CYP isoform identification 2. Sensitive assays involving nonspecific substrates can be used to measure CYP isoform activity, thereby allowing high throughput screening of inhibitory potential

tic failure is the major concern for drug-drug interactions due to enzyme induction.

Mechanism-based Approaches to the Evaluation of Drug-drug Interactions

In the past, information on drug-drug interactions has been available only because of the occurrence of adverse reactions in the patients. Since patients can be significantly compromised by drug-drug interactions, it is prudent to evaluate the drug-drug interaction potential of new drugs. Because the potential interaction of a new drug with all existing drugs is impossible to be evaluated empirically, a more practical approach is to define the drug-drug interaction potential of the new drug based on mechanistic evaluation, followed by clinical trials specifically designed to further define the mechanistic observation.

Based on the understanding of the mechanism of drug-drug interactions, three approaches have been adopted recently for the evaluation of new drugs: 1. identification of the drug metabolizing enzymes responsible for metabolism; 2. evaluation of inhibitory potential of the new drug towards drug-metabolizing enzymes; and 3. evaluation of the induction potential of the new drug towards drug-metabolizing enzymes.

Human *In vitro* Metabolic Systems for Studying Pharmacokinetic Drug-drug Interactions

Because of the major differences in drug-metabolizing enzymes between human and nonhuman animals, human *in vitro* metabolic systems have been developed as tools to study xenobiotic metabolism, toxicology, and drug-drug interactions. The major *in vitro* systems involve those that have been developed from human liver: hepatic microsomes,²³⁾ hepatocytes,²⁴⁻²⁷⁾ liver

slices,²⁸⁻³⁰⁾ and cDNA-expressed cell lines.³¹⁻³⁵⁾

A comparison of individual *in vitro* human drug-metabolizing systems for their strengths in their applications to drug metabolism and drug-drug interaction studies is shown in **Table I**. A comparison of human *in vitro* drug metabolism systems in their limitations for application in drug metabolism and drug-drug interaction studies is shown in **Table II**. Physiologically relevant data can be obtained if these experimental systems are used within their limitations.

Intact Cell Properties (ICP)

I would like to introduce the concept of intact cell properties (ICP), which I believe to be critical for the correlation of data obtained with human *in vitro* experimental system with human *in vivo*. Experimental systems with intact cells such as hepatocytes and liver slices have several advantages over cell-free systems such as microsomes because of the following ICP (**Table III**):

1. Plasma Membrane

The plasma membrane serves as a barrier between the plasma and the hepatocytes in the liver *in vivo*. The plasma drug concentration can be dramatically different from the intracellular concentration owing to the following reasons:

a. Membrane permeability: Membrane permeability plays an important role in the ability of the drug in the plasma to enter the hepatocytes. Compounds that are highly permeable would equilibrate across the membrane at a faster rate than less permeable compounds. Because of metabolism and excretion, the intracellular concentration of less permeable compounds may be significantly lower than the plasma concentration.

Table II A comparison of human *in vitro* drug metabolism systems in their limitations for application in drug metabolism and drug-drug interaction studies

System	Limitations
Hepatic microsomes	<ol style="list-style-type: none"> 1. Disrupted system, lack ICP 2. Nonphysiological enzyme and cofactor concentration 3. Mainly phase I oxidation activity, limited phase II and no phase III activities 4. Cannot be used for induction studies
Hepatocytes	<ol style="list-style-type: none"> 1. Experimentation with fresh hepatocytes (e.g. induction studies) dependent on availability of fresh human livers 2. Cannot use reagents (e.g. inhibiting antibodies) that cannot cross the plasma membrane 3. Cannot study substrates at cytotoxic concentrations
cDNA microsomes	<ol style="list-style-type: none"> 1. Only one isoform, may yield overly-simplistic results if multiple enzyme pathways are involved in metabolisms 2. Nonphysiological enzyme/cofactor/coenzyme levels 3. Not suitable for enzyme induction studies

Table III Intact Cell Properties (ICP): Properties of intact hepatocytes which allows this experimental system to be a physiologically relevant model of the liver

ICP	Relevance
Intact plasma membrane	Barrier between plasma and intracellular enzymes
Active transport	Bioaccumulation of drug leading to a higher intracellular drug concentration than that in the plasma
Active export (e.g. bile excretion)	Bioelimination of drug leading to a lower intracellular drug concentration than that in the plasma
Complete, physiological levels of enzymes and cofactors	Physiologically relevant rates of metabolism; metabolite formation by relevant pathways
Active gene expression, gene transcription and protein synthesis	Modeling of enzyme induction and gene expression

b. Active transport: Bioaccumulation against the concentration gradient has been observed for certain drugs, leading to an intracellular concentration higher than the plasma concentration.

c. Active excretion: Xenobiotics in hepatocytes are actively excreted in the bile via several energy-dependent processes, including Gp170, MOAT, and Gp110. If the rate of active excretion exceeds that of the rate of uptake, the intracellular drug concentration would be lower than that in the plasma.

Observations made with intact hepatocytes would be effects as a function of the drug concentrations outside the cells (extracellular concentrations) while those made with microsomes would represent effects of drugs directly on the microsomal enzymes (intracellular concentrations). As for most drugs, the plasma concentration, which is extracellular concentration, is readily available while the intracellular concentration is not, extrapolation of *in vivo* effects probably is more appropriate using data obtained with intact hepatocytes rather than data obtained with liver microsomes.

2. Complete Drug-metabolizing Enzyme Pathways

Intact cells have all the enzyme pathways present in an undisrupted manner, in contrast to microsomes,

which are prepared by cell disruption and centrifugation. The major difference between intact hepatocytes and microsomes is that phase II conjugation metabolism, e.g., sulfate- and glucuronide-conjugation, are retained in hepatocytes but to a significantly lower extent in microsomes. Not only that the enzymes are intact, the cofactors which are critical to enzyme activities are also present in hepatocytes at physiological levels. The presence of complete enzymes and cofactors at physiological levels allows both a physiological rate of drug metabolism as well as the biotransformation of the parent drug into metabolites by pathways similar to that *in vivo*.

3. Active Biosynthetic Pathways

Intact cells have active gene expression and protein synthesis, allowing the study of enzyme induction and gene expression. CYP induction, a major mechanism of drug-drug interactions, can only be studied in hepatocytes. In fact, because of the major differences between laboratory animals and human in drug-metabolizing enzymes, primary human hepatocytes may represent the only appropriate system for the evaluation of CYP induction potential of novel drugs before the performance of clinical trials. Gene expression analysis using gene chips

is a new technology which may have vast impact to our understanding of drug properties. Hepatocytes represent one of the most important cell system for the evaluation of the effects of drugs on gene expression.

4. Intact Signaling Pathways

Intact cells can reproduce physiological events that arise due to intracellular signaling (e.g., calcium ions; cyclic nucleotides). These events require active, living, and intact cells and therefore cannot be studied in microsomes.

Because of ICP, human hepatocytes represent a physiologically-relevant model of the human liver. In the past, most researchers could not work with human hepatocytes because of the general unavailability of human livers for research. The recent success in hepatocyte cryopreservation allows human hepatocytes isolated from one laboratory to be stored and used in other laboratories³⁸⁻⁴¹) and therefore greatly extent the availability and utility of this important research tool.

Mechanistic Approaches to the Evaluation of Drug-drug Interactions

The following are the rationale and approaches for the mechanistic evaluation of drug-drug interactions. Intact hypatocytes, liver microsomes, and cDNA-microsomes are the experimental systems involved (Table IV).

1. Identification of the Major Drug Metabolizing Enzymes

a. Rationale: Understanding the relative contribution of each CYP isoform can provide valuable information on the drug-drug interaction potential of the drug in question. If one isoform is found to be mainly responsible for its metabolism, it is likely that pharmacokinetic interactions would occur between the drug in question and drugs that are known to be inhibitors or inducers of the isoform. For example, if the new drug is metabolized mainly by CYP3A4, known CYP3A4 inhibitors such as itraconazole, ketoconazole, and troleandomycin would be expected inhibit the metabolism of the new drug. On the other hand, CYP3A4 inducers such as phenytoin, rifampin, phenobarbital would be predicted to enhance the drug's metabolism. Furthermore, the new drug may have interactions with other drugs that are metabolized by the same isoform because of competition for the same binding site of the CYP isoform. A CYP3A4 substrate, for instance, may cause competitive inhibition of metabolism with other CYP3A4 substrates such as erythromycin and cyclosporine.

b. Approach: To identify the major drug metabolizing enzyme pathway, one needs to know firstly the metabolite profile of the drug being evaluated. As described earlier, the most accurate approach to predict *in vivo* metabolite profile is to use an intact cell system such as intact hepatocytes which contain all hepatic

drug metabolizing enzymes and cofactors at physiological levels. The common approach of the use of liver microsomes and with NADPH as cofactor would only allow the generation of oxidative metabolites. From the metabolite profile, one can deduce the relative contribution of phase I and phase II pathways in the metabolism of the drug being studied. If oxidation is the major route of metabolism, the next step is generally to assumed that P450 isoforms are involved and therefore experiments are performed to define which CYP isoforms are involved in the metabolism. Microsomes and cDNA-expressed microsomes are routinely used for the identification of CYP isoform(s) involved in metabolism of a new drug. With microsomes, the effects of specific inhibitors for specific isoforms or inactivating antibodies are used. The inhibition of drug metabolism by a specific inhibitor would indicate that the inhibited isoform is responsible for metabolism. With cDNA-expressed microsomes (microsomes derived from cells engineered to express only one specific CYP isoform), microsomes with different individual isoforms are evaluated for metabolism of the new drug. The microsomes with the highest activity would be the ones that expresses the isoform mainly responsible for metabolism. With CYP isoforms that are inducible (e.g. CYP1A2, CYP2A6, CYP2B6, CYP2E1, CYP3A4), correlation of increased metabolism with induction of the isoforms in cultured hepatocytes can also aid the identification of specific isoforms involved in the metabolism of the new drug. The application of these approaches is illustrated in a recent review on CYP3A4 substrates.³⁶⁾

2. Inhibitory Potential

a. Rationale: A drug that can inhibit a specific CYP isoform would have the potential to inhibit the metabolism of drugs that are substrates of the specific isoform. For instance, a drug that is a potent inhibitor of CYP3A4 would have the potential to inhibit the metabolism of a co-administered CYP3A4 substrate such as terfenadine, erythromycin, and cyclosporin.

b. Approach: Microsomes, cDNA-expressed human CYP isoforms and hepatocytes can be used for the evaluation of the inhibitory potential of a drug for individual CYP isoforms. The *in vitro* metabolism systems are incubated with specific isoform substrates, and the effects of the new drug on the metabolic rate of the substrates are evaluated for potential inhibitory activities. Results are usually expressed by an inhibition constant (K_i), which can be derived using enzyme kinetic approaches. Based on the K_i values, the effect of the inhibitor *in vivo* can be calculated based on the known plasma concentration of the inhibitor, assuming linear pharmacokinetics. Using this approach, data have been obtained for drugs under development. Apparent K_i values obtained from human hepatocytes may be more relevant that the values obtained from the use of microsomes and cDNA-expressed enzymes, particularly for drugs with

significant differences in plasma and intracellular (intra-hepatocyte) concentrations *in vivo*. The application of human liver microsomes in the prediction of *in vivo* CYP inhibitory potential has been reviewed by Wrighton *et al.*³⁷⁾ That intact hepatocytes and microsomes may have significant K_i values is illustrated by the evaluation of drug-drug interaction between terfenadine and azole antifungals ketoconazole and itraconazole.^{11,38)} The K_i values for the inhibition of terfenadine metabolism by ketoconazole and itraconazole are found to be significantly lower in primary human hepatocytes than in human microsomes, illustrating the possibility of underestimation of *in vivo* inhibitory potential using microsomes. The application of cryopreserved human hepatocytes to evaluate CYP inhibition has been successfully illustrated using CYP isoform-specific inhibitors such as furfylline (CYP1A2), quinidine (CYP2D6), and ketoconazole (CYP3A4).³⁹⁾

3. Induction Potential

a. Rationale: A drug that can induce the activity of a specific CYP isoform would have the potential to enhance the metabolism of other drugs that are metabolized by that isoform. For instance, a drug that is an inducer of CYP3A4 would enhance the metabolism of a CYP3A4 substrate.

b. Approach: Human hepatocytes represent the only proven *in vitro* experimental system with which induction of human CYP can be studied. Because of the known examples of species differences in response to CYP inducers, primary human hepatocytes *in vitro* may represent a more relevant system than laboratory

animals *in vivo* as a preclinical experimental system to evaluate drug-drug interaction via enzyme induction.²⁶⁾ Multiple laboratories have demonstrated that known *in vivo* CYP inducers are also potent inducers in primary human hepatocytes.²⁷⁾ The hepatocytes are incubated with the new drug, and the CYP isoform activities are then measured to evaluate for potential induction. As of this writing, the CYP isoforms believed to be highly inducible in human liver include CYP1A1, CYP1A2, CYP2A6, CYP2E1, CYP2B6, CYP3A4, CYP3A5, and CYP3A7. The isoforms that are not yet found to be highly inducible but evidence exist that they can be induced include CYP2C9, CYP2C19. As of this writing, the isoforms with no substantial evidence of inducibility in human liver are CYP2D6, CYP4A9 and CYP4A11.

4. Empirical Evaluation of Drug-drug Interactions

a. Rationale: Drug-drug interaction potential revealed by the mechanistic approach as detailed above may be analyzed further *in vitro* using drugs that may be co-administered. This may allow a more accurate estimation of the degree of drug-drug interactions. Further, evidence now exist that, at least for CYP3A4, results with one substrate (e.g. testosterone) may not directly extrapolatable to another substrate (e.g. midazolam).

b. Approach: The effects of one drug on the metabolism of another drug can be evaluated in the *in vitro* systems as described above, except that in stead of using probe substrates, the actual drugs will be used. For the evaluation of inhibitory effects, the human hepatocytes or liver microsomes are incubated simultaneously (it may be important to first preincubate with

Table IV Mechanism-based approaches to the evaluation of pharmacokinetic drug-drug interactions using human *in vitro* drug metabolism systems

Experimental Objectives	Approaches
Identification of metabolites	Incubation of drug with intact human hepatocytes followed by LC/MS/MS identification of metabolites
Identification of CYP isoform (s) involved in metabolism	1. Evaluate the effects of isoform specific inhibitors on the rate of drug disappearance upon incubation with human liver microsomes 2. Compare the rate of metabolism of the drug with cDNA-expressed human microsomes
Evaluation of inhibitory potential for CYP isoform (s)	Evaluate the effects of the drug in the rate of metabolism of isoform-specific substrates in intact human hepatocytes or human liver microsomes
Evaluation of induction potential for CYP isoform (s)	Evaluate the effects of a 2–3 day drug treatment of primary cultured hepatocytes on the rates of metabolism of CYP isoform-specific substrates. Results can be further confirmed with western blotting to quantify the cellular content of isoform proteins.
Empirical evaluation of drug-drug interactions	Evaluate the effects of one drug on the metabolism of another drug in human hepatocytes. This experiment is usually performed to allow better estimation of interactions between two specific drugs.

the drug that is believed to be inhibitory, especially if the mechanism-based inhibition is expected). An example of this approach is our studies involving ketoconazole/terfenadine interactions.^{11,38)} For the evaluation of induction effects, human hepatocytes are first incubated with the inducing drug. After the induction period (usually 2–3 days), metabolism of the co-administered drug will be evaluated. An example of this approach is our studies involving rifampin/lidocaine interaction.¹⁰⁾

Conclusions

Because of the major differences in drug-metabolizing enzymes between human and nonhuman animals, human *in vitro* metabolism systems are invaluable tools for the evaluation of metabolic fate and toxicity of drugs. Recently, human *in vitro* systems have been applied towards the evaluation of drug-drug interaction potential of new and existing drugs. The *in vitro* systems are derived from the human liver and include microsomes, hepatocytes, slices, and cDNA-expressed enzymes. Three mechanism-based approaches are commonly used in the evaluation of the drug-drug interaction potential of a drug: 1. identification of metabolic pathways; 2. evaluation of the inhibitory potential for drug-metabolizing enzymes; 3. evaluation of the induction potential for drug-metabolizing enzymes; and 4. Empirical evaluation of drug-drug interactions using actual drugs which may be co-administered in patients. *In vitro* human hepatic are routinely used in these studies. cDNA-expressed enzymes and liver microsomes are useful in the evaluation of metabolic pathways, especially in the identification of isoforms involved in metabolism. While microsomes and hepatocytes are useful in the evaluation of the inhibitory potential, they have different applications. Microsomes allow one to evaluate the direct interaction of the xenobiotic being studied with the microsomal enzymes, therefore is more appropriate for the evaluation of the mechanism of inhibition. Hepatocytes, because of the presence of intact plasma membrane, allow the modeling of intracellular concentration, and therefore represent a relevant experimental model for the estimation of inhibitory potential based on extracellular (i.e. plasma) concentration. As of this writing, cultured hepatocytes represent the most useful experimental model for the evaluation of induction potential.

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