Drug Metabolism & Pharmacokinetics in Drug Discovery: A Primer for Bioanalytical Chemists, Part I

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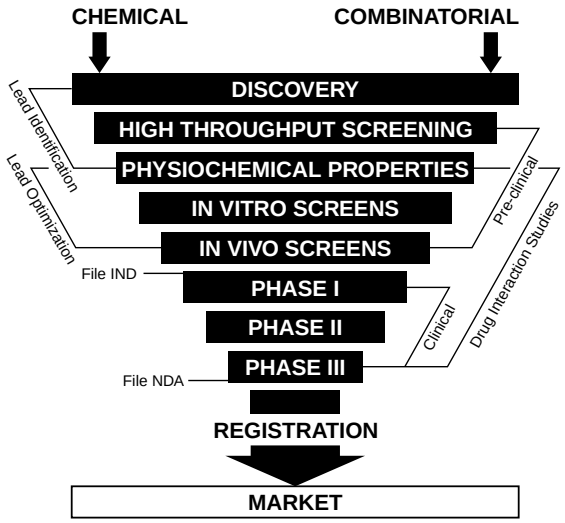
In the face of advancing technology in combinatorial synthesis and high throughput screening, the drug discovery process continues to evolve. Preclinical drug metabolism and pharmacokinetics studies play a key role in lead identification and optimization. This fast-paced development process has imposed an enormous burden on the analytical chemist to design faster and more sensitive assay techniques to aid the drug discovery and development. This article, Part I of a two-part series introduces the analytical chemist to the fundamentals of drug metabolism. Part II of this series will discuss the pharmacokinetics aspects and how drug metabolism data can be used to predict pharmacokinetic parameters.

Technological innovation and the pressures of competition have caused enormous changes in the drug discovery process. Progress in molecular biology and the Human Genome Project has contributed to the remarkable advances made in identification of new therapeutic targets. The drug discovery process is rapidly evolving due to the technological developments in target identification along with automation of combinatorial synthesis and high throughput screening (HTS). In light of these advances, improving efficiency in the optimization of desired pharmacological activity in humans while decreasing the reliance on animal studies has become a challenge. New chemical entities (NCEs) enter the drug discovery pipeline through combinatorial synthesis and rational drug design where information about the target of action is used to design the lead compound. HTS helps the identification of the leads that provide the required effect at high concentrations. In the secondary screening stage physicochemical properties such as solubility, liphophilicity and stability are determined by measuring the octanol-water partition coefficient and pK_a. These measurements are useful in predicting the protein binding, tissue distribution and absorption in the gastrointestinal tract (1).

The selected leads are further screened using in vitro tests during lead optimization. The goal of lead optimization is to select compounds with required biological activity in humans. Relevant pharmacokinetic parameters such as tissue penetration, stability, intestinal absorption, metabolism, and elimination are obtained using in vitro systems. These in vitro systems include microsomes, hepatocytes or tissue slices for metabolite identification and evaluation of metabolic pathways and rates, and caco-2 cell lines for evaluating transcellular absorption. Cytotoxicity data can be obtained by using organ-specific cell lines. Knowledge of the toxic potential of these early leads and their possible metabolites is essential for successful drug discovery. Most drug candidates fail at this stage and only a few will be judged sufficiently safe and efficacious to proceed further into development. Both in vitro

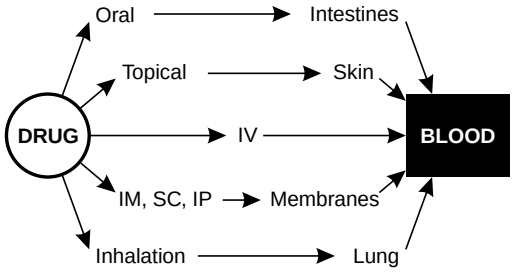
F1

Drug development process (IND: Investigational New Drug, NDA: New Drug Application)



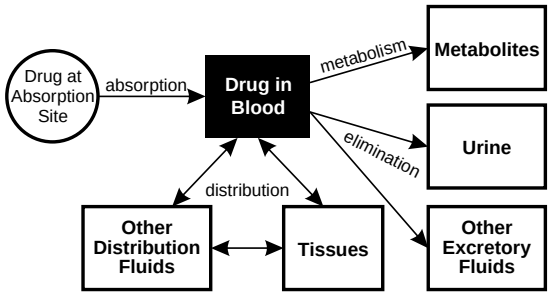
F2

Absorption of a drug after administration.



F3

Schematic representation of drug's path from blood.



and in vivo studies are then carried out on the active candidate compounds. The objective in these preclinical studies is not only to identify the most active leads with the most appropriate safety profiles but also, to select the closest animal species to the human for toxicity studies (2). Understanding of pharmacokinetic and metabolism characteristics of the selected compounds is needed in designing appropriate human clinical trials. Various stages of drug discovery are illustrated in ***F1***.

Meeting the objectives of drug metabolism research, whether it be in vitro or in vivo, requires the processing of a very large number of samples for the determination of drug candidates and metabolites. There is likewise a lot of structural chemistry to be done to identify metabolites. It is important for analytical chemists to understand that the vast majority of compounds (actually > 99.99%) will never become drugs. Thus the bioanalytical work must be fast. Often elegance must be traded for speed early in the process. Later on, for example in clinical trials, the number of samples for a particular compound will increase exponentially and carefully validated methods are both required and justified.

This article is intended to enlighten bioanalytical chemists on drug metabolism and pharmacokinetics aspects in drug discovery. Part 1 of the article covers the basics of drug metabolism. In Part II we will discuss the kinetics of drug metabolism and the relationship of kinetic data to the pharmacokinetics of a drug.

Path of a drug

After administration by any route, a drug will reach the blood stream as schematically shown in ***F2***. This process is known as *absorption*. The drug in the blood distributes rapidly between the plasma and blood cells and also between plasma proteins. Most drugs readily cross the capillaries and reach the extracellular fluid of every organ. Lipid soluble drugs cross the cell membranes and distribute into the intracellular fluid of various tissues. This process of transferring a drug from blood to various tissues is called *distribution*.

A drug is eliminated either directly through an excretory route such as urine, bile etc. which is known as *elimination*; or indirectly through enzymatic or biochemical transformation by the liver. The latter path of elimination is called *metabolism*. The study of this whole process of absorption, distribution, metabolism and elimination of a drug as schematically shown in ***F3*** is known as *ADME* studies.

Drug Metabolism

Interest in drug or *xenobiotic* (foreign compounds) metabolism can be dated back to the early 19th century. Metabolism then was known as a "detoxication" mechanism in the body. In late 1930s, with the discovery of the synthetic azo-dye Prontosil's metabolism to antibacterial agent sulfanilamide in the body, studying metabolism has become an important priority. This year BAS and the International Society for the Study of Xenobiotics (ISSX) produced a historical calendar celebrating many of the original contributions to our knowledge of the metabolism of organic compounds (3).

Metabolism is the mechanism of elimination of foreign and undesirable compounds from the body and the control of levels of desirable compounds such as vitamins in the body. Since information on the metabolism of a drug plays a significant role in selection and further characterization of the drug, an in-depth look at the mechanism of drug metabolism is worth the effort.

The major site of metabolism in the body is the liver. Metabolism in liver occurs in two stages: *Phase I* pathways in liver microsomes where the drug is functionalized and *Phase II* pathways in liver cells where the parent or the metabolite from Phase I gets conjugated. Liver microsomes are in the endoplasmic reticulum of liver cells or *hepatocytes*. Phase I reactions in microsomes are catalyzed by a group of enzymes known as the *cytochrome P450* system that plays a significant role in drug metabolism. The common chemical reactions involved in Phase I are aromatic hydroxylation, aliphatic hydroxylation, oxidative N-dealkylation, oxidative O-dealkylation, S-oxidation, reduction and hydrolysis. Most often this simple functionalization could be sufficient to make a drug more soluble, facilitating elimination

T1

Known Cytochrome P450 substrates.

Enzyme	Substrates
CYP1A2	Amitriptyline, Betaxolol, Caffeine, Clomipramine, Clozapine, Chlorpromazine, Fluvoxamine, Haloperidol, Imipramine, Olanzapine, Ondansetron, Propranolol, Tacrine, Theophylline, Verapamil, (R)-Warfarin
CYP2A6	Coumarin, Betadiene, Nicotine
CYP2C9	Amitriptyline, Diclofenac, Demadex, Fluoxetine, Ibuprofen, Losartan, Naproxen, Phenytoin, Piroxicam, Tolbutamide, (S)-Warfarin
CYP2C19	Amitriptyline, Citalopram, Clomipramine, Diazepam, Imipramine, Omeprazole
CYP2D6	Amitriptyline, Betaxolol, Clomipramine, Codeine, Clozapine, Desipramine, Fluoxetine, Haloperidol, Imipramine, Methadone, Metoclopramide, Metoprolol, Nortriptyline, Olanzapine, Ondansetron, Paroxetine, Propranolol, Risperidone, Sertraline, Timolol, Venlafaxine
CYP2E1	Acetaminophen, Caffeine, Chlorzoxazone, Dextromethorphan, Ethanol, Theophylline, Venlafaxine
CYP3A4/5	Alprazolam, Amiodaron, Amitriptyline, Astemizole, Bupropion, Buspirone, Caffeine, Carbamazepine, Cerivastatin, Cisapride, Clarithromycin, Clomipramine, Codeine, Cyclosporine, Dexamethasone, Dextromethorphan, DHEA, Diazepam, Diltiazem, Donepezil, Doxycycline, Erythromycin, Estradiol, Felodipine, Fluoxetine, Imipramine, Lansoprazole, Lidocaine, Loratadine, Lovastatin, Midazolam, Nicardipine, Nifedipine, Omeprazole, Orphenadrine, Paroxetine, Progesterone, Quinidine, Rifampin, Sertraline, Sibutramine, Sildenafil, Simvastatin, Tacrolimus, Tamoxifen, Terfenadine, Testosterone, Theophylline, Verapami, Vinblastine, (R)-Warfarin

through the kidneys. Further conjugation in Phase II occurs by glucuronidation, sulfation, amino acid conjugation, acetylation, methylation or glutathione conjugation to facilitate elimination.

Cytochrome P 450 system

Cytochrome P450 (CYP) enzyme system, a very large group of enzymes encoded by the P450 gene superfamily, is one of the widely studied topics in drug development. CYPs are membrane bound proteins with an approximate molecular weight of 50 kD, and contain a heme moiety. CYPs and other mixed function oxygenases are mainly found in the endoplasmic reticulum of the liver. The monooxygenase function of CYP450 involves a number of steps but the end reaction is the transfer of one oxygen atom to the substrate (R) that has a site for oxidation as shown below (4).

$$NADPH + H^+ + O_2 + R\text{-}H \xrightarrow{CYP450} NADP^+ + H_2O + R\text{-}OH$$

Genetic polymorphism

Cytochrome P450 enzymes are grouped into families and sub families based on their structural similarity (5). Families include CYPs with >40% amino acid sequence homology and are designated by a number after CYP. Subfamilies are the CYPs within a family that have >60% amino acid sequence homology and are designated by a letter following the number. For example CYP3A4 is a cytochrome P450 enzyme, belonging to family 3 and subfamily A. The last number 4, refers to the sequence of discovery.

Several of the drug metabolizing enzymes, for example the CYP2 family, are *polymorphic* (having more than one variant of the gene). Although the CYP isozymes generally have similar functional properties, each one is different and has a distinct role. This polymorphism forms a basis for interindividual differences in the efficacy of drug treatment, side effects of drugs and the toxic and carcinogenic action of xenobiotics.

There are about 30 human cytochrome P450 enzymes, out of which only six, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are the major metabolizing enzymes. CYP3A is the most abundant and most clinically important isozyme in humans. It metabolizes nearly 50% of the clinically available drugs. ***T1*** shows the major CYPs involved in the metabolism of some known drugs. From the table it can be seen that some drugs are metabolized by more than one isozyme. This multiple-substrate metabolism is the cause for metabolism-based drug-drug interactions (DDIs).

Some drugs can be inducers or inhibitors of specific isozymes but not necessarily substrates. **Enzyme inducers** increase specific enzyme levels by modulating the gene expression. Some drugs induce P450 enzymes that are not involved in their metabolism. For example, omeprazole induces human CYP1A2 but is metabolized by CYP2C19 and CYP3A4 (6). Administration of omeprazole can lower the effect of a drug normally metabolized by CYP1A2, e.g., acetaminophen.

Enzyme inhibitors function in different ways. The **competitive inhibitors** compete with the substrate for the active site, e.g., fluvoxamine and caffeine for CYP1A2 (7). The **non-competitive inhibitors** bind to the enzyme-substrate complex or to the heme group, e.g., ketoconazole. The third type, **irreversible inhibitors** inactivate the enzyme either by heme binding or protein binding. Enzyme inhibition can lead to higher systemic levels of a drug causing enhanced efficacy or toxicity. This should be considered when multiple drugs are simultaneously prescribed and/or when over-the-counter drugs or neutraceuticals are concomitantly administered with prescription drugs.

When several enzymes metabolize a drug, e.g. propranolol (8), administration of an enzyme inhibitor will not have a great effect since the

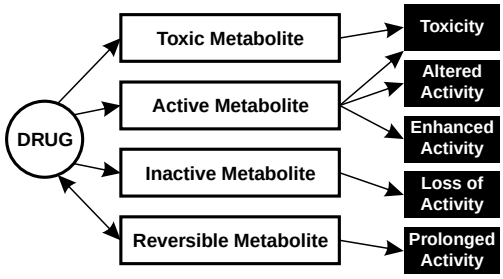
T2

Known inhibitors and inducers of CYP isozymes

Enzyme	Inducers	Inhibitors
CYP1A2	Cigarette Smoke, Phenobarbital, Ritonavir, Carbamazepine, Charbroiled Foods, Vegetables, Omeprazole	Enoxacin, Ciprofloxacin, Grepafloxacin, Fluvoxamine, Fluoxetine, Nefazodone
CYP2A6	Barbiturates	
CYP2C9	Rifampin, Carbamazepine, Ethanol, Phenytoin	Amiodarone, Fluvastatin, Fluvoxamine, Fluoxetine, Fluconazole, Miconazole, Metronidazole, Ritonavir, Sulfamethoxazole
CYP2C19	Rifampin	Fluvoxamine, Fluoxetine, Ticlopidine, Ritonavir
CYP2D6	Pregnancy	Quinidine, Fluoxetine, Paroxetine, Sertraline, Thioridazine, Cimetidine, Diphenhydramine, Haloperidol, Ticlopidine (Ticlid), Ritonavir
CYP2E1	Ethanol, Isoniazid, Ritonavir	Cimetidine, Watercress
CYP3A4/5	Carbamazepine, Dexamethasone, Rifapentine, Prednisone, Growth Hormone, Rifampin, Phenobarbital, Phenytoin,Troglitazone	Ketoconazole, Itraconazole, Erythromycin, Grapefruit Juice, Fluvoxamine, Fluoxetine, Diltiazem, Verapamil, Clarithromycin, Omeprazole), Ritonavir, Indinavir

F4

End results of drug metabolism



drug has an alternate pathway. ***T2*** shows some inducers and inhibitors of the CYP isozymes.

There is a wide variation in the expression, activity and concentrations of different isozymes among individuals, species and ethnic groups. The expression or the activity of these enzymes is influenced by factors such as species specificity, genetic polymorphism, gender- hormonal control, age, disease and environmental inducers (caffeine, cigarette smoke). The variability associated with the CYP450 enzymes in each individual results in marked differences in response when the same drug and the dose is administered to different individuals. Genetic polymorphism of CYP450 enzymes characterize the general population into three groups:

a) Extensive metabolizers (EM): normal population.

b) Poor metabolizers (PM): Individuals who inherit two inactive alleles (alternative forms of the gene) showing complete absence of enzyme activity.

c) Ultra extensive metabolizers (UEM): Individuals with one common allele and one amplified allele showing enhanced enzyme expression.

Ultra extensive metabolism can cause therapeutic failure due to reduced bioavailability or lack of activation of the drug whereas poor metabolism can lead to drug toxicity and sometimes death. For optimal drug therapy, the prescribing physician should have the knowledge of the genetic makeup of the CYP enzymes in the patient.

Outcome of drug metabolism

Various possibilities of the outcome of drug metabolism are illustrated in ***F4***.

Cytochrome P450 reactions make substrates more hydrophilic for easy elimination through the kidneys. Although most often this results in inactivation of the drug, some compounds form active metabolites. These active metabolites can enhance, modify, or inhibit the desirable activity of the drug. Sometimes the active metabolite initiates the pharmacological activity. This function is used in designing pro drugs. Pro drugs are defined as therapeutic agents that are inactive but are transformed into the active form by enzymatic reactions. This is very useful when the active form is unstable or poorly water soluble, making the formulation a challenge. Following oral administration, the hypotensive drug Enalapril maleate (Vasotec) undergoes ethyl ester hydrolysis to form enalaprilate, which is the active drug.

Some drugs have very little therapeutic potential but form a more pharmacologically active metabolite. For example codeine itself has very low analgesic activity. It forms morphine, the more active form when it is metabolized by CYP2D6. Poor metabolizers of CYP2D6 or patients who are taking CYP2D6 inhibitors, therefore, do not experience the analgesic property of codeine.

In some cases the metabolite exhibits the same pharmacological activity as the parent and is less toxic than the parent. One such example is the antihistamine drug fexofenadine (Allegra) which is a metabolite of

terfenedine (Seldane). Seldane was withdrawn from the market due to its fatal interactions with erythromycin and ketoconazole in some patients when concomitantly administered.

While there are many examples where both parent and the metabolite have the same pharmacological activity, some metabolites will show different pharmacological activity from the parent. This may lead to the discovery of a new drug. Loxapine is an antipsychotic drug that undergoes extensive metabolism. The N-demethylated metabolite, amoxapine however has anti depressant activity and is prescribed for that indication.

Metabolism can also result in toxic metabolites. Formation of reactive metabolic intermediates is one of the causes for drug toxicity. Oxidation to electrophilic intermediates or reduction to nucleophilic radicals that can attack DNA or RNA and induce carcinogenicity are two major reactions by which toxicity is exerted. Although many leads are abandoned early on in drug discovery stage due to the toxic metabolite formation, presence of a toxic metabolite does not always implies toxicity in a given drug candidate since there are other factors that can make the metabolite toxic or non-toxic. Presence of a toxic metabolite however raises a red flag, which must be extensively examined in animal toxicity studies.

Some drugs are metabolized reversibly. For example, sulindac, a nonsteroidal anti-inflammatory drug is reversibly metabolized to sulindac sulphide which has anti-inflammatory and analgesic properties and is irreversibly metabolized to sulindac sulphone which has been suggested to possess antiproliferative effects against tumors (9).

Factors Affecting Drug Metabolism

There are marked differences in drug metabolism across species. Selecting a species that closely represents the human is very crucial in drug discovery and in designing clinical trials later. Age, hormonal control (gender, pregnancy), genetic polymorphism, disease state, are all internal factors that affect the metabolism. Infants for example, lack Phase II enzymes whereas elderly patients have diminished metabolism and excretion due to the aging process. Although there is no evidence of clinically relevant gender differences in metabolism of humans, there have been studies showing the effect of rat sex hormones on bioavailability. Liver diseases such as hepatitis, liver cancer, or cirrhosis impair drug metabolism either due to the decreased number of functional hepatocytes or to the altered NADPH/NAD ratio in the liver. If the drug is cleared only by the liver the impaired metabolism can result in drug overdose.

Genetic or hereditary factors are the most significant factor in drug metabolism (10). Genetic differences among individuals or ethnic groups can lead to an excessive or prolonged therapeutic effect or toxic overdose. For example, the enzyme CYP2D6 which metabolizes a large number of drugs has 16 alleles. The activity of this enzyme varies widely among ethnic groups (11). About 1% of Arabics, 30% Chinese and 7-10% Caucasians are poor metabolizers of CYP2D6 drugs. Another example is CYP2C19, which contributes to the metabolism of anxiolytics (e.g. diazepam). About 14-22% Asians and 3-6% Caucasians are poor metabolizers of CYP2C19. Elevated plasma drug levels in these populations after drug administration can increase the sedative effect of the drug.

Environmental factors such as diet, smoking, alcohol consumption and concomitant drug therapy also influence the outcome of drug metabolism. Cigarette smoke produces poly aromatic hydrocarbons (PAH) which induce CYP1A2 (12). CYP1A2 metabolizes the PAHs to carcinogens responsible for lung and colon cancer. Grapefruit juice is a good example of dietary constituent that inhibits CYP3A4 (13). With the new boom in consumption of neutraceuticals and herbal medicines like St. John's Wort, Gingko Biloba, the possibility increases considerably for drug interactions to occur.

Influence of Drug Metabolism on Drug Development

In drug development it is important to have information on the enzymes responsible for the metabolism of a drug candidate as early as possible in the design phase. Knowledge of the metabolic pathways, metabolite stability, toxicity and the specific isozymes involved in the metabolism are all important information in the drug development process and in planning human clinical studies. The rate of metabolism affects the oral bioavailability and clearance in humans and preclinical species. As discussed before, polymorphic enzymes will lead to high interindividual variability and potential for DDIs. Genetic information is used to predict the response of individual patients and patient populations to drugs and to tailor drug selection and dosage to fit the individual's genetic constitution. Metabolite profiles are important for designing prodrugs and pharmacologically active metabolites and for selecting the right animal species for toxicology studies. Structural modification of the drug candidate can alter the metabolism. Highly hydrophilic or highly lipophilic compounds are not suitable because they result in poor bioavailability and very slow or very fast excretion rates. In these instances replacing an active group with another non-reactive group in the compound can achieve greater metabolic stability. For example, replacing a methyl group by a t-butyl group can prevent demethylation. Similarly, oxidation of aromatic rings can be prevented by substituting them with stronger electron withdrawing groups (e.g. CF_3). Information obtained from pre-clinical drug metabolism studies can be fed back to the design team to introduce functional groups which will alter the physical properties to make

T3

Information that can be obtained from in vitro studies.

In vitro studies can give information on:
• Metabolite stability
• Metabolite profile
• Metabolite identification
• Interspecies comparisons
• Toxicology species selection
• CYP induction/inhibition
• Drug/Drug interaction studies
• CYP isoform identification
• Phase II enzyme studies

the compounds more metabolically stable. Thus final selection of a successful drug lead depends immensely on the drug metabolism studies.

Pre-clinical Drug Metabolism Studies

It is important to know how the drug is eliminated early in the drug development process. If elimination is mainly by metabolism, then the metabolic pathways and products need to be understood. Knowing the toxicity of a drug and its metabolites before entering human clinical trials is essential to avoid failures later on in the process. Both in vitro and animal in vivo studies are done in the pre-clinical stage.

Most promising compounds are selected from in vitro studies and their pharmacokinetic parameters are obtained in two animal species, commonly in rat and dog in the in vivo animal studies. This article will focus on the in vitro studies and information obtained from them.

In Vitro Studies

The in vitro studies during pre-clinical screening are low-throughput systems. Primary in vitro metabolic systems used in drug metabolism involve hepatic enzymes or tissue preparations. Information (***T3***) obtained by incubating a test drug with these systems can be used as feedback to design safer and more metabolically stable drugs. Compounds can be ranked according to the metabolic stabilities. Mass spectrometry is used as a qualitative tool to identify the metabolites and the sites at which metabolism occur. A variety of hepatic in vitro systems differing in biological intricacy are now commercially available for metabolism studies. Most widely used systems are discussed below in detail.

Expressed Enzymes

Advances in molecular biology have enabled the identification and characterization of a large number of individual CYP genes. Specific cDNA sequences for particular CYP isozymes have been cloned and expressed heterologously. These expressed enzymes including human enzymes are now commercially available as pure systems. Since the conditions of reactions such as concentrations of enzyme, substrate and co-factor can be carefully controlled, enzyme systems have become a powerful tool to study drug metabolism. This system is very useful in the study of kinetics, specificity and the mechanism of the enzyme reaction. However, folding or the posttranslational modifications and enzyme activity of the expressed enzyme may differ from the native enzyme.

Microsomes

Microsomes can be prepared easily from frozen liver tissues. They contain most of the oxidative drug metabolizing enzymes. Their easy preparation and good long-term stability at -80 °C make microsomes the most frequently used in vitro system in drug metabolism studies. Microsomes are isolated from liver cells by disrupting the cellular contents and centrifugation at 100,000 × g. Liver microsomes can be manipulated by induction and inhibition to vary the activity or the levels of the isozymes. The ability to phenotype microsomes greatly increases the utility of this system in the identification of specific isozymes responsible. Metabolic information such as metabolic profiles, stability, metabolite identification and kinetics can be obtained from microsomal systems. Microsomal incubations are most often used to obtain information on Phase I reactions. One disadvantage is that the information is not complete as from the cellular systems.

Isolated Hepatocytes

Cell cultures or cell suspensions can be used to study multiple aspects of drug metabolism, drug transport across cell membranes, cytotoxicity and enzyme induction in an environment where enzymes and co-factors are present in normal physiological concentrations and cellular integrity is maintained. Hepatocytes are used to study both Phase I and Phase II reactions. Cells can be either primary or permanent cell cultures. Primary cell lines are most often used for drug metabolism studies because permanent cell lines possess very little or no enzyme activity. Primary cells are isolated from fresh liver tissue and can be used immediately after isolation or culture for long-term studies. However, cultured or cryopreserved cells lose the P450 activity rapidly with time (14). Also, hepatocytes cannot be frozen and thawed or be prepared from previously frozen liver. Therefore, there is a great need for research in improving cryopreservation technology and stabilization of P450 activity in primary cultures. With the increased availability of fresh human tissues from various commercial and non-profit institutions, human hepatocytes have become the most widely used and preferred in vitro system.

Tissue Slices

While tissue slices have been used from other organs like brain, heart, and kidney, liver is the most commonly used tissue type for drug metabolism experiments. Tissue slices have certain advantages over other systems. With intact cell-cell junctions, normal hepatic cellular architecture is retained in the tissue. Since they contain the complete complement of drug metabolizing enzymes with all the cofactors present in rele-

T4

Comparison of in vitro systems.

System	Advantages	Disadvantages	Future Needs
Expressed Enzymes	Pure system	Single system	Integration with other enzyme systems
Microsomes	Well-used, Long term storage at -80°C,Well-characterized	Limited information, Need cofactors	
Isolated cells (e.g. Hepatocytes)	Integrated cellular system	Short life time, Limited enzyme stability	Increased availability of human cells, Better preservation
Slices	Easy to prepare, Cellular integrity maintained	Limited medium penetration, Short-term viability	Greater availability of human tissues, Cryopreservation

vant concentrations, complete information on the metabolism reactions can be obtained. Liver slices can be easily and rapidly produced. In addition, liver slices are not exposed to proteolytic enzymes that can destroy important membrane receptors of the cell. Although liver slices are increasingly used now in drug metabolism studies, they have certain disadvantages. One drawback is the inadequate penetration of the medium. Liver slices cannot be cryopreserved and they have a limited useful experimental period.

Some advantages and limitations of these in vitro systems are summarized in ***T4***.

With greater availability in human tissues and recombinant enzymes it is now possible to predict potential DDIs before clinical trials. Identifying the major metabolic pathways of the drug and its metabolites and exploring the effect of the test drug on the metabolism of other drugs and vice versa are two major goals of the in vitro studies. In vitro studies also could help to decide that a particular drug is not a substrate for certain pathways. This reduces or eliminates the need to study the possible inhibitory effects of that drug on other drugs metabolized by that pathway.

Despite the progress in the in vitro assays, the general consensus is that in vitro models are too simplistic to substitute for in vivo studies. In vitro studies are best suited to determine the types of clinical trials needed to assess potential DDIs. FDA guidelines suggest first using in vitro studies to assess the effect of drugs on metabolic pathways and if the results indicate possible DDIs, to follow up with in vivo assays (15).

The eventual goal of the in vitro studies is to predict the in vivo outcome in humans. Utility of human in vitro models to predict drug-drug interaction potential and pharmacokinetic variability has been demonstrated successfully for the anti psychotic drug, olanzapine (16). Methodology of scaling in vitro data to predict in vivo outcome, or in *vitro-in vivo correlation*, is expanding due to the increasing availability of human in vitro systems (17). Part II of this article will discuss the significance of in vitro enzyme kinetics data in the evaluation of in vivo pharmacokinetic data.

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