CASE STUDY

PharmaPendium®

Dr. Kevin Lustig, President and CEO, The Assay Depot, Inc. Greater San Diego Area

Understanding Key Determinants of Drug Activity

SUMMARY
This article highlights the increasing role that drug metabolism and transport proteins have in the drug approval process.

ELSEVIER

The dynamics of drug metabolizing enzymes and transporters is critical to the development of personalized medicine strategies.

Dr. Kevin Lustig, Author
President & CEO, The Assay Depot Inc.
Greater San Diego Area

Abstract / Summary

The dynamics of drug metabolizing enzymes and transporters is critical to the development of personalized medicine strategies. A multitude of factors regulate enzymes and transporters, which in turn affect the pharmacokinetic properties of drugs and mediate drug interactions. Evaluation of drug interaction profiles is a critical step in drug development and necessitates detailed in vitro and in vivo studies along with predictive modeling.

PharmaPendium® via its Metabolizing Enzymes and Transporters Module provides unprecedented depth of data on drug metabolizing enzymes and transporters and offers a unique platform for modeling advanced drug interactions. This Module will prove to be a valuable resource capable of improving workflows and accelerating development by enabling intelligent, in silico drug design. By providing scientists with all of the available knowledge about drug metabolism, we can significantly reduce the need for costly and time-consuming lab work and animal models. Requiring these only to answer the true unknowns where no one else has gone before.

Introduction

Every successful drug discovery and development effort needs to factor in drug efficacy and safety, both of which are intimately linked to drug metabolism. Hence knowledge and understanding of drug transport and metabolism is crucial during drug development to predict potential drug interactions and interindividual variations. The Food and Drug Administration recommends, "pharmacokinetic interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness." Additionally, a successful personalized medicine approach requires an in-depth understanding of the dynamics of drug transport and drug metabolism.

Drug Transport

Membrane-bound carriers or transporters are the primary mediators of drug transport and directly affect drug absorption, distribution, metabolism and excretion (ADME) for the majority of compounds. Though hundreds of membrane transporters have been identified, most of those associated with drug transport belong to the ATP-binding cassette (ABC) or the solute carrier (SLC) superfamilies. Drug transporters are critical determinants of drug efficacy and toxicity. For instance, transporters in the gastrointestinal tract affect drug absorption by increasing uptake (uptake transporters) or by limiting drug absorption (efflux transporters). Alternatively, the P-gp transporter in the blood-brain barrier prevents drugs from entering the central nervous system by efflux mechanisms.

Dr. Maria Thompson, Co-Author
Principal Consultant

Drug Metabolism

Drug metabolism is an important aspect of clinical pharmacology and is the primary mechanism by which drugs are converted into their inactive metabolites. Equally important is the effect of metabolism on some drugs that are converted into pharmacologically active metabolites or in some cases, into reactive, toxic, and carcinogenic metabolites. Drug metabolizing enzymes are broadly categorized into two distinct classes: phase I or functionalizing enzymes and phase II or conjugating enzymes.

Among the phase I enzymes, cytochrome P450 (CYP) monooxygenases are the most-important enzyme class involved in drug metabolism. Three subfamilies, CYP 1A2, 2C and 3A4 together are responsible for over half of the drug metabolic reactions. Non-CYP enzymes may also mediate biotransformation. Phase II biotransformation of drugs involves conjugation or covalent addition of endogenous moieties to the drug that converts a lipophilic substrate into a polar product.

Drug metabolizing enzymes are found in most body tissues with the highest levels in liver, small and large intestines and some expression in the nasal mucosa and lung. Notably, a single enzyme pathway can drive the metabolism of multiple drugs. Similarly, metabolism of one drug can involve multiple pathways acting in series or in parallel.

Regulation of Drug Transport and Metabolism

Drug transporters and metabolizing enzymes show significant differences based on gender, age, race/ethnicity, genetics (polymorphisms) and a variety of external factors (such as diet and lifestyle). It is important to understand these variations not only during drug development but they also form an important aspect of designing individualized medicine strategies. A few representative examples below demonstrate how these proteins are differentially regulated and affect pharmacokinetic profiles of drugs.

- The metabolizing enzyme CYP3A4 shows higher activity in females than males. Hence, drugs that are metabolized via CYP3A4 will show higher clearance in females, and doses may have to be adjusted accordingly. On the other hand, males have a higher activity of the enzyme CYP2D6 and hence show higher clearance rates for drugs metabolized by this enzyme.

- Most drug transporters, such as P-gp and MRP-1 follow a developmental pattern. The activity of transporters increases during the first few months after birth and reach adult levels at 2 years of age. Similarly, most drug metabolizing enzymes are immature in neonates. For examples, levels of CYP enzymes at birth are only ~30% that of normal adult levels and the different CYP enzyme families attain adult levels at different ages.

- An extraneous factor such as smoking increases CYP1A2 and can in turn increase the clearance of caffeine and theophylline.

- Polymorphisms in transporters and enzymes can affect drug distribution and elimination. For example, mutations decrease CYP2D6 activity whereas multiple gene copies produce increased enzyme activity. A wide variety of drugs used clinically can be affected by variations in CYP2D6. For one such drug, tamoxifen, the CYP2D6 status can affect drug efficacy and toxicity and can dictate what patient groups respond optimally to tamoxifen.

It is important to take into account all the above-mentioned variations, not only in clinical practice but also during drug discovery and development. During clinical management of patients, these physiological and pharmacological factors will drive decisions such as drug dosage and coadministration of drugs to exploit drug interactions favorably for the patient.

PharmaPendium®'s new Metabolizing Enzymes and Transporters Module is rich source of information and a platform that provides access to preclinical and clinical data on drug metabolizing enzymes and transporters, in addition to dynamic pharmacokinetic parameters on all approved drugs.

The ability to quickly search a single comprehensive source of data (stretching back to 1938) is invaluable. To identify information salient to a compound of interest can dramatically impact the time and cost of drug development, enabling researchers to deliver higher quality compounds to the clinic in a reduced time.

Drug Interactions

Drug interactions occur when one drug affects the pharmacokinetics of other drugs and/or metabolites. Two factors that can produce drug interactions are modulation of absorption/distribution and metabolism driven by the combined activity of drug transporters and metabolizing enzymes.

Drug Absorption and Distribution

Inhibition of transporters can affect drug absorption and/or distribution to different sites. For example, the transporter P-gp through efflux mechanisms reduces drug distribution across the blood-brain barrier. Similarly, the transporter OATP monitors or limit drug distribution to the liver. The clinical implications for drug administration are that concomitant use of other drugs that activate or inhibit these transporters may affect distribution of the first drug. A frequently cited example is that of quinidine, an inhibitor of P-gp; when quinidine is coadministered with loperamide, the former inhibits P-gp and allows higher concentrations of loperamide to cross the blood-brain barrier, which can lead to dangerous neurotoxicity, including respiratory depression.

Drug Metabolism

Drugs can interact by induction or inhibition of metabolizing enzymes. The most prominent enzyme thus regulated and involved in drug interactions is the CYP group. Enzyme inhibition leads to decreased drug metabolism, which in turn, increases the

drug levels in the body. Identifying the type of inhibition may help counter it during clinical drug administration. On the other hand, a more complex phenomenon that can lead to drug interactions is enzyme induction. This entails increased transcription and synthesis of enzyme proteins, resulting in augmented catalytic activity. Enzyme induction often leads to enhanced clearance of drugs leading to a reduced activity. In some rare cases, induction is associated with increased production of the active metabolite(s) of the drug. A clinically relevant example is the induction of CYP2E1 that increases metabolism of acetaminophen to produce a hepatotoxic metabolite. A key difference between enzyme inhibition and induction is that inhibition may be seen following even one dose of the drug whereas induction typically requires chronic drug administration. This is relevant due to different approaches required during acute and chronic drug therapies.

Interactions between metabolizing enzymes and transporters

Since most drugs are substrates, inhibitors or inducers of both metabolizing enzymes and transporters, metabolism may affect transport and vice versa when enzymes and transporters are in close physical proximity. For example, both P-gp and CYP3A4 are expressed in intestinal enterocytes, and both these proteins have common substrates. Hence, this proximity can limit drug bioavailability either by intestinal first-pass metabolism of the drug via CYP3A4 or by P-gp-mediated efflux of the drug. These interactions have important clinical implications when co-administering different drugs with variable effects on transporters and metabolizing enzymes.

PharmaPendium® provides a unique data module that can serve as a major resource for modeling drug interactions resulting from the effects of drug metabolizing enzymes and transporters. It is a rich source of information for researchers and clinicians, providing data on phase I and phase II metabolizing enzymes, transporters and dynamic pharmacokinetic parameters. This repository offers detailed data on interactions between drugs, enzymes and transporters including the role of the drug as a substrate, inducer or inhibitor. In addition, PharmaPendium®'s module provides comparative data on drug-drug interactions not widely available in the scientific literature.

Drug Discovery and Development: Importance of Drug Transport and Metabolism

A critical element of drug discovery and development involves determining the exact role of drug transport and metabolism and its impact on pharmacokinetic parameters of the drug candidate. An inadequate focus on this aspect during drug development has previously led to several late-stage failures or even withdrawals from the market. In depth interaction studies during new drug development determine whether potential drug interactions might require dose adjustments of the new drug or other drugs concurrently administered. It is also necessary to determine if additional therapeutic monitoring is required or if some drug combinations would be entirely contraindicated. The three major interactions that need to be examined for new drugs are:

"Now, instead of spending weeks gathering data from scientific articles which would then have to be standardised, we now spend half a day working with PharmaPendium; the hard work is all done for us."

– **Dr. Kevin Lustig**, President & CEO
The Assay Depot Inc.

1. **Metabolism-based drug interactions:** It is necessary to determine if the new drug is a substrate of an enzyme that can be modulated by other drugs during co-administration and also whether the new drug is likely to affect metabolism of other drugs already in use clinically.

2. **Transporter-based drug interactions**: Similarly, it needs to be determined whether transporters can affect absorption and disposition of the new drug and whether the new drug affects transporters to modulate absorption and disposition of other drugs in the clinic.

3. **Multiple drug-drug interactions:** These involve complex interactions involving multiple metabolizing enzymes and transporters being modulated in different ways by co-administration of different drug combinations.

Detailed and comprehensive experiments involving in vitro and in vivo interaction studies are currently used for such analyses. Early in the process of drug development, a compound's interaction profile is evaluated in vitro by determining whether the drug is a potential substrate, inducer, or inhibitor of enzymes and transporters. To study drug metabolism, candidate compounds are analyzed by exposing them to cellular extracts expressing drugmetabolizing enzymes, such as microsomes, hepatocytes and liver slices. In addition to cell extracts, studies also use recombinant enzymes to determine metabolic profiles of drugs. It is important to assess pathways affected and the potential of the drug candidates to induce or inhibit enzymes. Drug transporters are similarly studied using a variety of in vitro assays and screens. In addition, population-pharmacokinetics is considered a useful adjuvant to in vitro screens.

For the in vivo aspect, researchers have traditionally resorted to the use of specialized animal models. Candidate molecules are administered at high doses to animals in order to determine basic ADME. Animal models that are traditionally used to study metabolizing enzymes and transporters include native, knockout, humanized, chimeric and disease mouse models, in addition to rats, rabbits, dogs and monkeys (Salyers and Xu 2012). However, direct application of these data to humans can be tricky owing to the significant differences in the expression, specificity and activity of these metabolizing enzymes among species (Guengerich 1997).

Potential drug-drug interactions are examined using rats or rhesus and cynomolgus monkey models for induction-based interactions and mice, rats and monkeys for inhibitionbased interactions. Transporters are studied during drug development for potential drug interactions using in vivo mouse, rat, rabbit or dog models (Salyers and Xu 2012).

Accurate analyses of drug metabolism and transport for novel drug candidates and elucidation of potential drug-drug interactions require a combination of in vitro assays and in vivo models. A recent development in the analyses of drug metabolism is the use of metabolomics. This enables elucidation of toxicity and efficacy of drug candidates by examining body fluids such as plasma, CSF, urine and stool from drug-treated animals. Another upcoming trend in drug development is the use of in silico modeling. This approach may, in the future, be tailored to predict effects on drug transport and metabolism and in turn, on drugdrug interactions. Various algorithms and models can supplement these studies, such as the Physiologically Based Pharmacokinetic (PBPK) model or other in silico models that provide comprehensive information about drug interactions. Such studies can help predict clinical consequences of drug-drug interactions and design strategies during the early phases of drug development. However, even modeling approaches require accurate datasets covering expression profiles of these proteins.

To this end, PharmaPendium®, via its Metabolizing Enzymes and Transporters Module provides a resource to obtain reliable data on drug interactions and prioritize the safest, most promising candidates for further drug development. Using the case of quinidine, loperamide, and the P-gp transporter cited as an example above, with just a few keystrokes we find a list of carefully curated data relating to the effect of P-gp on drug transport. This represents a wealth of information for the drug development scientist, enabling an understanding of all that has already been done and providing a platform for the rational, intelligent design of future experiments. PharmaPendium® offers risk assessments for drug development projects in areas such as drug interaction-induced toxicity. Additionally, this module sheds light on past regulatory issues, providing an opportunity to avoid pitfalls and streamline the regulatory submission process. This wealth of detailed, filterable data can guide drug development decisions, avoiding costly late stage drug failures and delivery benefits to the pharmaceutical industry both in terms of dollars saved as well as enabling redirection of finite resources to pursue other more viable compounds.

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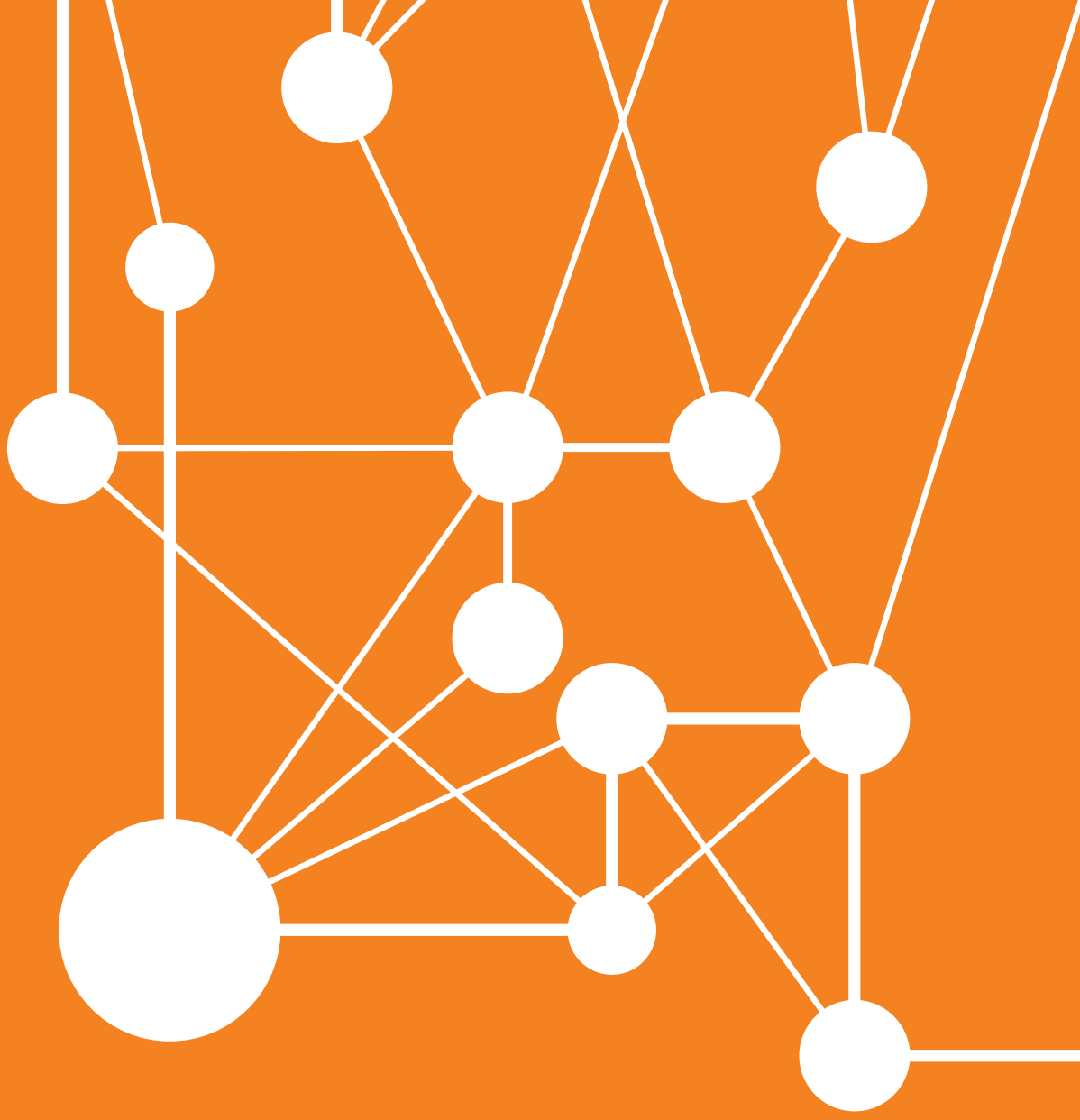

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