Predicting Drug Disposition by Integrating In Vitro and In Silico Methodology

by

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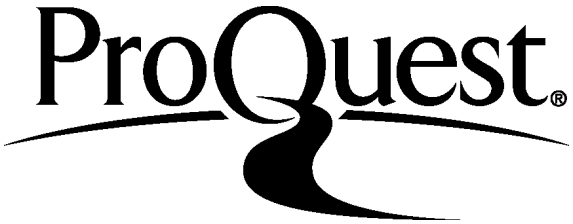

DEDICATION

For Mom, Dad, David, Erica, and Parker for always supporting me, loving me, and keeping life interesting.

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ABSTRACT

The safety and efficacy of drugs depend upon appropriate dosing of drugs made possible by understanding the dispositional profile a drug will follow. A drug's disposition includes its absorption from an administration site, its distribution throughout the body, and its elimination from the body, characterized by metabolism and excretion. Disposition is often mediated by drug metabolizing enzymes and drug transporters. Alterations in the expression or activity of metabolizing enzymes and transporters can therefore affect the safety or efficacy of a drug and it is necessary to characterize their impact on every drug. The Biopharmaceutics Drug Disposition Classification System (BDDCS) uses the extent of metabolism and solubility of drugs to predict drug disposition, including when transporters and metabolizing enzymes are clinically relevant. Here, we utilized observations from this system to predict the three major routes of drug elimination (metabolism, renal excretion of unchanged drug, and biliary excretion of unchanged drug). These predictions were made by integrating *in vitro* measurements of permeability rate to predict the extent of metabolism with an *in silico* logistic regression model we developed that uses calculated polarizability and predicted metabolic stability to predict when poorly metabolized compounds will be eliminated in the urine or the bile. This approach correctly identified 72 ± 9%, 85 ± 2%, and 73 ± 2% of extensively metabolized, biliarily eliminated, and renally eliminated drugs, respectively. We discuss the physiological context through which permeability, polarizability, and metabolic stability may inform the major elimination route. We further developed a model predicting BDDCS class using commercially available *in silico* models of permeability rate to predict the extent of metabolism and dose number to predict the solubility class. This approach correctly identified 54.1%, 57.8%, 69.3%, and 45.2% of class 1, 2, 3, and 4 drugs, respectively, while *in vitro* approaches predict with greater accuracy. We correct previously misclassified drugs, discuss reasons for misclassification, incorporate more than 175 additional drugs into the system, and discuss how BDDCS can self-correct when observed and predicted dispositional effects are not aligned. We

conclude by reflecting on the demonstrated and potential applications of BDDCS and the importance of predicting drug disposition.

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CHAPTER 1. INTRODUCTION

Pharmacotherapy has become a crucial aspect of improving and correcting human health. Humans have seemingly always used natural resources to modify their physical and mental health. The ancient Egyptians recorded a variety of herbal remedies to treat various ailments in the Ebers Papyrus, for instance recognizing that herbs could be heated and inhaled to treat asthma, while the Greeks used the lethal poison hemlock as capital punishment for condemned prisoners, most notably Socrates. However, many substances do not so easily fall into treatment versus poison categories. Foxglove (digitalis) can be used to treat congestive heart failure, yet has also been used as a homicidal agent in higher doses. Some substances are safe in very large quantities, such that it would be nearly impossible for a human to consume toxic amounts, while others are so toxic that even the slightest dose can be fatal. Yet, the puzzle that pharmaceutical scientists must solve is finding compounds and a dosing strategy that maximize therapeutic benefit, while limiting risk.

The most fundamental understanding of dosing, then, relates to understanding the balance between dose, effect, and toxicity. Every dosing scenario carries a degree of benefit and risk. If a given dose is too small, there may be little risk of off-target effects, yet the drug may be inefficacious. Alternatively, too large of a dose may give the desired effect, but could be toxic, either because the drug has overcompensated for the defect it was attempting to correct, or because it established too much off-target toxicity. A well-established and consistent dose is therefore necessary to mediate the appropriate, yet safe, response. In fact, as early as 1240, Frederic of Sicily ordered apothecaries to standardize their remedies(1). As time has progressed, so too has drug standardization, such that the dose and contents of drug products are now well-studied prior to dosing in humans and well-regulated before reaching the market.

By understanding these principles, scientists are able to predict appropriate doses that will strike an appropriate balance between the benefits a drug can provide with the potential

risks that a drug can incur. The ability to evaluate parameters of drug exposure, handling and response has evolved into the fields of pharmacokinetics and pharmacodynamics.

Absorption, Distribution, Metabolism, and Elimination

A drug's safety and efficacy depends upon how the body handles the drug, referred to as pharmacokinetics, as well as the effects that the drug has on the body, commonly called pharmacodynamics. Scientists assess pharmacokinetics using the principles of ADME, an acronym for absorption, distribution, metabolism, and elimination of drug. Together these principles help develop safe and efficacious doses.

For a drug to be effective, it must become available at the target site. However, target sites are often inaccessible and it is necessary to dose from a convenient location. The drug must therefore be absorbed from a dosing site, often from the gastrointestinal tract since many drugs are orally administered. Subsequently, the drug must distribute throughout the body until some drug reaches the target site. However, because drugs are xenobiotics, the body will attempt to protect itself by eliminating the drug; either by directly excreting it as the parent drug, usually in the urine or the bile, or by metabolizing it to something generally easier to excrete. Pharmaceutical scientists attempt to optimize each of these processes to ensure that drugs reach their targets safely and efficaciously, yet with few off-target effects, and can be dosed on a convenient schedule. These are affected by physiological factors and chemical properties of drugs. Pharmaceutical scientists have invested a lot of research into understanding these properties in order to predict disposition prior to developing a drug and testing it in humans.

ADME can be assessed with plasma concentrations over time. The area under this curve (AUC) represents drug exposure, which is generally correlated with therapeutic response. However, for some drugs, antibiotics for example, the maximum concentration (C_{max}) better predicts therapeutic response. In other cases a relatively immediate response is required, when using sleep-inducing hypnotics, for instance. Drugs of this nature must enter the systemic

circulation and be able to access their target quickly, which means that the time to maximum concentration (t_{max}) must be short and that the drug must have properties that facilitate rapid distribution to the target tissue (e.g. the brain).

The primary goal of pharmacokinetics is to select an appropriate dose. AUC is the clinical output from which clearance and volume of distribution, the primary pharmacokinetic parameters, can be calculated. Clearance and volume of distribution contribute to dose selection and regimen along with bioavailability. When conditions are non-normal, pharmaceutical companies, physicians, or pharmacists must know how to adjust the dose. Changes in AUC reflect changes in clearance or distribution. By understanding how different factors impact disposition, we can deduce the mechanism of the observed exposure change and appropriately amend the treatment regimen.

Each aspect of ADME can be regulated by the active impact of metabolizing enzymes and/or drug transporters. Other factors such as blood flow, membrane permeability, pH, protein binding, and endogenous substances all play a significant role as well. There is significant variation in the expression and activity of metabolizing enzymes and drug transporters in healthy individuals. The impact of this variation is studied in a field called pharmacogenomics. In addition to native variation in the behavior of metabolizing enzymes, transporters, blood flow, protein binding, permeability, and pH, other drugs, supplements, disease, and even food can alter a drug's activity. These factors can also result in transient physiological changes that may impact a drug's disposition.

Certain diseases such as chronic kidney disease can have a large impact on drug disposition by affecting the concentrations of endogenous compounds, which can interact with drugs, or affecting physiological aspects like blood flow or protein binding. For instance, in renal disease, the ability to eliminate and clear drug is directly impacted by decreased renal function. Meanwhile, structural and physiological changes and decreased CYP3A expression(2) in patients with Celiac's disease may potentially alter drug absorption(3). Other diseases including,

but certainly not limited to, diabetes, cystic fibrosis, cancer, and congestive heart failure can significantly impact a drug's disposition.

Understanding how physiological systems change with disease and how interindividual differences, either biological or due to external factors such as copharmacy, will impact which drugs helps pharmaceutical companies develop safer, more efficacious prescriptions with a clearer understanding of necessary alterations in dosing recommendations. In fact, pharmacokinetics was once a leading cause of drug failure during development, but is no longer a significant concern due to our improved understanding of drug disposition(4,5).

With greater understanding of pharmacokinetics and disposition comes the ability and convenience of predicting drug disposition prior to dosing in humans. Each process is associated with quantitative predictive preclinical animal models, *in silico* models varying in complexity from simple physicochemical predictors to complex machine learning methods, or *in vitro* models generally representing a simplified model of a dispositional organ, e.g. hepatocytes for predicting metabolism.

Current Issues and Challenges in Pharmacokinetics

Drugs can be administered through a myriad of routes. These include, but are not limited to, topical, optical, injections (including intramuscular, subcutaneous, or intravenous), or oral administration. Intravenously administered drugs are directly administered to the blood and therefore the entire dose is available for distribution in the body. However, drugs administered by any other route must pass some barriers before entering the blood, in a process called absorption. Naturally, a percentage of the drug cannot pass through the barriers and is lost between the site of absorption and the blood. This loss depends on both the barrier that must be crossed as well as properties of the drug.

Some membranes are leakier than others, while some are perfused by higher blood flow. Other differences include higher or lower fat content, pH differences and variation in the

expression of transporters and metabolizing enzymes. This makes a difference in how a drug can be administered. For instance, insulin is generally administered subcutaneously, but can also be inhaled because of the relatively high permeability of the alveolar epithelium(6). Insulin cannot, however, be absorbed from the gastrointestinal tract due to degradation by proteolytic enzymes and an inability to be transported(7).

Metabolism dominated the understanding of drug disposition for a very long time. Eventually, scientists began to realize that drug transporters, initially called phase III metabolism, have an equally important role in drug absorption, distribution, and elimination. Hundreds of drug transporters have been identified in humans(8), but currently at least 7 are considered clinically important in regulating drug disposition. The FDA 2012 guidance on drug interactions(9) recommends determining if a drug is a substrate, inhibitor, or inducer of P-gp and BCRP for all drugs; OATP1B1 and OATP1B3 if a drug is hepatically eliminated; and OAT1, OAT3, and OCT2 when a drug is renally eliminated. However, other transporters such as MATEs are considered clinically relevant by the International Transporter Consortium (ITC), which recommends prospectively studying MATE interactions. The ITC recommends that MRP2 and BSEP be evaluated in retrospective studies depending on clinical and preclinical observations. Other transporters such as OATP2B1, ENTs, and PEPTs are also considered clinically relevant(8,10). Other drugs, high-fat food or components in food, endogenous substrates, disease, and genetics can alter the function of these and other transporters. Since transporters often highly regulate drug exposure in the systemic circulation and tissues as well as play a role in drug elimination, when their function is altered, the safety and efficacy of a drug can be compromised. We will discuss when transport is relevant to the clinical outcome of a drug, as well as many cases when it is not.

Predicting Oral Absorption and Availability

Most drugs are preferably administered by the oral route to increase patient compliance and facilitate delivery. Before a drug can enter the systemic circulation after oral dosing, it must a) be absorbed in the gut, where it may be affected by apical uptake and efflux transporters b) pass through gut epithelial cells (enterocytes) where it may be metabolized, and then c) escape from metabolism or biliary elimination in hepatocytes. The combination of these processes determines the bioavailability of the drug, or the fraction of the dose that enters systemic circulation.

In humans, bioavailability (F) can be readily measured by comparing exposure from intravenous and oral dosage forms: $F = AUC_{oral}/AUC_{iv}$, correcting for dose if necessary. However, it is very difficult to predict the fraction of the bioavailability due to absorption (F_A), and thus the extent of absorption because although the hepatic bioavailability (F_H) can be estimated when an intravenous dose is given and total and renal clearance is measured [$F_H = 1 - CL_H/Q_H$, where hepatic blood clearance (CL_H) equals total blood clearance (CL) minus renal blood clearance (CL_R) and Q_H is than estimate of hepatic blood flow rate], separating the fraction of the dose that is absorbed and the fraction of the dose that escapes gut metabolism since $F = F_A*F_G*F_H$ where F_G is the gut bioavailability, requires invasive methods such as sampling from the portal vein. The rate and extent of absorption depends upon the physicochemical properties of the active component in a drug product, the formulation and release of the drug-product, and physiological traits of the gastrointestinal system.

Additionally, microbiotic metabolism and luminal degradation of drug can reduce the proportion of the parent drug that is available for absorption. Even after initial absorption, a drug can be effluxed by transporters in the enterocytes, effectively reducing absorption.

Drug absorption can be mediated through passive or active permeation across (primarily) enterocytes. Passively absorbed compounds can transcellularly diffuse (through the cell) or paracellularly diffused (between the cells). Active permeation requires the intercession of

drug transporters, which can move a drug across either side of a polarized cell membrane. Active transporters are responsible for either bringing the drug into a cell or ejecting it from a cell.

Physicochemical Determinants of Absorption

It is generally presumed that lipophilicity correlates with cell permeability, within a reasonable boundary and when considering structurally similar compounds. In 1997, Lipinski *et al*.(11) developed a set of rules that aided in understanding the properties of drugs that are readily absorbed. Poor absorption is more likely when a drug has greater than 5 hydrogen bond donors (OH and NH), a molecular weight > 500 Da, cLogP > 5, or greater than 10 hydrogen bond acceptors (oxygens and nitrogens). However, this rule does not apply when transporters mediate drug absorption.

Passive Absorption

Passive absorption generally refers to the diffusion of compounds that have properties that allow them to cross through a cell (transcellular). Drugs can only pass transcellularly if they are small and relatively lipophilic. Compounds that instead pass between cells (paracellular permeation) may be small and hydrophilic.

Transported-Mediated Absorption

Most compounds are known or presumed to be substrates of transporters, even if they can also be passively absorbed. Uptake transporters are responsible for mediating absorption into a cell, while efflux transporters help to remove a drug from a cell. In gut absorption, apically expressed uptake transporters facilitate absorption, while apically expressed efflux transporters counteract absorption. However, highly permeable, highly soluble compounds are not dependent upon transporters for their absorption even if they are substrates and will not be

impacted by disruptions of transporter function. This will be discussed in great detail throughout this chapter.

In Vitro Predictions of Absorption

The Biopharmaceutics Classification System, as we will discuss later, opened the door to predicting absorption with surrogate *in vitro* systems, such as Caco-2 and MDCK. The Biopharmaceutics Drug Disposition Classification System (BDDCS) allowed us to do the same with artificial membranes such as PAMPA. Compounds with a high *in vitro* permeability rate are expected to be well absorbed.

Caco-2, an immortal cell line derived from colorectal adenocarcinoma cells, come from human enterocytes. They confer the advantage of being human in nature with a microvillus surface. However, these cells take 2-3 weeks to culture, and even then do not fully express transporters or metabolizing enzymes. Additionally, tight junctions predominate and resistance is high compared to *in vivo* morphology(12). This can lead to significant underprediction of permeability rate and absorption. The *in vitro* lack of expression of highly expressed transporters in humans can greatly underpredict the extent of absorption(13).

MDCK (Madin Darby Canine Kidney) cells are immortal cells that come from the kidney of dogs. These cells have a shorter culture time than Caco-2 and have lower resistance than Caco-2 cells, a condition more similar to the human gut. However, these cells are not human in nature and, similar to Caco-2, they poorly express CYP3A.

PAMPA (Parallel artificial membrane permeability assay) is an artificial membrane that does not express transporters and has no cells to create tight junctions or cellular pores. It is representative of passive permeability through a lipid bilayer.

All *in vitro* models lack the flow of gut contents and blood on either side of enterocytes. Portal blood flow constantly removes drug from the basolateral membrane of enterocytes, resulting in “sink conditions”, a downhill concentration gradient that facilitates drug absorption *in*

vivo. The fluidity of gut contents means that drug in the gut lumen will be exposed to the different morphologies and expressions in different segments of the gut *in vivo* that is not simulated in *in vitro* cell studies.

While highly permeable compounds are almost all extensively absorbed, *in vitro* permeability rate predictions often under-predict absorption. This is because many compounds are actively absorbed, but have low passive permeability. Predicting extent of absorption is improved by including active drug transport. Larregieu *et al*.(13) show that when transporter expression is decreased more than 10 fold in Caco-2 cells compared to humans, absorption of compounds that are substrates of highly expressed transporters such as PEPT1, amino acid transporters, and nucleoside transporters are poorly predicted.

Predicting Distribution

Once a drug is systemically available, it is distributed throughout the body. Some drugs are liable to remain in circulation, with little distribution, while others have an affinity toward promiscuous distribution throughout many tissues, even those that are poorly perfused by blood flow. A particular challenge for pharmaceutical scientists is understanding to which tissues a drug may or may not be distributed, and either targeting or avoiding those tissues to maximize effect or minimize off-target toxicity. The central nervous system is a common concern due to difficulty in obtaining exposure when necessary, or undesired exposure resulting in central side effects for peripherally acting drugs.

Drug distribution is determined by physiologic characteristics such as cardiac output, tissue blood flow and volume, and capillary permeability, as well as tissue permeability and drug transporters. Following drug dosing, well-perfused tissues such as the liver and kidney initially receive a high drug concentration. This initial distribution phase is apparent when considering the shape of a plasma concentration profile. A secondary distribution phase, characterized by slowly decreasing plasma concentrations, reflects drug distribution to the more poorly perfused

tissues. Additionally, protein-bound drugs cannot traverse cellular membranes and therefore protein binding has an impact on drug distribution. In the plasma, drugs primarily bind to either albumin, if the drug is acidic, or α-1-acid glycoprotein, if the drug is basic. Protein binding can be modified by disease and drug-drug interactions. For instance, patients with cystic fibrosis often have hypoalbuminemia(14). However, Benet and Hoener(15) have shown that changes in protein binding are only important for high clearance, narrow therapeutic index drugs that are dosed intravenously, e.g. lidocaine.

Volume of Distribution

Distribution can be characterized by the theoretical pharmacokinetic term, the apparent volume of distribution**.** This term characterizes the apparent space in the body into which a drug distributes. That is, systemic concentration multiplied by the apparent volume of distribution is equal to the amount of drug in the body. A large volume of distribution indicates that a compound is predominantly located outside of the sampling space (plasma); that is outside of systemic fluids flowing to the organs of elimination. Volume of distribution depends on how much of a drug binds to receptor sites, plasma proteins, and tissues, as well as the lipophilicity of a drug. Volume of distribution measures can be determined from plasma concentration-time curves. While the volume of distribution can be calculated a few ways, the volume at steady state or V_{ss}, is the most useful measure of the apparent space available in the body into which drug may distribute, since it is not affected by elimination. V_{ss} measures can be determined from plasma concentration-time curves using the following equation:

$$V_{ss} = CL\ x\ \frac{AUMC}{AUC}$$

Here, AUMC is the area under the moment curve, or the area under the curve of the product of concentration and time versus time.

Additionally, physiologically based PK (PBPK) models such as GastroPlus™ and Simcyp provide estimates of V. Age, percent of body fat, sex, and disease can all affect the volume of distribution. Accurately predicting volume of distribution is vital in predicting C_{max} and can be important in defining clinically relevant half-life ($t_{1/2}$) measures. Volume of distribution is also predicted in animal models, which include physiological features like blood flow and organ topology. The volume of distribution can be estimated by collecting plasma concentrations over time and using allometric scaling approaches to predict the volume of distribution in humans.

Predicting Metabolism and Elimination

Most drugs are eliminated by metabolism, renal elimination of unchanged drug, or biliary elimination of unchanged drug. To ensure safety, pharmacokinetic studies are conducted with mass balance, or collection of the entirety of a dose in eliminated equivalents (parent drug or metabolites). Ideally, the entirety of the dose is eliminated in either the urine or the bile. This provides evidence that the compound is not sequestered and accumulating in a peripheral tissue, potentially resulting in unanticipated toxicity, and lends support to the validity of the calculated pharmacokinetic parameters. Incomplete recovery sometimes indicates that a drug is eliminated by another organ (e.g. the lungs). However, mass balance may not be as simple as it sounds. Realistically, the entirety of the dose often cannot be collected. Some drugs have very long half-lives, which makes collections in a clinical setting unrealistically arduous.

Many metabolites and some parent drugs are eliminated in the bile, which is a difficult fluid to accurately obtain and analyze. The bile drains into the lumen of the intestine and its contents are eventually eliminated in the feces. Fecal samples could be collected to estimate the fraction of the dose that is eliminated in the bile. However, the feces also contain orally administered material that was never absorbed from the lumen of the intestine. For this reason, it is impossible to differentiate between parent drug that is unabsorbed from an oral administration and parent drug that is eliminated in the bile in fecal samples. This means that,

unless a drug was administered non-orally and all of the drug in the feces must therefore come from biliary excretion, direct bile collection approaches such as collection from t-tubes or nasobiliary tubes are necessary to account for the elimination of unchanged drug in the bile. However, such procedures are rarely conducted and are done during surgeries. The patients often have hepatobiliary disease, so the donor samples do not necessarily represent healthy conditions. Other methods such as the bile string or duodenal collection studies are slightly less invasive and can be conducted with healthy volunteers. Duodenal collection studies are difficult to conduct however, and are still invasive.

It is much easier to determine the extent of urinary elimination of unchanged drug or the extent of metabolism. Urine samples are almost always collected during pharmacokinetic studies to account for mass balance and the parent drug and metabolites can be readily quantified. Parent drug collected in the urine represents absorbed drug only since the drug can only reach the kidneys after entering the systemic circulation. Metabolites can be quantified in the urine and may also be collected in feces. If the drug was not degraded or metabolized by bacteria in the gut, we can assume that the drug was absorbed since most metabolism occurs post-absorption. Degradation and presystemic metabolism can be confirmed with stability studies as will be described in a later section.

Prior to conducting trials in humans, pharmaceutical scientists predict what will be the major route of drug elimination. *In silico*, *in vitro*, and *in vivo* models of drug elimination have been developed to predict elimination routes and their potential liabilities. For instance, biliarily eliminated drugs may be subject to enterohepatic recycling, which exposes the drug to the intestine and liver multiple times and may result in several plasma concentration peaks. Metabolism may produce pharmacologically active or toxic metabolites that can alter pharmacodynamics or need to be evaluated for safety. A developer may want to avoid renal elimination if the drug is likely to be dosed to patients with failing kidneys. Alternatively, an

eliminating organ may be the desired site of action and a developer may attempt to target that route.

Recently there have been many efforts to associate the chemical properties of drugs with their major elimination routes. Certain trends have been noted for a long time, with properties such as lipophilicity, molecular weight, protein binding, and ionization state lauded as harbingers of elimination route. However, these rules are sometimes considered too simple, or were applied to small datasets of compounds that are often structurally similar. With the advent of “big-data” methodologies, more complicated and/or thorough analyses are possible.

Characteristics of Metabolism

Most drugs are designed to be sufficiently lipophilic to cross biological membranes during absorption or distribution and to achieve biochemical potency by encouraging binding to a target site through hydrophobic interactions. Coincidentally, most drugs are metabolized, and indeed, lipophilicity is historically considered a characteristic of drug metabolism.

There at least a couple of reasons why lipophilicity and metabolism are associated with each other. The first is that relatively lipophilic drugs may be able to be passively reabsorbed across membrane barriers surrounding excretory fluids including the aqueous bile and urine(16). Metabolic enzymes generally convert a lipophilic substance into a more hydrophilic substance, which aids in retention in and thus elimination from the body in aqueous bile or urine. Secondly, lipophilicity is correlated with protein binding(16), and may aid in binding to enzymatic proteins through hydrophobic interactions(16), which will convert a drug to a more hydrophilic molecule.

Many very lipophilic molecules are indeed metabolized, and in fact we do not know of any marketed poorly metabolized drugs with a measured or calculated LogP > 5(17). As always, it is important to note that these are trends and not rules. Despite the common assumption that metabolized compounds are lipophilic and vice-versa, metabolized compounds cover a vast

physicochemical space and not all lipophilic compounds are metabolized. In particular, drugs that are eliminated unchanged in the bile also exhibit relatively high LogP values(17) (Chapter 3), with no significant LogP difference from metabolized compounds as determined by the t-test and no rank-ordered differentiability as indicated by receiver operating characteristic curves[1]. Furthermore, a large proportion of metabolized drugs have a low LogP. This may be because drugs eliminated unchanged in the bile require uptake and efflux transporters in the hepatocyte, and therefore must be sufficiently lipophilic to bind to these transporters. Alternatively, almost all compounds eliminated as unchanged drug in the urine have a low LogP and are poorly bound to proteins.

However, given the somewhat ambiguous predictability of LogP, it is necessary to predict which compounds will be metabolized by other methods. Metabolism can be assessed using *in vitro*, *in vivo*, or *in silico* methodology.

In Vitro Predictions of Metabolism

The extent, rate, and mechanisms of metabolism are often initially evaluated *in vitro*. Ideally, human hepatic and enterocytic tissues can be utilized to evaluate metabolism. However, these merely serve as predictive tools, and are fraught with errors.

Microsomes are a subcellular fraction containing the contents of the endoplasmic reticulum including CYPs and UGTs. CYP metabolism accounts for about 70% of the metabolism of extensively metabolized drugs of the top 200 drugs(18), while UGT metabolism accounts for about 14% of the metabolism of the top 200 drugs prescribed in the United States(19).

In vitro microsomal experiments can determine the intrinsic clearance (CL_{int}), representing the capacity of metabolizing enzymes to eliminate a compound in the absence of

[1] The receiver operating characteristic is a plot that illustrates how well a continuous features (e.g. LogP) classifies a binary outcome (e.g. biliary versus metabolic elimination). When the area under the ROC curve is greater than 0.8, the continuous features is expected to differentiate between the classes well.

other factors like blood flow, protein binding, membrane permeability, and competing elimination mechanisms.

Intrinsic clearance can be expected to correlate with clinical clearance in humans, particularly for drugs that are primarily metabolized by CYP enzymes in the liver. Prior to the acknowledgment of the contribution of transporters in drug disposition, human clearance was estimated from intrinsic clearance in microsomes generally using the well-stirred model. However, Miyauchi *et al*. proposed an extended clearance concept to include the effect of transporters on hepatic clearance(20).

Microsomes provide a reliable estimate of metabolic kinetics (clearance). However, microsomes have been used to predict the extent of metabolism by measuring the percent of dosed drug that is unmetabolized after a set period of time. They may be unreliable predictors of the extent of metabolism(21-23) since presumably most drugs will be metabolized if they are left to incubate in the presence of a variety of enzymes without interference. As we will discuss in detail later, *in vitro* permeability rate can predict the extent of metabolism in humans.

Supersomes, expressing only one enzyme, may be used to predict the metabolic intrinsic clearance by a single enzyme and to identify metabolites formed by a specific enzyme.

A major concern of predicting the extent or rate of metabolism *in vitro*, perhaps especially in microsomes, is that it assesses metabolism in isolation of competing processes. *In vivo*, transport-limited clearance into the bile or passive or transport-limited clearance into the urine may prevail. However, microsomal incubations cannot tell a researcher what is the major route of elimination and metabolic clearance does not necessarily relate to the extent of metabolism. Isolated or sandwich cultured hepatocytes are more complex tools that incorporate transporters and may be used to predict metabolic or hepatic clearance.

In Vivo Predictions of Metabolism

In vivo approaches confer the advantage by including factors such blood flow, sequestration due to transport, and membrane permeability. *In vivo* approaches include humanized animals and allometric predictions. Humanized animals, like supersomes, can help assess the impact of a single enzyme or transporter.

Allometry applies scaling factors based on body size to pharmacokinetics across species. Simple allometry from a single species is commonly used early in drug development, requiring only clearance data from preclinical species. Modifications to simple allometry have been proposed to improve the predictability of these models(24).

These predictions require sampling plasma concentrations over time in preclinical animal models and may incorporate other physiological parameters such as plasma protein binding and the blood to plasma ratio. They can be supplemented with physicochemical drug properties such as molecular weight or LogP.

The most obvious disadvantage to using preclinical animal models are physiological differences. While allometry attempts to correct for differences in body weight, protein binding, blood flow, etc., animals often have different patterns of metabolizing enzyme and transport expression and substrate specificity. While pharmacokinetics are frequently similar between species, marked differences can be seen for a variety of substrates. Consider, for instance, digoxin or zidovudine. Digoxin is extensively metabolized in rats(25), but is primarily eliminated as unchanged drug in humans(26). Zidovudine is extensively metabolized in humans(27), but is primarily eliminated unchanged in rats(28).

In Silico Predictions of Metabolism

Several *in silico* methods predict aspects of metabolism, including understanding the affinity for a particular enzyme(29), the site of metabolism on the molecule(29), predicting metabolic clearance(30), identifying metabolites(31), or predicting metabolic stability(32).

Unfortunately, many of the published methods rely on proprietary descriptors or are derived from small or structurally similar compound datasets(33). Since many compounds are metabolized by several enzymes and/or are sequentially metabolized, it is crucial to integrate many predictive models. Several reviews discuss the challenges and applications of *in silico* predictions of drug metabolism in depth and discuss available predictive software(29,31,33).

Second to metabolism, renal elimination of unchanged drug is responsible for the elimination of most drugs.

Renal Elimination of Parent Drug

Renal elimination of drugs is dependent upon three renal processes: glomerular filtration, renal secretion, and renal reabsorption. Glomerular filtration is a passive process where free (unbound) small molecule compounds are drained from blood in the afferent arteriole and collected in the filtrate. Large molecules, including drugs bound to proteins, cannot sieve through the glomerulus and remain in circulation. While glomerular filtration rate = 120 mL/min, urine is only formed at 1 mL/min, so 119 mL of water is reabsorbed from the kidney tubules every minute. For this reason, many compounds, especially metabolized compounds, are passively reabsorbed from the filtrate as water is actively retained in the body. Several compounds are actively secreted into the filtrate directly from the blood via drug transporters expressed on the proximal tubule.

Renal clearance of drugs tends to decrease with increasing lipophilicity(34). This is intuitive, since highly lipophilic compounds are often susceptible to reabsorption. Additionally, lipophilic compounds are more likely to be protein bound(16). Unsurprisingly, compounds that are primarily eliminated as unchanged drug in the urine are expected to be small and polar, having low protein binding. However, Hosey *et al*. demonstrated that while this holds true for orally dosed compounds, many non-orally dosed (generally intravenously administered) compounds could be renally eliminated even if the molecular weight is high. Alternatively,

protein binding of compounds primarily eliminated in the urine was low for both orally and non-orally administered medications(23). This is likely because all small molecules (<10,000 Da)(35) can be filtered through the glomerulus, but compounds bound to proteins are always filtered out. Therefore, highly protein bound drugs must be eliminated by other routes and protein binding is a determinant of renal elimination.

While charge does not determine if renal elimination is the primary route of elimination(23), it does trend with renal clearance. Anions and cations are primarily secreted, whereas neutral compounds are primarily reabsorbed. Additionally, lipophilicity tends to decrease with increasing renal clearance, while polar descriptors increase with renal secretion(34).

Biliary Elimination of Parent Drug

Biliary elimination of unchanged drug accounts for the third major route of drug elimination. Biliary elimination is an active process requiring both uptake transporters on the hepatic basolateral membrane facing blood in the portal vein and efflux transporters on the hepatic apical membrane facing the bile canalicula.

Historically, it was hypothesized that high molecular weight (> 500 Da) anions would preferentially be eliminated in the bile, and that biliary excretion was selective for these properties. This was likely derived by considering the weight and molecular species of the primary endogenous substrates, e.g., bile salts. Millburn *et al*.(36) suggested that drugs with a molecular weight less than 500-600 g/mol were less susceptible to biliary elimination. More recently, Yang *et al*.(37) predicted when anions have molecular weights greater than 475 Da, 10% or more of the dose is likely to be eliminated in the bile. We demonstrate in chapter 2 that drugs whose major route of elimination is unchanged drug in the bile were poorly permeable, and had a high polarizability, which is highly correlated with molecular weight, and a low predicted metabolic stability(17,23). Our study also points out that high molecular weight is

descriptive of biliarily eliminated drugs, but that high molecular weight does not qualify biliary elimination. In other words, almost all drugs that are predominantly eliminated as unchanged drug in the bile have a high molecular weight, but the primary route of elimination is not biliary excretion for the majority of high molecular weight drugs

Other properties associated with biliary elimination have been less clearly defined and in some cases exhibit contesting associations between studies. Greater hydrogen bond interactions have been associated with increased biliary excretion(37,38). Some studies indicate that biliarily eliminated compounds are primarily anions(37,38), while some indicate that cations are also eliminated in the bile(39), and yet others suggest that ionization is not an important characteristic(23). Greater dipole moments(37,39), the presence of carboxylic acid group(37,39,40), and more rotatable bonds(38,40) have also been associated with increased biliary excretion. Lipophilicity results yield varying indications between studies, with some indicating that biliarily eliminated drugs are hydrophilic(38,39), some indicating they are lipophilic(17,41) and others discussing both lipophilic and polar regions, and some studies finding no relationship between lipophilicity and extent of biliary elimination(37,42). While the bile is a hydrophilic medium, compounds likely require a degree of lipophilicity to bind to and be transported by drug transporters such as P-gp. This may be especially true as the most widely accepted mechanism of P-gp transport relies on an initial partition into the membrane(43). These relationships likely depend on how “major” biliary elimination was defined and which drugs were included in the study.

As we mentioned earlier, biliary elimination is difficult to gather and quantify in humans. However, *in vitro*, *in vivo*, and *in silico* approaches may provide a reasonable quantitative or qualitative understanding of biliary elimination.

Perhaps the most widely accepted *in vitro* approach to predict biliary clearance is the use of sandwich-cultured hepatocytes, isolated from humans or rats, which maintain the polarity of cell membranes, a crucial condition to determine canalicular efflux. These hepatocytes are

plated on a collagen platform and maintained in the presence of calcium. After several days, transporter expression is optimized for vectorial transport. After introducing the test drug, the tight junctions are ruptured by removal of calcium and differences in accumulated intracellular concentrations can be measured(44).

In vivo, one of the most common approaches to estimate the contribution of biliary elimination is with bile duct cannulation in an isolated perfused rat liver. Unfortunately, rats have a higher bile flow (relative to body weight)(45), efflux transporter expression(46), and rate of efflux(47) so more compounds are eliminated in the bile at greater concentrations and rats are not altogether reliable models.

The approaches described above are useful and necessary tools to predict drug disposition prior to human dosing. These approaches can eliminate drugs from development that are unlikely to be successful in the clinic for reasons such as insignificant absorption, distribution to undesired tissues or a lack of distribution to necessary tissues, unacceptably fast elimination, which might require too frequent dosing, or unacceptably slow elimination, which may result in drug accumulation. BDDCS can supplement and improve upon these predictive approaches by making qualitative ADME predictions.

BIOPHARMACEUTICS DRUG DISPOSITION CLASSIFICATION SYSTEM

Using empirical observations of clinical data, Wu and Benet(48) developed a system that has the ability to make many qualitative predictions for each process in ADME. The Biopharmaceutics Drug Disposition Classification System (BDDCS) is a model that uses known disposition characteristics of currently or previously approved drugs to predict what biological and external factors can alter a drug's ADME and how they will do so. Perhaps the most significant advance of BDDCS is predicting when a drug transporter will be clinically relevant in regulating the disposition of a drug.

In 1995, Amidon *et al*.(49) proposed the Biopharmaceutics Classification System (Figure 1-1), a scheme that used “permeability rate” and solubility to characterize drugs into 4 classes, and then employed *in vitro* dissolution methodology to predict drug bioavailability. This was based on an apparently good correlation between human jejunal permeability in single-pass perfusion studies and the fraction of dose absorbed across the gut wall(49). Since its development, the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and World Health Organization (WHO) have implemented its principles to grant biowaivers to some highly soluble drugs. While most drugs require clinical bioequivalence studies to demonstrate similar exposure to the original product any time a drug is manufactured at a new site, is formulated differently, or is synthesized by an altered method, biowaivers grant regulatory approval to certain immediate release drug products based on solubility and dissolution studies and permeability criteria.

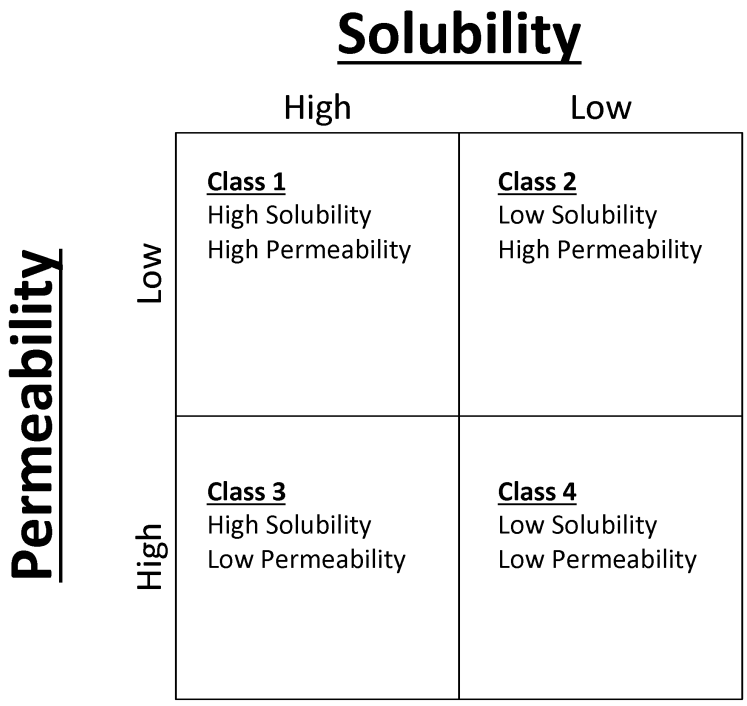

Figure 1-1. The Biopharmaceutics Classification System.

BDDCS was initially proposed when Wu and Benet(48) recognized that compounds with a high passive intestinal permeability rate as defined by BCS were extensively metabolized, while drugs eliminated in an unchanged form in the urine or bile were primarily poorly permeable in BCS. In this seminal publication, they suggested that extent of metabolism might serve as an appropriate surrogate for absorption and/or intestinal permeability when those data are unavailable, since the extent of metabolism is easier to assess than intestinal permeability/absorption, thus expanding the number of class 1 drugs available for a biowaiver. Therefore this system substituted the extent of metabolism for permeability in its classification (Figure 1-2). Importantly, the modified system was also used to predict drug disposition,

especially when predicting when transporters or metabolizing enzymes are clinically relevant, for which it is most appreciated today.

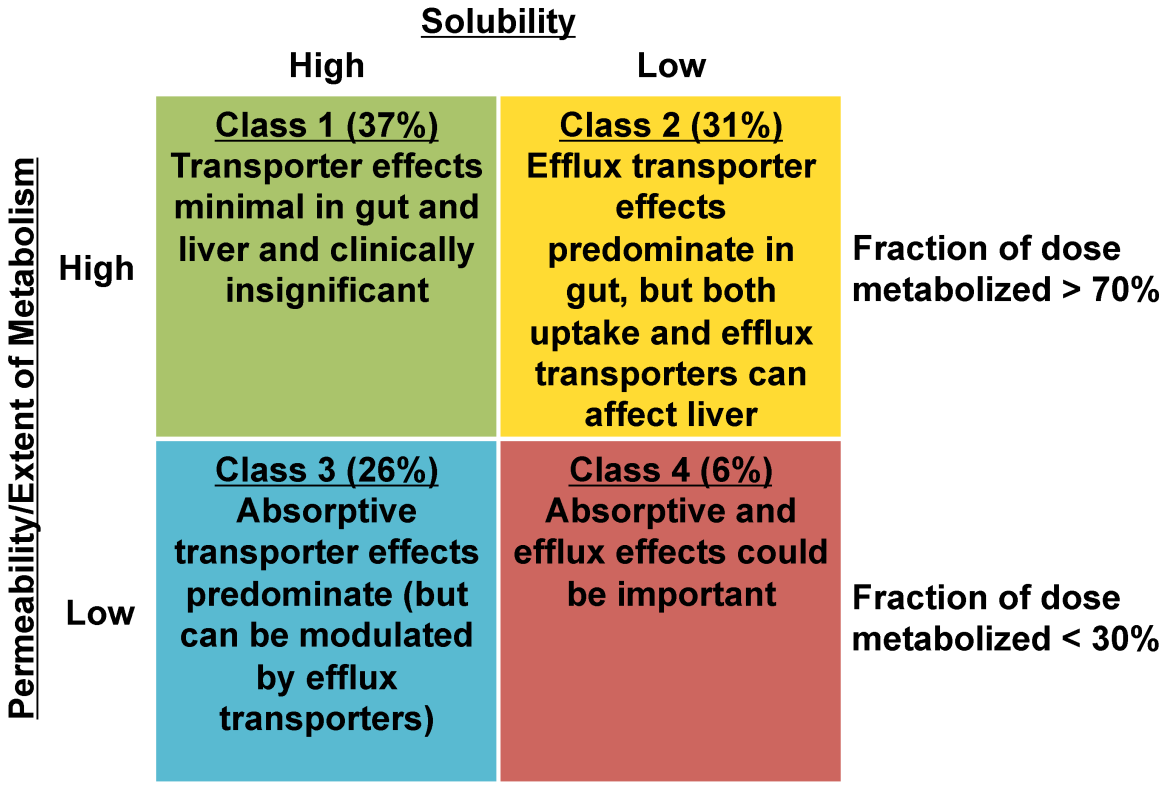

Figure 1-2. The Biopharmaceutics Drug Disposition Classification System.

The extent of drug metabolism is often quantified during phase I pharmacokinetic/mass balance studies(50). Tabulating absorption, on the other hand, requires invasive intestinal perfusion studies in man or portal blood sampling. Absorption is a prerequisite to enzymatic metabolism, which occurs intracellularly in the endoplasmic reticulum or cytosol. Therefore, we can assume that enzymatically metabolized drugs are absorbed. Since metabolism is easier to quantify than absorption/intestinal permeability rate, Wu and Benet proposed that metabolism be used as an alternative measurement to predict absorption. The EMA and recently the

FDA(51) have incorporated this suggestion into the guidance recommendations for granting biowaivers and highly soluble compounds with ≥85% metabolism are eligible for biowaivers.

When BDDCS was developed, a primary observation was that there was a very dichotomous extent of metabolism. Drugs tend toward a primary route of elimination. Specifically, metabolism tends to contribute greater than 70% of total drug elimination for extensively metabolized drugs, or less than 30% for drugs that are eliminated as unchanged drug. There are few (< 5%) examples of drugs being eliminated with an intermediate extent of metabolism.

BDDCS differs from BCS in two major aspects: 1) the primary goals of the systems and 2) the definition, interpretation, and relationship of “highly permeable drugs”.

The primary goal of BCS is to grant biowaivers using *in vitro* methodology to predict drug absorption and its limiting steps. Alternatively, while BDDCS provides the basis for recommending biowaiver extension to extensively metabolized compounds, the primary goal of BDDCS is to predict drug disposition.

Via application of BCS, biowaivers are approved based on extent of absorption, which may not always correlate well with intestinal permeability rate. While high permeability rate predicts a high extent of absorption, the opposite is not necessarily true. There are many examples of highly absorbed drugs that have a poor passive permeability rate, not reflecting their high absorption extent(52,53). For example, sotalol, a BDDCS class III drug, is poorly permeable in Caco-2 cells, but has an absolute bioavailability of 98%, and thus, is highly absorbed(54-56). Its high absorption is likely mediated by transport(57) since it has a poor *in vitro* permeability rate, but is a substrate for the gut uptake transporter OATP1A2(58). While some drugs are considered highly permeable in BCS because of their high absorption, they are not, in fact, highly permeable. This is, in fact, the basis of a major difference between BCS and BDDCS. Specifically, BCS class 1 and 2 compounds may be class 3 or 4 in BDDCS, since

drugs are classified by metabolism extent, and not absorptive extent. These differences are crucial in predicting drug disposition.

BDDCS is invaluable during drug development because understanding the effect of transport and metabolizing enzymes is now essential for drug approval. Specifically, new drug applications (NDAs) must include the major routes of drug elimination, the quantitative contributions of enzymes and transporters, and drug-drug interaction studies(9). BDDCS can alert developers to which enzymes and transporters are likely important, and may even justify negating some studies.

BDDCS does not predict quantitative values of drug disposition. It can, however, provide qualitative information about the absorption of some compounds, the extent of metabolism, the extent of biliary or renal elimination of unchanged drugs, and distribution. More accurately, it predicts what processes, i.e. transport at specific membranes and/or metabolism, will affect each aspect of disposition and the direction of the effect.

BDDCS predicts when a transporter or a metabolizing enzyme can clinically regulate the disposition of a drug, whether or not the drug is a substrate. When BDDCS predicts that drug transport at a membrane is not clinically relevant for a particular drug, it does not presume that the drug is not a substrate for a transporter. In fact, it is likely that almost all drugs are substrates for transporters. Instead, BDDCS predicts if a transporter significantly contributes to the disposition of a drug compared with passive diffusion. These effects are perhaps most obvious in clinical studies examining the effect of transport inhibition on drug absorption, distribution, metabolism, and elimination. In cases where a transporter or metabolizing enzyme is important in a drug's disposition, affecting one, e.g. by inhibiting transport, can cause clinically significant pharmacokinetic changes to elimination, bioavailability, or distribution, observed as changes in the plasma concentration versus time curve (AUC, C_{max}, t_{max}) and altering the parameters CL, V, or F that define dose. These changes may impact the safety or efficacy of the drug, resulting in a dose change. If inhibition of transport does not cause dispositional

changes enough to necessitate a dosage change, the transporter is not considered clinically significant in the drugs' disposition.

BDDCS predicts that extensively metabolized/highly soluble class 1 drugs are not clinically relevant substrates of drug transporters, even if *in vitro* evidence shows an affinity (Figure 1-2). In other words, while these drugs may have a biochemical affinity to transporters, the contribution of the active transporter to permeation across a membrane is minor compared to passive permeability, and any functional discrepancy of the transporter will not result in a significant change that requires dose adjustment to achieve safety or efficacy. For class 1 drugs, *in vitro* studies can provide a false positive predictive transporter interaction that studies *in vivo* or in humans are unlikely to replicate these results(59). *In vivo* or clinical interaction studies are costly and time restrictive. The FDA guidance recommends that P-gp and BCRP be evaluated as transporters for every drug, yet acknowledges that it may not be necessary for BCS class 1 drugs and sponsors may submit class 1 drugs without transporter data(9). This would more appropriately be acknowledged for BDDCS class 1 drugs, since some BCS class 1 drugs (e.g. sotalol) may be subject to transporter interactions.

Furthermore, BDDCS does not presume to predict that there *will* be an interaction for every drug in a class, but rather that an interaction *could* exist, and should be tested during development. Finally, BDDCS makes no predictions regarding inhibitor or inducer status.

Predicting Absorption via BDDCS

From an evolutionarily protective standpoint, enterocytes are equipped with metabolizing enzymes, which can change a xenobiotic into a generally less toxic and easy to secrete substance, and efflux transporters, which can help pump xenobiotics back into the gut. However, successful absorption and subsequent drug bioavailability must overcome these processes. Uptake transporters are also present in the enterocyte, presumably to facilitate absorption of nutrients. One such example is PEPT1, which is responsible for bringing

oligopeptides in, but also helps to absorb compounds such as cefadroxil, a β-lactam antibiotic(60).

Food, other drugs, and endogenous substrates can affect the environment in which the drug is dissolved through intrinsic chemical properties (e.g. percent fat, pH, water content) and by stimulating physiological changes in the gut and stomach. They may also interact biochemically with transporters and metabolizing enzymes, which can regulate the rate and extent to which a drug is absorbed. Factors such as pharmacogenomics can likewise alter drug absorption. Since oral administration is preferred due to compliance, convenience, and stability reasons, food, other drugs, endogenous substrates, and pharmacogenomics can be major barriers during drug development. Predicting absorption and how internal and external variations can change absorption, is therefore very important when selecting drug candidates.

BDDCS predicts that all class 1 and 2 drugs will be well absorbed, but that some class 3 and 4 may also be well absorbed if they are substrates for gut uptake transporters.

BDDCS class 1 and 2 drugs can so readily permeate enterocytes that gut apical uptake transporters provide only a minor contribution to their absorption. Therefore BDDCS predicts no effect when uptake is affected for highly permeable compounds since class 1 and 2 drugs can enter enterocytes unaided by transporters.

Class 3 and 4 drugs are poorly permeable and require active uptake transporters to be absorbed, and therefore alterations to their activity or expression will result in clinical differences in absorption and bioavailability. Specifically, decreased uptake transport functionality results in decreased absorption, and increased uptake function results in increased absorption. Uptake transport in the gut must therefore be evaluated for BDDCS class 3 and 4 drugs.

Gut Apical Efflux and Transporter-Enzyme Interplay

Apical efflux transporters counteract net xenobiotic absorption from the gut. Apical efflux transporters include P-gp, MRP2, and BCRP(61). After a drug is absorbed into an enterocyte,

substrates of apical efflux transporters are pumped back into the gut, reducing the effective absorption. Not only can efflux transporters affect parent drug absorption, they can also regulate the extent of metabolism of some drugs.

- BDDCS class 1 drugs are clinically unaffected by changes in transporter expression or activity in the gut, even if they are substrates. These drugs will be affected only by changes in metabolism, and the degree of metabolism is unaffected by transporters.
- BDDCS Class 2: Apical efflux transporters can have a clinical impact on the absorption of class 2 drugs. When efflux is inhibited, an increase in absorption may be observed. BDDCS class 2 drugs are in a unique position because efflux transporters in the gut can impact both parent drug absorption and their intestinal metabolism. Wacher *et al.*(62,63) discovered that inhibition of P-gp, even in the absence of CYP3A4 inhibition, decreases intestinal CYP3A4 metabolism, the enzyme that accounts for approximately 70% of CYP expression in the gut(64,65). One might expect that inhibiting efflux in the gut would increase metabolism by forcing a drug to interact with metabolizing enzymes for longer. One might also expect that metabolism would not be affected, but that inhibiting efflux would increase absorption and therefore bioavailability. However, because CYP3A4 and P-gp are co-regulated and share so many substrates(66), P-gp substrates are also likely to be metabolized by CYP3A4. Metabolizing enzymes are located just below the microvillus border in enterocytes(67). P-gp and CYP3A4 work in concert to eliminate drug from the body. Efflux transporters recycle xenobiotics that have not yet been metabolized by CYP3A4, pumping them back into the gut lumen and allowing them to be absorbed multiple times, giving the drug multiple opportunities for drug exposure, but also multiple opportunities for metabolism, a process called enzyme-transporter interplay. Therefore, when an enteric efflux transporter is inhibited, a class 2 drug may have decreased metabolism and increased bioavailability greater than would be expected by inhibiting absorption alone. This is specific to enterocytes and inhibition of

efflux transporters in hepatocytes leads to increased concentrations of parent drug and increased metabolism.

- BDDCS Classes 3 and 4: Apical efflux transporters play a protective role against poorly permeable class 3 and 4 drugs by effectively limiting absorption of poorly permeable drugs that are substrates for an efflux transporter. Class 3 and 4 drugs that are substrates of apical efflux transporters may see an increase in drug absorption when these transporters are inhibited.

Gut Basolateral Transporters

Little has been explored regarding basolateral transporters expressed on the enterocyte(61). It is unlikely that basolateral efflux is extremely important since concentrations in the portal vein will be very low compared to the cell, encouraging passive diffusion. Apical uptake transporters may be necessary for more hydrophilic class 3 and 4 drugs to enter the cell, but leaving the cell requires passage through the hydrophilic portion of the membrane and is likely not a limiting factor.

Effects on Absorption Rate: Flip-flop Kinetics

When a drug is given as an extended release formulation, absorption rate is often slower than elimination. Alternatively, the absorption of an immediate release drug is generally a relatively quick process compared to elimination. For most immediate release drugs, elimination is the rate-limiting step. However, a very small number of immediate release drugs exhibit flip-flop kinetics, where the rate of absorption is the rate-limiting step in the disposition of a drug, instead of elimination. Flip-flop kinetics may be a developmental concern when a compound is poorly permeable/poorly metabolized and also has a relatively short $t_{1/2}$, specifically if it is shorter than gastrointestinal transit time. For drugs displaying flip-flop kinetics, the terminal slope actually reflects absorption processes because absorption rate is not limited for highly

permeable/highly soluble drugs. We expect that only class 3 and 4 drugs would demonstrate flip-flop kinetics. Specifically, the absorption rate is probably limited by the affinity to and velocity of gut uptake transporters. Garrison *et al*. recently evaluated this hypothesis(68). For 19 drugs exhibiting flip-flop kinetics, 16 were indeed class 3 or 4. While the absorption of class 2 drugs is unlikely limited by uptake transporters, their absorption may be slow as a result of poor dissolution and very slowly dissolving class 2 drugs may display flip-flop kinetics.

Pharmacogenomics Affecting Absorption

Genetic factors can directly impact a person's ability to absorb a drug. For instance, patients with inflammatory bowel disease have increased MRP1 expression in the intestine(69), which can potentially result in decreased absorption of class 2, 3, or 4 drugs. Genetic differences within healthy populations can also result in variation in absorption. The variant SLCO2B1*3, which codes for OATP2B1 and has decreased uptake activity, has an allele frequency of 30.9% in Japanese(70,71). When fexofenadine, a BDDCS class 3 drug, was dosed to a Japanese population, those with the allele had a 37% lower AUC than those without the allele, indicating that genetic differences can impact drug absorption. Alternatively, there was no significant difference observed in AUC when midazolam, a class 1 drug, was dosed(72). Genetic differences are sometimes highly related to race, highlighting the importance of selecting an appropriate population of healthy volunteers representing common genotypes for dosing in certain countries. Genetics, expression, and activity of metabolizing enzymes and transporters can directly impact absorption and other dispositional functions of a drug and increased or decreased functionality follow the predictions outlined for each class.

Genetic differences in expression or activity of a metabolizing enzyme or transporter can significantly impact the metabolism or elimination of drugs. For example, a significant percentage of people are poor CYP2C19 metabolizers. Therefore, when a CYP2C19 substrate is prescribed, we would expect increased exposure in these patients. In fact, poor metabolizers

have greater acid suppression and ulcer healing than extensive metabolizers when they take proton pump inhibitors such as omeprazole as a result of their increased exposure(73).

Non-transport Mediated Interactions Affecting Drug Absorption

Finally, concomitant food, drug, or supplement administration can potentially alter drug solubility. For instance, some tyrosine kinase inhibitors (TKIs), which are anticancer agents, or the malignancy itself, can cause gastric distress such as gastroesophageal reflux disease (GERD). To combat this unpleasant side effect, many patients take proton-pump inhibitors, which increase gastric pH. Unfortunately, this can have the effect of decreasing the solubility of some of these weakly basic TKIs, thereby decreasing drug absorption(74). Yago *et al*.(75) showed that absorption, presumably by improving drug solubility, could be improved in healthy volunteers with elevated gastric pH (hypochloridria) by pre-dosing betaine hydrochloride that acidified the stomach prior to dosing the TKI dasatinib. Solubility-based drug interactions are likely to affect poorly soluble BDDCS class 2 and 4 drugs only, since the solubility class is defined by the lowest solubility condition possible in the stomach and gut.

Food can have a significant impact on drug absorption by influencing drug solubility and active absorption. These interactions will be explored in detail in a later section.

Role in Predicting Metabolism and Hepatic Elimination

The majority of drug metabolism follows drug absorption. While expressed in smaller concentrations than in the liver, the gut wall contains relatively high concentrations of metabolizing enzymes compared to other organs. Gut metabolism is a component responsible for decreasing a drug's bioavailability. The fraction of the absorbed dose that escapes gut metabolism is represented by F_G. Gut metabolizing enzymes are also largely responsible for the bioactivation of prodrugs.

CYP3A is only expressed at 1.4% of that in hepatocytes(76,77), but accounts for 70-80% of CYP expression in the gut (76) and more than 50% of metabolized drugs are substrates for CYP3A4(78) and may be presystemically metabolized. As a low-affinity, high-capacity enzyme, it may be particularly susceptible to drug concentration differences(67), influenced by permeability, transport, or solubility.

BDDCS class 1 and 2 drugs are, by definition, subject to extensive metabolism, while class 3 and 4 are poorly metabolized. Therefore, inhibition of gut or hepatic metabolism will significantly increase the bioavailability of class 1 and 2 drugs, but have little effect on class 3 and 4 drugs. Induction of metabolism is expected to decrease bioavailability of class 1 and 2 drugs.

Hepatic Basolateral Uptake

After oral absorption, the liver is the first organ to process drugs and so hepatic transport is important. Hepatic drug exposure is often regulated by hepatic basolateral uptake. While at least 7 major transporters (OATP1B1, OATP1B3, OATP2B1, NTCP, OCT1, OCT3, and OAT2) and the bidirectional transporters ENT1, ENT2, OAT7, OCTN2, and OSTα-OSTβ facilitate hepatic distribution, the FDA considers OATP1B1 and OATP1B3 to be the most clinically relevant(9) and recommends evaluating hepatically eliminated drugs for their potential to interact with these transporters as substrates, inhibitors, or inducers.

As in the gut, BDDCS predicts that uptake transport will be clinically irrelevant for class 1 drugs. BDDCS predicts that hepatic basolateral uptake transporters may play a significant role in class 2 drugs, which differs from predictions for the gut, and are necessary for hepatic exposure of poorly permeable class 3 and 4 drugs. The gut has “leakier” membranes (composed of epithelial cells) than the liver, which is composed of endothelial cells. This may possibly explain the difference in observed uptake transporter effects for class 2 drugs(50).

Metabolism in hepatocytes is the major eliminating function of class 1 and 2 drugs. Since class 1 and 2 drugs are extensively metabolized and the massive portion of metabolism occurs hepatically, it follows that hepatic transporters (for class 2 drugs) and/or metabolizing enzymes (for class 1 and 2 drugs) will be significant determinants of their disposition.

Class 1: Class 1 drugs do not depend on uptake for their hepatic access and transporters will have no impact on their disposition. However, hepatic metabolizing enzymes mediate the majority of elimination of class 1 drugs and their function will affect the disposition of class 1 drugs. If an hepatic metabolizing enzyme contributing to the clearance of a class 1 compound is inhibited, drug exposure (AUC) is expected to increase, and a lower dosage may be required to avoid toxicity. Alternatively, if the metabolizing enzyme is induced, clearance may be greater than expected, resulting in poor exposure and a potential for drug inefficacy.

Class 2: The systemic and metabolic disposition of class 2 drugs can be affected by both transport and metabolism. For BDDCS class 2 drugs, BDDCS predicts that decreased function of a hepatic basolateral uptake transporter may result in increased portal vein concentrations and decreased hepatocyte concentrations. Subsequently, when uptake is inhibited, decreased metabolism may be observed, while induction may lead to increased metabolism. Obviously when metabolism is inhibited, there may be increased plasma or hepatic concentrations of parent drug and decreased elimination, while metabolic induction will result in decreased plasma or hepatic concentrations of parent drug and increased metabolite concentrations.

Classes 3 and 4: Class 3 and 4 drugs are primarily eliminated by either renal or biliary elimination of unchanged drug. We expect that the poorly permeable class 3 and 4 drugs require a transporter to enter hepatocytes, while biliarily eliminated drugs require active canalicular efflux into the highly concentrated bile. Thus, especially if a compound is biliarily eliminated, inhibition of uptake transporters in the liver may result in increased AUC and

increased half-life as a result of decreased clearance. A lower dose may be required for biliarily eliminated compounds whose hepatic uptake has been inhibited.

For instance, rosuvastatin and pravastatin are primarily eliminated as unchanged drug in the bile. They are clinically relevant substrates of OATP1B1 and polymorphisms in the gene encoding OATP1B1, SLCO1B1, or drugs inhibiting 1B1 have been shown to increase plasma concentrations and decrease hepatic concentrations of these drugs(79,80). Not only may this decrease the efficacy of these statins(81), whose mechanism of action is in the liver, it also increases the risk of rhabdomyolysis, a severe muscle toxicity(82).

Hepatic Apical Efflux

Apical efflux transporters regulate parent drug and metabolite entry into the bile. Apical efflux transporters in the liver can contribute to the disposition of some class 2, 3, and 4 drugs. Drugs that are eliminated as parent drug in the bile must be actively transported into the bile by canclicular efflux transporters and follow bile flow through the biliary tree until the biliary contents are dumped back into the duodenum. Some drugs may be reabsorbed through the gut. Drugs that are not reabsorbed in the gut will be eliminated as part of the feces. Apical efflux can regulate biliary efflux as well as hepatic retention.

Class 1: Apical efflux will have no effect on the disposition of class 1 drugs.

Class 2: When apical efflux is inhibited, concentrations in hepatocytes are increased. For class 2 drugs, this may result in increased metabolism. You may notice that apical efflux inhibition in the gut results in the opposite effect: decreased metabolism. We hypothesize that this is because the drug is exposed to the apical transporter after metabolizing enzymes in the hepatocyte, while the drug interacts with efflux transporters prior to metabolizing enzymes in the gut.

Class 3 and 4: It has been hypothesized that canalicular efflux does not contribute to the systemic clearance of poorly metabolized drugs(83). If this is true, there will not be

increased systemic exposure of class 3 and 4 drugs. In this case, decreasing the dose may not be necessary to reduce toxic systemic exposure. However, accumulation within hepatocytes sometimes mediates hepatotoxicity, and a decreased dose may be required for this mechanism. There is a scarcity of data regarding the effect of apical efflux inhibition on systemic concentrations, however.

Predicting Bioavailability

Bioavailability depends upon the extent of absorption, the extent of metabolism in the gut and the liver, and drug loss due to first pass biliary elimination. In addition to understanding the metabolic component of bioavailability, all of these processes can potentially be affected by drug transport for class 2-4 drugs.

Because bioavailability depends on sequential processes, high absorption does not necessarily predict high bioavailability, since many highly absorbed drugs are also extensively metabolized. As such, BDDCS predicts that highly permeable class 1 and 2 drugs will have good absorption, but not necessarily good bioavailability. BDDCS assumes that metabolized compounds were absorbed compounds. However, there may be some compounds that are metabolized by non-enzymatic routes or by bacteria in the gut lumen. When over 900 drugs were classified into BDDCS, however, extensively metabolized drugs were categorized regardless of the mechanism(54). However, this is a nascent field and few drugs are currently known to be metabolized in this manner.

Class 1: The bioavailability of class 1 compounds can be affected by metabolizing enzymes and inhibition of metabolism will increase the bioavailability.

Class 2: Class 2 compounds can be affected by both transporters and metabolizing enzymes. Inhibition of efflux transporters in the gut can lead to increased absorption, decreased metabolism, and increased bioavailability. Inhibition of hepatic basolateral uptake may lead to decreased metabolism and increased bioavailability, while inhibition of hepatic basolateral efflux

may lead to increased hepatic metabolism and decreased bioavailability. Inhibition of metabolizing enzymes may increase bioavailability.

Classes 3 and 4: Metabolism is not a significant factor in the bioavailability of class 3 and 4 drugs. However, uptake and efflux transporters can potentially regulate bioavailability in both enterocytes and hepatocytes. One would expect decreased bioavailability if an enteric uptake transporter responsible for a drug's uptake was inhibited due to decreased absorption. Conversely, inhibition of enteric apical efflux would result in increased absorption and bioavailability. Additionally, first-pass biliary excretion may play a role. Hepatic uptake is generally considered the rate-limiting step of biliary elimination. Therefore, inhibited hepatic uptake of class 3 and 4 drugs would likely see a decrease in biliary elimination and an increase in bioavailability. It has been suggested that hepatic canalicular efflux does not regulate systemic clearance(83), and therefore inhibition of hepatic canalicular efflux would not likely have an effect on bioavailability, however, given the lack of clinical studies on biliary elimination, there is little data to confirm or deny this hypothesis.

Below we tabulate pharmacokinetic changes that may be expected when the function of an enterocytic drug transporter is decreased for any number of reasons including chemical inhibition from other drugs, food, or endogenous substrates and genetic mutations.

Table 1.1. Effects of Gut Apical Transporters on Pharmacokinetic Parameters

BDDCS Class	Decreased Functionality of Apical Uptake					Decreased Functionality of Apical Efflux				
	F	k_a	t_{max}	C_{max}	AUC	F	k_a	t_{max}	C_{max}	AUC
1	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
2	⇔	⇔	⇔	⇔	⇔	↑	↑	↓	↑	↑
3	↓	↓	↑	↓	↓	↑	↑	↓	↑	↑
4	↓	↓	↑	↓	↓	↑	↑	↓	↑	↑

Predicting Food Effects

Eating a meal can have a substantial impact on pharmacokinetics. In fact, many drug labels advise that the drug be taken either with a meal or separate from a meal. The act of eating causes a cascade of physiological changes in the gastrointestinal system. These can greatly affect the solubility and the transit time of the drug. In turn, the transit time can affect the exposure to intestinal fluids and membranes, as well as the location of drug-membrane exposure, where different segments of the intestine have different properties including membrane tightness, transporter and metabolizing enzyme expression. Additionally, food, drinks, and supplements can have a biochemical impact on the drug, where components of each can serve as inhibitors of transport or metabolism. Obviously, the effect of food on pharmacokinetics is a multi-faceted problem, which makes predicting food effects *a priori* quite difficult. However, some general predictions have been proposed using BDDCS principles.

Food chemistry is extremely complex. Even a single food can have multiple molecular components that inhibit uptake, efflux, metabolism, or any combination, forcing a complex interaction. For example, grapefruit juice, famous for its ability to inhibit CYP3A4, includes flavonoids and furanocoumarins. While flavonoids were initially expected to be the perpetrators of this interaction, it was ascertained that certain furanocoumarins are the culprits(84). When taken with antihistamines such as terfenadine or astemizole, grapefruit juice increased drug exposure to dangerous concentrations that caused cardiotoxicity, and, in some cases, death. Both drugs were eventually removed from the market. Many drugs metabolized by CYP3A4 are now labeled with cautions against consuming grapefruit juice. Additionally, components in grapefruit juice have been shown to inhibit uptake transporters(85) and P-gp(86).

Meanwhile, human gastrointestinal physiology is incredibly variable, as are the contents of the gastroinstestinal tract. Baseline gastric efflux on a fasted or fed stomach and pH can vary significantly between humans. The microbiome, which can break down drugs, having a direct effect, or influence food digestion, causing an indirect effect, is signature to each person.

Despite this complexity and variability, BDDCS is able to predict the effects of food for approximately 70% of drugs(87).

After eating, some physiological changes occur that can result in dispositional changes to drugs. The predicted dispositional changes as a result of physiological response to food, and more specifically high fat meals, are tabulated below.

Table 1.2. Dispositional Impacts due to Physiological Changes after a Meal

Physiological Change	Effect on Absorptive Component	Class 1	Class 2	Class 3	Class 4
Gastric fluid increases from 300-500 to 800-900 mL, while intestinal fluid volume increases from 500 mL to 900-1000 mL (88-90)	Increase volume for drug dissolvation and solvent drag		F↑		F↑
Phospholipids and bile salts increase 4-5 fold(91,92)	• Bile salts increase breakdown of fats into monoglycerides • Bile salts inhibit P-gp • Bile salts inhibit uptake • Bile salts increase solubilization • Monoglycerides and lipids liberated by bile salts can inhibit uptake and efflux as well	F⇔	F↑	F↓↑⇔[a] The majority of class 3 drugs will exhibit decreased F	F↓↑⇔
pH decreases with some foods such as juices	Weakly acidic drugs have decreased solubility, while weakly basic drugs have increased solubility	F ⇔	F↓ for acidic drugs F↑ for basic drugs	F ⇔	F↓ for acidic drugs F↑ for basic drugs
pH generally increases with food	Decreases solubility for weakly basic drugs(90,93)		F↓ for basic drugs F↑ for acidic drugs		F↓ for basic drugs F↑ for acidic drugs

Physiological Change	Effect on Absorptive Component	Class 1	Class 2	Class 3	Class 4
Splanchic blood flow is increased	Improve bioavailability(90,94)	F↑	F↑	F↑	F↑
Gastric emptying is delayed	Increases time before drug can be absorbed in the gut Increases interactions with gastric acid, which may contribute to drug decomposition (49,95)	t_{peak}↑ F↓	t_{peak}↑ F↓	t_{peak}↑ F↓	F↓
Gastric secretions including bile are increased by up to 5 fold(96)	• Increased bile volume allows for increased micellular solubilization and increased drug wetting of poorly soluble drugs • Increased bile concentrations • Break down fats into lipids • Bile salts and lipids can interfere with drug transport (97-99) • Aggregation into micelles which help with solubilization		F↑		

[a]Uptake inhibition may decrease bioavailability, efflux inhibition may increase bioavailability, and the effects combined may result in no change in bioavailability. However, the majority of class 3 drugs appear to have decreased bioavailability with a high-fat meal, presumably due to uptake inhibition.

Biochemical Inhibition

Components in high fat meals[2] may inhibit intestinal transport. Lipids consumed, monoglycerides and fatty acids liberated during fat digestion, and bile salts released to aid in digestion have all been shown to inhibit transport, especially that of P-gp(90,97-99,101), but also of uptake(90).

Components in any food, whether or not it is part of a high-fat meal, have the potential to serve as biochemical inhibitors of transport or metabolism and, if consumed daily, inducers. Certain fruit juices, teas, beer, and wine can cause biochemical inhibition of transporters and/or metabolites. For instance, orange, grapefruit, and apple juice have been shown to inhibit OATPs and P-gp in the gut(85,86,102).

The specific inhibitors in food can be quite difficult to identify since foods contain small concentrations of many compounds and compounds may have an additive effect.

Chemical Solubility Effect

Drug solubility depends on the pH of fluid, temperature, volume, and contents of fluid. The lipophilicity of a drug is also correlated with water solubility. The rate and extent of absorption can be altered by food.

Factors that increase the amount of drug solubilized are particularly important for BDDCS class 2 and 4 drugs, whose absorption is limited by their poor solubility. Foods can increase solubility by increasing the volume into which a drug can be solubilized, changing the pH of the fluid, and increasing concentrations of bile salts 4-5(91,92) fold**.** After a meal, the volume of intestinal fluids increases 2-3 fold, which can potentially increase the amount of drug that is solubilized, meaning more drug may be available for absorption. Bile salts can improve the solubility of some drugs by acting as surfactants(103). Changes in the pH of gastrointestinal

[2] A high fat meal contains 800-1000 calories with 50-65% from fat and 25-30% from carbohydrates and 15-20% protein(100)

fluids can alter the solubility of drugs. Acidic drugs will be more poorly soluble in acidic media, while basic drugs will lose solubility in basic media and vice versa.

Overall Predicted Food Effects After Consuming High Fat Meals for Each BDDCS Class:

Class 1: The overall bioavailability is unlikely to change for BDDCS class 1 drugs since increasing solubility will have no effect and class 1 drugs are not subject to transporter inhibition. Since gastric emptying will be delayed after eating, however, these drugs may be more slowly absorbed, and t_{peak} may be later.

Class 2: When class 2 drugs are administered with a high fat meal, the bioavailability will likely increase while time to reach peak concentrations may shift in either direction.

High-fat meals may inhibit P-gp, resulting in increased bioavailability. As we discussed earlier, P-gp inhibition can also limit metabolism of BDDCS class 2 drugs. Therefore, by decreasing both efflux and metabolism, BDDCS class 2 drugs are likely to be more bioavailable when P-gp is inhibited. Additionally, fatty food and the release of bile acids can form micelles, promoting drug solubilization.

The time to reach a maximum concentration for a class 2 drug can be affected by a multitude of factors and no single trend is predicted. By delaying gastric emptying, a high fat meal can increase the amount of time it takes for the drug to be absorbed in the intestine, increasing t_{peak}. The time to reach a maximum concentration may also decrease due to the inhibition of efflux cycling by high fat meals. Additionally, these processes may compete, causing no effective change in t_{peak}.

Class 3: High fat meals, bile salts, and chemical components in food can inhibit uptake transporters, which class 3 drugs rely on to be absorbed. Patients taking a poorly permeable class 3 drug might experience decreased bioavailability and poor exposure when administered with a high-fat meal. Because their uptake is inhibited, the time to reach C_{max} may also increase. T_{peak} may also increase as a result of delayed gastric emptying after a high-fat meal.

Class 4: BDDCS class 4 drugs are very difficult to predict because many interacting effects including increased solubility, increased gastric emptying time, and inhibited uptake compete. With so few class 4 drugs, it is difficult to predict a single trend.

Role in Predicting Distribution

Wu and Benet(48) observed that the volume of distribution was somewhat higher in the highly permeable class 1 and 2 drugs compared to those in classes 3 and 4.

Transporters can drastically affect the volume of distribution by concentrating drug in tissues. When certain major transporters in the liver or kidney are inhibited, Grover and Benet(104) noticed certain trends in distribution.

The liver has a primary effect on the volume of distribution. In peripheral tissues, altered transporter function may have a pharmacodynamic effect and the compound may be attenuated in tissues, but the calculated volume of distribution does not appear to change.

When hepatic uptake is inhibited, there is an increase in plasma concentration coupled with a decrease in hepatic distribution, leading to a decrease in volume of distribution. When hepatic canalicular efflux is inhibited, there is also a decrease in the volume of distribution (or it is not predictable). Inhibition of hepatic basolateral efflux results in an increase in the volume of distribution.

However, when renal uptake is inhibited, there is generally no effect on the volume of distribution. When renal efflux is inhibited, the volume of distribution often increases. They hypothesize that the discrepancy between changes in volume of distribution due to inhibition of uptake in the liver versus the kidney is likely a result of the larger mass of the liver, coupled with increased capacity for transporter expression and drug sequestration(104).

Finally, gut transporters will not have an effect on the volume of distribution because volume of distribution is a systemic parameter.

Since class 1 drugs have no clinically relevant transporter effects, we expect no changes in the volume of distribution of class 1 drugs when a transporter has increased or decreased function or expression. Alternatively, since uptake and efflux transporters can affect class 2, 3, and 4 drugs in the liver, we would expect changes to the volume of distribution described above.

Distribution into the Brain

Distribution to various tissues can be predicted by BDDCS. Specifically, we now understand the conditions necessary for central nervous system penetration. This is a particularly difficult problem during drug development of CNS-targeted drugs, as the brain is well-protected from xenobiotics with tight junctions and high efflux transporter expression. Understanding and predicting brain penetration is also important to avoid central side effects for a peripherally acting drug. P-gp, BCRP, and various MRPs are expressed on the apical membrane of brain capillary endothelial cells, poised to extrude drugs that gain entry across its membrane. In development, substrate specificity for efflux transporters is a cue that the drug will be unable to successfully penetrate the brain. When the brain is the intended site of action, lipophilic compounds with a low polar surface area are expected to be available to the CNS(105,106).

The brain is also a particularly concerning tissue for drug resistance. Some diseases, including some cancers and epilepsy, are resistant to drug penetration in the brain as a result of overexpressed P-gp or other efflux transporters. This overexpression is sometimes innate to the disease and sometimes acquired, potentially due to drug treatment. To overcome drug resistance, some scientists have proposed co-dosing with efflux inhibitors. Instead, dosing class 1 drugs may be a more thorough and facile approach.

Mahar Doan *et al*.(105) suggested that highly permeable, non-P-gp substrates were likely to cross the blood-brain barrier, while poorly permeable and P-gp substrates are less likely to cross the blood-brain barrier. While this holds true for a majority of compounds, an analysis

by Wager *et al*. revealed that 20% of CNS drugs were both poorly permeable and P-gp substrates(107). Broccatelli *et al*.(108) incorporated BDDCS classifications, correctly predicting the CNS distribution of greater than 90% of their dataset. Ninety-eight percent of class 1 drugs in their dataset were able to cross the CNS, whether or not they were a substrate for P-gp. In fact, after correcting for a misclassified drug, all of the BDDCS class 1 P-gp substrates were able to distribute into the CNS(59,108). Even when P-gp was able to partially efflux the drug, there was significant brain penetration. Contrarily, 75% of P-gp substrates in classes 2, 3, and 4 were unable to traverse the blood brain barrier. While presumably all of the class 1 drugs have CNS exposure, even if they are P-gp substrates, clearly 25% of P-gp susbtrates in other classes were still able to access the brain, likely because they are good substrates for uptake transporters at the brain. While Broccatelli *et al*. only considered P-gp substrate specificity, other efflux transporters such as BCRP are expressed at the blood-brain barrier and are responsible for extruding drugs. The same principles should apply to substrates of other efflux transporters. Based on these findings, each class is predicted to behave as follows:

Class 1: Transporter effects are minimal and drugs are expected to penetrate the CNS

Class 2: Efflux transporters at the blood-brain barrier may affect class 2 drugs

Class 3: Uptake transporters at the blood-brain barrier (OATP1A2, OATP2B1) are required for brain penetration; while efflux transporters can extrude drugs from the brain

Class 4: Uptake transporters at the blood-brain barrier are required for brain penetration; while efflux transporters can extrude drugs from the brain

Therefore, when developing a drug with a CNS indication, a class 1 drug may be preferable for candidate selection, since it will penetrate, regardless of transporter affinity. Class 2 drugs may be developable as long as they are not substrates for efflux transporters. Class 3 and 4 drugs have more stringent requirements. For a class 3 or 4 drug to be effective as a CNS agent, it must be a substrate for an uptake transporter in the gut (if it is orally administered) and

at the blood-brain barrier, and should not be a substrate for efflux transporters at the blood-brain barrier.

Alternatively, when developing peripherally acting drugs, class 1 drugs may have potential CNS side effects, even if they are substrates for efflux transporters. Class 2 drugs may have central effects if they are not substrates of efflux transporters. To avoid central effects for class 3 and 4 drugs, it is best to avoid substrates of uptake transporters at the blood-brain barrier. Non-class 1 drugs will need to be evaluated as substrates of CNS-expressed transporters to predict brain penetration.

Table 1-3. Effect of Apical Transporters on Central Nervous System Distribution

BDDCS Class	Apical uptake	Apical efflux	CNS exposure
Class 1	No effect	No effect	Yes
Class 2	• May be necessary for some class 2 drugs to penetrate the brain • Inhibition may lead to decreased CNS exposure	• May decrease CNS exposure • Inhibition may lead to increased CNS exposure	If a non-substrate for efflux and may possibly require an uptake transporter
Class 3	• Required for CNS exposure • Inhibition will lead to decreased CNS exposure	• Will prevent exposure to the CNS • Inhibition may lead to increased CNS exposure	If a substrate for an uptake transporter and a non-substrate for an efflux transporter
Class 4	• Required for CNS exposure • Inhibition will lead to decreased CNS exposure	• Will prevent exposure to the CNS • Inhibition may lead to increased CNS exposure	If a substrate for an uptake transporter and a non-substrate for an efflux transporter

Potential Extensions to Other Membranes

While distribution and transporters regulating distribution outside of the gut, liver, and brain have not been analyzed with respect to BDDCS dispositional predictions, we expect that most internal tissues (composed of tightly regulated endothelial cells) will behave in a similar manner to the liver and brain and not like the epithelial-based gut. Therefore, we expect that class 1 drugs will distribute to a tissue regardless of transporter function, while class 2 drugs may be affected by the function of uptake transporters regulating drug entry and may have less tissue penetration if they are a substrate of a relevant efflux transporter. Class 3 and 4 drugs will almost certainly require active transport into the tissue and their distribution will be significantly impacted by efflux transporters expressed in the tissue. For class 1 drugs, there may be some concern about undesired distribution to off-target organs. Scientists can potentially use this information to aid in drug delivery to target tissues, including the heart and skeletal muscles. Distribution across the placenta could also potentially be predicted, which may be advantageous because its distribution cannot be studied for ethical reasons.

Distribution to the Kidney

As we discussed earlier, renal elimination is a combination of passive filtration processes, reabsorption, and active secretion. Reabsorption is primarily passive. The vast majority of water and solutes are reabsorbed along the tubule, resulting in only 1 mL of urine production every minute. There are some transporters responsible for active reabsorption expressed primarily in the proximal tubule, however. While a number of secretory transporters are expressed along the proximal tubule, OAT1, OAT3, and OCT2 are currently considered the most clinically significant and the FDA recommends studying renally eliminated drugs for interactions with these transporters(9). All drugs should be evaluated as substrates of P-gp, as well. However, the ITC lists a number of renal transporters that they consider important to evaluate during drug development, including the bidirectional transporters ENT2, expressed on

the basolateral membrane, and ENT1, OCTN1, and OAT4, expressed on the apical membrane. Secretory transporters including OAT2 and OATP4C1, which are expressed on the basolateral membrane and MATE1, MATE2-K, MATE2, MRP2 and MRP4 all of which are expressed on the apical membrane are considered relevant by the ITC. They also include the absorptive transporter URAT1, which is expressed on the apical membrane(10).

Class 1 and 2 drugs are likely to be reabsorbed from the tubule, as we have discussed. We expect that class 2 drugs may interact with basolateral uptake and apical efflux transporters, similar to hepatic predictions. We expect that uptake and efflux transport will be required to contribute to net secretion of class 3 and 4 compounds. However, renal elimination can also be completely passive, and class 3 and 4 compounds are not necessarily substrates of renal transporters even if they are eliminated as unchanged drug in the urine.

Renal Impairment

Chronic kidney disease (CKD) is a serious condition, affecting more than 10% of adults in the United States(109). Since the kidney eliminates many drugs and metabolites, impaired renal function can also seriously decrease renal clearance of these drugs, mandating dose adjustments in patients. One may understandably, but mistakenly, conclude that renal dysfunction should only affect renally eliminated drugs. In fact, metabolism can be dangerously altered in CKD patients, particularly as disease progresses. When the kidneys begin to lose their function, endogenous compounds that are eliminated by the kidneys in healthy people accumulate in toxic concentrations. These compounds are called uremic toxins. Initially, it was hypothesized that uremic toxins inhibited metabolizing enzymes. Investigations showed that uremic toxins inhibited some, but not all, CYP metabolizing enzymes(110-115). As it became clear that drug transporters also played a role in controlling drug access to metabolizing enzymes, Reyes and Benet questioned if perhaps uremic toxins could also inhibit transporters, potentially reducing metabolic clearance *in vivo*. They concluded that uremic toxins could inhibit

uptake transporters in hepatocytes. Since the disposition of a class 1 drug is unlikely to be affected by drug transporters, they tested whether uremic toxins inhibited hepatic exposure of propranolol, a class 1 drug. While uremic toxins did not inhibit the uptake of propranolol, some uremic toxins did inhibit the uptake of losartan, a class 2 drug and eprosartan, a class 4 drug.

Interestingly, in this study, uremic toxins were unable to inhibit phase I metabolism when human uremic serum was incubated with microsomes dosed with propranolol or losartan, both of which are extensively metabolized(116).

Given previous evidence, it would be unwise to suggest that metabolizing enzymes are uninhibited by uremic toxins. Therefore, decreased metabolism may be observed for class 1 and 2 drugs taken by patients with CKD. Alternatively, uptake transporters are likely inhibited by uremic toxins, which may decrease the metabolism of class 2 drugs and decrease hepatic clearance of class 3 and 4 drugs. All drugs should be tested for increased parent drug exposure, though the mechanism of inhibition will differ between classes.

This is a critical prediction that may a) increase the safety of drugs in ESRD patients, many of whom require several drugs and b) ease the developmental burden. The FDA now recommends that most new molecular entities be evaluated in ESRD patients, excepting drugs predominantly cleared by the lungs, monoclonal antibodies, and drugs intended for single-dose administration(117). Unfortunately, generating these studies and recruiting patients is difficult, costly, and variable. Applying BDDCS concepts to pharmacokinetic studies in renal disease may help prioritize what studies are necessary and help understand if physicians should be concerned about inhibition of transporters, metabolizing enzymes, or both in administering one or multiple drugs.

CONCLUSIONS: THESIS AIMS

The goal of this thesis is to address some of the current challenges in pharmacokinetics. We specifically aim to more fully understand mediators of dispositional processes, in particular

the role of transporters, metabolizing enzymes, and their integrative mechanisms. We largely focus on the role of transporters and metabolizing enzymes in the context of drug elimination, but also discuss their roles in absorption and distribution.

In chapter 2, we address current challenges in understanding biliary elimination. Since measuring biliary elimination is difficult, sometimes unreliable, and the extent of biliary elimination is often unknown, we develop a model that predicts which drugs are likely to exhibit biliary elimination of parent drug. We additionally discuss our current understanding of how drug transport mediates biliary or renal elimination of parent drug.

In chapter 3, we consider how permeability rate may serve as a mechanism that determines which drugs are extensively metabolized and which drugs are poorly metabolized and eliminated in the bile or the urine as unchanged drug. Although we show that it is particularly difficult to understand when a drug is eliminated as unchanged drug in the bile versus metabolized in chapter 2, the study carried out in chapter 3 demonstrates that permeability rate can effectively discriminate between these two elimination routes.

Throughout this thesis, we refer to the predictive utilities of BDDCS. While this system can be powerfully applied to predict transporter and metabolizing enzyme interactions and dispositional effects in absorption, distribution, and elimination, it currently relies on *in vitro* and partially on clinical data. Its predictive utility during development would be greatly enhanced by successful *in silico* models predicting BDDCS class. In chapter 4, we develop an *in silico* model to predict BDDCS class, compare its predictive ability to a successful *in vitro* model, and discuss the *in silico* model's strengths and limitations.

BDDCS makes predictions regarding every aspect of disposition: absorption, distribution, metabolism, and excretion. BDDCS provides many valuable predictions that can be useful in guiding drug development decisions. It currently relies on the extent of metabolism, which cannot be assessed until phase I studies, and solubility studies. It would be extremely useful to have accurate high-throughput methods to predict BDDCS class prior to phase I trial.

In this project, we aimed to develop new methods of predicting aspects of drug disposition. In chapters 2 and 3, we utilize observations from BDDCS to integrate *in vitro* information with *in silico* models that we developed to predict the major routes of drug elimination. In chapters 4 and 5, we extend the utilities of BDDCS by developing and analyzing an *in silico* model that predicts BDDCS class and discuss its growing list of applications.

CHAPTER 2. PREDICTING WHEN BILIARY EXCRETION OF PARENT DRUG IS A MAJOR ROUTE OF ELIMINATION IN HUMANS[†]

ABSTRACT

Biliary excretion is an important route of elimination for many drugs, yet measuring the extent of biliary elimination is difficult, invasive, and variable. Biliary elimination has been quantified for few drugs with a limited number of subjects, who are often diseased patients. An accurate prediction of which drugs or new molecular entities are significantly eliminated in the bile may predict potential drug-drug interactions, pharmacokinetics, and toxicities. The Biopharmaceutics Drug Disposition Classification System (BDDCS) characterizes significant routes of drug elimination, identifies potential transporter effects, and is useful in understanding drug-drug interactions. Class 1 and 2 drugs are primarily eliminated in humans via metabolism and will not exhibit significant biliary excretion of parent compound. In contrast, class 3 and 4 drugs are primarily excreted unchanged in the urine or bile. Here, we characterize the significant elimination route of 105 orally administered class 3 and 4 drugs. We introduce and validate a novel model, predicting significant biliary elimination using a simple classification scheme. The model is accurate for 83% of 30 drugs collected after model development, with 100% of biliarily eliminated drugs correctly predicted and 79.2% of renally eliminated drugs correctly predicted. The model, which incorporates calculated polarizability and metabolic stability, corroborates the observation that biliarily eliminated drugs have high molecular weights, while demonstrating the necessity of considering route of administration and extent of metabolism when predicting biliary excretion. Interestingly, a predictor of potential metabolism significantly improves predictions of major elimination routes of poorly metabolized drugs. This model successfully predicts the major

[†] Modified from Hosey CM, Broccatelli F, Benet LZ. Predicting when biliary excretion of parent drug is a major route of elimination in humans. AAPS J. 2014;16:1085–96.

elimination route for poorly permeable/poorly metabolized drugs and may be applied prior to human dosing.

INTRODUCTION

Drugs are primarily eliminated via metabolism, biliary excretion of unchanged drug, or renal elimination of unchanged drug in the urine. During development, predicting how a drug will be eliminated from the body can help to assess potential toxicities, drug-drug interactions (DDIs), and pharmacokinetics, including possible exposure to the target site. Extent of metabolism and urinary excretion are readily quantifiable. However, biliary excretion is difficult to quantify in humans, and is often predicted in preclinical animal models, which perform poorly, especially when hepatic uptake transporters mediate biliary clearance(118). It would therefore be ideal to model when biliary excretion will be a primary elimination route in humans prior to human dosing.

Transporter-mediated drug interactions can alter the exposure of drugs, resulting in toxicity or lack of efficacy. For example, cyclosporine inhibits the uptake of rosuvastatin, a biliarily eliminated drug, by OATP1B1, resulting in a sevenfold increase in AUC(119), which may result in life-threatening rhabdomyolysis. It is now considered essential to determine possible transporter-mediated drug interactions and develop respective guidances during drug development(120). The Biopharmaceutics Drug Disposition Classification System (BDDCS) predicts when drug-drug interactions may be a concern utilizing extent of metabolism, which is qualitatively correlated with passive intestinal permeability rate, and solubility(48).

Biliary elimination is a vectorial process mediated by transport on the basolateral and apical membranes of hepatocytes, which may both cause interactions and affect disposition. To access the liver, drugs in the portal vein must traverse the hepatic basolateral membrane, requiring active transport for biliarily eliminated drugs, which are poorly permeable. Notably, Varma *et al*.(38) observed a large overlap in the physicochemical space between human OATP

substrates and drugs where biliary excretion accounts for ≥ 10% of the administered dose in rats. Human OATP substrates and biliarily excreted compounds both tended toward MW ≥ 400 Da, cLogD7.4 < 2.0, and RPSA (polar surface area normalized by molecular mass) ≥ 20%. Subsequent to hepatic uptake, biliarily eliminated drugs are actively effluxed across the canalicular membrane to the highly concentrated bile by transporters such as P-gp, BCRP, MRP2, MDR3, BSEP, or MATE1. Drugs that are not eliminated in the bile can reenter the systemic circulation by permeation back across the basolateral membrane or be metabolized. As the most promiscuous efflux transporter, biliarily eliminated drugs are frequently P-gp substrates and therefore might be expected to exhibit physicochemical properties that overlap with those of P-gp substrates. Recently, Broccatelli determined that P-gp nonsubstrates have a calculated surface area (S) < 400 $Å^2$ (121). Transport efficiency can be inhibited by xenobiotics, endogenous substrates, disease states, or genetic polymorphisms, resulting in decreased hepatic clearance of drugs and endogenous compounds such as bilirubin and bile salts and may result in unpredictable, possibly toxic exposure.

The major route of elimination can dictate a drug's observed pharmacokinetics and therefore may be targeted or avoided. For instance, drugs that are excreted into the bile may be subject to enterohepatic circulation, resulting in variable plasma concentrations with multiple peaks(122), and a longer half-life. Drugs eliminated in the bile may not be appropriate or may require extra pharmacokinetic monitoring for patients with certain diseases or genetic polymorphisms, such as those with Dubin-Johnson syndrome, where a mutation in MRP2 results in poor biliary elimination of bilirubin glucuronides and drug substrates. On the other hand, biliary elimination, because of enterohepatic circulation, could be usefully targeted to treat diseases in the enterohepatic system, such as Crohn's disease or liver cancers. Alternatively, renal elimination should be targeted for drugs that need to reach the systemic circulation or to treat conditions where the kidney is the target organ such as urinary tract infections. Overall, understanding the major routes of elimination can help predict drug-drug interactions, toxicities

and pharmacokinetics during development, and may be useful in predicting substrates of efflux transporters in other tissues.

While human liver microsomes generally provide reliable predictions of human metabolic clearance for extensively metabolized drugs(123-128) and renal clearance is not difficult to determine, predictions of human biliary clearance are difficult and scarce, despite ongoing efforts(129). Clinical methods include bile duct cannulation during surgery, collection of duodenal fluid in healthy volunteers, biliary string or fecal collections. These procedures are difficult, uncommon, and variable. Additionally, much of the data are collected from patients who do not necessarily have a healthy hepatobiliary system and are under anesthesia, which may result in physiological changes. *In vitro* predictions can be carried out in sandwich-cultured rat or human hepatocytes, which preserve cell polarity and bile canaliculi(130,131). However, uptake transporter expression is not well preserved in sandwich-cultured rat hepatocytes(132) and biliary clearance can be rate limited by uptake(133). Several studies have demonstrated that *in vitro* measures correlate with, but underpredict, *in vivo* biliary clearance(134-136). Bile duct cannulation in rats is often performed, but may not scale to humans, especially since rats have greater rates of bile flow(45) and canalicular efflux transport(46), as well as increased expression(47,137) of canalicular efflux transporters.

Historically, a molecular weight cut-off of 500–600 Da was proposed for minimizing biliary clearance in humans(138). More recently, Yang *et al*. published a model including 97 drugs, which as a part predicted that anionic drugs with molecular weights greater than 475 Da are likely to be significantly (> 10% of parent dose) excreted in the bile(37).

While in our study we initially consider only poorly metabolized drugs and drugs that can be administered orally, their dataset also included extensively metabolized drugs, as well as drugs that cannot be orally administered. Their study and others implicated hydrogen bond interactions(37,38), charge state(37-39), the presence of polar groups or a large polar surface

area(37-41), and the presence of dipole or quadrupole moments(37,39), while others have implicated hydrophilicity(38,39,41), carboxylic acid groups(39,40), and rotatable bonds(38,40).

Since our objective was to predict when biliary excretion is a major route of elimination (> 35% of parent dose), we initially did not consider extensively metabolized drugs, as their disposition is unlikely to be greatly affected by changes in biliary excretion. Wu and Benet(48) proposed the Biopharmaceutical Drug Disposition Classification System (BDDCS), which segregates extensively metabolized (class 1 and 2) drugs in humans from those that are eliminated primarily via renal or biliary routes (class 3 or 4). They noted that very few drugs have an extent of metabolism between 30% and 70%, and that high permeability rate drugs were extensively metabolized in humans, while low permeability rate drugs were primarily eliminated unchanged. The classification system makes predictions about transporter effects and disposition for drugs in each class, based on extent of metabolism and solubility. In particular, highly soluble and extensively metabolized class 1 drugs will not exhibit clinically relevant alterations in disposition due to transporters. Transporters may affect class 2 drugs, but their disposition changes would primarily reflect changes in metabolite formation and parent drugs are unlikely to be greatly affected by biliary excretion. Drugs that are significantly eliminated in the bile or urine fall within classes 3 and 4, and may exhibit altered disposition due to drug-drug interactions affecting transporters in the gut and/or liver. Benet *et al*. have compiled a dataset of over 900 drugs and provided the BDDCS class for each of these drugs(54).

Here, we combine BDDCS's observations about major routes of elimination with easily obtained urinary excretion data (see "Methods") to characterize drugs significantly eliminated in the bile (> 35% of available parent drug). As class 1 and 2 compounds exhibit less than 30% elimination into the bile and urine, biliary or renal excretion may only need to be evaluated for extensively metabolized drugs with a narrow therapeutic range. Indeed, Varma *et al*.(139) recently reported that drugs in their data set with MDCK permeability rates greater than 5×10^{-6} cm/s contribute less than 30% of parent drug to human renal elimination and are unlikely to be

affected by renal DDIs, while rat biliary elimination and permeability rate were inversely related. Finally, low permeability rate compounds were highly represented as substrates of hepatic basolateral uptake transporters(139). Importantly, their data indicate that *in vitro* permeability rate can be used as a surrogate for extent of metabolism for new molecular entities (NMEs) when clinical data is unavailable, as has been proposed by our laboratory(54,87,140). Specifically, compounds with permeability rates equal to or exceeding a standard, e.g., labetalol, are likely extensively metabolized *in vivo* in humans, while those with permeability rates lower than the standard are likely eliminated primarily as unchanged drug in either the bile or the urine.

Lipinski *et al*.(11) published guidelines for predicting which drugs are likely to be absorbed upon oral dosing. However, these rules do not apply when transporters mediate the intestinal uptake of drugs, i.e., class 3 and 4 drugs that are eliminated in the bile or the urine.

Here, we initially evaluate the molecular properties associated with significant biliary elimination of orally administered drugs. We then evaluate the importance of considering routes of administration (oral versus non-oral) and elimination when developing predictive models and discuss the interesting relationship between absorbed drugs that are eliminated in the bile and non-orally administered drugs, presumed as poorly absorbed, eliminated in either the bile or urine. We discuss a surprising and novel observation that poorly metabolized drugs can be classified by qualitative *in silico* predictions of CYP3A4 metabolism, and discuss the overlap in molecular properties of hepatically cleared compounds. The classification model outlined here can be applied to predict the major route of elimination of poorly metabolized drugs and provides guidelines to determine if a drug predicted to be poorly absorbed should be evaluated for active intestinal uptake.

Dataset

BDDCS classification of 927 drugs was assigned by Benet *et al*. as previously described(54). Briefly, compounds were classified as highly soluble if the highest dose strength was soluble in 250 mL of water over the pH range of 1–7.5 at 37 ° C. Compounds with greater than 70% metabolism in humans were classified as highly metabolized. From this dataset, we selected orally administered BDDCS class 3 and 4 drugs. Two clear outliers, tenofovir disoproxil, a prodrug, and vancomycin, which is rarely administered orally, were removed. Finally, drugs that fell into the primary excretion route criteria outlined below were selected for analysis, leaving a dataset of 105 drugs. An external dataset of 6 biliarily eliminated and 24 renally eliminated drugs was developed by considering clinical data of orally administered BDDCS class 3 and 4 or poorly metabolized drugs that did not meet the initial criteria based on fraction excreted unchanged, but had clinical data supporting biliary or renal elimination.

Class 3 and 4 drugs were classified as primarily excreted renally, with no significant biliary contribution, or significantly excreted in the bile as follows:

$$Total\ Absorbed\ Dose = 100 = f_e + f_b + f_m$$

$$f_m < 30$$

$$70 < f_e + f_b$$

Here, f_e represents percentage of absorbed dose excreted unchanged in the urine, f_b represents percentage of absorbed dose excreted unchanged in the bile, and f_m represents percentage of absorbed dose eliminated via metabolism. Assuming less than 30% of the absorbed dose is metabolized for class 3 and 4 drugs allows calculation of the minimum amount of drug excreted in the bile. Therefore, class 3 and 4 drugs with 35% or less of the parent drug excreted unchanged in the urine are presumed to be significantly excreted in the bile (≥ 35% dose), while drugs with 65% or greater of the dose excreted unchanged in the urine are

primarily excreted renally, with biliary elimination presumed to be insignificant. Drugs with $35 < f_e < 65$ were removed due to mathematical uncertainty of the significance of biliary excretion, since we wanted to initially operate on a set of drugs where preferential biliary or renal elimination were well differentiated. Drugs excreted in the bile were considered the positive class.

Model Creation

Using VolSurf+(141-143) at pH=7.5 and default options, 128 descriptors and charge state at pH=7.5 were calculated for the dataset. Physicochemical properties were calculated in ADMET Predictor™(30) with default settings at pH=7.4.

The open software R(144) was used for principal component analysis, partial least squares analysis(145), logistic regression(144), and receiver operating characteristic curves(146). The open machine-learning software Orange(147) was used for variable selection.

Principal component analysis of the VolSurf+ features was performed using the stats package in R. The data were scaled and centered. Scores for each component were obtained and compared between classification groups with the t-test.

The number of variables was minimized to avoid overfitting the data and to physiologically interpret the results. Variables were ranked according to information gain, which is an algorithm that assesses the entropy a variable provides to the dataset, and the top 15 variables were selected for analysis. The classification accuracy, specificity, and sensitivity of variable combinations were assessed for Naïve Bayes, k-Nearest Neighbors (kNN), and logistic regression models with fivefold cross-validation by adding variables in order of information gain. Variables were left in the model if one or more of the evaluations (classification accuracy, specificity, or sensitivity) increased for one or more of the models. Optimal variable combinations were assessed with the VizRank tool in Orange with the following settings: six attributes, tenfold cross-validation of 100% of the dataset, and were evaluated by average

accuracy in kNN. Variable selection data are not shown, as the methods were used only for variable reduction, and not model development and validation.

Partial least squares discriminant with scaling of all variables and six selected variables was performed using the pls package in R(145). Models were validated by 10×5-fold cross-validation. Cross-validation training and test sets were randomized and stratified.

Logistic regression models were developed and used to define a decision boundary to predict drugs excreted in the bile from drugs excreted in the urine using the stats package in R(144). The default fitting characterized by iteratively reweighted least squares was employed. Models were validated by 10×5-fold cross-validation. Cross-validation training and test sets were randomized and stratified. An external dataset was collected, selected from compounds expressed in the literature as having significant biliary or renal elimination, but which were not available in the BDDCS classified compounds, or did not meet our initial criteria ($f_e \leq 35$ or $f_e \geq 65$), which was developed to instill certainty in our classifications.

Receiver operating characteristic (ROC) plots were created in the ROCR package(146) in R. The true positive rate was plotted against the false positive rate and an area under the ROC curve (AUC) obtained. Thresholds of each model depicting optimal separation between classes were defined at the minimum distance to the ROC curve from (0, 1) where sensitivity and specificity were each greater than 0.8. Drugs were assigned class predictions by considering the value of the feature or model evaluator of a drug in relation to the threshold, and sensitivity $\frac{TP}{TP+FN}$, specificity $\frac{TN}{TN+FP}$, positive predictive value (PPV) $\frac{TP}{TP+FP}$, negative predictive value $\frac{TN}{TN+FN}$, and accuracy $\frac{TP+TN}{TP+TN+FP+FN}$ were calculated, where TP represents true positives, FP represents false positives, TN represents true negatives, and FN represents false negatives.

Additional Considerations

Models trained initially on orally administered drugs were tested with non-orally administered drugs. A model encompassing all routes of administration was created. Differences in physicochemical properties between orally administered and non-orally administered drugs were detected with principal component analysis.

P-gp substrate data were collected from Broccatelli's dataset(121) and compared with renally and biliarily eliminated drugs. The search was extended to other sources for biliarily eliminated drugs(58,148-154). Drugs were considered non-substrates for efflux ratios < 1.8 and substrates if the efflux ratio was > 2.2.

RESULTS

From the dataset, 105 of 188 orally administered class 3 and 4 drugs met the primary excretion class criteria. Of these, 27 were significantly excreted in the bile and 78 were primarily excreted in the urine. Categorized by ionization state at pH 7.5, 29 drugs were anionic, 26 were cationic, 33 were neutral, and 17 were zwitterionic. It was noted during analysis that ranitidine was listed in the database with a fraction excreted unchanged in the urine of 30, but the correct value is 69, and this adjustment was made(155).

Principal component analysis including all features revealed a clear segregation between the excretion classes and there was a significant difference between biliarily and renally eliminated drugs along the first component ($p < 1x10^{-9}$) (Figure 2-1).

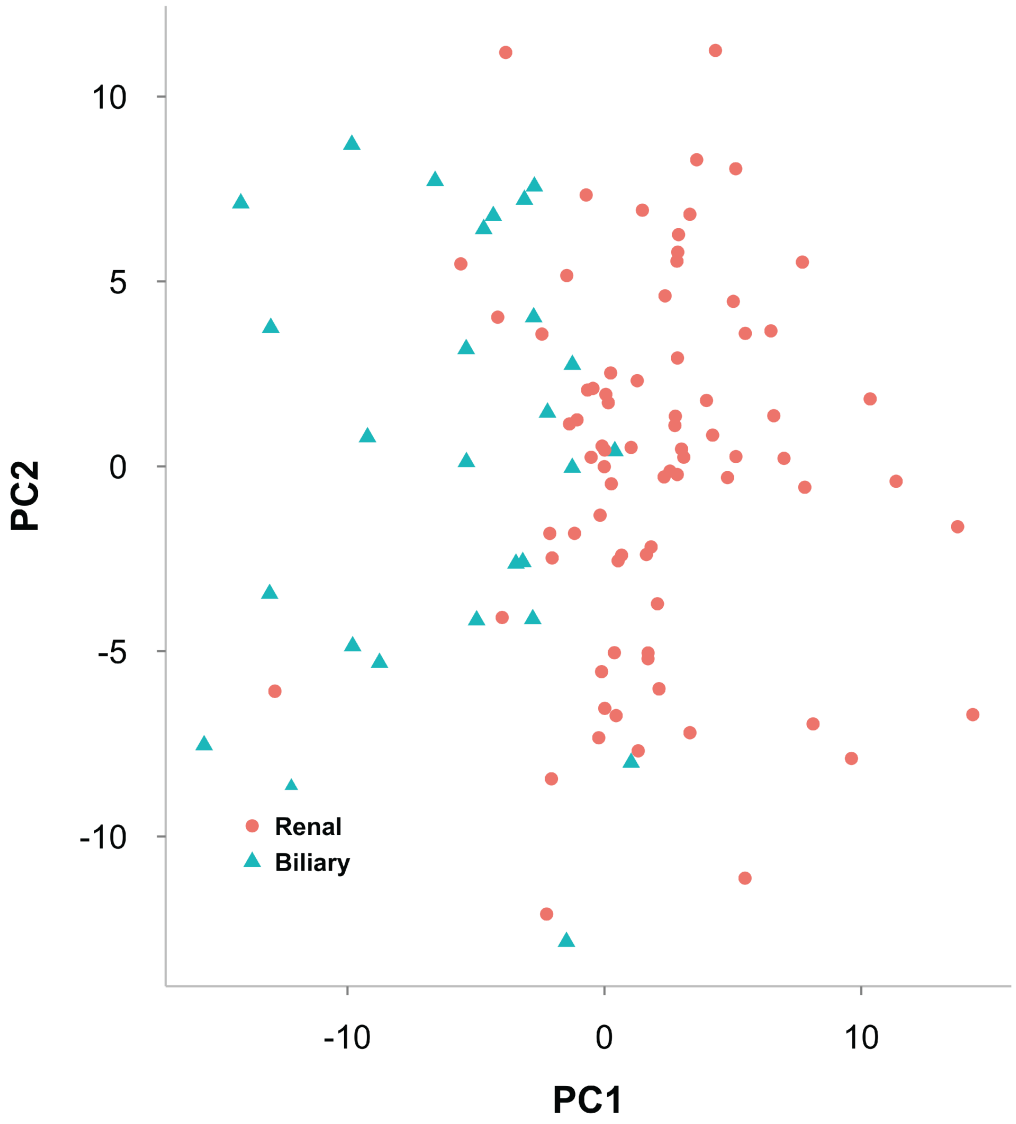

Figure 2-1. First Two Principal Components Including Information from All Features Calculated by VolSurf+. The first two components contributed 0.457 cumulative variance and the first-component scores were significantly different between elimination routes ($p < 1x10^{-9}$).

Feature Selection

The following features from VolSurf+ were selected for evaluation: molecular weight (MW); metabolic stability (MetStab), a calculated prediction of the percent of parent drug

remaining after metabolism in CYP3A4 supersomes; intrinsic solubility (SOLY); polarizability (POL), which describes the extent to which a molecule can form an induced dipole in an electric field(156); hydrophobic surface area (HSA); and rugosity (RUG), a ratio of molecular volume to surface area. The parameter values for MW, SOLY, POL, HSA, and RUG were the generally accepted units as follows: MW: Da, SOLY: mol/L at 25°C, POL: $Å^2s^4/kg$, HSA: $Å^2$, and RUG:Å, while MetStab ranges from 0 to 100%. All of the features except MetStab were highly correlated with each other (Pearson's R values>0.8) (Table 2-1). The following features were selected in ADMET Predictor: natural population analysis partial charge on hydrogens (NPAh), number of CYP Sites (NCYPSites), and LogD (pH=7.4).

Table 2-1. Pearson R Values of Correlations between Features

	POL ($Å^2s^4/kg$)[b]	**SOLY** (mol/L)[c]	**RUG** (Å)[d]	**HSA** ($Å^2$)[e]	**MetStab** (%)[f]
MW (Da)[a]	0.96	-0.82	0.88	0.87	-0.63
POL ($Å^2s^4/kg$)[b]		-0.86	0.91	0.94	-0.71
SOLY (mol/L)[c]			-0.81	-0.90	0.76
RUG (Å)[d]				0.84	-0.58
HSA ($Å^2$)[e]					-0.78

Abbreviations used defining VolSurf+ descriptors: [a]molecular weight, [b]polarizability, [c]solubility, [d]rugosity, [e]hydrophobic surface area, [f]metabolic stabilty

Partial Least Squares Discriminant Model

MetStab+POL was 92.5±0.1% accurate in 10×5-fold cross-validation and was more accurate than other models ($p < 0.01$). Table 2-2 highlights the performance of the model testing sets.

Table 2-2. Performance Statistics of Top Logistic Regression or Partial Least Squares Models Developed Using VolSurf+ or ADMET Predictor™ Parameters Validated by 10x5-Fold Cross-Validation

Model[a]	Sensitivity	Specificity	PPV[b]	NPV[b]	Accuracy	AUC
MetStab+POL (PLS)	0.90±0.10	0.93±0.06	0.84±0.12	0.97±0.04	0.92±0.05	0.95±0.04
MetStab+POL (LogReg)	0.85±0.14	0.87±0.09 $^{+++}$	0.73±0.15 $^{+++}$	0.95±0.05	0.87±0.08 $^{+++}$	0.94±0.05
MetStab+MW (PLS)	0.86±0.15	0.88±0.08 $^{+++}$	0.72±0.14 $^{+++}$	0.95±0.05	0.87±0.07 $^{+++}$	0.94±0.04
MetStab+MW (LogReg)	0.81±0.15 $^{+++}$	0.86±0.08 $^{+++}$	0.68±0.13 $^{+++}$	0.93±0.05 $^{+++}$	0.85±0.07 $^{+++}$	0.93±0.05
NCYPSites+PolG	0.75±0.17 $^{+++}$	0.84±0.11 $^{+++}$	0.65±0.17 $^{+++}$	0.91±0.07 $^{+++}$	0.82±0.10 $^{+++}$	0.90±0.08 $^{+++}$
NPAh+LogD	0.81±0.18 $^{+++}$	0.90±0.08	0.77±0.17	0.93±0.06 $^{+++}$	0.88±0.08 $^{+++}$	0.90±0.08 $^{+++}$
NPAh+LogD+NCYPSites	0.80±0.17 $^{+++}$	0.91±0.08	0.79±0.17	0.93±0.06 $^{+++}$	0.88±0.08 $^{++}$	0.90±0.09 $^{+++}$

Compared to MetStab+POL (PLS): $^{+}$ $p<0.05$, $^{++}$ $p<0.01$, $^{+++}$ $p<0.001$

[a]MetStab: metabolic stability, POL or PolG: polarizability, MW: molecular weight, NCYPSites: number of CYP sites on the molecule, LogD: distribution coefficient at pH=7.4, NPAh: natural population analysis partial charge on hydrogens. [b]PPV: positive predictive value, NPV: negative predictive value. The threshold represents where optimal average between sensitivity and specificity occurs such that by using only information from the individual feature, a prediction of elimination route can be made with the above certainty.

Logistic Regression Model

Logistic regression resulted in slightly lower performance, but is more appropriate for classification problems and was selected as the primary model. Sensitivity, specificity, and accuracy exceeded 0.8 for all models, except NCYPSites + POL (designated as PolG in ADMET Predictor), which was developed as a comparative model (Table 2-2). Logistic regression, when molecular polarizability and calculated metabolic stability are considered, predicts the probability of biliary elimination, given by:

$$\Pi(x) = \frac{1}{e^{-(0.217POL - 0.0745MetStab - 2.28)} + 1}$$

and the optimal threshold predicts biliary elimination when ∏(x) > 0.237. This can be transformed into the linear equation depicted as a decision boundary in Figure 2-2: 0 = 0.344 × MetStab - POL+ 5.14. When the combination of MetStab and POL gives a result < 0, the compound is predicted to be eliminated in the bile. From the external dataset collected after review, 6/6 biliarily eliminated compounds (100% sensitivity) and 19/24 renally eliminated compounds (79.2% specificity) were correctly predicted, resulting in 83% accuracy overall (Appendix Table 1).

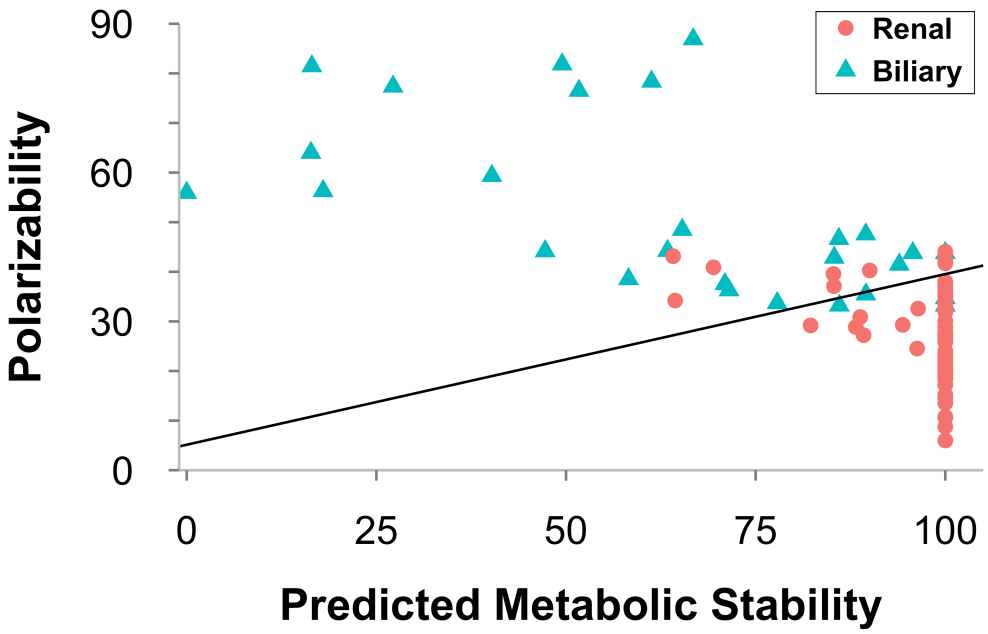

Figure 2-2. Calculated Metabolic Stability and Polarizability of 105 Orally Administered, Poorly Metabolized Drugs. The decision boundary represents the average threshold (Π(x) = 0.237) of best average sensitivity and specificity of the training group that predicts the probability of biliary excretion. It is represented by: 0 = 0.344 × MetStab - POL + 5.14.

The predictive ability of individual variables was assessed with ROC plot analysis (Table 2-3 and Figure 2-3).

Table 2-3. Receiver Operating Characteristic Curve Analysis of Individual Features Validated by 10x5-fold Cross Validation

Feature	Sensitivity	Specificity	PPV	NPV	Accuracy	Threshold
MetStab	0.832±0.154	0.855±0.075	0.678±0.140	0.939±0.055	0.849±0.071	96.8±2.3
POL	0.768±0.167	0.823±0.105	0.630±0.164	0.914±0.060	0.809±0.084	36.2±1.2
MW	0.761±0.170	0.820±0.095	0.614±0.136	0.912±0.056	0.806±0.079	379±10.
NCYPSites	0.802±0.183	0.910±0.069	0.766±0.178	0.931±0.062	0.882±0.084	22.0±0.2
SLogD	0.783±0.192	0.782±0.086	0.561±0.141	0.916±0.069	0.782±0.082	0.187±0.101
NPAh	0.775±0.163	0.863±0.076	0.678±0.152	0.918±0.060	0.840±0.078	5.16±0.08

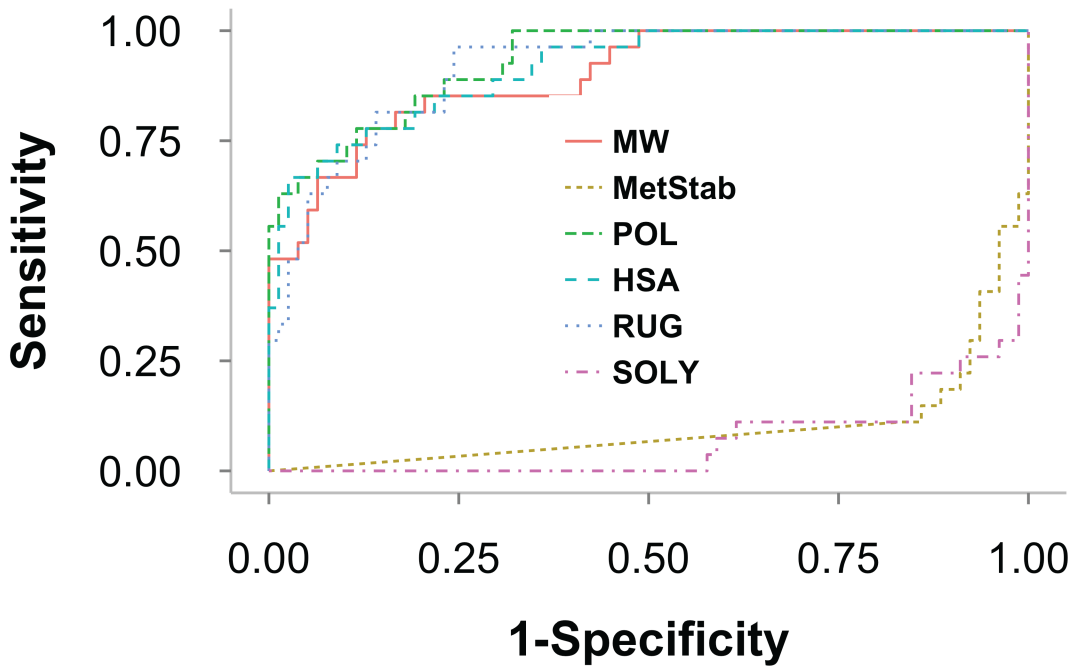

Figure 2-3. Receiver Operating Characteristic Curves of 6 Selected Descriptors. The graphs reflect sensitivity vs (1-specificity) at each value for every descriptor.

Boxplots assessed the distribution of descriptors within each excretion class (Figure 2-4). The minimum predicted metabolic stability observed in a renally eliminated drug was 71.8% (levocetirizine), while norfloxacin and leucovorin, drugs eliminated in the bile, were predicted to be 100% metabolically stable. The median weight of drugs excreted in the bile was 434 Da, with a lower limit of 288 Da. The median weight of drugs excreted in the urine was 282 Da, with an upper limit of 461 Da.

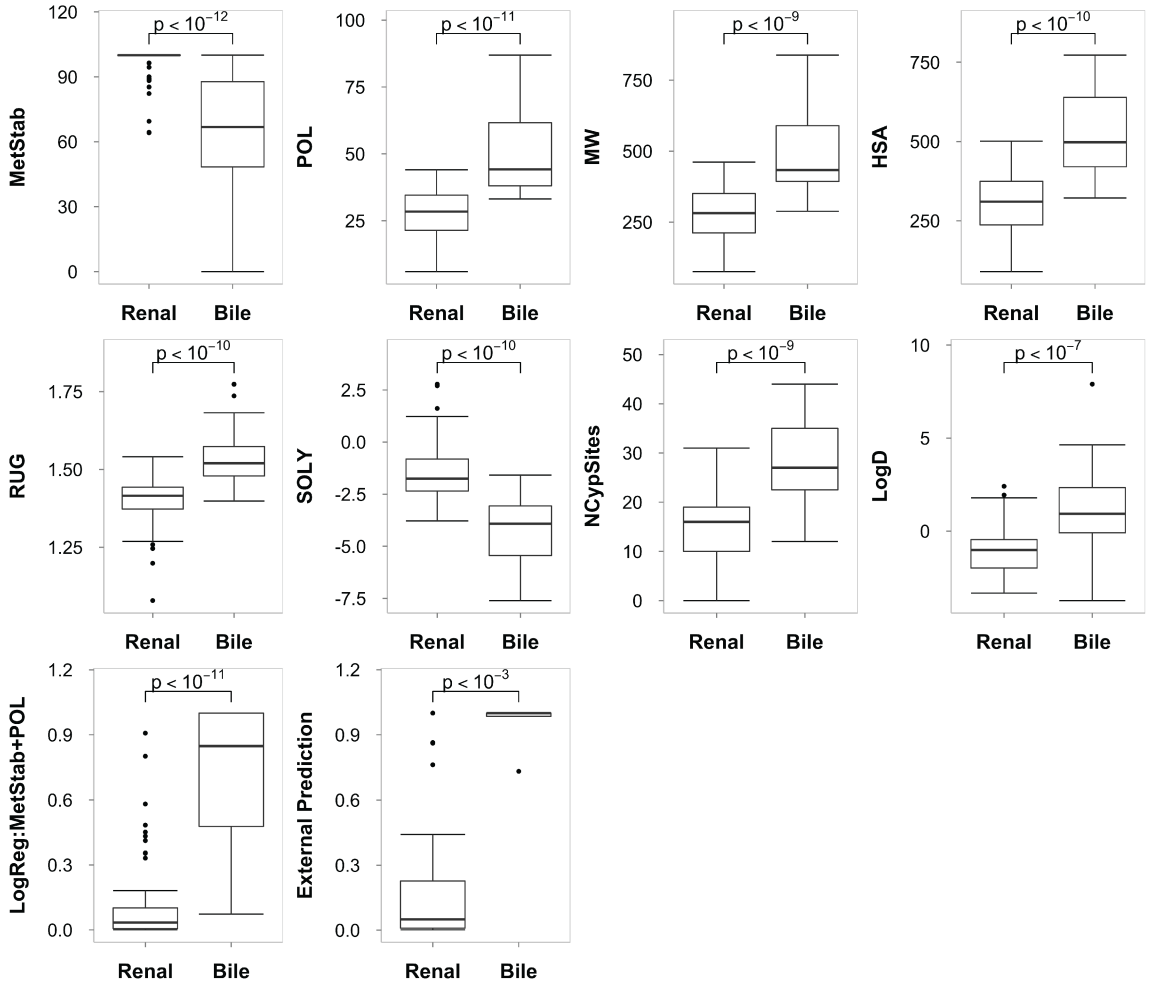

Figure 2-4. Boxplots of the Selected Variables, Model, and External Validation of the Model such that the box represents the values between the 25th and 75th percentile and the median. Tukey-defined extremes are represented by the whiskers and outliers are represented as individual datapoints.

Although historically biliary excretion was predicted for high molecular weight anionic drugs(37,138), segregating drugs into ionization classes provided somewhat better performance of MW as a predictor of excretion class for cationic, neutral and zwitterionic compounds compared to anionic compounds (Table 2-4).

Table 2-4. AUC of ROC Curve Representing Molecular Weight When Orally Administered Compounds Were Segregated into Ionization State at pH 7.5

Ionization	AUC	Accuracy
Anion	0.858	0.759
Cation	0.917	0.846
Neutral	0.957	0.970
Zwitter	0.808	0.824

Models Including Non-orally Administered Drugs

Using the same methods outlined for orally administered drugs, PLS models were developed that included non-orally administered drugs only or all administration routes, but satisfactory performance was not achieved. Significant differences were observed between the PCA first-component scores of orally and non-orally administered drugs ($p < 1x10^{-6}$). The distributions of the variables selected to represent the differences, largely indicative of hydrophilicity/lipophilicity, size/shape, or permeability, are depicted in Appendix Figure 1.

Eight of 27 orally administered, biliarily cleared and 1/78 renally cleared (methotrexate) drugs violate Lipinski's Rule of Five. Alternatively, 4/11 biliarily eliminated compounds and 17/49 renally eliminated drugs given via the intravenous route violated the Rule of Five.

Clinical Validation of the Classification Scheme and Transporter Effect

Our classification system was compared to clinical data from Yang *et al*.(37) Ten of the 11 drugs falling within our selection criteria (BDDCS Class 3 or 4, orally administered, with $f_e \leq 35$ or $f_e \geq 65$ depicting biliary or renal elimination, respectively) were in agreement with the clinical classifications. To further validate that BDDCS classification and low f_e indicate biliary elimination, we extended the search for clinical data of biliary elimination. In total, there were 18 drugs that we classified as biliarily excreted for which clinical information provided some indication of presence or lack of biliary elimination. Fifteen of these drugs (83%) indicated likely biliary excretion from clinical data (Appendix Table 2).

Efflux data were found for 16 of the 27 biliarily eliminated, orally administered compounds. Twelve of these 16 drugs were P-gp substrates. Of the remaining four, two were MRP2 substrates, and one was a BCRP substrate. Six of 15 renally eliminated drugs present in the Broccatelli dataset were P-gp substrates and nine were non-substrates. Thirty-seven compounds in our orally administered dataset had S < 400 $Å^2$ and were all excreted renally. Calculated metabolic stability is significantly lower ($p < 0.0001$) for P-gp substrates in this subset (Figure 2-5).

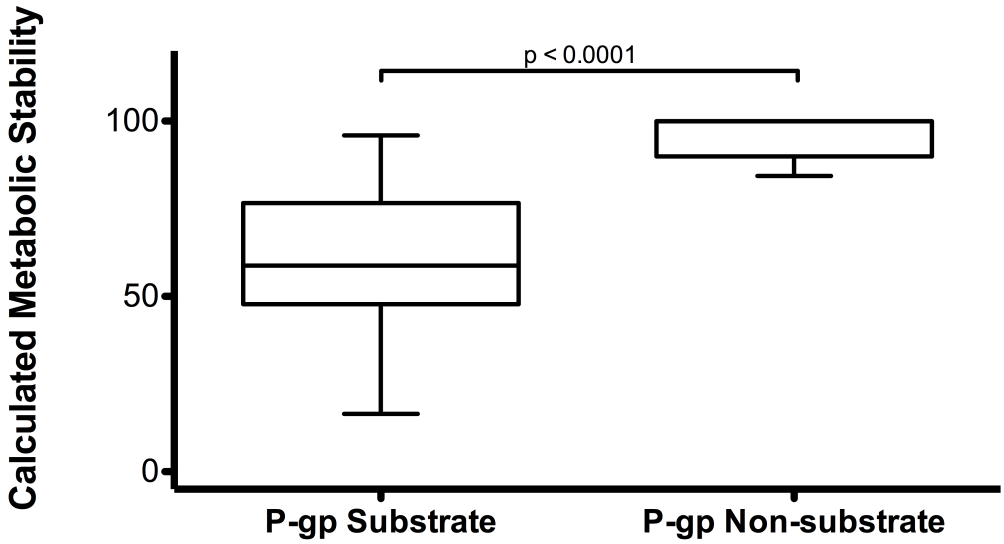

Figure 2-5. Boxplots of Calculated Metabolic Stability of BDDCS Class 3 and 4 P-gp Substrates and Nonsubstrates. The box represents the values between the 25th and 75th percentile and the median. Tukey-defined extremes are represented by the whiskers and outliers are represented as individual datapoints.

Projecting Non-orally Administered Drugs on the Model

Non-orally dosed drugs tested on the MetStab + POL logistic regression model developed for orally administered drugs yielded AUC = 0.659, sensitivity = 0.889, specificity = 0.429, and accuracy = 0.541. AUC determined for POL was 0.818 when all

administration routes were considered, but drops to 0.671 when only non-orally dosed drugs are considered. The AUC for MetStab of orally and non-orally dosed compounds is 0.806, while the AUC of non-orally dosed drugs is 0.673. Figure 2-6 depicts the metabolic stability and molecular weight of orally and non-orally administered drugs.

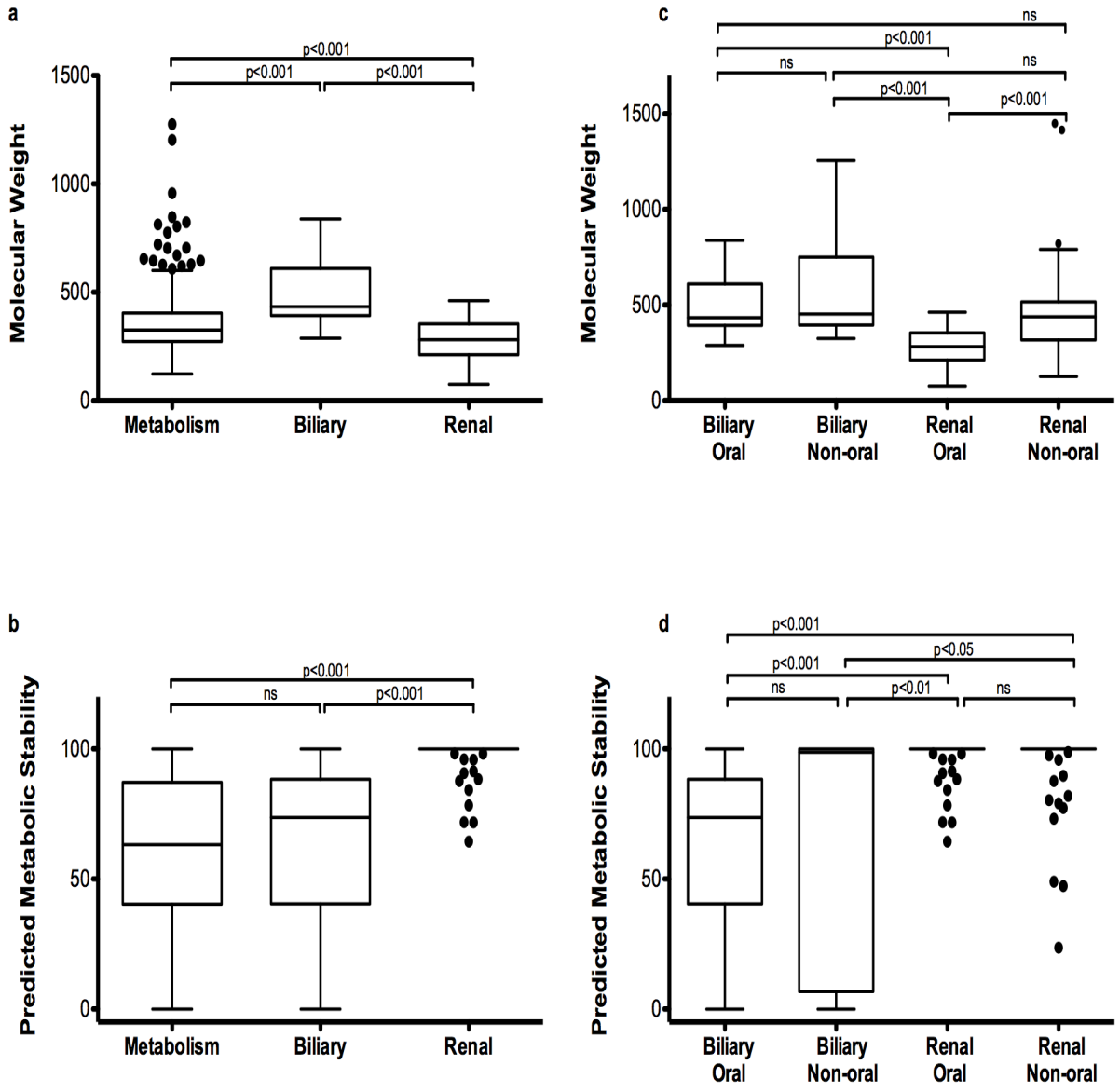

Figure 2-6. Distribution of Calculated Molecular Weight and Metabolic Stability of Orally Administered Drugs by major route of elimination (a and b) or by route of administration (c and d).

Predicting Extensively Metabolized Drugs on the Biliary and Renal Excretion Discriminating Model

When projected on the MetStab + POL logistic regression and PLS models, respectively, 70.0 ± 5.6% and 73.2 ± 0.4% of extensively metabolized parent drugs were predicted as eliminated in the bile. The AUC of MetStab as an indicator of biliary or metabolic elimination was 0.478 and the p value of the t-test was 0.710. Figure 2-6 depicts the metabolic stability and molecular weight of drugs by major routes of elimination.

Applicability to Other Software

ADMET Predictor™ has metabolic features including intrinsic clearance for various CYP isoforms, as well as "number of CYP atoms" and "number of CYP sites". The AUC for the number of CYP sites (NCYPSites) predicting primary route of elimination on the subset of poorly metabolized drugs was 0.858. The number of CYP atoms and CYP sites correlated with MetStab (Pearson's R = 0.725 and 0.712, respectively). Polarizability calculations were reproducible in ADMET Predictor (Pearson's R > 0.99). The number of CYP sites is correlated with MW (Pearson's R = 0.821). Table 2-2 depicts the models' performance.

DISCUSSION

Classification Scheme

We classified the major route of elimination of drugs using easily obtained and reliable urinary excretion data (f_e), initially filtered by removing highly permeable/extensively metabolized BDDCS class 1 and 2 drugs. Our classification scheme reliably identifies which poorly permeable/poorly metabolized drugs are eliminated in the bile, independent of biliary excretion data. In fact, of the 11 drugs for which direct comparison (orally administered, BDDCS class 3 and 4, $f_e \leq 35$ or $f_e \geq 65$) with the human dataset compiled by Yang *et al*.(37) was

possible, all classifications except methotrexate were in agreement, but this one discordant classification is expected. While we considered methotrexate's primary route of elimination as renal (f_e = 81), Yang *et al*.(37) classified this drug as having significant biliary elimination, since this group utilized > 10% biliary elimination as the criteria for significant elimination by this route. However, measurements of parent methotrexate eliminated in the bile range from 3 to 26%, so variations in classification are expected(37,157-160). Of the drugs we defined as eliminated in the bile using BDDCS class and $f_e \leq 35$, 83% agreed with available clinical data (Appendix Table 2). We expected agreement with clinical data, as BDDCS class 3 and 4 drugs attribute < 30% of their disposition to metabolism, the fraction excreted as unchanged drug in the urine was known, and other routes of elimination only impact a small number of drugs. This demonstrates the utility of BDDCS to characterize the major routes of elimination when permeability/extent of metabolism and the fraction excreted unchanged in the urine are known.

Application to New Molecular Entities

Prior to any studies in animals or humans for an NME, *in vitro* permeability data, as demonstrated by Varma *et al*.(139) and initially proposed by our laboratory(48,87,140), can identify which drugs are primarily eliminated by metabolic or non-metabolic (biliary, renal) routes. Highly permeable drugs are likely extensively metabolized *in vivo*. Of the poorly permeable, poorly metabolized drugs, consideration of metabolic stability and polarizability may be applied to predict the primary route of excretion (biliary or renal), using the relationship defined below and depicted in Figure 2-2, such that (MetStab, POL) combinations above the line are predicted as eliminated in the bile, while combinations below the line are predicted as eliminated in the urine. The probability of biliary elimination is given by

$$\Pi(x) = \frac{1}{e^{-(0.217POL - 0.0745MetStab - 2.28)} + 1}$$ ($p < 1\times10^{-11}$) and predicts biliary elimination when

$\prod(x) > 0.237$. This equation can be transposed into a linear equation depicted as a decision

boundary in Figure 2-2 using the optimal threshold of ∏(x) = 0.237. Our external dataset was collected and tested with 83% accuracy, indicating that this model can be applied to compounds that were not included in the dataset and may perform well on NMEs.

Importance of Metabolic Stability

The VolSurf+ calculated metabolic stability model was created from a PLS of 94 parameters calculated in VolSurf+ and was initially created using *in vitro* data from 1800 compounds incubated with CYP3A4 human cDNA microsomes. The value of calculated MetStab represents the fraction of a drug predicted to remain unmetabolized by CYP3A4 *in vitro*. These calculations have been validated, and accurately predict the *in vitro* metabolic stability of 85% of the tested drugs(32). With a large number of parameters contributing to the model, individual descriptors do not contribute greatly, but trends of lipophilicity and size appear to drive the MetStab model. Metabolically unstable compounds tend to have wide hydrophobic interactions, high amphiphilicity, as well as high polarizability and size, and hydrogen bond acceptor groups. Stable compounds, on the other hand, tend to have dense polar regions, large polar surfaces, and high hydrophilic-lipophilic balances, descriptors that largely indicate that stable compounds are more hydrophilic than unstable compounds(32). With no significant difference in MetStab between metabolized and biliarily eliminated compounds (Figure 2-6b), we believe that this term reflects hepatic access. Specifically, we expect that this finding could be explained by one or more of the following phenomena: 1) biliarily eliminated compounds with low MetStab may be substrates of metabolizing enzymes *in vitro*, but are stronger substrates of transporters *in vivo* and therefore are eliminated unchanged in the bile, 2) as 70% of metabolized compounds were predicted as eliminated in the bile, we expect many metabolized compounds are in fact partially biliarily eliminated *in vivo*, but a third condition, high permeability rate, allows the drug to be reabsorbed into hepatocytes, such that hepatic metabolism is the

ultimate mode of elimination, and/or 3) low metabolic stability predicts which class 3 and 4 drugs are P-gp substrates.

Predictions of extensively metabolized drugs in a naïve dataset were heavily skewed toward biliary elimination, partially due to overlapping MetStab (Figure 2-6b). Some studies have identified compounds that are metabolically unstable *in vitro*, but are primarily eliminated as unchanged drug *in vivo*(21,22). Our data indicate that this may be because biliarily eliminated drugs may be metabolically unstable CYP substrates *in vitro*, but competition with transporters on the canalicular membrane *in vivo* partially determines the molecule's fate.

Permeability of hepatically available compounds likely plays a great role in the observed disposition of a drug. Highly permeable/extensively metabolized drugs may be capable of being partially eliminated in the bile initially, but are sufficiently permeable to be reabsorbed into hepatocytes, resulting in low excretion of unchanged drug, but extensive metabolism *in vivo*. Gustafson and Benet showed that 46% of a phenolphthalein glucuronide dose administered directly to the bile duct in cannulated rats was available in the plasma, indicating the possibility of molecular reabsorption from the bile duct (161). The same phenomenon is predicted with highly permeable drugs initially filtered or secreted in the urine(87), following the observation that extensively metabolized drugs show high permeability rates.

The final hypothesis is based on the well-known substrate overlap between CYP3A4 and P-gp(66). Although the MetStab model was developed to predict CYP3A4 substrates, considering the intrinsic overlap of substrates, low MetStab may also tend to predict affinity for P-gp. Correspondingly, we saw that class 3 and class 4 P-gp substrates had low calculated MetStab (predicted *in vitro* CYP3A4 substrate) (Figure 2-5) while nonsubstrates had significantly higher MetStab ($p < 1\times10^{-4}$). To point, compounds that were incorrectly predicted by the model in part due to a MetStab value uncharacteristic of the compound's excretion route were either substrates of hepatic canalicular efflux transporters besides P-gp (norfloxacin is a substrate of BCRP) or were P-gp substrates and renally eliminated (acrivastine, levocetirizine). Renally

eliminated compounds that are substrates for P-gp, which is present in both the liver and kidney among many other tissues, might not be substrates for hepatic uptake transporters, and are therefore not biliarily accessible. As others have hypothesized, hepatic uptake, particularly by OATPs, may be a rate-limiting step in biliary excretion(38,123). Indeed, acrivastine and levocetirizine were not found to be substrates of major hepatic uptake transporters in the literature.

We believe that each of these previously noted observations play a role in the surprising finding that biliarily eliminated compounds are predicted to be metabolically unstable.

Polarizability and MW

Polarizability, which is highly correlated with MW, was the best secondary predictor, and may describe the physiological recognition of biliarily eliminated compounds better than the conventional molecular weight (Table 2-3). This simple property quantifies the ability of the molecule to distort its own electron density when interacting with other molecular entities; polarizability is therefore a measure of the non-specific weak intramolecular dispersion forces and has been shown to correlate with a number of biological properties by Hansch and Kurup(162), and has been found to contribute to biliary excretion by others(39,41). Polarizability could account for non-specific weak interactions between a drug molecule and transporter proteins(163). Highly polarizable molecules may be more apt to interact with hepatic uptake and efflux transporters on hepatic membranes.

Historical and Current Relevance of Molecular Weight and Correlated Features

Drugs eliminated in the bile tend to have a high POL, MW, RUG, and HSA, or a low SOLY compared to renally cleared compounds (Figure 2-4). Physiologically, these descriptors may predict that hydrophilic molecules are more likely to remain in blood and less likely to

partition into the basolateral membranes of hepatocytes. Small hydrophilic molecules may bypass hepatic elimination and be filtered through the glomerulus at the kidney.

As historically proposed, molecular weight may be a reliable surrogate for physiological processes that contribute to the excretion route of poorly metabolized/poorly permeable drugs (Figure 2-4 and Table 2-3). In our dataset, this occurs regardless of charge state (Table 2-4). Predicting that poorly metabolized, orally administered drugs with a MW > 380 Da are significantly eliminated in the bile, while those with a MW < 380 Da are renally eliminated will correctly predict the excretion route greater than 80% of the time. Poorly permeable, orally administered drugs with a MW < 288 Da will almost certainly be eliminated in the urine, while those with MW > 462 Da will almost certainly be eliminated in the bile. This is similar to the proposed cut-off for anions at 475 Da by Yang *et al*.(37).

We believe we are the first to emphasize that these molecular weight cutoffs only apply for orally administered, poorly metabolized drugs (i.e., BDDCS classes 3 and 4). As we and others(37) have demonstrated, low molecular weight compounds will largely be eliminated by extra-biliary routes no matter the route of administration or BDDCS class. However, there are many high molecular weight compounds that are extensively metabolized (Figure 2-6a) or non-orally administered, renally eliminated drugs (Figure 2-6c). These compounds represent false positives that overwhelm the true positives when using high MW as a basis of predicting biliary elimination (Table 2-5). Demonstrating this principle, tiotropium bromide, a renally eliminated compound, was incorrectly classified as biliarily eliminated by the model, but upon investigation, was incorrectly classified as orally administered(54) and is actually an inhaled drug. Additionally, metabolized drugs account for 73% of drugs on the market and 72% of NMEs(87), and the proportion of high molecular weight drugs is skewed toward metabolism. While the MW of extensively metabolized drugs slightly increases over the years, as defined by CAS number (Pearson's R = 0.38), the MW of biliarily excreted drugs is not changing over time (Pearson's R = 0.05).

Table 2-5. Population of Compounds in Molecular Weight Groups by Route of Administration and Elimination

	Molecular Weight (Da)			
Major Elimination Route	> 380	< 380	> 475	< 475
		Oral Administration		
Biliary	22	5	13	14
Renal	13	65	0	78
Metabolism	153	345	53	445
		Nonoral Administration		
Biliary	11	1	7	5
Renal	42	21	21	42
Metabolism	42	50	29	63

Active Efflux in Biliary Excretion

We hypothesize that active transport results from both necessity (highly protein-bound compounds cannot be passively filtered) and convenience (unbound drugs with high POL may be good *in vivo* transporter substrates). P-gp is a promiscuous transporter that is expressed on the bile canalicular membrane and in various other tissues. Due to its promiscuity and a relative deficit of known substrates for other ABC transporters, we hypothesize that many biliarily eliminated drugs must be substrates of P-gp. Available data of P-gp substrates indicate that this is true, and that active efflux is a mandatory process of biliary elimination, excepting saxagliptin. Further investigation indicated that the f_e may have been incorrectly reported for saxagliptin, as the bioavailability was not reported(164). Unsurprisingly, P-gp efflux does not overwhelm the transport of renally eliminated drugs. Poorly permeable, poorly metabolized drugs that are not substrates for P-gp or other hepatic efflux transporters are likely eliminated renally. As biliarily eliminated compounds are poorly permeable, as predicted by BDDCS, and observed by others(139), we expect that hepatic uptake must be an active process. Varma *et al*. showed the overlap in physicochemical properties between drugs that were biliarily eliminated in rats and drugs that are substrates for human OATP, including high MW(38). As biliarily cleared drugs

have high MW/POL, they may be substrates for OATPs, while many renally cleared drugs may not be. While a number of the drugs that we classified as biliarily eliminated are P-gp substrates and there are many biliarily eliminated compounds that are substrates for OATP, other transporters can also play a role, and, although they were not investigated, may correlate with properties such as high polarizability and low metabolic stability.

Administration Route

Physicochemical differences exist between orally and non-orally dosed drugs and considering the groups together can confound model predictions. Specifically, orally administered drugs are more permeable and lipophilic, while non-orally administered drugs tend to be more polar, hydrophilic, and larger (Appendix Figure 1). Specifically, high MW/high POL appears necessary for biliary elimination, but low MW/ low POL is not necessarily indicative of renal elimination, particularly when a drug is not orally administered. The glomerulus begins filtering out molecules when MW > 10,000 Da(35) and thus, molecular size of unbound small molecule drugs may be unimportant at the kidney. Instead, protein binding may be a key deciding factor of elimination route at the kidney. Protein-bound compounds cannot be filtered through the glomerulus (albumin MW = 67,000 Da) and passively eliminated by the kidney, so will require active transport into an eliminating organ. We hypothesize that, by requiring an active process, many of these highly protein-bound compounds would be eliminated in the bile. We noticed that biliarily eliminated compounds were indeed more highly bound to plasma proteins than renally cleared compounds, for both orally and non-orally administered compounds (Appendix Figure 2).

Almost 1/3 of biliarily excreted drugs that are orally administered, and therefore presumed to be reasonably well-absorbed, violate Lipinski's Rule of Five. These rules do not predict oral absorption when transporters mediate absorption, which is presumed to always be true for poorly permeable drugs, including those eliminated in the bile(87). Our model suggests

that prior to dosing an NME to animals or humans, the major unknown in predicting whether biliary or renal excretion will be the major route of excretion of the NME, will be knowledge of whether the compound can achieve its desired effects following oral dosing. If oral dosing is feasible, the accuracy of the prediction of biliary versus renal excretion should be quite good. There would be less confidence in this prediction if oral dosing is not feasible. However, as noted above in characterizing drug disposition in humans, probably the most difficult mechanism to define is the differentiation between poor absorption and biliary excretion of parent drug. This is, however, the issue addressed here. Therefore, we recommend that drugs predicted to be poorly absorbed using Lipinski's Rule of Five be evaluated for intestinal uptake.

Ionization Status

Although other groups have suggested that charged groups play a role in biliary elimination(37-39), our data indicate that charge is a relatively unimportant factor distinguishing primary modes of elimination, perhaps because transporters exist for each charged state in both the kidney and the liver and charge is not a limiting factor for active transport. For instance, OATPs can transport anions, amphipathic compounds, and some cations, while OCTs, OCTNs, and MATEs specifically transport cations and OATs specifically transport anions. The efflux transporter MDR1 can transport both cationic and amphipathic compounds, while MDR3, MRPs, and BCRP are responsible for transporting anions. Interestingly, cations exhibit the greatest range of molecular weights, including the lowest and highest MW for drugs eliminated in the bile. This may be a result of P-gp efflux into the bile, which is well known to be a promiscuous transporter.

CONCLUSIONS

- We have developed a novel classification scheme and model predicting significant biliary excretion. This model does not rely on unreliable animal models and is not limited by

scantily available human biliary excretion data. This model is supported by analyses developed from *in vivo* data.

- The model proposed here takes advantage of the BDDCS system, which allows identification and classification of highly metabolized (high permeability rate) drugs versus poorly metabolized (low permeability rate) drugs. Biliary and renal elimination of unchanged drug will not be significant for high permeability compounds. Thus, the methodology here is useful for differentiating biliary versus renal elimination for poorly metabolized/poorly permeable BDDCS class 3 and 4 drugs.
- We show that *in silico* determinations of metabolic stability may provide a simple mechanism for predicting significant biliary elimination, especially when co-employed with polarizability.
- This model, utilizing polarizability and metabolic stability, can be applied to new molecular entities to predict the major route of elimination when the extent of metabolism is known or predicted from *in vitro* permeability data, but its accuracy will be poorer for NMEs that cannot be dosed orally.
- Compounds that violate Lipinski's Rule of Five should be evaluated for intestinal uptake, as these compounds may be well-absorbed and eliminated in the bile.

CHAPTER 3. PREDICTING THE EXTENT OF METABOLISM USING IN VITRO PERMEABILITY RATE MEASUREMENTS AND IN SILICO PERMEABILITY RATE PREDICTIONS[†]

ABSTRACT

The Biopharmaceutics Drug Disposition Classification System (BDDCS) can be utilized to predict drug disposition, including interactions with other drugs and transporter or metabolizing enzyme effects based on the extent of metabolism and solubility of a drug. However, defining the extent of metabolism relies upon clinical data. Drugs exhibiting high passive intestinal permeability rates are extensively metabolized. Therefore, we aimed to determine if *in vitro* measures of permeability rate or *in silico* permeability rate predictions could predict the extent of metabolism, to determine a reference compound representing the permeability rate above which compounds would be expected to be extensively metabolized, and to predict the major route of elimination of compounds in a two-tier approach utilizing permeability rate and a previously published model predicting the major route of elimination of parent drug. Twenty-two *in vitro* permeability rate measurement data sets in Caco-2 and MDCK cell lines and PAMPA were collected from the literature, while *in silico* permeability rate predictions were calculated using ADMET Predictor™ or VolSurf+. The potential for permeability rate to differentiate between extensively and poorly metabolized compounds was analyzed with receiver operating characteristic curves. Compounds that yielded the highest sensitivity-specificity average were selected as permeability rate reference standards. The major route of elimination of poorly permeable drugs was predicted by our previously published model and the accuracies and predictive values were calculated. The areas under the receiver operating curves were > 0.90 for *in vitro* measures of permeability rate and > 0.80 for the VolSurf+ model of permeability rate, indicating they were able to predict the extent of

[†] Modified from Hosey CM, Benet LZ. Predicting the extent of metabolism using *in vitro* permeability rate measurements and *in silico* permeability rate predictions. Mol Pharm. 2015;12:1456–66.

metabolism of compounds. Labetalol and zidovudine predicted greater than 80% of extensively metabolized drugs correctly and greater than 80% of poorly metabolized drugs correctly in Caco-2 and MDCK, respectively, while theophylline predicted greater than 80% of extensively and poorly metabolized drugs correctly in PAMPA. A two-tier approach predicting elimination route predicts 72±9%, 49±10%, and 66±7% of extensively metabolized, biliarily eliminated, and renally eliminated parent drugs correctly when the permeability rate is predicted *in silico* and 74±7%, 85±2%, and 73±8% of extensively metabolized, biliarily eliminated, and renally eliminated parent drugs correctly, respectively when the permeability rate is determined *in vitro*. These data suggest that while *in silico* permeability rates can predict extensively metabolized and renally eliminated drugs reasonably well, *in vitro* permeability rate data are necessary to confidently predict biliary elimination of parent drug.

INTRODUCTION

Absorbed drugs are predominately eliminated from the body via metabolism or secretion of unchanged drug in the bile or the urine. Elimination is a multi-factorial process mediated in part by passive permeability, drug transport, and substrate specificity to transporters and metabolizing enzymes. Understanding which route predominates in the disposition and elimination of a drug can help pharmaceutical scientists anticipate potentially dangerous interactions with other drugs, endogenous molecules, and food. Additionally, processes associated with drug elimination can be utilized to aid in drug delivery. For instance, a drug that is eliminated in the bile can undergo enterohepatic recycling, exposing the drug to the liver and intestine multiple times, while a concern for extensively metabolized drugs may be susceptibility to extensive first pass metabolism.

In 1995, the development of the Biopharmaceutics Classification System (BCS) recognized that drug permeability can predict the extent of drug absorption(49). Ten years later, Wu and Benet(48) proposed the Biopharmaceutics Drug Disposition Classification System

(BDDCS), which recognized that drugs exhibiting a high passive intestinal permeability rate were also extensively metabolized, while low permeability rate drugs were primarily eliminated as unchanged drug in the bile or the urine. This may be because highly permeable drugs are passively reabsorbed from the urine or the bile, and require metabolism to a more polar compound to be successfully eliminated from the body. BDDCS classifies drugs based on their extent of metabolism and solubility. BDDCS class 1 and 2 drugs are extensively metabolized, while poorly metabolized drugs, which are primarily eliminated as parent drug in the bile or the urine, populate classes 3 and 4. The BCS is used by the FDA and the EMA to grant biowaivers to certain highly permeable, highly soluble drugs(55). Therefore, a number of assays are outlined to qualify a drug as highly permeable, including determining the *in vitro* permeability rate in monolayer-cultured epithelial cells(51). Ideally, this principle could be applied to predict the extent of metabolism prior to *in vivo* studies. Recently, Varma *et al*. demonstrated that BDDCS class can be provisionally classified by *in vitro* permeability rate, measured in MDCK-II cells in their study, and solubility(139). Cell-based *in vitro* permeability rate is typically measured in human colorectal adenocarcinoma cells (Caco-2) or Madin-Darby canine kidney (MDCK) cells, epithelial cell lines that are cultured as monolayers. Alternatively, permeability rate can be measured in the parallel artificial membrane permeability assay (PAMPA). Permeability rate is often expressed as an absorptive rate, in the apical to basolateral direction. We expect that the permeability rate measured in this direction will relevantly predict the extent of metabolism, as we hypothesize that reabsorption of high permeability-rate drugs across the apical membranes of the kidneys (i.e. from the tubule) or the liver (i.e. from the bile) result in poor excretion of unchanged drug and a high extent of metabolism. Permeability rate measurements vary significantly between laboratories due to differences in experimental conditions such as cell source, passage number, culture media, cell density, monolayer age, or transport buffer(165). As a result, permeability rate measurements should be carried out in single laboratories and compared with a reference standard to categorize if a drug is highly or poorly permeable.

Metoprolol is widely used as a reference compound to define highly permeable or highly absorbed drugs(166), but studies have suggested that it is too conservative(13,167), resulting in incorrect classification of drugs that would otherwise be considered highly permeable and potentially subject to a biowaiver in BCS, or correctly classified as extensively metabolized in BDDCS. Furthermore, normalization of permeability rate to metoprolol's permeability rate does not reduce the variability of quantitative measurements to predict absorption between laboratories(140).

In the previous chapter, we presented an *in silico* logistic regression model utilizing polarizability and predicted metabolic stability. This model successfully predicted the major route of elimination of poorly metabolized parent drugs, i.e., biliary versus renal(23). When we tested extensively metabolized drugs on this model, we noted that many extensively metabolized drugs shared similar *in silico* properties with poorly metabolized drugs that are primarily eliminated as unchanged drug in the bile, i.e., a high polarizability or molecular weight and a low predicted metabolic stability. Although high molecular weight was historically predictive of biliary elimination, we showed that greater than 80% of orally administered drugs with MW > 380 Da, the molecular weight threshold that we calculated(23), and greater than 80% of orally administered drugs with MW > 475 Da, the molecular weight threshold for anions calculated by Yang *et al.*(37), were extensively metabolized. Although high molecular weight/polarizability and low predicted metabolic stability identify both biliarily eliminated and many extensively metabolized drugs, we expected that *in vitro* permeability rate measurements or *in silico* permeability rate predictions could differentiate poorly metabolized drugs, including those eliminated in the bile, from extensively metabolized drugs.

This study aims to demonstrate the utility of *in vitro* permeability rate measurements and *in silico* permeability rate predictions in defining the extent of metabolism using 22 *in vitro* permeability rate datasets drawn from the literature and BDDCS classification as defined by Benet *et al.*(54). Additionally, we evaluate lipophilicity as a surrogate estimation of permeability

rate. We aim to determine a less conservative permeability rate reference compound than metoprolol that produces the most accurate predictions of the extent of metabolism. Finally, we predict the major route of drug elimination by combining extent of metabolism predictions based on permeability rate with a logistic regression model(23) predicting the elimination route of unchanged drugs.

METHODS

Datasets

Caco-2, MDCK, and PAMPA data were obtained from the literature(139,168-185). We required experimental values in each dataset to be determined in the same laboratory. Datasets considering only one therapeutic drug class (e.g. fluoroquinolones) were not selected for analysis. To be included in our analysis, at least 4 extensively metabolized and 4 poorly metabolized drugs were required to be in the dataset. The data were reported as P_{app} (x 10^{-6} cm/s) in the apical to basolateral direction. *In silico* permeability predictions were calculated in ADMET Predictor™ (Simulations Plus, Inc.) with default settings at pH = 7.4 or in VolSurf+(142,143) with default options at pH = 7.5 using the predefined models S+ MDCK and S+ P_{eff} from ADMET Predictor™ (available from http://www.simulations-plus.com) or CACO2 from VolSurf+ (available from http://www.moldiscover.com). Measured octanol/water partition coefficients (mLogP) were obtained from Benet *et al*.(54), calculated octanol/water partition coefficients (cLogP) were determined in ADMET Predictor and VolSurf+, and calculated cyclohexane/water partition coefficients were determined in VolSurf+. BDDCS class was assigned using the classifications assigned by Benet *et al.*(54). BDDCS classes 1 and 2 are extensively metabolized, while classes 3 and 4 are poorly metabolized. Drugs were removed from the permeability rate datasets when BDDCS class and therefore extent of metabolism was not categorized by the Benet *et al.* dataset. *In vitro* measured permeability rate, predicted *in*

silico permeability rate, and measured or calculated LogP was assessed with bootstrapped area under the receiver operating characteristic (ROC) curve (AUC) for their abilities to differentiate the extent of metabolism (extensively *versus* poorly metabolized). AUCs > 0.8 are considered representative of significant differentiability, while values approaching 0.5 represent a lack of discrimination.

Optimal Permeability Rate Reference Standard Determination and Classification Statistics

For analysis, extensively metabolized drugs were considered the positive class, while poorly metabolized drugs were considered the negative class. Drugs present in 3 or more datasets were evaluated for their effect on the sensitivity (ratio of true positives to all positives, representing how accurately extensively metabolized drugs are predicted), specificity (ratio of true negatives to all negatives, representing how accurately poorly metabolized drugs are predicted), positive predictive value (PPV, the ratio of true positives to predicted positives, representing how accurately high permeability rates describe extensively metabolized drugs), and negative predictive value (NPV, the ratio of true negatives to predicted negatives, representing how accurately low permeability rates describe poorly metabolized drugs).

Optimal permeability rate standards were selected for each cell line by choosing the drug giving the maximum average of sensitivity and specificity, with the requirement that sensitivity, specificity, negative predictive value, and positive predictive values must all be greater than 0.7.

Two-tier Approach to Predicting Major Elimination Route

Two datasets(139,170) (Varma, Skolnik) met the initial dataset inclusion criteria and included at least 4 drugs from each of the three major routes of elimination, as previously defined(23). To expand the analysis, we included the Pham-The dataset that reports an average permeability rate from many sources(186). As the logistic regression model can only usefully be

applied to orally administered drugs(23), we reduced each dataset to orally administered drugs only. We applied the previously defined logistic regression model(23) using predicted metabolic stability and polarizability to poorly permeable compounds and calculated the accuracy and predictive ability of a two-tier classification approach (Figure 3-1).

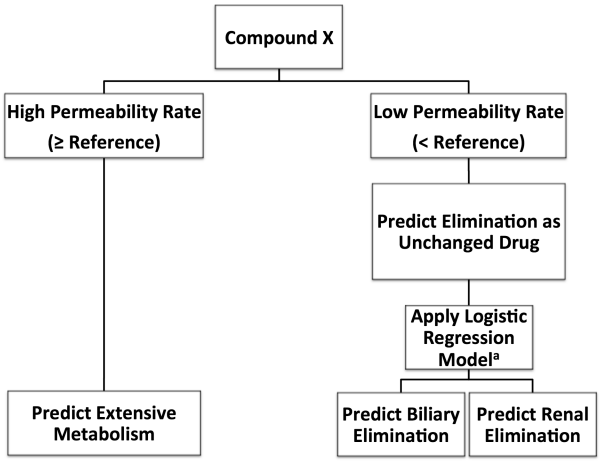

Figure 3-1. Two-tier Approach to Predicting Major Route of Elimination utilizing *in vitro* permeability rate to determine extent of metabolism and the previously defined logistic regression model to predict major route of elimination of poorly metabolized drugs. [a]Logistic regression model including calculated polarizability and metabolic stability published by Hosey *et al*.(23): $\Pi(x) = \frac{1}{e^{-(0.217POL-0.0745MetStab-2.28)}+1}$. When $\Pi(x) > 0.237$, the drug is predicted to be eliminated in the bile.

In silico permeability rate models were evaluated for their performance in the two-tier approach. Permeability rates were predicted in ADMET Predictor with the S+ MDCK model and the S+ P_{eff} model and in VolSurf+ with the CACO2 model. 100x5 fold cross validation was performed as follows: the stratified dataset was randomly assigned to 5 groups 100 times. For each of the 100 randomizations, a numeric permeability rate threshold giving the maximum average between sensitivity and specificity was calculated from 4/5 of the stratified data. The threshold was applied to predict the extent of metabolism of each compound and the previously

published logistic regression model predicting the major route of elimination of poorly permeable drugs was applied. The performance of this process was tested on the remaining 1/5 of the stratified data. This process was repeated 5 times, using each progressive 1/5 of the data as a test set. After sampling through 100 randomizations, the threshold and the performance values were averaged to represent the selected numeric threshold and performances specific to each *in silico* model.

Improving Permeability Rate Rredictions

To detect regions of permeability rate with very high predictability, we selected a "low" permeability rate standard, such that drugs with permeability rates less than this standard were very likely to be eliminated unchanged (NPV > 0.8). We also selected a "high" standard, which reflected a permeability above which drugs were very likely to be extensively metabolized. We considered drugs that were present in all of the Pham-The, Skolnik, and Varma datasets, and that gave a high predictive value (PPV or NPV > 0.8) among the datasets predicting extent of metabolism.

RESULTS

Dataset

Eleven Caco-2 datasets, 5 MDCK datasets, and 6 PAMPA datasets met the criteria for dataset inclusion, with *in vitro* permeability rate measurements obtained for 214 drugs. Appendix Table 3 details the population of compounds by cell line and extent of metabolism. When biliarily eliminated drugs were listed as part of the dataset, the table details the population of compounds via the major routes of elimination.

In Vitro Permeability Models

The area under the ROC curve is a metric that is independent of threshold selection (in this case, the permeability rate of the selected reference compound), but portrays the ability of a feature (e.g. permeability rate) to discriminate between two classes (e.g. poorly or extensively metabolized drugs). Table 3-1 reports the ROC AUCs of *in vitro* permeability rate measures and *in silico* permeability rate predictions as discriminators of the extent of metabolism when comparing extensively metabolized drugs to: all poorly metabolized drugs, drugs primarily eliminated as unchanged drug in the urine, and drugs primarily eliminated as unchanged drug in the bile. The last column exhibits the AUC when comparing the permeability rates of drugs primarily eliminated as unchanged drug in the bile to those eliminated as unchanged drug in the urine.

Table 3-1. Area Under the Receiver Operating Characteristic Curve for Bootstrapped Sampling of Measured or Predicted Permeability Rate as a Predictor of Extensively Metabolized and Poorly Metabolized Drugs Eliminated Primarily as Unchanged Drug in Either the Bile or Urine

	Extensive Metabolism vs			
	Elimination as unchanged drug (N)	**Renal elimination of unchanged drug (N)**	**Biliary elimination of unchanged drug (N)**	**Biliary vs Renal**
***In Vitro* Model**				
Caco2	0.93±0.07 (11)	0.90±0.11(11)	0.82 (1)	0.53 (1)
MDCK	0.91 ± 0.03 (5)	0.95 ± 0.02 (5)	0.89 (1)	0.53 (1)
PAMPA	0.93 ± 0.05 (6)	0.95 ± 0.04 (6)	---	0.71 (1)
***In Silico* Model**				
AP MDCK	0.78±0.03	0.82±0.04	0.81±0.05	0.56±0.09
AP Peff	0.74±0.03	0.76±0.04	0.69±0.07	0.58±0.09
VS+ CACO2	0.82±0.03	0.81±0.07	0.87±0.03	0.61±0.10

(N) represents the number of datasets
AP: ADMET Predictor
VS+: VolSurf+

Permeability Standard Selection and Validation

The drugs that met the criteria for standard reference drug selection, listed in order of decreasing average between sensitivity and specificity were: labetalol, dexamethasone, and methylprednisolone for the Caco-2 cell line; zidovudine and labetalol for the MDCK cell line; and theophylline and metoprolol for PAMPA. The drugs selected as permeability rate reference compounds for each *in vitro* method and the mean classification statistical values are reported in Table 3-2a. Table 3-2b indicates the number of drugs used in the datasets to generate the performance measures listed in Table 3-2a. Performance of the selected standards in alternative cell lines is also shown.

Table 3-2a. Performance Measures of Reference Compounds Selected to Compare Experimental *In Vitro* Permeability Rate of Extensively and Poorly Metabolized Compounds

	Primary Standards			Secondary Standards		
	Labetalol	**Zidovudine**	**Theophylline**	**Dexamethasone**	**Methylprednisolone**	**Metoprolol**
Number of Datasets in Selection Set (Cell Lines)	3(Caco-2)	3 (MDCK)	5 (PAMPA)	7 (Caco-2)	3 (Caco-2)	6 (PAMPA)
Number of Datasets in Validation Set (Cell Lines)	4 (MDCK) 1 (PAMPA)	2(Caco-2) 2 (PAMPA)	5 (Caco-2) 2 (MDCK)	2 (MDCK) 3 (PAMPA)	2 (MDCK) 3 (PAMPA)	8(Caco-2) 3 (MDCK)
Caco-2						
Sensitivity	0.83±0.13	0.88±0.12	0.45±0.13	0.78±0.11	0.75±0.13	0.52±0.18
Specificity	0.87±0.03	0.86±0.04	1.00±0.00	0.87±0.10	0.90±0.10	0.99±0.01
Positive Predictive Value	0.92±0.02	0.92±0.02	1.00±0.00	0.92±0.07	0.91±0.10	0.99±0.03
Negative Predictive Value	0.77±0.11	0.84±0.10	0.54±0.12	0.70±0.14	0.72±0.10	0.56±0.10
MDCK						
Sensitivity	0.76±0.12	0.90±0.09	0.55 ± 0.00	0.75±0.03	0.80±0.05	0.28±0.07
Specificity	0.90±0.04	0.81±0.13	0.97 ± 0.04	0.84±0.08	0.78±0.16	1.00±0.00
Positive Predictive Value	0.90±0.04	0.90±0.04	0.97 ± 0.04	0.89±0.02	0.87±0.05	1.00±0.00
Negative Predictive Value	0.75±0.15	0.85±0.14	0.62 ± 0.15	0.67±0.05	0.70±0.05	0.57±0.11
PAMPA						
Sensitivity	0.64	0.69±0.12	0.88 ± 0.11	0.46±0.22	0.54±0.24	0.72±0.23
Specificity	0.87	0.82±0.06	0.81 ± 0.24	0.93±0.07	0.93±0.07	0.90±0.13
Positive Predictive Value	0.90	0.85±0.06	0.90 ± 0.12	0.93±0.06	0.94±0.06	0.94±0.07
Negative Predictive Value	0.57	0.65±0.15	0.81 ± 0.10	0.56±0.10	0.60±0.13	0.70±0.17

Table 3-2b. The Number of Drugs Populating the Datasets Used for Analysis Above

Cell Line	Labetalol	Zidovudine	Theophylline	Dexamethasone	Methylprednisolone	Metoprolol
Caco-2	43,22,41	43, 41	23, 24, 17, 22, 15	23, 24, 43, 83, 15, 41, 22	24, 43, 84	23, 24, 43, 83, 18,17, 22, 41
MDCK	45, 20, 101, 26	27, 45, 101	20, 101	27, 45	27, 45	45, 20, 101
PAMPA	85	58, 85	32, 32, 58, 17, 85	17, 58, 85	17, 58, 85	32, 32, 17, 58, 17, 85

High (metoprolol) and low (chlorothiazide) permeability rate standards were selected to provide more discriminating predictability in extreme permeability rate regions. The low permeability rate reference standards selected were: chlorothiazide, hydrochlorothiazide, nadolol, furosemide, atenolol, and pravastatin. Selecting chlorothiazide as the standard resulted in the highest predictive performance of drugs predicted to be eliminated in the bile (4/6, 3/7, and 8/22 in Varma, Skolnik, and Pham-The, respectively), with the highest retention (specificity) of renally cleared drugs, which are 100% predictive. Metoprolol was selected due to its historical relevance as a permeability rate reference compound, with evidence of high positive predictive values (Table 3-2a). Among all datasets including either metoprolol or chlorothiazide, 97±5% (n=20) of the compounds with permeability rate greater than that of metoprolol were metabolized, while 90±14% (n=8) of the compounds with permeability rates less than that of chlorothiazide were poorly metabolized. Table 3-2a shows the PPV and NPV of selected intermediate standards. For extensively metabolized drugs, the intermediate permeability rate standards (e.g. labetalol, zidovudine) approach the PPV of metoprolol, but for poorly metabolized drugs, the intermediate permeability rate standards do not approach the NPV of chlorothiazide. Selecting metoprolol and chlorothiazide as additional standards allowed us to consider the regions of permeability rate that are highly predictive (low and high permeability rates), as well as regions of permeability rate with a higher degree of uncertainty in the predictability (low-intermediate and high-intermediate permeability rates). Table 3-3 depicts the predictive values when a compound has a low-intermediate permeability rate (predicted to be poorly metabolized), bounded by the permeability rates of chlorothiazide and the selected reference compound, or high-intermediate permeability rate (predicted to be extensively metabolized), bounded by the permeability rates of the selected reference compound and metoprolol.

Table 3-3. Predictive Values of Intermediate Regions of Permeability Rates

Reference	NPV[a] of permeability rates (Chlorothiazide to Reference)	N[b]	PPV[c] of permeability rates (Reference to Metoprolol)	N
Labetalol	0.59±0.27	7	0.87±0.07	7
Zidovudine	0.63±0.25	6	0.77±0.29	5
Dexamethasone	0.50±0.19	6	0.85±0.08	9
Theophylline	0.38±0.25	4	0.83±0.26	11
Methylprednisolone	0.52±0.19	5	0.86±0.10	5
Salicylic Acid	0.55±0.17	4	0.94±0.06	4
Hydrocortisone	0.48±0.11	5	0.88±0.12	9

[a]NPV is the negative predictive value. [b]N represents the number of datasets including both the reference compound and chlorothiazide or metoprolol. [c]PPV is the positive predictive value.

Two-tier Predictions

Table 3-4 depicts the predictive values and accuracies for each elimination route and the accuracy of predicting the major route of elimination when utilizing a two-tier prediction. The *in vitro* two-tier prediction first uses a drug's permeability rate as compared to a standard reference compound to predict the extent of metabolism, and then applies the previously published logistic regression model(23) to predict the major route of elimination (biliary or renal) of compounds predicted to be poorly metabolized parent drugs.

Table 3-4. Accuracy and Predictive Values of Two-tier Elimination Route Predictions

A. *In Vitro* Permeability Rates

			% of Drugs Correctly Predicted			Predictive Value (%)			Accuracy
			Metabolism	*Biliary*	*Renal*	*Metabolism*	*Biliary*	*Renal*	
Group[a]	**Reference**	**Dataset**							
I	Zidovudine	Varma	82	83	80	93	26	100	82
I	Dexamethasone	Pham-The	68	86	74	90	19	78	70
I	Dexamethasone	Skolnik	73	86	64	83	32	90	73
II	Metoprolol, Chlorothiazide	Varma				94	67	100	
II	Metoprolol, Chlorothiazide	Skolnik				90	43	100	
II	Metoprolol, Chlorothiazide	Pham-The				90	36	100	
III	Labetalol	Varma	67	83	87	96	19	87	71
III	Methylprednisolone	Skolnik	68	86	64	82	29	90	69
III	Methylprednisolone	Pham-The	77	79	68	86	22	82	75
IV	Indinavir	Varma	94	67	80	91	40	100	90
IV	Indinavir	Skolnik	90	57	64	80	40	90	81
IV	Indinavir	Pham-The	87	64	41	78	28	88	79

[a]*Group I represents analyses of the drugs selected as single-cutoff permeability standards. Group II represents the predictive abilities of a high permeability standard (metoprolol) and a low permeability standard (chlorothiazide). Group III represents the performance of alternative standards. Group IV represents the performance of indinavir, which gives the highest average accuracy in the two-tier prediction for these datasets.*

B. *In Silico* Permeability Rates

		% of Drugs Correctly Predicted			Predictive Value (%)			Accuracy
		Metabolism	*Biliary*	*Renal*	*Metabolism*	*Biliary*	*Renal*	
Threshold	**Dataset**							
105.92 ± 9.48	S+ MDCK	0.73 ± 0.05	0.56 ± 0.17	0.59 ± 0.12	0.46 ± 0.01	0.15 ± 0.04	0.31 ± 0.04	0.69 ± 0.10
0.90 ± 0.06	S+ Peff	0.63 ± 0.04	0.37 ± 0.17	0.72 ± 0.11	0.46 ± 0.01	0.08 ± 0.03	0.31 ± 0.03	0.64 ± 0.05
0.05 ± 0.09	VS+ CACO2	0.80 ± 0.07	0.54 ± 0.19	0.66 ± 0.11	0.45 ± 0.01	0.17 ± 0.05	0.35 ± 0.04	0.77 ± 0.03

Figure 3-2 provides a visualization of the permeability rates of drugs by elimination route, compared with selected high (metoprolol), intermediate (zidovudine, dexamethasone), and low (chlorothiazide) permeability rate standards, and shows the predicted excretion route of parent drug.

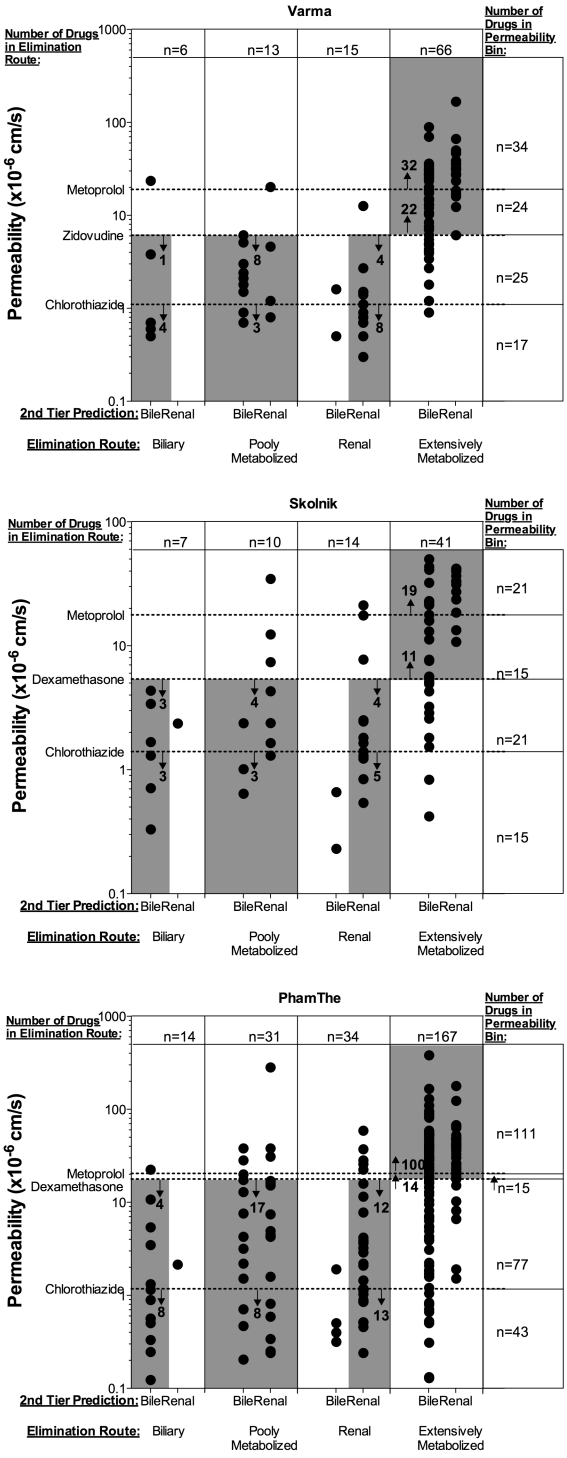

Figure 3-2. Two-tier Predictions of Major Elimination Route using *in vitro* permeability rate and *in silico* predicted elimination route of parent drug, segregated by the actual elimination route. Points within the grey boxes represent accurately predicted drugs. The number of

correctly predicted drugs is labeled within the bounds of the permeability rate reference standard compounds for each elimination route.

The two-tier approach using *in vitro* permeability rate data and the selected reference compounds as noted in group I in Table 3-4A resulted in an accuracy of 74±7%, 85±2%, and 73±8% for extensively metabolized, biliarily eliminated, and renally eliminated drugs, respectively, while choosing alternative compounds, listed in group III in Table 3-4A resulted in an accuracy of 71±6%, 83±4%, and 73±12% for extensively metabolized, biliarily eliminated, and renally eliminated drugs, respectively. Group II represents the predictability of permeability rate in very high (≥ metoprolol) or very low (≤ chlorothiazide) permeability rate regions. Group IV depicts the accuracy and predictability when indinavir, which gave the highest accuracy among the three datasets, but did not meet initial standard reference selection criteria, was selected as an intermediate reference compound.

Table 3-4B shows the performance of the two-tier prediction approach using *in silico* models to predict permeability rate/extent of metabolism utilizing the numeric threshold selected via 100x5 fold cross validation for each model, accurately predicting 72±9%, 49±10%, and 66±10% of metabolized, biliarily eliminated, and renally eliminated compounds respectively, where the VolSurf+ CACO2 model resulted in the highest predictability for biliary and renal elimination, and comparable predictability of metabolized compounds with the ADMET Predictor models.

Extreme Outliers

Table 3-5 shows the compounds classified as extensively metabolized, but having a very low (< chlorothiazide) permeability rate in at least one dataset and compounds classified as poorly metabolized, but having a very high (> metoprolol) permeability rate in at least one dataset.

Table 3-5. Compounds Exhibiting Permeability Rates Uncharacteristic of the Extent of Metabolism in One or More Datasets

Drug	Classified Extent of Metabolism	Outlier Frequency[a]	Dataset	Notes[b]	Ref
A. Outlier Compounds with Reported Permeability Rate < Chlorothiazide					
Bromocriptine	Extensive	1/2	A	Extensive first pass metabolism	(187)
Clofibrate	Extensive	1/1	A	Prodrug	(188)
Cyclosporine	Extensive	1/1	A	Extensively Metabolized	(189)
Enalapril	Extensive	1/2	A	Prodrug	(190)
Ketoconazole	Extensive	1/3	A	Extensively Metabolized	
Saquinavir	Extensive	2/2	B, C	Gut metabolism or Poor Bioavailability	(191)
Sulfasalazine	Extensive	3/7	A, B, D	Metabolized by bacteria	(192)
B. Outlier Compounds with Reported Permeability Rate > Metoprolol					
Atenolol	Poor	1/16	F	Eliminated Unchanged	
Captopril	Poor	1/1	A	40-50% unchanged, remainder is the disulfide dimer of captopril and captopril-cysteine disulfide	(193)
Cephalexin	Poor	1/4	B	Eliminated unchanged	(194)
Ciprofloxacin	Poor	1/4	E	Eliminated unchanged	(195)
Clonidine	Poor	2/3	A, B	Extensively Metabolized	(196)
Disopyramide	Poor	1/3	F	50% eliminated unchanged, 30% metabolized	(197)
Flecainide	Poor	1/1	E	Extensively Metabolized	(198)
Metoclopramide	Poor	1/1	C	Extensively Metabolized	(199)
Phenazopyridine	Poor	1/1	C	Extensively Metabolized	(200)
Pindolol	Poor	1/9	A	Extensively Metabolized	(201)
Procainamide	Poor	1/1	E	40-70% eliminated unchanged, approximately 50% acetylated with a large range; acetylation may depend upon acetylator phenotype	(202)
Trimethoprim	Poor	1/6	A	60-80% unchanged	(203)

[A]Zhu; [B]Skolnik; [C]Varma; [D]Wang; [E]Sugano; [F]Teksin

[a] Outlier frequency represents the number of times the compound exhibited an uncharacteristic permeability rate per the number of times the compound and the reference compound (chlorothiazide or metoprolol) were in the same dataset.

[b] Notes reflect characteristics of the compound, which may be valuable in understanding the uncharacteristic permeability rate.

We considered the regions of high permeability rate in each dataset that were uniquely occupied by extensively metabolized drugs and the regions of low permeability rate that were uniquely occupied by poorly metabolized drugs as a proportion of the extensively metabolized drugs or poorly metabolized drugs in the dataset, respectively. On average, 60±30% of the extensively metabolized drugs and 45±32% of poorly metabolized drugs occupied their respective unique permeability rate regions ($p < 0.01$). We additionally considered the range of permeability rates occupied by metabolized compounds or poorly metabolized compounds. Metabolized compounds covered 75.9±56.3 x 10^{-6} cm/s on average, while poorly metabolized compounds covered 24.6±24.7 x 10^{-6} cm/s on average ($p < 0.0001$), and the permeability rate range metabolized drugs covered was greater than the range of poorly metabolized compounds for every dataset.

Lipophilicity

Extensively metabolized compounds are significantly more lipophilic than poorly metabolized compounds (Figure 3-3). However, when poorly metabolized compounds are separated into major routes of elimination, there is no significant difference in mLogP or cLogP calculated in VolSurf+ between extensively metabolized compounds and compounds primarily eliminated as unchanged drug in the bile, although 21% of the metabolized compounds have a mLogP greater than the maximum mLogP (4.02) of biliarily eliminated compounds. There was a significant difference in the cLogP calculated by ADMET Predictor for extensively metabolized and biliarily eliminated drugs, but the area under the ROC curve = 0.63. The Pearson correlation coefficient of permeability rate to mLogP is 0.48±0.26.

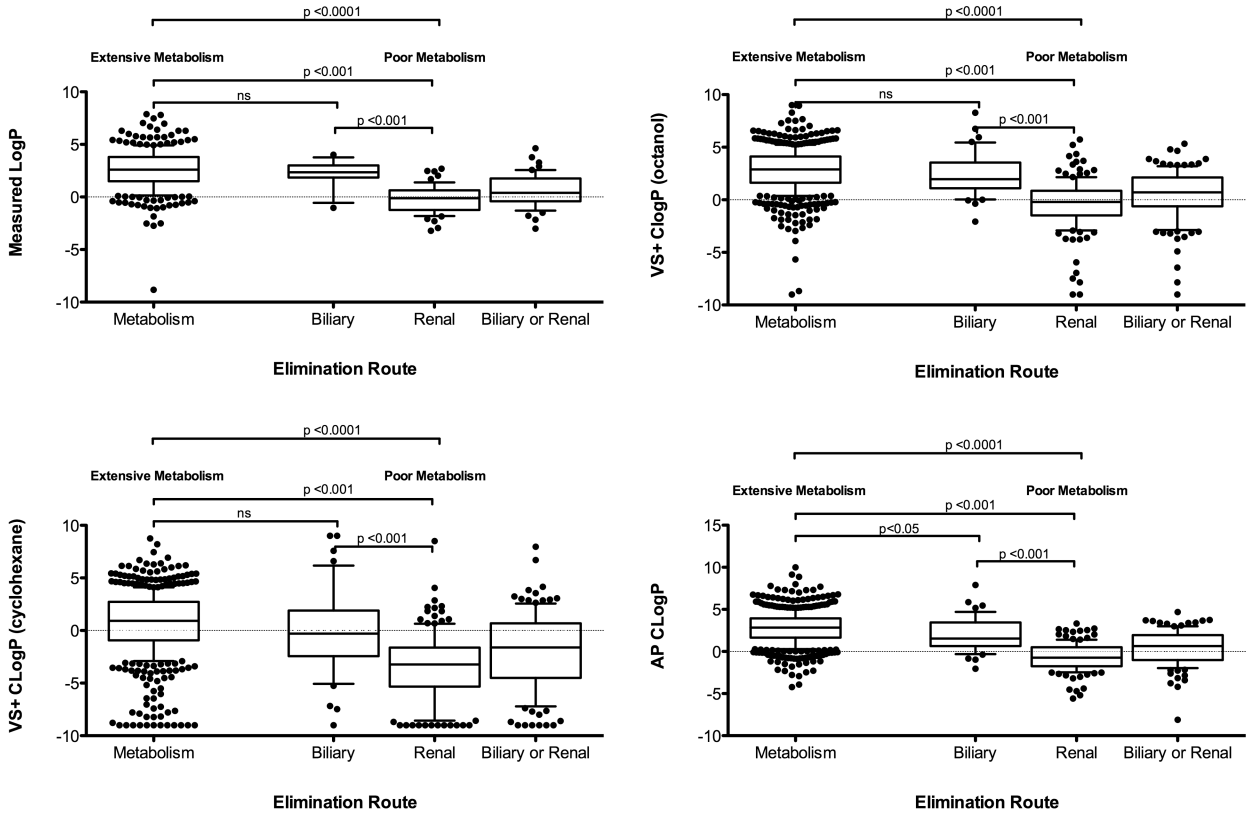

Figure 3-3. The Measured and Calculated Logarithms of Partition Coefficients by Major Route of Drug Elimination.

DISCUSSION

As our lab has proposed, *in vitro* measurements of permeability rate can predict when extensive metabolism is a major route of drug elimination. Varma *et al*.(139) demonstrated this principle while developing a provisional BDDCS classification based on *in vitro* measures. As permeability rate measurements between laboratories are notoriously variable, we wanted to extend the analysis to many datasets amongst Caco-2 and MDCK cell lines and PAMPA. We used BDDCS classes previously curated from clinical data by Benet *et al*.(54) to represent the extent of metabolism. *In vitro* permeability rate is differentiable among extensively and poorly metabolized compounds as demonstrated by the AUCs > 0.8 shown in Table 3-1, and this

differentiability persists when poorly metabolized drugs are deconstructed into their major routes of elimination of parent drug. As expected, *in vitro* permeability rate does not discriminate between the major elimination routes of poorly metabolized compounds. *In silico* permeability rate predictions can provide a prediction of the extent of metabolism quickly, but with less differentiability (Table 3-1).

We proposed that an alternative compound to metoprolol could serve as a permeability rate reference compound, such that drugs with permeability rates greater than the selected standard are predicted to be extensively metabolized in humans, while lower permeability rate drugs are predicted to be eliminated primarily unchanged in the bile or the urine. As a conservative reference, metoprolol is ineffective at identifying many metabolized compounds by their permeability rate. While compounds with permeability rates greater than metoprolol are almost certainly metabolized, using metoprolol's permeability rate as a standard in Caco-2 or MDCK studies predicts many extensively metabolized drugs as eliminated as unchanged drug, as indicated by low sensitivity values of 28% in MDCK and 52% in Caco-2 (Table 3-2a). Our goal in choosing a reference standard, then, was to increase the negative predictive value, or the proportion of compounds with a low permeability rate relative to the reference that are truly poorly metabolized, while preserving the positive predictive value as much as possible. Our analysis indicated that labetalol or zidovudine might best serve the purpose of a single permeability discriminator for Caco-2 or MDCK cells, and theophylline might best serve the purpose of a single permeability discriminator for PAMPA. Although labetalol was not selected as the optimal standard reference compound for permeability rate studies in MDCK cells, it met the criteria for a standard reference compound for both MDCK and Caco-2 cell lines. Labetalol has previously been proposed as an alternative permeability rate standard(87), and has been used as a reference standard in studies to predict BCS class(176,182), but we are unaware of any studies that have rigorously tested its performance in multiple laboratories as a standard predictor of metabolism. In Caco-2 and MDCK cells, using labetalol, zidovudine,

dexamethasone, or methylprednisolone as a reference compound results in correctly identifying a higher proportion of extensively metabolized drugs (an increase in sensitivity) than metoprolol, while increasing the negative predictive value, the confidence that a poorly permeable drug is poorly metabolized. The standards appear transferable between Caco-2 and MDCK cells, while these standards perform poorly for PAMPA. This difference is understandable as Caco-2 and MDCK are biological membranes that include uptake and efflux transporters and tight junctions for paracellular transport. However, there is little difference in predictive performance of the cell lines or PAMPA (Table 3-2a), assuming that the experimenter selects a standard substrate recommended for that system.

Additionally, there are a number of acceptable standards for the cell lines (Caco-2, MDCK). While the methodology we used provides confidence that these standards are preferable alternatives to metoprolol, discrepancies exist in the drugs used to develop each dataset. Therefore, this list is not exhaustive, and while we have provided a ranking of performance, any of the standards listed may be acceptable choices.

Metoprolol was selected naively as an alternative to theophylline in PAMPA, but remains a more conservative reference compound. While using less stringent reference compounds compared to metoprolol penalizes the positive predictive value of high permeability rate drugs and the specificity, the proportion of poorly metabolized drugs correctly identified, they still result in > 90% positive predictive value and > 80% specificity for the selection cell line (Table 3-2a).

Including additional reference compounds provides more informative predictions. When the permeability rate of drugs was broken into 4 sectors with permeability rate relative to high (metoprolol), intermediate (labetalol, zidovudine, dexamethasone, or methylprednisolone), and low (chlorothiazide) standards, an interesting pattern emerged. We noted that in many cases (14/18 combinations of reference standard with *in vitro* method) a single segregation by intermediate permeability references resulted in greater specificities than sensitivities (Table 3-2a). While only around 50% of the low-intermediate permeability rate drugs were

correctly identified as poorly metabolized (NPV), greater than 85% of the high-intermediate permeability rate drugs are extensively metabolized (PPV) (Table 3-3). In addition, a smaller proportion of poorly metabolized drugs populated the low permeability rate regions unique to poorly metabolized drugs than uniquely highly metabolized dugs populated the high permeability regions. Finally, the range of permeability rates for metabolized compounds vastly exceeds the range observed for non-metabolized drugs. These may indicate that while highly permeable drugs require metabolic elimination, as we have hypothesized(87), high permeability rate may not be mandatory for drug metabolism. Rather, a drug with a low-intermediate permeability rate is equally likely to be eliminated unchanged or by metabolism. As new molecular entities follow a similar distribution of extent of metabolism (extensive or poor metabolism)(87), we expect that *in vitro* permeability rate will be an indicator of the extent of the metabolism for future compounds.

When predicting the major route of elimination of orally administered drugs with a two-tier approach, the uncertainty in each tier is naturally multiplicative, and therefore excellent results (> 80% accuracy) are difficult to obtain. We were able to obtain accuracy > 79% in all three datasets when indinavir was used as the reference compound (Table 3-4). However, accuracy is skewed by the success of predicting the highly populated extensively metabolized drugs, while zidovudine and dexamethasone provide more balanced accuracy across the major routes of elimination. On the other hand, indinavir provides well-balanced and higher predictive values, and may be a useful reference compound. It was only present in the Varma (MDCK), Skolnik (Caco-2), and PhamThe (Caco-2) datasets and therefore did not meet the minimum number of datasets per cell line as a selection criteria for standard reference compounds. It would therefore be useful to consider indinavir as a reference compound in future studies.

While the previously defined model(23) almost always correctly assigns renally and biliarily eliminated drugs, extensively metabolized drugs invade low-permeability rate compounds. For this reason, there may be little value in assessing metabolic clearance of low-

permeability rate compounds *in vitro*. Renally cleared compounds are unlikely to be metabolized *in vitro,* while biliarily eliminated compounds may be metabolized in microsomes(23) and may be confounded with lower-permeability rate metabolized compounds. By utilizing a high, intermediate, and low standard, regions of uncertainty can be better characterized, and regions of high predictive value can be prioritized. Therefore, if the permeability rate is greater than metoprolol, it is safe to assume that the drug is extensively metabolized. If the drug has a very low permeability, i.e. less than chlorothiazide, the drug is very likely poorly metabolized, and the *in silico* model predicting biliary elimination may be applied. In the three datasets considered, all of the low permeability rate compounds predicted as renally eliminated were correctly predicted. When the compound exhibits a permeability rate between chlorothiazide and metoprolol, the intermediate "best standard" can predict the extent of metabolism, followed by the *in silico* model for a prediction, although *in vivo* experiments may still be required, particularly if the drug is predicted to be eliminated in the bile. This is, however, still an improvement in predicting which compounds are likely to be eliminated as unchanged drug in the bile.

Two-tier performance was evaluated with permeability rate in reference to the standard with the highest average sensitivity and specificity among all datasets in the cell line containing the standard. Therefore, zidovudine was selected as the MDCK cell line standard for the Varma dataset. Labetalol was the highest ranking permeability rate standard for Caco-2, but was not available in the Skolnik dataset, so the second highest ranking standard, dexamethasone, was selected. We selected dexamethasone as the standard reference compound for the Pham-The dataset as the permeability rate of labetalol was greater than that of metoprolol. This only occurred in one other dataset (Zhu), of the eight datasets, including the Pham-The dataset. It is important to note that the permeability rates given in the Pham-The dataset are an average from several datasets and therefore do not meet our initial selection criteria and may not be representative of permeability rates in a single lab. Additionally, the Zhu dataset(183) had the greatest percentage of outliers in its dataset (13% of the orally administered drugs).

While threshold independent evaluations of *in silico* permeability rate predictions indicated that the VS+ CACO2 model could significantly differentiate extensively from poorly metabolized compounds and the MDCK model approached significant differentiability, the loss of differentiability compared to *in vitro* methods may contribute to the poor sensitivity, specificity and predictive values in the two-tier approach compared to *in vitro* methods (Table 3-4). We therefore recommend that initial permeability rate studies be conducted *in vitro.*

As we have recognized previously(23), molecular weight is an inadequate predictor of biliary excretion, as biliarily eliminated drugs encompass only 12% of orally administered drugs with MW > 380 Da and 20% of orally administered drugs with MW > 475 Da and this number drops significantly when including non-orally administered drugs. However, combining *in vitro* permeability rate and the logistic regression model vastly improves the success rate, achieving up to 67% predictability for biliary excretion being the major route of elimination when comparing permeability rate to a conservative reference (i.e. chlorothiazide).

Despite the success of this two-tier approach, we noted that there were BDDCS class 1 and 2 drugs with reported very low permeability rates (< chlorothiazide), and BDDCS class 3 and 4 drugs with reported very high permeability rates (> metoprolol). We therefore reviewed these compounds for discrepencies between the listed BDDCS classes, and conflicting literature (Table 3-5). This table indicates the number of times the compound was an outlier per the number of datasets containing the compound and the reference compound (cholorothiazide in part A, metoprolol is part B). Notably, in the BDDCS classification publication, extensively metabolized compounds were not limited to compounds metabolized by metabolic processes subsequent to absorption, such as cytochrome P450 or phase II metabolism, as was initially proposed(48,55), but was extended to all extensively metabolized drugs (≥ 70% metabolism). Therefore, drugs such as sulfasalazine, which is metabolized by bacteria in the gut, may not follow the high permeability/ extensive metabolism relationship. No extensively metabolized drugs appear to have been misclassified by BDDCS. Of the high permeability rate poorly

metabolized drugs, there were five BDDCS class 3 and 4 compounds (clonidine, flecainide, metoclopramide, phenazopyridine, and pindolol) that may have been misclassified, and may be extensively metabolized. Interestingly, 4/5 of these compounds (all except metoclopramide) were listed with intermediate fractions excreted unchanged in the urine ($35 < f_e < 65$), which may indicate multiple elimination routes and variable reports regarding the major elimination route. Other notes reported in Table 3-5 indicate additional possibilities of incorrect prediction due to permeability rate. We note that many of the outlier compounds have a low frequency of incorrect prediction based on permeability rate (e.g. atenolol is only an outlier in 1/16 datasets), and subsequent evaluations of permeability rate may indicate that the compound in question is correctly identified by permeability rate. More than half of the outlier drugs (10/19) were found in the Zhu *et al*. dataset(183).

Lipophilicity

Using both measured and calculated LogPs, we have shown that while extensively metabolized drugs are more lipophilic than poorly metabolized drugs, this relationship deteriorates by considering biliarily eliminated drugs as a subgroup of the poorly metabolized drugs (Figure 3-3). While there is a significant difference in the LogP calculated by ADMET Predictor of extensively metabolized versus biliarily eliminated compounds, the area under the ROC curve = 0.63, indicating no differentiability. Indeed, for the measured LogP or the VolSurf+ calculated LogPs (where the nonpolar phase is either octanol or cyclohexane), there is no significant difference in lipophilicity between metabolized and biliarily eliminated drugs, and biliarily eliminated drugs are significantly more lipophilic than renally eliminated drugs. Additionally, some groups have found no difference between the lipophilicity of biliarily and non-biliarily eliminated compounds(37,42), while others found that biliarily eliminated compounds are more hydrophilic(38,39). Uncontested, urinary excretion is negatively correlated with lipophilicity(204). High lipophilicity is often considered a surrogate for high passive membrane

permeability, and it has been observed that highly lipophilic compounds have high affinity for metabolizing enzymes/are extensively metabolized by cytochrome P450(16,204,205) and UGTs(206). We found a modest correlation between the measured LogP and *in vitro* permeability rates, though with a large standard deviation. The active sites of CYP enzymes are localized on the cytosolic side of the endoplasmic reticulum, while the active site of UGT enzymes are localized on the luminal side of the endoplasmic reticulum(207). The binding region of P-glycoprotein (P-gp), a transporter responsible for biliary efflux, is located in the transmembrane region(208). Increased lipophilicity has been hypothesized to be required for successful permeation across membranes encasing UGT enzymes within the endoplasmic reticulum(206), or P-gp within the plasma membrane(38). However, due to the localization of CYP enzymes and other transporters, it is unlikely that increased lipophilicity in metabolism and biliary excretion is due to enzyme or transporter access across a membrane. The presumed relationship between permeability rate and lipophilicity might indicate that highly lipophilic drugs are metabolized due to reabsorption from the bile or urine. However, since biliarily eliminated compounds are highly lipophilic, it is more likely that lipophilicity, while slightly correlated with permeability, actually represents increased hydrophobic interactions that allow metabolized compounds and biliarily eliminated compounds to interact with metabolizing enzymes(204) and transporters(42), respectively. Considering the large variability in the relationship between mLogP and *in vitro* permeability rates, as well as overlapping lipophilicities of metabolized and biliarily eliminated compounds, lipophilicity is not an appropriate predictor of permeability rate and/or extent of metabolism.

CONCLUSIONS

In vitro permeability rate of compounds compared to reference compounds such as labetalol, dexamethasone, or methylprednisolone are acceptable predictors of the extent of metabolism in Caco-2 cells; zidovudine or labetalol are acceptable predictors of the extent of

metabolism in MDCK cells; and theophylline or metoprolol serve as appropriate references for PAMPA. Highly permeable drugs, especially those with permeability rates greater than metoprolol are very likely to require metabolic elimination, and while extensively metabolized drugs tend to be more highly permeable than poorly metabolized drugs, high permeability rate may not be required for a compound to be metabolized. The major route of elimination of a drug intended for oral administration may be predicted using a two-tier approach by predicting extent of metabolism using permeability rate, and parent drug excretion of poorly metabolized drugs with a logistic regression model incorporating calculated metabolic stability and polarizability. This two-tier approach correctly predicts 72±9%, 49±10%, and 66±7% of extensively metabolized, biliarily eliminated, and renally eliminated parent drugs, respectively when permeability rates are predicted *in silico*, but 74±7%, 85±2%, and 73±8% of extensively metabolized, biliarily eliminated, and renally eliminated parent drugs, respectively when permeability studies are carried out *in vitro.* Thus, *in silico* permeability rates can predict extensively metabolized and renally eliminated parent drugs reasonably well, but to have confidence in predicting biliary excretion of an NME, a simple *in vitro* permeability study appears necessary.

CHAPTER 4. PREDICTING BDDCS CLASS USING IN SILICO METHODS

ABSTRACT

BDDCS currently relies on clinical measures of metabolism and *in vitro* measures of solubility to categorize drugs. The goal of our study was to develop an *in silico* model predicting BDDCS class. While an *in vitro* predictive model of BDDCS has been established, an accurate *in silico* model would minimize laboratory requirements and could help in its implementation in early-phase development. Here, we demonstrate the ability of commercially available models to predict the extent of metabolism, using *in silico* predictions of permeability rate, and the solubility class, using *in silico* predictions of dose number. The GastroPlus™ P_{eff} model is able to differentiate extensively from poorly metabolized drugs as demonstrated by an area under the receiver operating characteristic curve (ROC AUC) = 0.80 ± 0.04, while the GastroPlus™ D_o model is able to differentiate highly from poorly soluble drugs with a ROC AUC = 0.87 ± 0.03. We additionally show that a dose of 100 mg adequately predicts BDDCS class, independent of highest dosage strength. By combining P_{eff} and D_o predictions, 69.7%, 70.5%, 50.8%, and 19.8% of drugs predicted as class 1, 2, 3, or 4, respectively, were true members of each class. 86% of the drugs predicted to be class 1 and 95% of the drugs predicted to be class 2 are extensively metabolized (class 1 or 2). While 87% of the drugs predicted to be class 3 are highly soluble, 36% of the drugs predicted to be class 3 are actually extensively metabolized, class 1 drugs. Drugs that are predicted to be extensively metabolized are unlikely to be poorly metabolized and may not need to be evaluated as substrates for absorptive transporters in the gut. Drugs that are predicted *in silico* to be poorly metabolized, highly soluble (class 3) are unlikely to be poorly soluble and may not require further solubility characterization though *in vitro* permeability should still be assessed to predict the impact of transporters and/or enzymes, as many of the drugs predicted as class 3 are extensively metabolized. Drugs predicted to have a low solubility and permeability rate (i.e. BDDCS class 4) are unreliable and should be further

investigated *in vitro* or *in vivo*. While *in silico* predictions of BDDCS class cannot supplant *in vitro* predictions, we outline valuable insights that arise from *in silico* predictions.

INTRODUCTION

BDDCS was developed based on marketed compounds and has demonstrated substantial utility for understanding the effects of transporters and metabolizing enzymes for these compounds. Ideally, this system can be applied to drugs in development in order to predict which transporter and metabolizing enzyme effects will be relevant in the clinic. These predictions may be useful in limiting unnecessary experiments, which may decrease development time and cost, benefitting both the consumer and the pharmaceutical company. However, the current classification system depends upon clinical metabolism data, which generally correlates with *in* vitro measures of permeability, as well as *in vitro* solubility measurements. Scientists must also know the highest dose strength to classify solubility, which is unknown until after clinical studies.

Wu and Benet observed that compounds that are extensively metabolized are also highly permeable in humans(48). We and others have shown that *in vitro* permeability rate predicts the extent of metabolism well, as outlined in the previous chapter(17,139). This can be a useful tool in predicting the extent of metabolism as a component of BDDCS class using *in vitro* or *in silico* methods.

Companies such as Pfizer have already made great strides in predicting BDDCS class prior to human studies. Varma *et al*.(139) have shown that BDDCS class can be predicted well using *in vitro* apparent permeability rate as measured in MDCK-LE cells at pH 6.5 for acids and pH 7.4 for bases and solubility measured at pH 1.2 in PBS for acidic compounds and in FassIF for all other compounds. They used an internally developed permeability rate cut-off of $5x10^{-6}$ cm/s, above which, compounds were predicted to be extensively metabolized, and below which, compounds were predicted as poorly metabolized. Dose strength is generally determined prior

to and during phase I trials. This makes it difficult to accurately predict the dose number of a drug. This group proposed a solubility cut-off of 200 µg/mL, which corresponds to a 50 mg dose being entirely soluble in 250 mL of water. This approach correctly predicted 84% of the compounds in their dataset, specifically 83%, 83%, 88%, and 67% of class 1, 2, 3, and 4 drugs, respectively. Additionally, over 90% of the drugs predicted as class 1 or class 2 actually belonged to those classes and over 80% of the drugs predicted to be class 3 were actually class 3, while 40% of the drugs predicted to be class 4 actually were class 4. The small number of drugs that actually are class 4 may have contributed to the poor predictions of class 4 molecules.

Pharmaceutical companies can universally apply this approach, yet slight modifications will be required. Since measured permeability rate is extremely variable between laboratories(165) and each laboratory may choose a different method of permeability rate evaluation, each laboratory will need to develop a permeability rate standard to predict the extent of metabolism. We have investigated compounds that perform well as standards depending on the method of investigation (i.e. labetalol for Caco-2, zidovudine for MDCK, or theophylline for PAMPA)(17). Additionally, each company will need to decide upon a predicted highest dose strength prior to assigning a solubility class. As mentioned above, Varma *et al*. decided to use 50 mg. Here we analyze different dose strengths as an initial predictor of dose in order to predict solubility.

To ease the time and cost of these predictions during development, an *in silico* approach is preferable. There have been at least two attempts to predict BDDCS class *in silico*. In 2007, Khandelwal *et al*.(209) developed models using machine learning methods including recursive partitioning, random forest, and support vector machines. They used molecular features to assign drugs to one of the four BDDCS classes, predicting 33.3% correct overall. In 2012, using the extended dataset published by Benet *et al*.(54), Broccatelli *et al*.(210) used a binary approach to predict the solubility and the extent of metabolism of the drugs before making a

class prediction. Solubility was predicted using Naïve Bayes, k-nearest neighbor, and support vector machine models, where the solubility class was assigned using a consensus model, which predicted the class based on how it was predicted in a majority of the models. This model was 77% accurate. The extent of metabolism was predicted from a consensus model of a Naïve Bayes and two support vector machine models. This model was 79% accurate. When combining the solubility and extent of metabolism models to predict BDDCS class, however, this approach was 55% accurate.

We selected a similar approach as Broccatelli *et al*.(210), predicting extent of metabolism and solubility separately, but we decided to use validated commercially available models that predict *in vitro* permeability rate, which serves as a surrogate for the extent of metabolism and that predict solubility and its derived parameter, dose number. We have shown that we can reliably predict the extent of metabolism using *in vitro* methods(17), but an *in vitro* provisional classification system has already been successfully developed by Varma *et al.*(139). We therefore set out to use a previously developed, commercially available *in silico* model to predict the extent of metabolism. Since we know that *in vitro* permeability rate methods can predict the extent of metabolism well, we expected that *in silico* permeability rate methods may also be able to predict the extent of metabolism. We therefore considered the GastroPlus™ effective permeability rate model (GP P_{eff}) as a predictor of the extent of metabolism (BDDCS classes 1 and 2 versus BDDCS classes 3 and 4). Additionally, we evaluated the GastroPlus™ dose number model (GP D_o) as a predictor of the solubility classification.

METHODS

Predicting Extent of Metabolism

We assigned extensively metabolized compounds a 1 as the positive class and poorly metabolized compounds a 0 as the negative class. We evaluated how well the GP P_{eff}

predictions were segregated between extensively and poorly metabolized compounds, with the expectation that poorly metabolized compounds would have low predicted *in silico* permeability rates and that extensively metabolized compounds would have high predicted *in silico* permeability rates, using a receiver operating characteristic curve (ROC). When the area under the ROC curve (AUC) was greater than 0.8, the permeability rate model was considered capable of segregating extensively from poorly metabolized compounds.

The receiver operating characteristic curve is a method of determining how well a continuous feature predicts a binary classification outcome. In this case, the continuous feature is *in silico* permeability rate, while the binary classification outcome is extent of metabolism (extensive versus poor). The continuous feature is rank-ordered and the true positive rate (sensitivity) is plotted against the false positive rate, which is equal to 1-true negative rate (specificity) at each continuous value, resulting in high AUCs (> 0.8) when there is good segregation between the continuous values allotted to the classifications, or low AUCs (0.5-0.8) when the continuous values are not well segregated between the classifications. An AUC of 0.5 indicates complete integration of the continuous values between the segregated classes where essentially every other rank-ordered value belongs to one class. We further investigated specific performance measures at a threshold that would maximize the average between sensitivity and specificity.

- Sensitivity: the percent of highly metabolized compounds that were correctly assigned an extensive metabolism classification by high GP P_{eff}
- Specificity: the percent of poorly metabolized compounds that were correctly assigned a poor metabolism classification by low GP P_{eff}
- Positive Predictive Value: the percent of high GP P_{eff} compounds (thus predicted to be extensively metabolized) that are extensively metabolized

- Negative Predictive Value: the percent of low GP P_{eff} compounds (thus predicted to be poorly metabolized) that are poorly metabolized
- Accuracy: the percent of all compounds that were correctly assigned their metabolism class
- The average between sensitivity and specificity, and the average between positive and negative predictive value were also evaluated.

Predicting Solubility

We evaluated the dose number predictions in GastroPlus™ (GP D_o) for their ability to predict the actual dose number and solubility classification. We used known doses for the predictions, and when doses were unknown, we used 100 mg, which is the recommended dose prediction by the program, and is the dose that we selected for predictions based on dose analysis. The ability of GP D_o to predict solubility was evaluated with ROC curves. Because a low dose number (≤ 1) indicates a highly soluble compound, while a high dose number (> 1) indicates a poorly soluble compound, when we evaluated predicted dose number, we classified poorly soluble compounds as the positive class to generate the ROC plot, but calculated the performance parameters by assigning highly soluble compounds the positive class. We further investigated specific performance measures at a threshold that would maximize the average between sensitivity and specificity.

- Sensitivity: the percent of highly soluble compounds that were correctly assigned a high solubility classification
- Specificity: the percent of poorly soluble compounds that were correctly assigned a poor solubility classification
- Positive Predictive Value: the percent of compounds assigned a high solubility classification (by a low dose number) that are truly highly soluble

- Negative Predictive Value: the percent of compounds assigned a poor solubility classification (by a high dose number) that are truly poorly soluble
- Accuracy: the percent of all compounds that were correctly assigned their solubility class
- The average between sensitivity and specificity, and the average between positive and negative predictive value were also evaluated.

Evaluating Measured Solubility as an Indicator of FDA Solubility

Measured solubility as reported by Benet *et al.*(54) or Hosey *et al.*(59) was compared between BDDCS classes using Kruskal-Wallace one-way analysis of variance and comparing each class against one another with Dunn's multiple comparison test.

Evaluating Dose

We evaluated how simulated doses of 50, 75, 100, and 200 mg would affect the solubility classification of orally administered drugs. We first calculated what the dose number would be given a known experimentally measured solubility using the following equation:

$$Dose\ Number = \frac{Highest\ Dose\ Strength\ (mg)}{250\ mL\ x\ Minimum\ Solubility\ (\frac{mg}{mL})}$$

We then evaluated the performance of solubility assignment at various simulated doses compared to actual solubility assignment. When dose number ≤ 1, the drug is considered highly soluble, and when dose number > 1, the drug is considered poorly soluble. Performance of the simulated dose was evaluated with the following:

- Sensitivity: the percent of highly soluble compounds that were correctly assigned a high solubility classification at the simulated dose
- Specificity: the percent of poorly soluble compounds that were correctly assigned a poor solubility classification at the simulated dose

- Positive Predictive Value: the percent of compounds assigned a high solubility classification at the simulated dose that are truly highly soluble
- Negative Predictive Value: the percent of compounds assigned a poor solubility classification at the simulated dose that are truly poorly soluble
- Accuracy: the percent of all compounds that were correctly assigned their solubility class
- ROC AUC, the average between sensitivity and specificity, and the average between positive and negative predictive value were also evaluated. The measured solubility at which the greatest average between sensitivity and specificity was obtained and associated with the dose that would determine the boundary between extensively and poorly metabolized compounds (Dose number = 1) using the dose number equation given above.

We additionally evaluated the accuracy of predicting each class and the predictive value of each class, assuming the extent of metabolism was already known.

Predicting BDDCS Class

The BDDCS Class was predicted using the P_{eff} model to predict the extent of metabolism with the Predicted Dose Number model from GastroPlus™ to predict the solubility class. The thresholds that delineate the classifications were selected using optimal thresholds based on maximum averages between sensitivity and specificity. Accuracy and predictive values of each class were calculated.

Evaluating Measured Solubility as an Indicator of FDA Solubility

Significant differences were found between the measured solubility of high FDA solubility (classes 1 and 3) and low FDA solubility (classes 2 and 4) drugs (Figure 4-1).

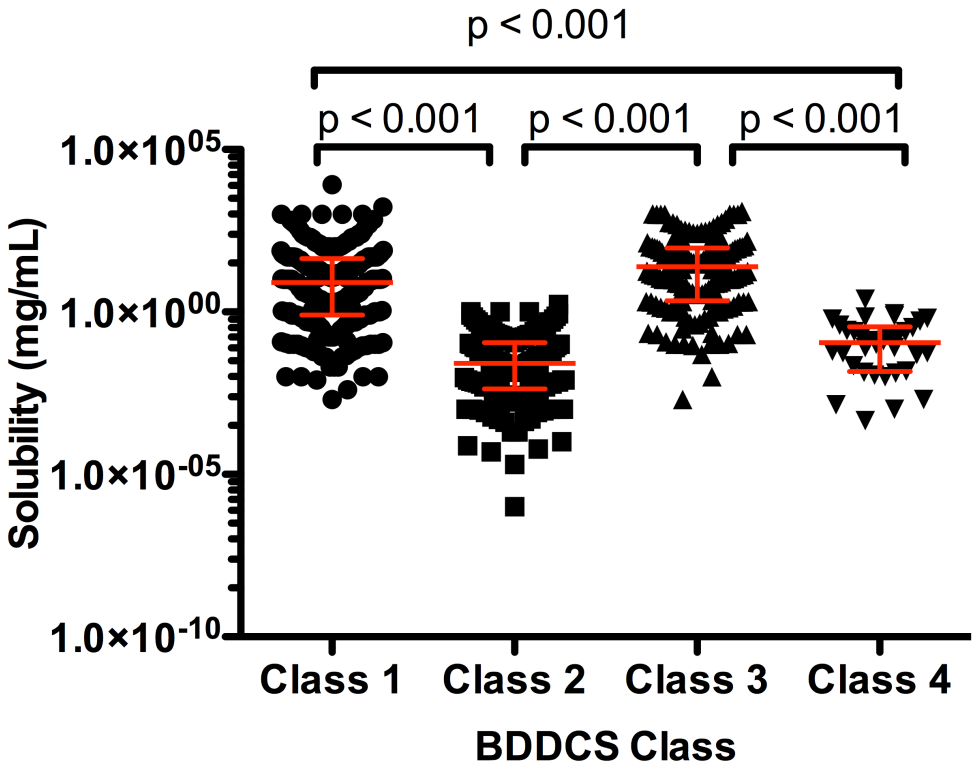

Figure 4-1. Distribution of Measured Solubility Between BDDCS Classes 1-4.

Additionally, a significant difference was observed between classes 1 and 3 ($p < 0.05$). The ROC AUC between class 1 and 3 is 0.61. The solubility boundary conditions of classes 1 and 3 versus 2 and 4 are detailed in Table 4-1. This indicates what dose would be required under certain conditions to change the FDA solubility classification of a drug.

Table 4-1. Boundary Conditions of Currently Classified Drugs

BDDCS	Boundary Solubility	Dosing Condition
Class 2 or 4	2.5 mg/mL maximum	If solubility is > 2.5 mg/mL, the drug will only be poorly soluble if requiring a dose > 625 mg
Class 1 or 3	0.002 mg/mL minimum	If solubility is < 0.002 mg/mL, the dose must be < 0.5 mg to be a high solubility drug

Evaluating Dose

Class 4 drugs had significantly higher doses than each of the other classes for orally administered drugs as seen in Figure 4-2. The dose of class 4 drugs was also significantly higher than class 1 and 2 drug for intravenously administered drugs, and had a higher mean and median dose value than class 3 drugs, although the difference was insignificant. Alternatively, class 1 drugs had the lowest doses for orally administered and intravenously administered drugs, although there was no significant difference in the doses of class 1 and 2 intravenously administered drugs.

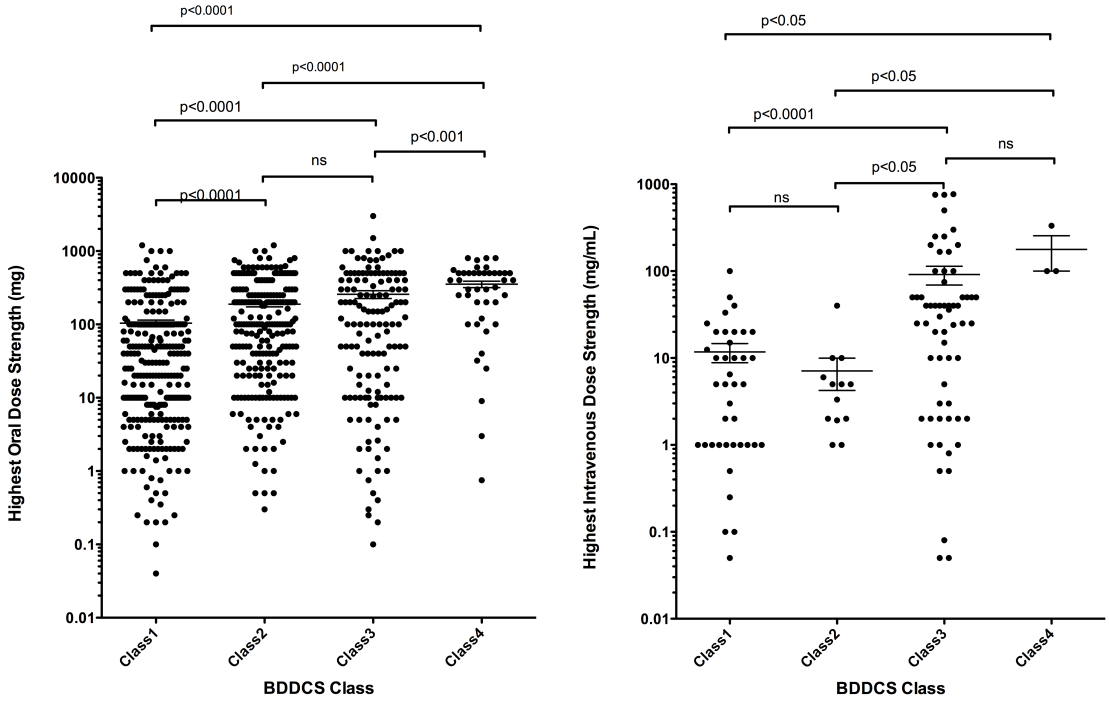

Figure 4-2. Highest Dosage Strength of Orally and Non-orally Administered Compounds by BDDCS Class.

The Effect of Dose Changes on Dose Number

Table 4-2 shows how changing a dose (from 50, 75, 100, or 200 mg) affects how well the solubility class (1 and 3 versus 2 and 4) was predicted using the measured solubility and with a theoretical dissolution volume of 250 mL.

Table 4-2. The Effect of Dose Changes on Dose Number

Performance Measure	**Dose (mg)**			
	50	75	100	200
% of Highly Soluble Compounds Correct (Sensitivity)	0.87	0.84	0.82	0.77
% of Poorly Soluble Compounds Correct (Specificity)	0.78	0.84	0.89	0.97
% of Those Predicted to Be Highly Soluble Correct (PPV)	0.88	0.90	0.93	0.98
% of Those Predicted to Be Poorly Soluble Correct (NPV)	0.77	0.75	0.74	0.70
Average between Sensitivity and Specificity	0.83	0.84	0.86	0.87
Average between PPV and NPV	0.82	0.82	0.83	0.84
Accuracy	0.84	0.84	0.85	0.84
ROC AUC	0.82	0.84	0.85	0.87

Table 4-3 shows how changing the dose will affect the accuracy of the solubility class predictions for classes 1-4 and the predictive value assuming the extent of metabolism is known. For example, the predictive value of drugs predicted to be class 1 when the dose is 50 mg is the percentage of class 1 and 2 drugs having dose number ≤ 1 that belong to class 1.

Table 4-3. Performance Measures When Various Doses are Estimated for Each BDDCS Class

	Class 1		Class 2		Class 3		Class 4	
Dose (mg)	Accuracy*	Predictive Value**	Accuracy	Predictive Value	Accuracy	Predictive Value	Accuracy	Predictive Value
50	0.84	0.87	0.82	0.78	0.92	0.89	0.64	0.72
75	0.82	0.90	0.86	0.77	0.87	0.91	0.73	0.65
100	0.80	0.93	0.91	0.76	0.86	0.93	0.79	0.65
200	0.74	0.98	0.97	0.72	0.81	0.98	0.94	0.62

* Accuracy represents the percent of the compounds in the class that were correctly assigned solubility class for each dose

** Predictive value represents the percent of predictions that are correct when the extent of metabolism is known

The ROC AUC of solubility as a predictor of solubility class when dose was not estimated was 0.93. The optimal average between sensitivity and specificity was found at 0.4 mg/mL, which corresponds to a 100 mg dose to achieve a dose number = 1.

Using In Silico Models to Predict the Extent of Metabolism and Solubility Class

Figure 4-3 shows the ROC plots and performance measures for the GP P_{eff} model as a predictor of the extent of metabolism and the GP D_o as a predictor for solubility class. Since AUC values were ≥ 0.80, each of these models significantly discriminated their predicted classes. A threshold of 1.72 x 10^4 cm/s resulted in the highest average between sensitivity and specificity for the GP P_{eff} model, while a threshold of 1.11 resulted in the highest average between sensitivity and specificity for the GP D_o model. The performance measures are listed at these thresholds on Figure 4-3.

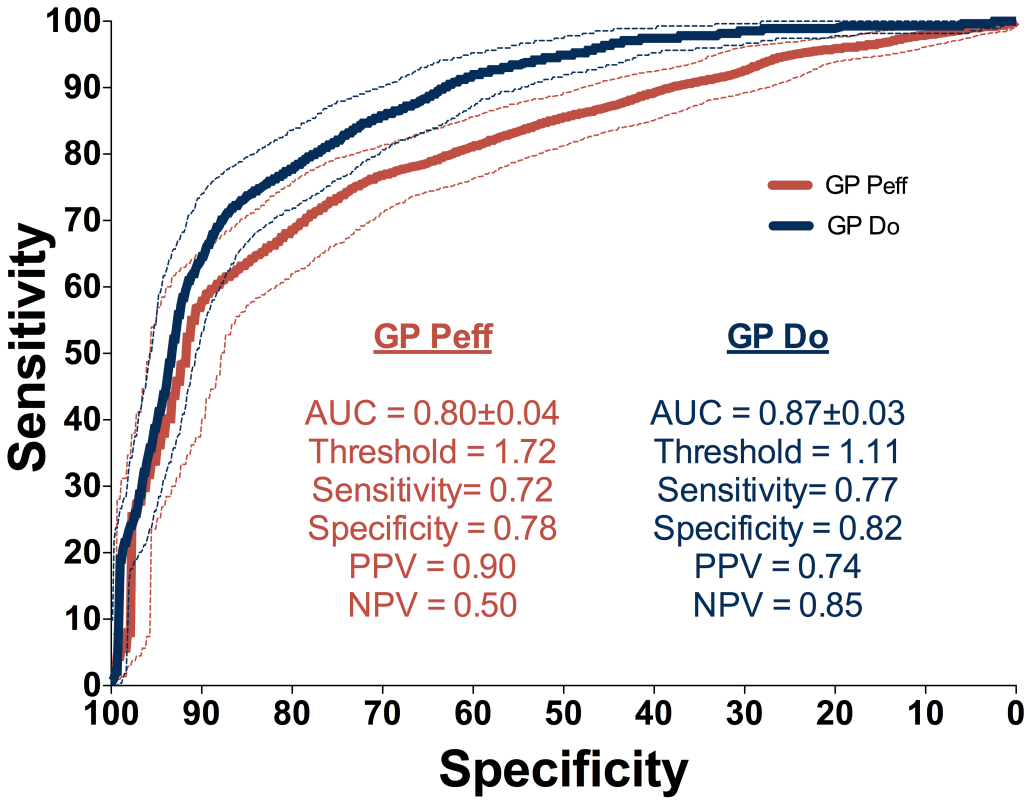

Figure 4-3. Receiver Operating Characteristic Curves of GastroPlus™ Predicted Dose Number and Effective Permeability.

Predicting BDDCS Class

Figure 4-4 depicts the predicted P_{eff} versus the predicted dose number, as calculated in GastroPlus™ for the drugs in our dataset. The results of these predictions are outlined in Table 4-4. Table 4-5 shows how drugs were predicted compared to their actual class.

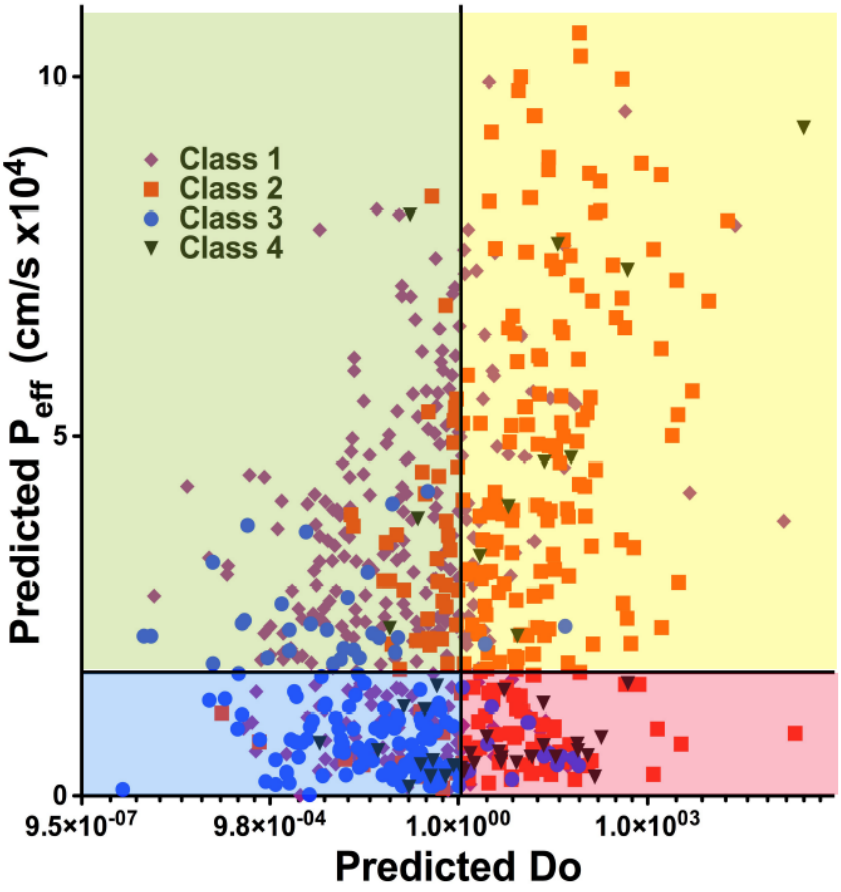

Figure 4-4. Predicted BDDCS class. Drugs with properties falling within each box are classified according to their predicted permeability rate and predicted dose number. Compounds in the green box are predicted as class 1, yellow as class 2, blue as class 3, and red as class 4. The legend shows the actual class of each drug.

Table 4-4. Performance of BDDCS *In Silico* Predictions

BDDCS Class	Predictive Value	Accuracy
1	69.7	54.1
2	70.5	57.8
3	50.8	69.3
4	19.8	45.2

Table 4-5. Confusion Matrix of BDDCS Predictions

Actual	Predicted			
	1	**2**	**3**	**4**
1	152	46	69	14
2	36	134	13	49
3	27	2	97	14
4	3	8	12	19

DISCUSSION

BDDCS is a powerful system that predicts when transporters are clinically irrelevant. We expect that almost all drugs are substrates for some transporters, and that *in vitro* experiments will often predict that a drug is a substrate for a transporter. However, we are unaware of any examples of highly soluble, extensively metabolized class 1 drugs that exhibit clinically relevant transporter effects. That is, the disposition of the drug is independent of the function of transporters. This is extremely powerful in predicting potential drug-drug interactions and understanding barriers to organ access. For instance, Broccatelli *et al.*(108) have shown that while efflux transporters can effectively decrease the central nervous system concentrations of class 2 drugs and uptake transporters and efflux transporters affect central nervous system access for class 3 and 4 drugs, class 1 drugs have no barriers to central nervous system access. Since transporters can be so important in mediating systemic and organ drug exposure, they must be evaluated during development. However, successful BDDCS class prediction, particularly of class 1 drugs, could be used to reduce the time and cost of development by eliminating unnecessary transporter studies. Alternatively, it can be used to inform which transporter studies may be necessary for class 2, 3, and 4 drugs and alert the developer to possible transporter interactions.

While BDDCS classes have been successfully predicted *in vitro*, there are currently no *in silico* predictive methods that are sensitive enough to apply during drug development. Therefore, we examined the potential to predict BDDCS class using commercially available *in*

silico methodology. We used predicted dose number from GastroPlus™ to predict solubility and predicted P_{eff} as a surrogate predictor of the extent of metabolism.

During early development, it is advantageous to predict transporter effects, yet dose is frequently unknown until clinical studies. Varma *et al*.(139) have suggested utilizing a 50 mg dose (equivalent to a solubility of 200 µg/mL at a dose number = 1) as an initial prediction to predict BDDCS class. We analyzed 4 doses to determine their effect on predicting BDDCS class when solubility is known. The performance is relatively stable across the dosages. This is likely because there is a significant difference in measured solubility independent of dose (Figure 4-1) and thus only large changes in dose will have an effect on the dose number of many drugs. Currently, transporter studies are carried out for all drugs. Because BDDCS predictions could potentially be used to eliminate transporter studies, which are unnecessary for class 1 drugs, but are important to ensure the safety and efficacy of other drugs, we wished to be conservative with the false prediction rate of class 1 drugs. At 100 mg, only 7% of the compounds that are predicted to be class 1 when the extent of metabolism is known to be extensive are false positives, while 80% of the class 1 compounds were still correctly predicted when 100 mg was used as the dose (Table 4-3). When we evaluated how measured solubility is segregated between classes 1 and 3 versus classes 2 and 4 using ROC analysis, we found that a dose of 100 mg maximized the average between sensitivity (the percent of class 1 and 3 drugs correctly predicted by measured solubility alone) and specificity (the percent of class 2 and 4 drugs correctly predicted by measured solubility alone). Thus, we selected 100 mg as an estimated dose when dose is unknown.

Predicting BDDCS Class

While GP D_o predicts solubility class well and GP P_{eff} predicts the extent of metabolism well (Figure 4-2), combining these to predict BDDCS class results in poor predictability and

accuracy for each class (Table 4-4). However, by analyzing where the errors occurred, these predictions may still be useful.

Of class 1, 2, 3, or 4 drugs, 95%, 94%, 99%, and 93% are correctly predicted by at least one property, respectively. Additionally, 90% of the drugs that are predicted as extensively metabolized class 1 or 2 drugs by a high *in silico* P_{eff} actually are extensively metabolized (Table 4-5). Since class 1 and 2 drugs do not require gut uptake transporters for absorption and are not clinically relevant substrates of them, it is unlikely that drugs predicted to be class 1 or 2 will need to be evaluated for gut uptake. Of the drugs predicted to be class 3, 87% are highly soluble (actually class 1 or class 3), but 36% of the drugs predicted to be class 3 are extensively metabolized. Since such a large proportion of these drugs are actually extensively metabolized, it may be advantageous to carry out *in vitro* permeability rate studies to predict the extent of metabolism and potentially eliminate unnecessary transporter studies, if the drug is indeed a class 1 drug. Solubility characterization, however, is likely unnecessary at this stage. Finally, since only 20% of the drugs predicted to be class 4 *in silico* are actually class 4 drugs, and only 40% predicted to be class 4 by *in vitro* measures are actually class 4, a BDDCS classification may only be assigned to these drugs after clinical studies and dose selection.

While using *in silico* methods to predict BDDCS class may not predict the exact BDDCS class well, we have analyzed the data with respect to how predictions may influence generalized transporter studies. More than 70% of drugs predicted as class 2, 3, or 4 actually belong to one of those classes. While class 2 drugs do not require gut uptake studies, but class 3 and 4 do, gut efflux studies, as well as hepatic and brain transporter studies are necessary for all class 2, 3, and 4 drugs. Therefore, by carrying out transporter studies for drugs predicted to be in classes 2, 3, or 4 by the *in silico* methodology outlined here, only 30% of the transporter studies are ultimately unnecessary and “wasteful”. This is still better than needlessly testing all class 1 drugs. Unfortunately, improving *in silico* predictability of class 1 drugs is necessary to eliminate transporter studies for even drugs predicted to be class 1 since 30% of the drugs

predicted to be class 1 are actually class 2, 3, or 4 drugs. This is problematic since, if transporters were not evaluated, 30% of the drugs may have transporter effects that need to be evaluated prior to human dosing.

In Figure 4-5, we show a chart that can be used to interpret which studies need to be carried out when *in silico* predictions of certain classes are made. Additionally, uptake transport studies should be conducted for drugs that are predicted to be class 3 after considering permeability rate.

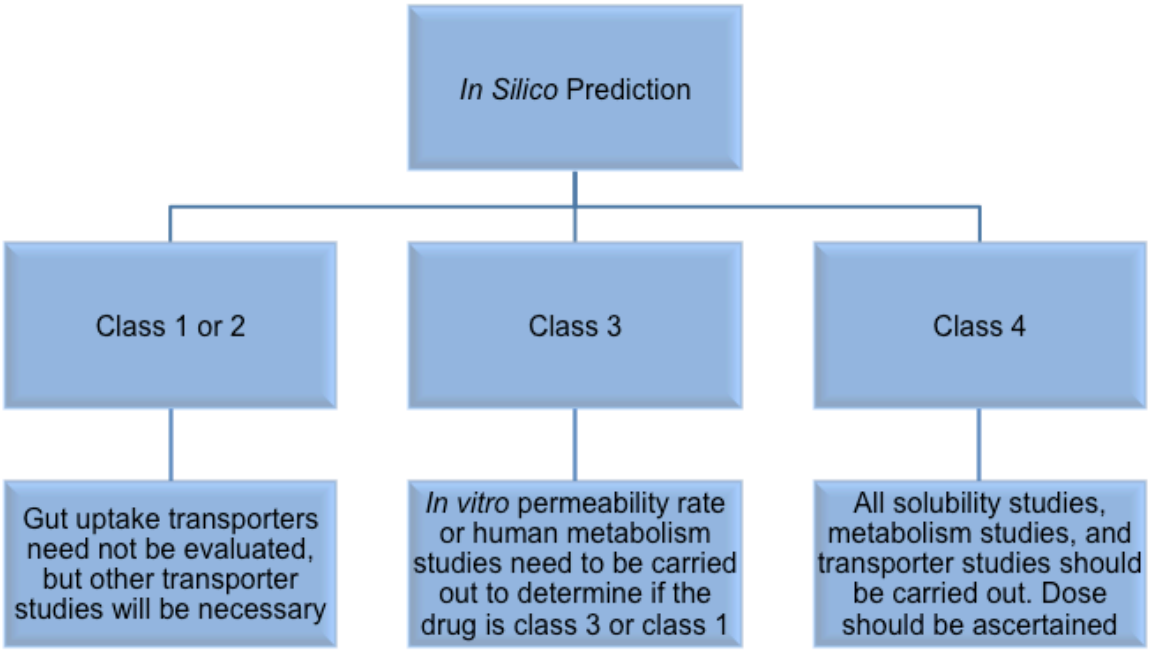

Figure 4-5. Interpreting Necessary Further Studies Given an *In Silico* BDDCS Prediction.

Alternative Methods

We have envisioned several other methods of predicting BDDCS class *in silico*. Data-mining approaches that predict each of the four classes individually (a quartenary classification

approach) such as support vector machines may be useful. However, our preliminary attempts at this classification have been less successful than using the binary approach outlined here. Alternatively, we could develop models that predict the [binary] extent of metabolism and the continuous solubility, continuous dose number, or binary solubility. However, several attempts have been made at predicting continuous solubility finding that continuous solubility is not useful in predicting BDDCS class without knowing the dose. Therefore, predicting dose number or a binary solubility classification would likely be the most effective remaining approaches.

Benet *et al*.(54) showed that *in silico* predictions of the minimum solubility of drugs over the pH range 3-7.5 are well segregated between class 2 and 3 drugs, but are unexpectedly similar when comparing class 1 and 4 drugs. Similarly, CLogP, serving as a permeability rate surrogate, is able to differentiate between classes 2 and 3, but confounds classes 1 and 4. These relatively simple *in silico* parameters are therefore able to predict when a drug is likely to be class 2 or 3, but a drug having a more moderate LogP ($0 < \text{LogP} < 2$) or predicted minimum solubility is unable to be accurately classified. Additionally, we have shown that there is no significant difference in the measured or calculated LogP of extensively metabolized class 1 and 2 compounds and class 3 and 4 compounds primarily eliminated as unchanged drug in the bile, although both are significantly higher than the LogP of renally eliminated compounds(17). Therefore, LogP is an unreliable indicator of BDDCS class. While we continue to investigate these confounding factors, currently the best prediction approach remains *in vitro*. These *in vitro* measures can reasonably predict BDDCS class prior to *in vivo* studies.

CONCLUSIONS

BDDCS has been successfully applied to understand and predict the disposition of currently marketed drugs. It could be applied with extensive utility prior to carrying out clinical studies during development, but would require non-clinical information. *In vitro* approaches have been successfully developed to predict the BDDCS class of new molecular entities, but *in silico*

approaches thus far have limited predictive utility, although some information may be garnered to direct transporter studies.

CHAPTER 5. BDDCS PREDICTIONS, SELF-CORRECTING ASPECTS OF BDDCS ASSIGNMENTS, BDDCS ASSIGNMENT CORRECTIONS, AND CLASSIFICATION FOR MORE THAN 175 ADDITIONAL DRUGS[†]

ABSTRACT

The Biopharmaceutics Drug Disposition Classification System was developed in 2005 by Wu and Benet as a tool to predict metabolizing enzyme and drug transporter effects on drug disposition. The system was modified from the Biopharmaceutics Classification System and classifies drugs according to their extent of metabolism and their water solubility. By 2010, Benet *et al*. had classified over 900 drugs. In this chapter, we incorporate more than 175 additional drugs into the system and amend the classification of 13 drugs. We discuss further applications of BDDCS, which include predicting toxicity and environmental impacts of drugs. When predictions and classes are not aligned, the system detects an error and is able to self-correct, generally indicating a problem with initial class assignment and/or measurements determining such assignments.

INTRODUCTION

Pharmacokinetics and pharmacodynamics are mediated by drug transporters or passive processes as well as potentially drug metabolizing enzymes. Drug transporters regulate the ability of some drugs to be absorbed from the small intestine, where some drugs may be initially metabolized. The activity and expression of transporters and metabolizing enzymes can therefore affect the bioavailability of the drug, either independently or in concert with each other(62). Drug transporters are expressed in a variety of tissues, including the liver and kidney— the organs primarily responsible for drug elimination— and target tissues such as the

[†] Modified from Hosey CM, Chan R, Benet LZ. BDDCS predictions, self-correcting aspects of BDDCS assignments, BDDCS assignment corrections, and classification for more than 175 additional drugs. AAPS J. 2016;18:251–60.

brain and heart. The expression and activity of drug transporters can determine the degree to which a drug can access organs, impacting on-target efficacy, off-target toxicity, or elimination. Elimination can also be influenced by the activity and expression of metabolizing enzymes, which are responsible for changing a drug into a usually more hydrophilic, water-soluble metabolite that can be more easily eliminated in the bile or urine than the parent drug. Drug transporters and metabolizing enzymes can therefore significantly impact the disposition of drugs.

Understanding the disposition of drugs is crucial during drug development. Each major dispositional process (absorption, distribution, metabolism, and elimination) impacts the safety and efficacy of a drug. In turn, other drugs, endogenous substrates, pharmacogenomics, and food can affect each of these processes. Drug interaction studies are a critical component of clinical development. Since considering the impact of each transporter or metabolizing enzyme, which can be expressed in multiple organs, is too slow and expensive, pharmaceutical scientists have prioritized when interactions with transporters and enzymes are likely to be clinically important(9,120)

Defining whether enzymes and transporters are clinically important can be further simplified by considering only 2 properties of the drug in question: its extent of metabolism and its solubility (Box 1). These features are straightforward to obtain. The extent of metabolism is routinely obtained during phase I clinical trials, while solubility can be measured in a laboratory(211). These two features are demarcated by high and low values, classifying drugs into four categories. These classes are each associated with specific predictions regarding which interactions may be a clinical concern. This predictive system is called the Biopharmaceutics Drug Disposition Classification System. The Biopharmaceutics Drug Disposition Classification System (BDDCS) was developed in 2005(48) after Wu and Benet recognized that highly permeable compounds, as outlined by the Biopharmaceutics Classification System (BCS) developed by Amidon *et al*.(49), were extensively metabolized,

while poorly permeable drugs were poorly metabolized. We expect that the relationship between a high permeability rate and a high extent of metabolism is a result of ready reabsorption of highly permeable drugs from the bile or the kidney lumen. Indeed, Gustafson and Benet(161) demonstrated that reabsorption of drugs from the bile is possible, while a recent study by Dave and Morris(212) found that 82% of drugs that are reabsorbed from the kidney tubule were BDDCS class 1 and 2 drugs. Analyzing a dataset published by Varma *et al*.(34) that included whether a drug was reabsorbed, secreted, or passively filtered by the kidneys, 52% of the class 1 and 2 compounds were reabsorbed compared to 19% of the class 3 and 4 compounds, while 69% of class 3 and 4 compounds were secreted in the tubule compared to 37% of class 1 and 2 compounds.

Box 1. BDDCS Predictions by Class

$$\text{Dose Number} = \frac{\text{HDS (mg) / 250 mL}}{\text{Minimum Solubility (mg/mL)}}$$

	BDDCS Classification	
	High Solubility (DN≤1)	Low Solubility (DN>1)
Extensive Metabolism	1	2
Poor Metabolism	3	4

Predicted Effect Resulting From:	BDDCS Class			
	Class 1	Class 2	Class 3	Class 4
Inhibition (induction) of metabolizing enzymes	Decreased (increased) metabolism; increased (decreased) parent drug exposure	Decreased (increased) metabolism; increased (decreased) parent drug exposure	Minimal effect	Minimal effect
Inhibition (induction) of intestinal apical absorptive transporters	No effect	No effect	Reduced (increased) exposure of parent drug	Reduced (increased) exposure of parent drug
Inhibition (induction) of intestinal apical efflux transporters	Minimal effect	Reduced (increased) metabolism; increased (decreased) exposure of parent drug	Increased (decreased) exposure of parent drug	Increased (decreased) exposure of parent drug
Inhibition (induction) of hepatic basolateral absorptive transporters	Minimal effect	Reduced (increased) metabolism; increased (reduced) exposure of parent drug	Reduced (increased) biliary excretion and increased (decreased) exposure	Reduced (increased) biliary excretion and increased (decreased) exposure
Inhibition (induction) of hepatic canlicular efflux transporters	No effect	Increased (reduced) metabolism; reduced (increased) exposure of parent drug	Decreased (increased) biliary excretion and increased (decreased) hepatic exposure of parent drug	Decreased (increased) biliary excretion and increased (decreased) hepatic exposure of parent drug
Inhibition (induction) of hepatic basolateral efflux transporters	Minimal effect	Increased (reduced) metabolism; reduced (increased) exposure of parent drug	Reduced (increased) exposure of parent drug	Reduced (increased) exposure of parent drug
Distribution to the central nervous system	Exposure clinically independent of transporter substrate status at therapeutic doses	Exposure if non-substrate for P-gp or BCRP	Exposure if substrate for uptake transporter and non-substrate of efflux transporter	Exposure if substrate for uptake transporter and non-substrate of efflux transporter
Inhibition (induction) of central nervous system absorptive transporters	No clinically relevant effect at therapeutic doses	Decreased (increased) CNS exposure	Decreased (increased) CNS exposure	Decreased (increased) CNS exposure
Inhibition (induction) of central nervous system efflux transporters	No clinically relevant effect at therapeutic doses	Increased (decreased) CNS exposure	Increased (decreased) CNS exposure if a substrate for uptake transporters	Increased (decreased) CNS exposure if a substrate for uptake transporters
[Predicted elimination]	Primarily metabolism	Primarily metabolism	Primarily eliminated as unchanged drug in the bile or the urine	Primarily eliminated as unchanged drug in the bile or the urine
High-fat meal*	No AUC effect	Increase AUC	Decrease AUC	No noted trend
Uremic toxins resulting from renal failure	No transporter effect, but possible increased exposure due to enzyme inhibition	May inhibit hepatic uptake transporters, resulting in increased parent drug exposure and decreased metabolism, but also may inhibit enzymes	May inhibit hepatic uptake transporters and reduce biliary excretion; increase exposure	May inhibit hepatic uptake transporters and reduce biliary excretion; increase exposure

*Predicted effects are accurate for ~70% of drugs

There is a marked distinction between extensively and poorly metabolized compounds: compounds in class 1 and 2 tend to attribute ≥ 70% of their disposition to metabolism, while classes 3 and 4 are primarily eliminated as unchanged drug and tend to attribute ≤ 30% of their elimination to metabolism, with few drugs having an intermediate extent of metabolism. Solubility is defined by FDA standards. While solubility was classified by dose number of the minimum solubility of the highest dose strength of the formulated drug at 37 ˚C over the pH range of 1 to 7.5 initially, the pH range has recently been adjusted to 1 to 6.8(51), which more accurately reflects the physiology of the gut. When the dose number ≤ 1 the drug is considered highly soluble and when the dose number > 1 the drug is considered poorly soluble(211). The classification system and predictions are detailed in Box 1.

It is important to recognize that the predictions Wu and Benet(48) proposed with regard to BDDCS were based on observations, not theory. These observations were supported by a broad knowledge of the pharmacokinetics of drugs including major elimination route and an understanding of metabolizing enzymes and transporters and their interactions. From these observations, they proposed 22 dispositional predictions for approved drugs belonging to each class(48). Wu and Benet were unable to identify any clinically relevant transporter effects in the gut or the liver for the BDDCS Class 1 drugs for the 153 drugs initially classified in the BDDCS. Briefly, class 1 drugs are expected to experience potentially clinically relevant dispositional changes when metabolizing enzymes are affected, but not when transporters are affected. As extensive metabolism necessitates extensive absorption, the BDDCS may be useful in granting biowaivers of some class 1 drugs, which has been implemented in EMA guidances(213), was supported by FDA scientists(55), and has recently been incorporated into a guidance(51). Class 2 drugs may experience clinically relevant changes from both metabolizing enzymes and efflux transporters in the gut, liver, and brain and uptake transporters in the liver and brain. Class 3 and 4 drugs are unlikely to be affected by changes in metabolism, but may be affected by

uptake or efflux transporters in the gut, liver, or brain. Clinically relevant transporter effects in the kidney have yet to be ascertained, though we have discussed the likely effects(87).

Recent work in our laboratory and others has progressed toward expanding the applications of BDDCS and applying the predictions to new molecular entities. The utilities of BDDCS are enumerated in various publications(48,50,87). BDDCS can be used in both discovery and development. Predictions include drug-drug interactions (DDIs), pharmacogenomic effects, food effects, endogenous substrate effects, distribution, and elimination route. As our understanding of drug transporters and metabolizing enzymes progresses, so do the applications of this system. BDDCS may predict toxicity, transporter-mediated drug resistance, and environmental impacts, and may inform drug delivery and dosage. Indeed, BDDCS could be a powerful predictive tool any time a drug transporter is involved in a physiological process.

CURRENT BDDCS PREDICTIONS

Predicting Drug-Drug Interactions

Of all Americans, 21.7%, and of Americans older than 65 years, more than 65%, take 3 or more prescription drugs(214). When taking 2 or more drugs, the safety or efficacy of one or more drugs may potentially be compromised by one of the other drugs (DDIs). BDDCS can qualitatively predict when the inhibition or induction of metabolizing enzymes or uptake or efflux transporters in the gut or liver may alter a drug's pharmacokinetic profile and therefore efficacy and safety. Concomitantly administered drugs and endogenous compounds may induce and/or inhibit transporters and/or enzymes, while genomic differences can alter the expression or activity of transporters or enzymes.

As BDDCS Class 1 drugs are unaffected in a clinically relevant manner by the inhibition or induction of drug transporters, one obvious and major advance of BDDCS is waiving

substrate transporter studies for an extensively metabolized, highly soluble compound. BDDCS class 1 drugs do not need to be evaluated as substrates of transporters and, if they are substrates *in vitro*, clinical studies do not need to be conducted. As BDDCS class 1 drugs comprise 40% of marketed drugs and 18% of new molecular entities(87), waiving transporter substrate studies would substantially reduce the developmental burden. However, while BDDCS class 1 drugs are unlikely to be victims of a transporter-mediated DDI, their status as inhibitors or inducers of transporters should be assessed, as they may still perpetrate transporter inhibition or induction and may clinically affect a non-class 1 drug.

BDDCS class should inform substrate selection in DDI studies. The FDA interaction guidance recommends metabolizing enzyme and transporter substrates to test if an NME is an inhibitor or an inducer of an enzyme or transporter(9). While the enzyme substrates are all extensively metabolized BDDCS class 1 and 2 substrates and are therefore appropriate for *in vitro* and *in vivo* interaction studies, some of the substrates listed for transporter interaction studies are class 1 compounds. Use of class 1 compounds as substrates *in vivo* may incorrectly suggest that the transporter in question is not inhibited or induced, which in fact may be apparent when using a class 2, 3, or 4 substrate (victim) drug.

Impact of Pharmacogenomics, Endogenous Substrates, and Food Effects

BDDCS can predict when pharmacogenetic variants or endogenous compounds may have an impact on a drug's pharmacokinetics (Box 1). For instance, a poorly permeable BDDCS class 3 or 4 drug will not be clinically impacted by genetic variants of CYP2C19, while a high permeability rate drug will need to be evaluated for CYP2C19 metabolism, since about 20% of Asians lack expression of CYP2C19 and do not metabolize its substrates(73), while pharmacogenomic differences in transporters are unlikely to impact the safety and efficacy of a class 1 drug. Concentrations of endogenous compounds can be increased or decreased by disease, and can act as inhibitors or inducers of transporters and metabolizing enzymes.

Importantly, the FDA recommends that investigational drugs be evaluated for pharmacokinetic changes in patients with impaired renal function or end-stage renal disease as transporter and/or enzyme inhibition from high concentrations of uremic toxins may alter pharmacokinetics, even if the compound is not renally eliminated(117). Additionally, diet can impact a drug's pharmacokinetics. BDDCS can correctly predict effects of high-fat meals on bioavailability for about 70% of drugs(90).

Predicting Distribution and Elimination

Central Nervous System Effects

During discovery and development, BDDCS can predict when central effects may or may not occur. P-gp has the potential to modify brain concentrations. It was hypothesized that for a drug to successfully penetrate and reside in the brain to achieve a pharmacodynamic effect, a drug should not be a P-gp substrate, while to avoid a central effect, e.g. drowsiness with antihistamines, a drug can be designed as a P-gp substrate. However, we have recently demonstrated that highly permeable/extensively metabolized, highly soluble (BDDCS class 1) compounds can have a central pharmacodynamic effect at clinically approved doses, even if the drug is a substrate for P-gp, regardless of whether the effect is desired(108). Therefore, it is preferable for a peripherally acting drug to be either poorly permeable and a non-substrate for uptake transporters in the brain, or poorly soluble and a P-gp substrate— or both— while efflux is not a concern in the efficacy of highly permeable/highly soluble drugs intended for central effects.

Predicting Elimination Routes

As BDDCS recognizes that compounds with a high intestinal permeability rate will be extensively metabolized, we can usefully predict which of the three major routes of elimination:

metabolism, renal excretion of unchanged drug, or biliary excretion of unchanged drug, will predominate in a drug's elimination. We have shown that the primary elimination route can be well predicted using *in vitro* permeability rates to predict the extent of metabolism, segregating classes 1 and 2 from 3 and 4, while two computed molecular features of a drug: metabolic stability and polarizability, can then predict if a poorly metabolized drug is eliminated in the bile or the urine as unchanged drug(17,23). These predictions may be very valuable during drug discovery and development. The major route of elimination can significantly impact if a drug can be safely and effectively administered to patients. For instance, renal elimination of unchanged drug should be avoided in patients with kidney failure. As such, drugs intended for treatment of a disease with significant comorbidity with renal failure, e.g. diabetes, should be designed with the expectation that they are eliminated by metabolism or in the bile. Alternatively, discovery scientists could adopt prediction of the major elimination route as a means of delivery to a target organ, such as the liver.

ADDITIONAL APPLICATIONS OF BDDCS

Toxicity Predictions

Additionally, BDDCS may predict when certain drug-induced toxicities, such as Torsade de Pointes (TdP)(215), Drug Induced Liver Injury (DILI)(216), and anti-epileptic drug cutaneous hypersensitivity(217), may be a clinical concern. BDDCS has linked a major role of intestinal metabolism and intestinal transporters in drug induced toxicity. For example, BDDCS helped schematize for which drugs hERG (human *Ether-à-go-go Related Gene*) voltage-gated potassium channel inhibition is likely to result in TdP(218,219) from drug−drug interactions due to CYP or P-gp inhibition(210). For BDDCS class 2 hERG inhibitors that are also substrates of both CYP and P-gp, the dual inhibition of metabolism and transport could significantly increase the plasma concentration leading to more cases of severe toxicity. For BDDCS class 1 hERG

inhibitors, the effect of P-gp should be less pronounced and result in a more moderate toxicity. BDDCS class 3 and class 4 drugs are less likely to be hERG inhibitors and therefore less likely to cause TdP. BDDCS may help characterize drugs with severe toxicity potential by better understanding their extent of metabolism and transporter interplay with other physicochemical properties and/or biomarkers that can be associated with toxicity.

Drug Resistance

Conditions provoked by rapidly evolving cells, e.g. cancer cells or bacteria, can be subject to drug resistance. This resistance is often mediated by the increased expression or activity of drug efflux transporters on the target cell. BDDCS class 1 drugs, which are not clinically affected by transporters, may therefore be protected from drug resistance.

Environmental Implications and Dose Differences

Recently, Daughton(220) suggested that BDDCS could be used in an attempt to decrease environmental exposure of active pharmaceutical ingredients. In particular, BDDCS class 1 drugs are likely to leave smaller environmental levels due to good absorption and significant biotransformation, while hypothesizing that class 4 drugs require higher doses as a result of poor permeability and poor solubility and thus generally poor absorption and are primarily excreted unchanged, resulting in higher environmental levels. We therefore analyzed dosages between the four classes and noted significant differences in doses between the classes, such that class 4 compounds were dosed significantly higher than all the other classes when a compound was given orally and higher than classes 1 and 2 when a compound was administered intravenously (Chapter 4, Figure 4-2). A possible explanation for higher required doses would be a higher clearance for class 4 compounds, but we actually saw the opposite trend—that class 1 compound had higher clearance than the other classes—and therefore this is not a plausible explanation (data not shown). We also note that class 3 compounds are

significantly overrepresented in the intravenously administered compounds, likely a result of necessity of intravenous dosing due to poor permeability rate and ease of dissolution due to high solubility.

The Self-Correcting Aspects of BDDCS Assignments

Benet *et al*. compiled a list of over 900 drugs containing the BDDCS class, properties of the drug including administration route and fraction of the drug excreted unchanged in the urine, and various physicochemical parameters such as solubility, partition coefficient (LogP) and molecular weight(54). One advantage to understanding this system is that BDDCS class indicates if a drug is extensively (≥ 70%) or poorly (≤ 30%) metabolized, which, when combined with the fraction of the drug excreted unchanged in the urine, was used to create a dataset of compounds eliminated primarily as unchanged drug in the bile. As a result, we were able to develop a system presented in chapters 2 and 3 for predicting the major elimination route using *in vitro* permeability rate measurements to predict the extent of metabolism followed by a 2-feature logistic regression model including calculated metabolic stability and polarizability that predicts when a poorly permeable, orally-administered drug is likely to be eliminated primarily as unchanged drug in the bile or primarily as unchanged drug in the urine(23).

Here we show that BDDCS has a feedback quality whereby its properties make mistakes obvious to allow reflection of reported properties (i.e. metabolism and solubility) and correct itself. This may not be immediately apparent upon classification, but as drug studies progress, outliers become glaring and demand revisiting. BDDCS errors generally stem from poorly reported data. Given that BCS or BDDCS classification is becoming relatively common in the pharmaceutical industry, new molecular entities may be less susceptible to mistakes, since most drugs were initially classified from a variety of literature sources whose measurements were not developed to predict BCS or BDDCS class. Updated and selective methodology and experiments conducted at single sites may provide more accurate measurements and

predictions. We have not yet seen verified exceptions where the drug does not match the predicted dispositional rules.

Corrections Recognized by Discrepancies Between Permeability Rate and Extent of Metabolism

When we considered *in vitro* permeability rate as a predictor of the extent of metabolism, we discovered that flecainide(185), clonidine(170,183), metoclopramide(139), phenazopyridine(139), and pindolol(183), while listed as BDDCS class 3 and 4 compounds(54), were highly permeable *in vitro*. Thus, upon further investigation, it was noted that these compounds are extensively metabolized(196,198-201). Literature indicated that colchicine is a low permeability rate drug, while we initially classified colchicine as BDDCS class 1. We realized that this compound was eliminated in the bile (Table 5-1). Importantly, colchicine was also identified as the sole false negative of highly permeable BDDCS class 1 compounds that were P-gp substrates when predicting CNS exposure(210). Aliskiren and cefoperazone are poorly permeable and eliminated in the bile(221,222), although we initially classified them as extensively metabolized/highly permeable. We utilized aliskiren as an external validation compound in our model predicting when biliary elimination is the major route of elimination and adjusted its class to class 3 for further studies. Diclofenac was listed with a high solubility, but a much lower solubility has been reported(223) (Table 5-2), resulting in a dose number of 1.4, and therefore necessitated a classification change to class 2. Changes to BDDCS class are listed in Table 5-1. Changes to BDDCS class or listed properties are listed in Table 5-2.

Table 5-1. BDDCS Class Changes from Initial Publication

Compound	Listed Class*	Updated Class	Major route of elimination
Aliskiren	1	3	Biliary
Cefoperazone	1	3	Biliary
Clonidine	3	1	Metabolism
Colchicine	1	3	Biliary
Dabigatran	3	4	Renal
Diclofenac	1	2	Metabolism
Flecainide	3	1	Metabolism
Metoclopramide	3	1	Metabolism
Phenazopyridine	4	2	Metabolism
Pindolol	3	1	Metabolism
Pitavastatin	2	4	Biliary
Saxagliptin	3	1	Metabolism
Tiagabine HCl	2	1	Metabolism

* As listed in Benet *et al*.(54)

Table 5-2. Parameter Changes from Initial Publication

Compound	Parameter Value Listed*	Updated Parameter Value
	%Dose Excreted as Unchanged Drug in Urine	
Memantine	71	48
Pravastatin	20	47
Ranitidine	30	69
Rosuvastatin	5	30
	Solubility	
Atorvastatin	0.0000204 mg/mL	0.0204 mg/mL
Diclofenac	9 mg/mL	0.14 mg/mL
	Administration Route	
Enalaprilat	Oral	Intravenous
Vancomycin	Oral	Intravenous
Tiotropium Bromide	Oral	Inhaled

* As listed in Benet *et al*.(54)

Discrepancies in Predicted and Actual Elimination Route

During development of our model predicting the major route of elimination of orally administered BDDCS class 3 and 4 drugs, we believed ranitidine was misclassified, having a listed fraction excreted unchanged in the urine as 30%, but was predicted as primarily

eliminated in the urine. We discovered that the fraction of the bioavailable dose excreted in the urine was actually 69%, and was therefore correctly predicted(155). In this publication, we considered efflux transporters of biliarily eliminated drugs. Saxagliptin was incorrectly predicted by the model and was not noted to be a substrate of any efflux transporters, as expected. After inspecting its approval package, we realized that saxagliptin is extensively metabolized(224) and amended its class to class 1. In this same investigation, vancomycin was predicted to be eliminated in the bile, despite being primarily eliminated in the urine. Here, we realized that vancomycin was listed as orally dosed, despite primarily being administered intravenously and is unabsorbed and intended for pseudomembranous colitis when administered orally. A similar anomaly was observed with tiotropium bromide, which is an inhaled drug(225). We recognized that successful segregation of renally and biliarily eliminated drugs was limited to orally administered drugs by this model, where some non-orally administered drugs that are renally eliminated could be confounded with [orally or non-orally administered] biliarily eliminated drugs. Enalaprilat was correctly identified, but was initially listed as an orally administered compound, but is in fact given intravenously. Characteristic changes of drugs unrelated to BDDCS class are listed in Table 5-2.

Additions to BDDCS

While building this model, we also considered the fate of recently approved drugs. Three compounds were labeled with significant biliary elimination (afatinib, teriflunomide, vismodegib). These compounds will therefore be classified as BDDCS class 3 or 4. The following compounds were published in a dataset(37) compiling when biliary excretion was significant and have been assigned class 3 or 4: cefbuperazone , cephaloridine, emepronium, flomoxef, indocyanine green, and temafloxacin. More than 175 additions to BDDCS classification, including these listed above and other compounds recently classified(68,74) are listed in Table 5-3.

Table 5-3. Newly BDDCS Classified Drugs

Drug	Class
Afatinib	3
Alclofenac	2
Alpidem	1
Amifloxacin	3
Amineptine	1
Aminosalicylic Acid	1
Axitinib	2
Azimilide	1
Bendazac	2
Benoxaprofen	2
Benzarone	2
Benzbromarone	2
Benziodarone	2
Benzonatate	2
Benzphetamine	0
Benztropine	1
Betaine	1
Bethanechol	3
Bidisomide	3
Boceprevir	1
Brexpiprazole	2
Bromfenac	2
Brotizolam	1
Canagliflozin	2
Carbinoxamine	1
Carbovir	4
Carisoprodol	1
Cefbuperazone	3
Cefcanel	3
Cefmenoxime	3
Cefoperazone	3
Cefpirome	3
Ceftolazone	3
Cephaloridine	3
Chlorhexidine	3
Chlormezanone	1
Chlorpropamide	0
Cinchophen	2
Ciprofibrate	2
Clinafloxacin	3

Clomacran	2
Clometacin	2
Clopamide	3
Cobicistat	2
Cotinine	1
Crizotinib	2
Cyclofenil	2
Dabrafenib	2
Daclatasvir	4
Dapagliflozin	1
Dasabuvir	2
Deferasirox	2
Dexfenfluramine	1
Dexloxiglumide	1
Dihydralazine	1
Dihydroergotamine	1
Dolutegravir	2
Dopamine	1
Droxicam	2
Ebrotidine	1
Edoxaban	4
Eltrombopag	2
Emepronium	3
Empagliflozin	1
Encainide	1
Enprofylline	3
Fenclozic Acid	2
Fenoterol	1
Fenoprofen	2
Fialuridine	2
Finafloxacin	4
Fipexide	3
Flavoxate	2
Flibanserin	2
Flomoxef	3
Flucloxacillin	4
Flupirtine	1
Fosaprepitant	1
Fosfluconazole	1
Fosinapril	1
Fosinaprilat	3
Fusidic Acid	2
Genistein	1

Glafenine	2
Guanethidine	1
Ibufenac	2
Indocyanine green	3
Iproniazid	1
Isocarboxazid	1
Isoproterenol	1
Isoxepac	2
Ivacaftor	2
Ketotifen	1
Ledipasvir	4
Lesinurad	2
Levovirin	3
Licarbazepine	1
Liothyronine	2
Lofexadine	1
Lumiracoxib	2
Meclizine	1
Meclofenamic acid	2
Mepazine	1
Mephenytoin	2
Metaproterenol	1
Methapyrilene	1
Methimazole	1
Methoxsalen	2
Methysergide	1
Metolazone	3
Metyrapone	1
Metyrosine	4
Mibefradil	2
Mifepristone	2
Nedocromil	3
Nemonapride	2
Nialamide	1
Nisoldipine	2
Nomifensine	1
Olaparib	2
Ombitasvir	4
Oxandrolone	2
Oxymetholone	1
Oxyphenisatine	2
Oxytetracycline	3
Pargyline	1

Paritapravir	2
Paromomycin	3
Pasireotide	3
Pazopanib	2
Pelrinone	3
Pemoline	3
Penbutolol	2
Peramivir	3
Perampanel	2
Phencyclidine	1
Phendimetrazine	0
Phenformin	3
Phenoxybenzamine	1
Phentermine	3
Phentolamine	1
Physostigmine	1
Pinacidil	2
Pirprofen	2
Practolol	3
Pralidoxime	3
Procyclidine	1
Rebamipide	4
Roquinimex	2
Rilpivirine	2
Sabeluzole	2
Sapropterin dihydrochloride	1
Sertindole	2
Simeprevir	2
Sinitrodil	1
Sofosbuvir	3
Tasosartan	2
Telapavir	2
Temafloxacin	3
Temocaprilat	3
Teriflunomide	4
Tedizolid phosphate	1
Tesaglitazar	2
Thiotepa	1
Tiapride	3
Ticagrelor	2
Ticrynafen	2
Tolrestat	3
Tranexamic Acid	3

Troglitazone	2
Trovafloxacin Acid	3
Trovafloxacin Mesylate	1
Vandetanib	2
Vemurafenib	2
Vismodegib	4
Vorinostat	2
Xamoterol	3
Yohimbine	1
Zomepirac	2
Zotepine	2

In Table 5-3, we added boceprevir, a drug used to treat hepatitis C. The highest dose strength of boceprevir is a 200 mg capsule, although 4 of these capsules are indicated per administration. Therefore, although the summary basis of approval classifies this drug as a low solubility class 4 drug based on a dose of 800 mg, we have classified this drug as a BDDCS class 1 drug based on the highest dose strength of 200 mg. In cases like boceprevir, classifications can sometimes be misleading, but classification consistency is necessary. BDDCS uses the FDA definition of solubility, as indicated in bioequivalence guidelines(51). However, the solubility criteria differ between regulatory agencies. The EMA has recently recommended that the highest dose given in a single setting according to a drug's labeling be used to calculate the dose number for biowaivers(226,227). In general, this is some multiple of the highest dose strength. For instance, if 80 mg was dosed in a single setting, but as two 40 mg tablets, where tablets greater than 40 mg were not developed, the FDA would allow biowaivers on the basis of the 40 mg dose, while the EMA would require dose number calculation based on an 80 mg dose. This can impact a few high-solubility compounds, shifting their classification to a low-solubility class and different dispositional properties would be predicted, particularly if the compound is highly permeable and extensively metabolized. The approach recommended by the EMA is a more conservative approach and fewer drugs are qualified for a biowaiver. This approach would also limit the percentage of class 1 drugs,

imposing slightly stricter standards to predict when transport is clinically irrelevant. However, this results in a change for only a small percentage of drugs. Solubility is a relatively inherent property of the drug, and relatively few drugs have such a significant change in dose that will result in a change of solubility classification. Recently, Sediq *et al*.(226) examined 27 drugs for which a biowaiver monograph was published for changes in classification mediated by differences in dose definition. Of the 27, only 4 (15%) of the drugs required a classification change.

CAUTIONS

In their 2005 paper(48), Wu and Benet included a section under the heading "Cautions," where they stated, "There will always be exceptions to the broad general rules presented here." However, we have yet to see compelling evidence of drugs behaving outside of their predicted effects by class. One of the most useful predictions from BDDCS, as noted earlier, is that the clinical relevance of transporters for BDDCS class 1 drugs is negligible. While our outliers have been explained by incorrectly reported or interpreted data leading to misclassification, any predictive system will have some unexplained outliers. We expect that there may be violations of our statement that BDDCS class 1 drugs are unaffected in a clinically relevant manner by the inhibition or induction of drug transporters, but we are unaware at this time of documented examples. Additional data may indicate the need to amend and/or grow BDDCS and generate new hypotheses.

Most recently we have begun to consider the possibility of using BDDCS as a tool in evaluating toxicity potential(217). Therefore, the expanded list of BDDCS drug classification here (Table 5-3) includes many drugs that have been removed from the market as a result of toxic manifestations. Expansion of the BDDCS classification list was particularly challenging since for many drugs that came onto the market a number of years ago, and then removed because of toxicity, little reliable information both in terms of metabolism and solubility can be

found in the literature. Therefore, when a drug is on the border of two classes, the BDDCS class is selected based on expected or known drug interactions. Finally, one of the reasons for drugs' misclassification in BDDCS classes can be the simplified, binary, non-continuous structure of BCS and BDDCS. This is particularly so for drugs lying on the border of two classes. While BCS and BDDCS are classification systems based on binary decisions, each property is measured on a continuous scale. It is therefore expected that compounds that approach the binary boundaries may be more difficult to evaluate and inherently risk potential misclassification.

CONCLUSIONS

As we have developed models that confirm and inform BDDCS predictions, or utilized BDDCS predictions to guide methods and hypothesis development, we have naturally encountered drugs with surprising outcomes. In these cases, we can often explain outliers with a model specific limitation or a physiological mechanism that overcomes the base prediction. For instance, Broccatelli *et al*. predicted that highly permeable P-gp substrates that were not class 1 would not be exposed to the central nervous system(108). Yet, in many cases, an uptake transporter overwhelmed the effect of P-gp. However, when mechanistic explanations cannot be determined, we often found that a misclassification was present in the initial dataset, and when we reviewed the solubility or extent of metabolism, we realized that a correction to the BDDCS classification was warranted.

BDDCS can self-correct when discrepancies are seen between predicted and observed effects, as we have seen with drugs such as aliskiren, colchicine, and others. Results of a BDDCS-based experiment often inform the analyst of the true BDDCS class, and, if other factors cannot explain a discrepancy, the analyst should consider reviewing the extent of metabolism and solubility of the drug to determine if reclassification is necessary.

CHAPTER 6. CONCLUSIONS

The disposition and profile of each drug is dictated by the processes of absorption, distribution, metabolism, and excretion. These processes are variable among individuals, and even within individuals, depending on physiological factors including blood flow, pH, membrane permeability, and innate expression and activity of proteins. These and other factors can be affected by stimuli such as food, environment, diseases, or other drugs. Drugs may have properties that allow an average estimation of their rate, extent, or localization of absorption, distribution, metabolism, and excretion. Yet, since these processes can be easily disturbed from average behavior by internal or external factors, understanding each process and what can affect each process is crucial to ensuring the safety and efficacy of drugs.

The Biopharmaceutics Drug Disposition Classification System has incorporated decades of research and progress into a simple system that predicts which drugs may be subject to pharmacokinetic disruption from internal and external factors. It further has helped us to understand the innate conditions dictating drug disposition. In particular, we were able to utilize the simple observation of correlation between permeability rate and the extent of metabolism to successfully predict the extent of metabolism using only *in vitro* data or less successfully *in silico* predictions. Our investigation of this prediction has given us valuable insights into understanding the mechanism of metabolism as an eliminating process *in vivo*.

INSIGHTS INTO METABOLISM FROM BDDCS

BDDCS was pivotal in observing that drugs with a high intestinal passive permeability rate were also extensively metabolized. The rationale behind this observation is that highly permeable drugs, when excreted into hydrophilic secretions, i.e. urine and bile, are rapidly reabsorbed due to the high concentration gradients. This gives the drug multiple chances for metabolism, until eventually the compound is changed to a generally more hydrophilic substance that resides in fluidic secretions. Datasets examining reabsorbed compounds support

this hypothesis. In a dataset published by Dave and Morris(212), 82% of drugs that were reabsorbed from the kidney tubule were extensively metabolized drugs. In a dataset published from Pfizer(34), slightly more than half of the extensively metabolized drugs were reabsorbed from the kidneys compared to less than 20% of class 3 and 4 drugs(59). Renal reabsorption is primarily a passive process driven by high tubular concentrations compared to the blood, though reabsorptive transporters are functional and can play a role. By analyzing this dataset and using permeability rate values generated from the same group, higher permeability rate compounds tended to be reabsorbed, while lower permeability rate compounds were either passively filtered or secreted (ROC AUC = 0.80).

Highly permeable drugs might also be reabsorbed directly from the biliary tract. When phenolphthalein glucuronide was dosed directly into the bile and prevented from undergoing enterohepatic recycling, it was recovered in hepatocytes, indicating that reabsorption from the bile is possible(161). BDDCS predicts that if highly permeable BDDCS class 1 and 2 drugs are initially secreted into the bile, they will be reabsorbed. This reabsorption process means that drugs will eventually be metabolized as a necessary elimination step. This is particularly important for some low-clearance compounds such as diazepam, which are too lipophilic to remain in secretions.

Passive permeability, not active transport or the extent of absorption, correlates with the extent of metabolism. In this way, extensive metabolism can be predicted with immortal cell lines such as Caco-2 and MDCK, which express low transport levels, or even artificial membranes such as PAMPA. Artificial membranes do not express transporters and accurately reflect passive permeability. This reduces the need for human tissue in evaluating metabolism with microsomes, supersomes, or hepatocytes. Permeability rate does not necessarily correlate with metabolic clearance, however.

Hosey and Benet showed that the extent of metabolism can be predicted with either *in vitro* or *in silico* tools (Chapter 3 in thesis)(17). Extreme variability in permeability rate values

persists between labs(165), and therefore numerical permeability rate cut-offs are incapable of predicting the extent of metabolism or absorption. As a solution to this variability, metoprolol has generally been regarded as a standard to determine high-permeability rate(166), above which compounds were predicted to be extensively metabolized or highly absorbed. However, metoprolol's permeability rate is restrictively high when measured in MDCK or Caco-2(13,17,167), mispredicting many extensively metabolized drugs because they had lower permeability rates than metoprolol, and the alternative standards labetalol and zidovudine were assigned for Caco-2 and MDCK, respectively(17) and correctly predicted more compounds across several datasets. While metoprolol was far too conservative to be an effective standard compound in Caco-2 or MDCK, it performs well as a standard in PAMPA(17). Theophylline was selected as an optimal standard when permeability rate studies are conducted in PAMPA and is also too conservative in MDCK or CACO-2. While BDDCS predicts that drugs that are highly permeable *in vitro* will be extensively metabolized clinically, and therefore subject to changes in metabolizing enzymes, *in vitro* systems are not interchangeable and unique protocols must be established for each.

Additionally, from 20 datasets, 97±5% of compounds with a permeability rate greater than metoprolol were extensively metabolized(17). While most compounds with a permeability rate greater than the selected standards labetalol, zidovudine, or theophylline are extensively metabolized, in many cases 20-25% of the compounds with a permeability rate lower than these standards are also extensively metabolized. Therefore, while high permeability rate compounds are almost always extensively metabolized, not all extensively metabolized compounds have a high permeability rate. For increased predictability, a very low permeability rate marker, chlorothiazide, was established. Most compounds with a permeability rate less than chlorothiazide's are poorly metabolized. The predictability of compounds with a permeability rate between chlorothiazide and the reference standard is only around 50%, however, and these compounds should be investigated in humans.

INSIGHTS INTO THE ELIMINATION ROUTE OF UNCHANGED DRUGS

While we do not yet understand why compounds follow such dichotomous elimination routes as exemplified by the observation that very few drugs exhibit intermediate extents of metabolism, we were able to harness this information to predict the major route of elimination of unchanged drugs. While biliary elimination is notoriously difficult to quantify in humans, we have developed a system that predicts which compounds may be subject to biliary elimination. This can help us predict potential complications of biliary elimination or be utilized to target biliary elimination. Indeed, the system that we outlined in chapters 2 and 3 performs far better than previous predictions, which relied on molecular weight and correctly predicted compounds as biliarily eliminated only 12% of the time. While we have validated many of the drugs we presume to be biliarily eliminated with clinical data, the lack of clinical data forces one to extrapolate from metabolism and urinary excretion data. This system allows scientists to understand when to examine hepatic apical and canalicular transporters that may interact with drugs predicted to be eliminated in the bile, which may not necessarily be evaluated for drugs expected to be metabolized. Indeed, many compounds predicted to be eliminated in the bile may actually be metabolized. It also allows scientists to consider potential ramifications of enterohepatic circulation, which can result in multiple peak concentrations and extended residence time as well as multiple exposures to organs in the enterohepatic system.

PREDICTING BDDCS CLASS

There is great value in predicting BDDCS class prior to human dosing. BDDCS class predictions could help direct preclinical and clinical studies during development. For instance, substrate transporter studies are unlikely to be useful for BDDCS class 1 drugs, while metabolism studies are unlikely to be useful for poorly permeable class 3 and 4 drugs. Currently, the system relies on metabolic information gathered from clinical studies. Varma *et al*.(139) have made great progress in predicting BDDCS class using only *in vitro* studies, which

we have improved by ensuring the system can be extended to other laboratories. In chapter 3, we statistically selected compounds to be used as permeability rate standards to predict the extent of metabolism across various laboratories and permeability models. This was an essential study, since numeric permeability rate varies extensively between laboratories and must be standardized to control compounds. In chapter 4, we demonstrated that a dose of 100 mg will provide optimal solubility class predictions. Together these analyses can be universally applied throughout the pharmaceutical industry to predict BDDCS class *in vitro*.

Ideally, we could use *in silico* predictions of BDDCS class to make similar decisions. Unfortunately, attempts at predicting BDDCS class *in silico* have not been as successful as *in vitro* predictions. Yet, our *in silico* BDDCS prediction strategy led to a useful decision tree outlined in Figure 4-5. Despite the errors, this tree could still be applied to predict when some transporter interaction studies are necessary. For instance, since most drugs predicted to be class 1 or 2 actually belong to one of these classes, they do not need to be evaluated for gut uptake transporters.

As we continue to understand the extreme variability of drug disposition, it is becoming necessary to prioritize studies that are invaluable in providing safe and efficacious doses to every individual. BDDCS allows us to assess potential modifiers of drug disposition, which can be relevantly applied to populations, as well as to understanding and predicting interindividual differences. In this thesis, we have discussed the expanded predictive utilities of BDDCS, and developed methods of predicting various aspects of drug disposition. We have predicted major elimination routes using simple features and have shown that BDDCS can be used in several ways to predict every aspect of disposition: absorption, distribution, metabolism, and excretion. The results of the work presented in chapter 2 can be initially applied during drug development as a method of initially screening for potential biliary excretion of unchanged drug, with greater predictivity than has previously been accomplished. Further, chapters 2 and 3 provide valuable insights into potentially comprehending the difficult problem of predicting if a compound will be

metabolized versus eliminated in the bile and emphasize the need for a greater understanding of the intracellular processes that determine the fate of relatively poorly permeable drugs. Chapter 3 improves upon our understanding of what dictates metabolism from a physiological context and how we can predict major elimination routes prior to human dosing. Chapter 4 allows us the ability to potentially eliminate unnecessary transporter studies during drug development using *in silico* methods and discusses the preclinical utility of *in vitro* BDDCS predictions. Finally in chapter 5, we describe extensions of BDDCS that can be utilized at every stage of development to guide dispositional understanding and its potential effectors, drug selection, and even environmental decisions. BDDCS currently has extensive utility and we can only envision its many future applications.

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Appendix Table 1a. Properties of Training Dataset of Biliarily and Renally Eliminated Compounds

Compound Name	f_e	Major Elimination Route	Charge	POL	MW	MetStab	Predictive Value	Predicted as bile?
Hydroxyurea	80	Renal	Neutral	5.995	76.055	100	0.0002	0
Cycloserine	65	Renal	Neutral	8.765	102.092	100	0.0004	0
Dalfampridine	90.3	Renal	Cation	10.659	95.123	100	0.0006	0
Flucytosine	99	Renal	Neutral	10.740	129.092	100	0.0006	0
Fosfomycin Tromethamine	82	Renal	Anion	13.536	136.043	100	0.0011	0
Piracetam	70	Renal	Neutral	13.554	142.156	100	0.0011	0
Metformin	99	Renal	Cation	14.468	130.172	100	0.0014	0
Aminocaproic Acid	65	Renal	Zwitterion	15.174	131.173	100	0.0016	0
Levetiracetam	66	Renal	Neutral	17.224	170.209	100	0.0025	0
Pyridostigmine	85	Renal	Cation	18.431	181.212	100	0.0032	0
Pregabalin	90	Renal	Zwitterion	18.844	159.226	100	0.0035	0
Acetazolamide	90	Renal	Neutral	18.931	222.245	100	0.0036	0
Miglitol	80	Renal	Neutral	19.216	207.224	100	0.0038	0
Phenylpropanolamine	65	Renal	Cation	19.850	152.213	100	0.0044	0
Gabapentin	100	Renal	Zwitterion	19.905	171.237	100	0.0044	0
Clodronic Acid	80	Renal	Anion	19.980	241.868	100	0.0045	0
Amantadine	85	Renal	Cation	20.076	152.257	100	0.0046	0
Zalcitabine	65	Renal	Neutral	20.506	211.218	100	0.0051	0
Emtricitabine	73	Renal	Neutral	21.280	247.247	100	0.0060	0
Lamivudine	67	Renal	Neutral	21.371	229.256	100	0.0061	0
Acyclovir	75	Renal	Neutral	21.504	225.205	100	0.0063	0
Phenylethylmalonamide	79	Renal	Neutral	22.153	206.241	100	0.0072	0
Miglustat	85	Renal	Neutral	22.249	219.278	100	0.0074	0
Baclofen	69	Renal	Zwitterion	23.092	213.660	100	0.0088	0
Ganciclovir Sodium	91	Renal	Neutral	23.554	255.231	100	0.0098	0
Memantine	71	Renal	Cation	23.746	180.310	100	0.0102	0

Lenalidomide	66	Renal	Neutral	23.979	262.236	100	0.0107	0
Neostigmine	67	Renal	Cation	24.527	223.291	96.2848	0.0158	0
Chlorothiazide	92	Renal	Neutral	25.859	295.723	100	0.0160	0
Ethambutol	79	Renal	Cation	26.023	205.318	100	0.0166	0
Hydroflumethiazide	90	Renal	Neutral	26.036	331.292	100	0.0166	0
Hydrochlorothiazide	100	Renal	Neutral	26.402	297.739	100	0.0180	0
Sulfamethizole	86	Renal	Anion	26.674	269.323	100	0.0190	0
Pramipexole	90	Renal	Cation	26.769	212.335	100	0.0194	0
Varenicline Tartrate	92	Renal	Cation	27.099	212.270	100	0.0208	0
Pyrimethamine	65	Renal	Neutral	27.228	248.711	89.225	0.0466	0
Risedronate	87	Renal	Anion	27.547	280.088	100	0.0229	0
Entecavir	70	Renal	Neutral	27.878	277.279	100	0.0246	0
Vitamin B1 (Thiamine)	90	Renal	Cation	27.935	265.354	100	0.0249	0
Fluconazole	75	Renal	Neutral	28.876	306.271	88.2044	0.0701	0
Furosemide	66	Renal	Anion	28.932	329.736	100	0.0307	0
Cadralazine	75	Renal	Neutral	29.196	283.327	82.2459	0.1119	0
Procainamide	67	Renal	Cation	29.302	236.333	94.3902	0.0496	0
Topiramate	70	Renal	Neutral	30.045	339.362	100	0.0388	0
Trimethoprim	70	Renal	Neutral	30.881	290.318	88.7841	0.1004	0
Fleroxacin	72.5	Renal	Anion	31.922	368.330	100	0.0572	0
Atenolol	94	Renal	Cation	31.934	267.344	100	0.0573	0
Loracarbef	94	Renal	Anion	32.083	348.761	100	0.0591	0
Sotalol	85	Renal	Cation	32.106	273.372	100	0.0594	0
Chlorthalidone	65	Renal	Neutral	32.578	338.766	96.4174	0.0837	0
Cephalexin	91	Renal	Anion	32.855	346.381	100	0.0691	0
Famotidine	67	Renal	Neutral	32.992	337.445	100	0.0710	0
Cephradine	86	Renal	Anion	33.047	348.397	100	0.0718	0
Norfloxacin	29	Biliary	Zwitterion	33.192	319.331	100	0.0740	0
Saxagliptin	24	Biliary	Neutral	33.211	315.410	86.0345	0.1850	0
Cefadroxil	93	Renal	Anion	33.492	362.380	100	0.0785	0
Metoclopramide	20	Biliary	Cation	33.702	300.804	77.8446	0.3173	1

Ampicillin	88	Renal	Anion	33.947	348.397	100	0.0860	0
Ranitidine	69	Renal	Zwitterion	34.206	315.412	64.3809	0.5857	1
Ciprofloxacin	65	Renal	Zwitterion	34.253	331.341	100	0.0914	0
Carbenicillin	82	Renal	Anion	34.547	376.384	100	0.0968	0
Sitagliptin	78	Renal	Cation	34.581	408.322	100	0.0974	0
Amoxicillin	86	Renal	Neutral	34.584	364.396	100	0.0975	0
Cefdinir	15	Biliary	Anion	34.708	394.406	100	0.0999	0
Lomefloxacin	65	Renal	Zwitterion	34.936	351.348	100	0.1044	0
Ceftibuten	71	Renal	Anion	35.088	408.409	100	0.1076	0
Cefuroxime	96	Renal	Anion	35.450	423.377	100	0.1153	0
Ritodrine	10	Biliary	Cation	35.454	288.361	89.5334	0.2215	0
Levofloxacin	74	Renal	Zwitterion	35.725	361.367	100	0.1216	0
VitaminB2	75	Renal	Neutral	36.031	376.364	100	0.1288	0
Iopanoic Acid; Iodopanoic Acid	33	Biliary	Anion	36.286	569.924	71.4876	0.5667	1
Nadolol	73	Renal	Cation	36.667	310.408	100	0.1451	0
Cefprozil	73	Renal	Anion	36.970	388.418	100	0.1535	0
Sulpiride	70	Renal	Cation	37.109	342.434	85.3247	0.3580	1
Cefpodoxime	81	Renal	Anion	37.372	426.447	100	0.1652	0
Bendroflumethiazide	21	Biliary	Neutral	37.531	421.415	70.9384	0.6409	1
Sitafloxacin	75	Renal	Zwitterion	37.986	409.814	100	0.1844	0
Atovaquone	3	Biliary	Anion	38.535	365.829	58.241	0.8511	1
Tiotropium Bromide	74	Renal	Cation	39.572	392.512	85.2481	0.4890	1
Cloxacillin	75	Renal	Anion	40.271	434.873	90.0329	0.4382	1
Acrivastine	67	Renal	Zwitterion	40.902	348.438	69.436	0.8058	1
Moxifloxacin Hydrochloride	22	Biliary	Zwitterion	41.456	401.431	93.932	0.4300	1
Cilazaprilat	91	Renal	Zwitterion	41.705	388.437	100	0.3363	1
Cefamandole	96	Renal	Anion	42.208	461.495	100	0.3610	1
Eprosartan	30	Biliary	Anion	42.856	422.497	85.3619	0.6593	1
Levocetirizine	71	Renal	Zwitterion	43.141	388.887	64.1189	0.9093	1
Lisinopril	94	Renal	Zwitterion	43.314	404.480	100	0.4180	1
Leucovorin; Folinic Acid	10	Biliary	Anion	43.797	471.423	100	0.4437	1

Pravastatin	20	Biliary	Anion	43.797	423.520	95.7162	0.5233	1
Methotrexate	81	Renal	Anion	44.055	452.423	100	0.4576	1
Trospium Chloride	6	Biliary	Cation	44.161	392.510	47.2519	0.9777	1
Celiprolol	20	Biliary	Cation	44.216	380.502	63.3872	0.9304	1
Valsartan	13	Biliary	Anion	46.640	433.503	85.973	0.8078	1
Rosuvastatin Calcium	5	Biliary	Anion	47.554	480.530	89.5182	0.7974	1
Alvimopan	2	Biliary	Zwitterion	48.474	424.532	65.3194	0.9668	1
Orlistat	1	Biliary	Neutral	55.933	495.735	0	0.9999	1
Loperamide	0.5	Biliary	Cation	56.276	478.045	17.9779	0.9998	1
Fexofenadine	25	Biliary	Zwitterion	59.334	501.656	40.2224	0.9995	1
Candesartan Cilexetil	0	Biliary	Neutral	64.000	609.651	16.3724	1.0000	1
Erythromycin (Base)	5	Biliary	Cation	76.509	734.934	51.708	1.0000	1
Digitoxin	30	Biliary	Neutral	77.355	764.939	27.2032	1.0000	1
Clarithromycin	35	Biliary	Anion	78.344	748.961	61.2794	1.0000	1
Rifaximin	0.035	Biliary	Anion	81.451	784.870	16.4918	1.0000	1
Azithromycin	6	Biliary	Cation	81.823	751.000	49.4902	1.0000	1
Roxithromycin	12	Biliary	Cation	86.899	838.054	66.7689	1.0000	1

Appendix Table 1b. Properties of External Dataset of Biliarily and Renally Eliminated Compounds

Compound Name	f_b	Major Elimination Route	POL	MW	MetStab	Predictive Value	Predicted as bile?
Emepronium	45; 28	Biliary	35.948	282.443	43.9898	0.9041	1
Aliskiren		Biliary	62.840	552.766	45.8561	0.9996	1
Digoxin	30.6	Biliary	77.992	780.938	52.1676	1.0000	1
Irbesartan	80	Biliary	47.745	427.521	52.1458	0.9852	1
Telmisartan	98	Biliary	58.419	513.609	23.2558	0.9998	1
Pirenzepine		Biliary	37.763	351.402	65.8544	0.7327	1
Tetracycline	0.14	Renal	43.761	443.426	100	0.4418	1
Milnacipran		Renal	30.721	247.356	100	0.0446	0
Naratriptan		Renal	39.381	336.472	68.4166	0.7629	1
Clavulanic acid	0.04	Renal	16.376	198.153	100	0.0021	0
Bumetanide	2.16	Renal	36.350	363.408	91.4598	0.2304	0
Cefaclor	0.096	Renal	34.871	367.807	100	0.1031	0
Disopyramide	1.29	Renal	42.281	340.482	68.0525	0.8612	1
Cimetidine	1.8	Renal	27.174	252.339	86.6029	0.0555	0
Cefixime	10.6	Renal	38.494	451.434	100	0.2015	0
Terbutaline		Renal	25.794	212.265	100	0.0158	0
Guanfacine		Renal	23.473	247.101	100	0.0096	0
Penicillamine		Renal	16.339	149.211	100	0.0021	0
Captopril		Renal	20.903	216.277	100	0.0055	0
Stavudine		Renal	21.854	224.213	100	0.0068	0
Almotriptan		Renal	39.381	336.472	59.2564	0.8643	1
Lacosamide		Renal	26.460	250.294	82.9965	0.0617	0
Adefovir		Renal	28.887	333.238	100	0.0304	0
Alendronate		Renal	25.540	247.080	100	0.0150	0
Desmopressin		Renal	105.448	1070.220	100	1.0000	1
Acamprosaic Acid		Renal	15.745	180.202	100	0.0018	0

Enalaprilat	Renal	37.458	347.385	100	0.1678	0
Vigabatrin	Renal	14.982	129.157	100	0.0015	0
Nizatidine	Renal	33.523	332.465	83.568	0.2259	0
Tiludronic	Renal	29.139	316.592	100	0.0321	0

Appendix Table 2. Clinical Data in Support of Biliary Excretion

Compound Name	Correct?	Indication of biliary excretion or fraction of dose eliminated in bile	Indication of renal excretion or fraction of dose eliminated in urine	Reference
Candesartan Cilexetil	0			Gleiter CH, Morike KE. Clinical pharmacokinetics of candesartan. Clin Pharmacokinet. 2002; 41:7-17
Rifaximin	1	0.002	0.000009	Verardi S, Verardi V: Bile rifaximin concentration after oral administration in patients undergoing cholecystectomy. Farmaco. 1990; 45:131–135. Hong KS. Rifaximin for the treatment of acute infectious diarrhea.Therap Adv Gastroenterol. 2011;4:227-35.
Orlistat	1	The proportion of orlistat that is absorbed, and its metabolites are subject to biliary excretion; cholestasis is a contraindication due to biliary excretion		European Medicines Agency, Assessment Report for alli [Internet]. 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000854/WC500024114.pdf
Alvimopan	1	Bile:primary pathway		Cubist Pharmaceuticals, Inc. Product Information: ENTEREG® (alvimopan) oral capsules. NDA 21-775. 2013.
Atovaquone	1	Major route of elimination		Rolan PE, Mercer AJ, Tate E, Benjamin I, Posner J. Disposition of atovaquone in humans. Antimicrob Agents Chemother. 1997:41;1319-21.

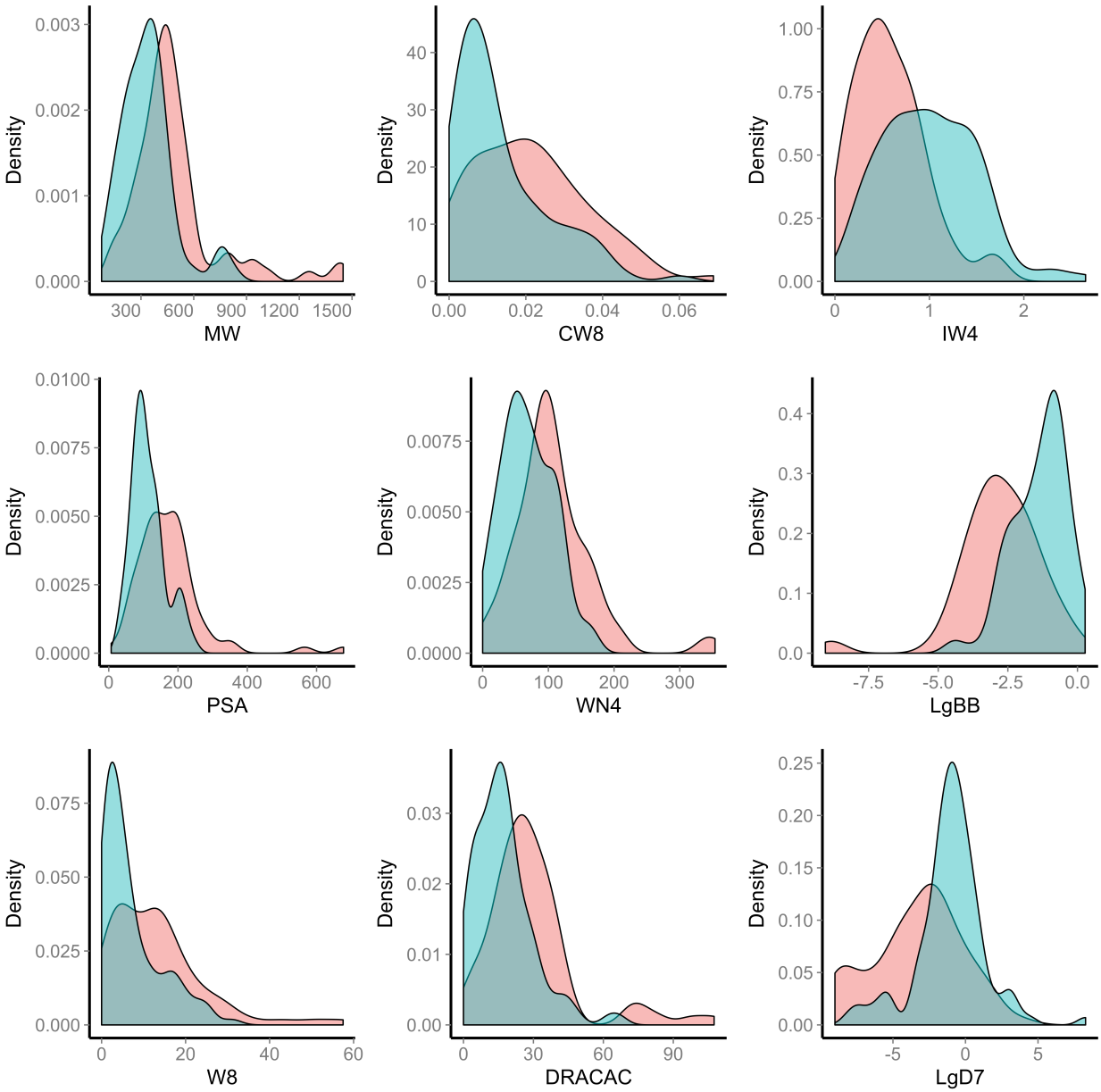
Erythromycin (Base)	1	12.6		Yang X, Gandhi YA, Duignan DB, Morris ME. Prediction of biliary excretion in rats and humans using molecular weight and quantitative structure–pharmacokinetic relationships. AAPS J. 2009;11:511–25.
Rosuvastatin Calcium	1	59		Yang X, Gandhi YA, Duignan DB, Morris ME. Prediction of biliary excretion in rats and humans using molecular weight and quantitative structure–pharmacokinetic relationships. AAPS J. 2009;11:511–25.
Azithromycin	1	Main elimination route	12	Singlas E. Clinical pharmacokinetics of azithromycin. Pathol Biol. 1995:43;505-11.
Trospium Chloride		36.8% of bioavailable dose	Primary route of elimination; 23.2% of bioavailable dose	Doroshyenko O, Jetter A, Odenthal KP, Fuhr U. Clinical Pharmacokinetics of Trospium Chloride. Clin Pharmacokinet. 2005;44:701-20. Allergan, Inc. Product Information: Sanctura® (trospium chloride) tablets, 20 mg. 2012.
Roxithromycin	1	53% fecal; well-absorbed		Puri SK, Lassman HB. Roxithromycin: a pharmacokinetic review of a macrolide. J Antimicrob Chemother. 1987;20 Suppl B:89-100.
Valsartan	1	70	30	Gleiter CH, Morike KE. Clinical pharmacokinetics of candesartan. Clin Pharmacokinet. 2002;41:7-17.
Cefdinir	0		Primarily eliminated via renal clearance of unchanged drug, but says 13 to 23%, doesn't say if it's the bioavailable dose or not; as dose increases, renal clearance decreases	Richer M, Allard S, Manseau L, Vallee F, Pak R, LeBel M. Suction-induced blister fluid penetration of cefdinir in healthy volunteers following ascending oral doses. 1995:39;1082-6. Guay DRP. Cefdinir: An expanded-spectrum oral cephalosporin. Ann Pharmacother. 2000;34:1469-77. Chen J, Ahmad J. Cefdinir-induced hepatotoxicity: potential hazards of inappropriate antibiotic use. J Gen Intern Med. 2008;23:1914-6.

Celiprolol	1	31% eliminated in the bile after IV dose		Hitzenberger G, Takacs F, Pittner H: Pharmacokinetics of the beta-adrenergic receptor blocking agent celiprolol after single intravenous and oral administrations in man. Arzneimittelforschung. 1983;33:50-52
Pravastatin	1	23 eliminated in feces	64% excreted as intact drug in bile (23) and urine (41) (IV); 30% metabolized	Hatanaka T. Clinical pharmacokinetics of pravastatin: mechanisms of pharmacokinetic events. Clin Pharmacokinet. 2000;6:397-412.
Moxifloxacin Hydrochloride	1	Extensively eliminated		Schwab D, Grauer M, Hahn EG, Muhldorfer S. Biliary secretion of moxifloxacin in obsturctive cholngitis and the non-obstructed biliary tract. Ailment Pharmacol Ther. 2005;22:417-22.
Saxagliptin	0		Primary route	Chacra AR. Saxagliptin for type 2 diabetes. Diabetes Metab Syndr Obes. 2010;3:325-35.
Fexofenadine	1	67%		Ayrton A, Morgan P. Role of trasnport proteins in drug absorption, distribution and excretion. Xenobiotica. 2001:31;469-97. Swift B, Tian X, Brouwer KLR. Integration of preclinical and clinical data with pharmacokinetic modeling and simulation to evaluate fexofenadine as a probe for hepatobiliary transport function. Pharm Res. 2009;26:1942-51. Lippert C, Ling J, Brown P, Burmaster S, Eller M, Cheng L, Thompson R, Weir S. Mass balance and pharmacokinetics of MDL 16,455A in healthy, male volunteers. Pharm Res. 1995;12:S-390.

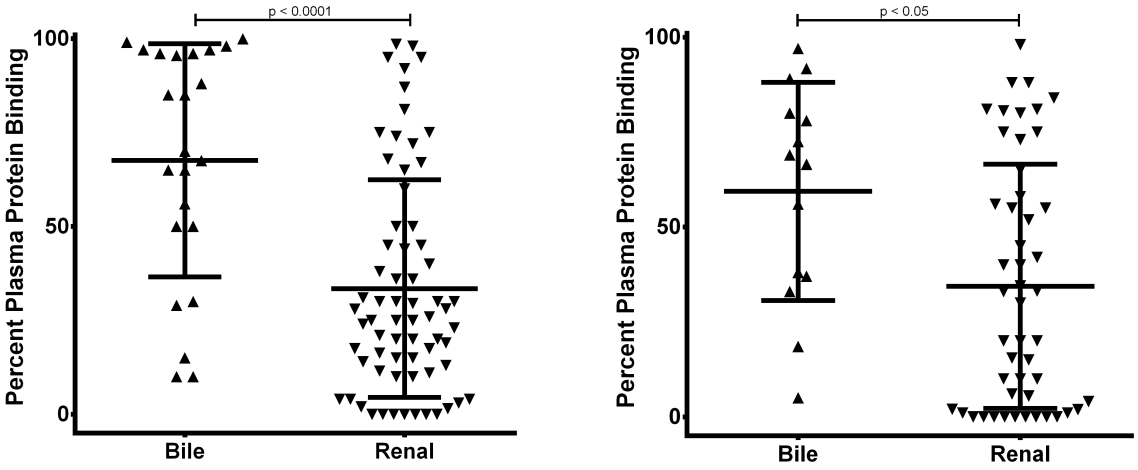
Norfloxacin		Distributes into the bile, but unlikely to contribute to elimination; cleared from the body through the kidneys, biliary excretion and metabolism.		Wise R. Norfloxacin—a review of pharmacology and tissue penetration. J Antimicrob Chemother. 1984;13 Suppl B:59-64. Sharma PC, Saneja A, Jain S. Norfloxacin: A therapeutic review. Int J Chem Sci. 2008:6:1702-13.
Digitoxin	1	"Bile concentration was higher than serum and urine concentration after 24 hr."		Storstein L. Studies on digitalis. III. Biliary excretion and enterohepatic crculation of digitoxin and its cardioactive metabolites. Clin Pharmacol Ther. 1975:17:313-20.
Eprosartan	1	90	10	Gleiter CH, Morike KE. Clinical pharmacokinetics of candesartan. Clin Pharmacokinet. 2002;41:7-17.

Appendix Table 3. Distribution of Drugs by Their Major Route of Elimination per Dataset

Author	Year	Cell Line	No. Metabolized	Total No. Non-Metabolized	No. Eliminated in Bile	No. Eliminated in Urine	Reference
Camenisch	1998	Caco-2	17	6			(171)
Chong	1995	Caco-2	4	6			(173)
Irvine	1999	Caco-2	25	18			(175)
Li	2008	Caco-2	15	9			(179)
Skolnik	2009	Caco-2	48	35	9	15	(170)
Stenberg	1998	Caco-2	11	7			(169)
Teksin	2010	Caco-2	11	6			(178)
Volpe	2007	Caco-2	13	9			(176)
Yamashita	2000	Caco-2	7	8			(172)
Yazdanian	1998	Caco-2	29	12			(168)
Yee	1997	Caco-2	15	7	2	2	(174)
Chen	2005	MDCK	18	9			(180)
Irvine	1999	MDCK	26	19			(175)
Varma	2012	MDCK-LE	67	34	6	15	(139)
Wang	2005	MDR-MDCK	13	13	2	6	(181)
Thiel-Demby	2009	MDR-MDCK	9	11			(182)
Chen	2008	PAMPA	22	10			(184)
Chen (2008)	2008	PAMPA	22	10			(184)
Li	2008	PAMPA	10	7			(179)
Sugano	2002	PAMPA	31	27	2	18	(185)
Teksin	2010	PAMPA	11	6			(178)
Zhu	2002	PAMPA	55	30	2	28	(183)
Pham-The	2013	Caco-2	169	83	15	36	(186)



Appendix Figure 1. Density *vs* Variables with Important Differences Between Orally and Non-orally Administered Drugs. The distribution of orally administered drugs is shaded blue, while the distribution of non-orally administered drugs is shaded red.



Appendix Figure 2. Boxplots of the Percent of Plasma Protein Binding of Drugs that Are Eliminated in the Bile or the Urine for orally administered (left) or nonorally administered (right) drugs. The box represents the values between the 25 and 75 percentile and the median. Tukey-defined extremes are represented by the whiskers and outliers are represented as individual datapoints.

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