Research Article

Effects of Drug Transporters on Volume of Distribution

Anita Grover¹ and Leslie Z. Benet^{1,2}

Received 12 February 2009; accepted 25 March 2009; published online 28 April 2009

Abstract. Recently, drug transporters have emerged as significant modifiers of a patient's pharmacokinetics. In cases where the functioning of drug transporters is altered, such as by drug-drug interactions, by genetic polymorphisms, or as evidenced in knockout animals, the resulting change in volume of distribution can lead to a significant change in drug effect or likelihood of toxicity, as well as a change in half life independent of a change in clearance. Here, we review pharmacokinetic interactions at the transporter level that have been investigated in animals and humans and reported in literature, with a focus on the changes in distribution volume. We pay particular attention to the differing effects of changes in transporter function on the three measures of volume. Further, trends are discussed as they may be used to predict volume changes given the function of a transporter and the primary location of the interaction. Because the liver and kidneys express the greatest level and variety of transporters, we denote these organs as the primary location of transporter-based interactions. We conclude that the liver is a larger contributor to distribution volume than the kidneys, in consideration of both uptake and efflux transporters. Further, while altered distribution due to secondary interactions at tissues other than the liver and kidneys may have a pharmacodynamic effect, these interactions, at least at the blood-brain barrier, do not appear to significantly influence overall distribution volume. The analysis provides a framework for understanding potential pharmacokinetic interactions rooted in drug transporters as they modify drug distribution.

KEY WORDS: distribution volume; drug-drug interaction; drug transporters; pharmacokinetics; pharmacogenomics.

INTRODUCTION

The pharmacokinetic parameter volume of distribution describes the relationship between the measured systemic concentrations and amount of drug in the body. It is a measure of the extent of tissue distribution, and it usually does not represent any physiological volume. Instead, it is considered a theoretical parameter that is dependent on a variety of drug properties: traditionally, lipophilicity (a measure of tissue affinity), plasma protein binding (1), and tissue components, including proteins. However, with the recent advances in the understanding of the importance of active drug transporters in pharmacokinetics, we sought to understand how transporter activity affects volume of distribution.

Drug transporters are found at numerous tissues in the body, implicating them as players in drug distribution. While a variety of transporters, including P-glycoprotein (P-gp), BCRP, and some members of the OATP family, are heavily expressed at the intestinal epithelium, they should not affect volume of distribution, as volume terms are related to the

behavior of the drug once it has entered the systemic circulation. Within the body, the liver and kidneys express the greatest variety and level of drug transporters. At these two organs, transporters modulate access to metabolizing enzymes and excretion processes, both biliary and renal. Consequently, they are likely to also have an effect on other pharmacokinetic parameters, particularly clearance and half life. The majority of published reports, therefore, focus on primary transporter interactions at either the liver or the kidneys.

In those cases where the functioning of drug transporters is altered, such as by a drug-drug interaction or by a genetic polymorphism in the transporter gene or relevant genetic control elements, the resulting change in volume of distribution can lead to a significant change in drug effect or likelihood of toxicity, as well as a change in half life independent of a change in clearance. Generally, because many transporters have a wide range of substrates, drug-drug interactions in this consideration are rooted in the inhibition of a transporter, leading to decreased functionality. Similarly, most polymorphisms will result in a reduced function transporter. Thus, here we primarily examine decreased transporter function and its effects on the distribution volume. It is possible, however, for an increase in transporter function to occur either by up-regulation of the transporter gene under multiple dosing conditions, or by a theoretical polymorphism that creates a transport protein with increased

¹Department of Biopharmaceutical Sciences, University of California, 533 Parnassus Ave, Room U-68, San Francisco, 94143-0912 CA, USA.

²To whom correspondence should be addressed. (e-mail: leslie.benet@ucsf.edu)

effectiveness or an increased amount of protein. Such transporter induction interactions have been reported to affect bioavailability in the gut; however, these should not affect distribution volume. While induction interactions have been reported at other tissues, including the liver and kidney, *in vitro* (2-4), clinical, pharmacokinetic interactions with increased functioning transporters have not yet been reported outside the gut.

The relationship between systemic concentrations and amount of drug in the body can differ depending on the dosing regimen and time at which the necessary parameters are measured. In particular, V_1 , the initial dilution volume, defined as the dose divided by the initial plasma concentration following an intravenous bolus dose, is likely to be relatively small, because equilibration to the other tissue spaces has not yet occurred. The volume at the terminal phase of elimination, V_{area} (also known as V_z), will be greater. Most commonly, this represents the phase when distribution is complete and elimination from the plasma is predominant, so drug is re-entering the circulation from the tissue spaces. V_{area} is defined as the clearance divided by the terminal rate of elimination, and is therefore heavily dependent on the terminal rate of elimination. This rate is often a more difficult parameter to estimate experimentally because it requires concentration data for a long time period following the dose, when concentrations may begin to fall below the limits of sensitivity for some analytical methodology. Finally, the volume at steady state, V_{ss} , is the sum of the distribution volumes of all the compartments in a pharmacokinetic model. It can also be calculated from a single dose as the product of clearance and the mean residence time in the body following non-compartmental analysis. Its value will be between V_1 and V_{area} , and V_{ss} is considered to be a more "accurate" measure of whole body distribution volume, as it is less directly dependent on changes in the elimination processes, a characteristic of V_{area} .

It is also important to note that calculations of volume are highly model-dependent. The three volume parameters as defined above assume elimination from the central compartment of a pharmacokinetic model (5). The typical compartmental model assumes the liver and kidneys are part of the central compartment, as they are highly perfused organs and assumed to be in rapid equilibrium with the plasma, thus providing for central compartment elimination. However, when elimination does not occur from the central compartment, these three measures will significantly under-predict distribution volume (6, 7). Therefore, it may be important to consider a possible case where transporter dysfunction means the liver or kidneys are not in rapid equilibrium with the plasma, such that elimination occurs from a peripheral compartment. We will return to this topic in the discussion.

Drug transporters can be loosely characterized as either uptake or efflux, denoting whether they facilitate drug entry into a cell or efflux out of a cell. Thus, an uptake transporter with reduced function prevents drug accumulation in the tissue expressing the transporter, while an efflux transporter with decreased function increases accumulation in the tissue expressing the transporter. The effect on total distribution volume depends on the tissue expressing the transporter, whether it is an uptake or efflux transporter, and where the transporter is expressed in this tissue.

Therefore, the objective of this work was to collect and analyze published reports evidencing changes to distribution volume in animals and humans due to drug-drug interactions, genetic polymorphisms, or knockout animals, to determine what conclusions could be drawn.

MATERIALS AND METHODS

Literature searches revealed a number of interactions at the transporter level that have been investigated and reported. A summary of thirty-seven such interactions, including the effects on the primary pharmacokinetic parameters, is presented in Table I. These thirty-seven interactions are all those available where a volume parameter was presented, or where a volume parameter could be calculated from the reported parameters: Where provided, the values in Table I reflect those in the reference article. Otherwise, the missing values were calculated: most commonly, assuming the provided half life is the terminal half life, V_{area} was calculated from Equation 1. When volume but not half life was provided, a half life was calculated also using Equation 1 under the assumption of a one compartment model. Finally, when clearance and mean residence time were provided, V_{ss} was calculated by the definition of V_{ss} as the product of clearance and mean residence time. Calculated values are indicated by an asterisk throughout this report.

RESULTS AND DISCUSSION

Further analysis of the interactions listed in Table I revealed a few interesting trends:

1. The magnitude of transporter mediated change in volume of distribution may differ depending on which measure of volume is used.
2. A transporter mediated change in volume of distribution may be independent of or correlated to a change in the drug's clearance and the associated half life.
3. In general, interactions at uptake transporters at the liver lead to a significant decrease in volume of distribution, while those at the renal tubules do not lead to a change in volume of distribution, although there are exceptions.
4. Interactions with efflux transporters at the liver generally lead to a decrease in volume of distribution, while those at the renal tubules lead to an increase in volume of distribution.
5. The primary location of the interaction (liver or kidneys) is a more important determinant of the change in distribution volume than the secondary change in tissue distribution, as evidenced by interactions that affect the integrity of the blood-brain barrier.
6. It is possible to predict the direction of the change in pharmacological effect given the mechanisms of action of the drug and the location of the interaction.

Each of these trends will be discussed in relation to the interactions presented in Table I.

The magnitude of transporter mediated change in volume of distribution may differ depending on which measure of volume is used.

As discussed above, there are three measures of distribution volume. The relative contribution of changes in transporter function to these three measures of volume may differ. The plasma concentration-time data from four studies (24, 30, 49, 52) were extracted and reanalyzed using WinNonlin version 2.1 (Pharsight Corporation, Mountain View, CA). For each study, the three measures of volume were calculated under both control and decreased transporter functionality conditions, as shown in Table II, to ensure consistency in calculation methods between the parameter estimates both within and between the four studies. V_1 changes less markedly than V_{ss} and V_{area} . This is expected since full tissue distribution is not likely to have occurred at the initial time points, and transporters not directly associated with very rapidly equilibrating organs may not have had the chance to exert their effects. In contrast, after all organs are in distribution equilibrium, V_{ss} and V_{area} show the full effect of transporter inhibition, exhibiting bigger changes than seen for V_1 . Thus, with respect to transporter effects on equilibrium volume measures, little difference is seen between the effects on V_{ss} and V_{area} .

A transporter mediated change in volume of distribution can be independent of or correlated to a change in the drug's clearance and the associated half life.

Half life is considered the most important parameter to the clinician for determining dosing changes due to drug-drug interactions or pharmacogenomic variability, as it is considered the parameter most closely associated with dosing interval and duration of drug effect. In the simplest relation, half life, clearance, and volume of distribution are related by Equation 1:

$$t_{1/2} \approx \frac{\ln(2) \cdot V}{CL} \quad (1)$$

Therefore, the change in half life is proportional to the change in distribution volume, and inversely related to the change in clearance. In this simple single-phase approximation, there will only be one volume term ($V = V_1 = V_{ss} = V_{area}$). In reality, most drugs exhibit multiple phases of distribution and/or elimination, and may have many half lives (53). However, changes in this single-phase approximation are still indicative of a general pharmacokinetic trend.

Further, as noted by Sahin and Benet (53), many different single value half lives can be reported for a drug that almost assuredly exhibits multi-compartment kinetics, including the single-phase approximation or the half life for the terminal phase. Therefore, for a number of drugs in Table I, the relationship between clearance, volume, and half life will not follow Equation 1.

In rats, digoxin is primarily metabolized in the liver by Cyp3a. It is a substrate for Oatp1a4 uptake and P-glycoprotein efflux in hepatocytes. When rats were dosed with dexamethasone, Cyp3a, Oatp1a4, and P-gp were induced. Following administration of a single dose of the Oatp-inhibitor rifampin to these dexamethasone induced rats, a decrease in steady state volume of distribution of 70.8% was observed together with a decrease in clearance of 54.2%, while no change in half life was evident in comparison to the induced, but not rifampin inhibited, controls. From previous studies, it is known that the concentration of inhibitor

achieved after the single dose had minimal effects on Cyp3a and P-gp. Therefore, inhibition of the uptake transporter led to the pharmacokinetic changes observed (24). Inhibition of Oatp1a4 prevents liver accumulation, decreasing distribution volume. Preventing liver entry also prevents metabolism, leading to the decrease in clearance. Possibly because Oatp1a4 is also expressed at the blood-brain barrier, choroid plexus, ciliary bodies, and retina, in addition to the liver (54), the decrease in volume is greater than the decrease in clearance. In this case, the changes in volume and clearance appear to be correlated.

In humans, however, digoxin is predominantly excreted unchanged in the urine. This process is mediated by P-gp. In patients concomitantly dosed with ritonavir, a P-gp inhibitor, steady state volume increased 76.7%, clearance decreased 41.8%, and half life increased 156% as compared to control (25). Here, inhibition of P-gp prevents efflux of drug from the renal epithelial cells into the urine, decreasing clearance. Inhibition of P-gp also prevents efflux of drug from other tissues protected by P-gp, such as the brain and heart. Therefore, the drug is more widely distributed in the body, and there is less drug in the systemic circulation, making less available to be cleared by the kidneys. Because the clearance rate is also decreased, both factors work towards increasing half life. In this case, the changes in volume and clearance are not correlated.

These examples elucidate mechanisms by which transporter inhibition can lead to significantly different pharmacokinetic patterns for the clinician to consider.

In general, interactions at uptake transporters at the liver lead to a significant decrease in volume of distribution, while those at the renal tubules do not lead to a change in volume of distribution, although there are exceptions.

Of twenty four interactions that involved uptake transporters with decreased function, nine did not cause a significant change in distribution volume. Each of these involved interactions documented at uptake transporters at the renal tubules for drugs that are primarily excreted unchanged in the urine. Three of twelve renal interactions did exhibit decreased volume. Conversely, the twelve interactions attributed to the liver all led to a decreased volume of distribution. A decrease in volume of distribution would be expected in these interactions, as inhibiting an uptake transporter prevents tissue accumulation. These uptake interactions are retabulated as either hepatic or renal in Table III.

From a physiological perspective, the liver is significantly more massive than the kidneys: in the average man, the kidneys weigh about 150 grams each, and the liver weighs about 1.5 kilograms (55). The liver also contains more cellular space available for transporter expression, while considerable kidney mass is interstitial fluid and tubule volume. Similarly, hepatocytes are more available to drug sequestration and storage than kidney epithelial cells. Therefore, preventing drug from entering the hepatocytes will have a greater relative effect on the entire body volume of distribution than will preventing drug from entering the epithelial cells at the renal tubules. Despite substantial decreases in renal clearance and associated increases in systemic concentrations, it appears that volume change due to interactions at the kidney level is not observable.

Table 1. Summary of transporter-based interactions and their effect on volume, clearance, and half life

Drug	Interaction	V	CL	t _{1/2}	Mechanism of clearance in reference organism	Related Transporter Comments	Reference
1. Adriamycin	Verapamil	↑ 31.2% (V _{ss})	↓ 32.6%	↑ 37.7%	Metabolism > Biliary > Renal	Inhibition of P-gp efflux in humans after iv dosing; in rats, renal transport is saturable but biliary is not, indicating a transporter is involved in renal elimination	(8, 9)
2. Atorvastatin	Rifampin	↓ 94.3% (V _{ss} /F)	↓ 87.0% (CL/F)	↓ 63.1%	Metabolism	Inhibition of OATP1B1 uptake into the liver in humans after oral dosing	(10)
3. Atorvastatin	OATP1B1 reduced function allele	↓ 57.6%* (V _{areal} /F)	↓ 59.2%* (CL/F)	↔	Metabolism	Reduced OATP1B1 uptake into the liver in humans after oral dosing	(11)
4. Cefazolin	Probenecid	↔ (V ₁)	↓ 32.1%	↑ 68.8%	Renal	Inhibition of OAT-mediated uptake into the renal tubules in humans after intramuscular or iv dosing, likely OAT2, OAT3	(12-15)
5. Cerivastatin	Cyclosporine	↓ 66.7% (V ₁ /F)	↓ 73.3% (CL/F)	↔	Metabolism	Inhibition of OATP1B1 uptake into the liver in humans after oral dosing	(16, 17)
6. Ceftriaxone	Pivlisicaine	↑ 102%* (V _{ss} /F)	↓ 28.9% (CL/F); ↓ 37.6% (CL _r)	↑ 188%	Renal > Metabolism	Inhibition of P-gp efflux in humans after oral dosing, possible inhibition of OCT2 as well, but P-gp effect is more significant	(18)
7. Ciprofloxacin	Probenecid	↔ (V _{ss})	↓ 41.2% (CL); ↓ 64.0% (CL _r)	↑ 51.5%	Renal > Metabolism	Inhibition of OAT-mediated uptake into the renal tubules in humans after iv dosing	(19)
8. Colchicine	SDZ PSC 833	↔ (V _{areal})	↓ 47.0%	↔	Biliary > Renal > Metabolism	Inhibition of P-gp efflux in rats after iv dosing	(20, 21)
9. Daunomycin	Verapamil	↓ 63.7%* (V _{areal})	↓ 89.1%	↑ 232%	Metabolism > Renal ~ Biliary	Inhibition of P-gp efflux in rats after iv dosing	(22, 23)
10. Digoxin	Rifampin	↓ 70.8% (V _{ss})	↓ 54.2%	↔	Metabolism	Inhibition of Oatp1a4 uptake into the liver under Cyp3a-induced conditions in rats after iv dosing	(24)
11. Digoxin	Ritonavir	↑ 76.7% (V _{ss})	↓ 41.8%	↑ 156%	Renal	Inhibition of P-gp efflux in humans after iv dosing	(25)
12. Famotidine	Probenecid	↓ 39.5% (V ₁ /F)	↓ 64.0% (CL _r)	↔	Renal	Inhibition of uptake into the renal tubules, likely OAT3, in humans after oral dosing	(26, 27)
13. Glyburide	Rifampin	↓ 67.4% (V _{ss} /F)	↓ 54.6% (CL/F)	↔	Metabolism	Reduced OATP2B1 uptake into the liver in humans after oral dosing	(28)
14. Metformin	OCT1 reduced function allele	↓ 53.9% (V _{areal} /F)	↓ 37.5% (CL/F)	↔	Renal	Reduced OCT1 uptake into the liver in humans after oral dosing; OCT1 does not mediate elimination at the renal tubules	(29)
15. Methotrexate	Pantoprazole	↓ 21.6%* (V _{areal})	↓ 45.7%	↑ 44.4%	Biliary > Renal	Inhibition of Berp mediated efflux in mice after iv dosing	(30)
16. Penicillin G	oat3 ^{-/-} mice	↓ 33.5% (V _{ss})	↓ 44.8%	↑ 72.9%	Renal > Metabolism	Decreased renal clearance in mice after iv dosing	(31)
17. Plisicaine	Ceftriaxone	↑ 106%* (V _{ss} /F)	↓ 26.6% (CL/F); ↓ 41.3% (CL _r)	↑ 206%	Renal >> Metabolism	Inhibition of P-gp efflux after oral dosing in humans, possible inhibition of OCT2 as well, but P-gp effect is more significant	(18)
18. Plisicaine	Cimetidine	↔ (V ₁ /F)	↓ 26.4% (CL/F); ↓ 28.0% (CL _r)	↑ 24.5%	Renal >> Metabolism	Inhibition of uptake into the renal tubules, likely OCT2, in humans after oral dosing	(18, 32)
19. Pitavastatin	OATP1B1 reduced function allele	↓ 27.6% (V _{areal} /F)	↓ 44.3%* (CL/F)	↑ 29.9%	Biliary	Reduced OATP1B1 uptake into the liver in humans after oral dosing	(33, 34)
20. Procainamide	Cimetidine	↓ 12.4%* (V _{areal} /F)	↓ 30.5%* (CL/F); ↓ 43.4% (CL _r)	↑ 26.0%	Renal > Metabolism	Inhibition of OCT-mediated uptake in humans after oral dosing; not likely a gut interaction	(35, 36)
21. Procainamide	Ciprofloxacin	↔ (V _{areal} and V _{ss})	↔ (CL); ↓ 14.8% (CL _r)	↔	Renal > Metabolism	Inhibition of OCT-mediated uptake in humans after iv dosing	(35, 36)
22. Procainamide	Levofloxacin	↔ (V _{areal} and V _{ss})	↓ 17.2% (CL); ↓ 25.9% (CL _r)	↑ 18.5%	Renal > Metabolism	Inhibition of OCT-mediated uptake in humans after iv dosing	(35, 36)
23. Repaglinide	Cyclosporine	↓ 59.0%* (V _{areal} /F)	↓ 59.0%* (CL/F)	↔	Metabolism	Inhibition of OATP1B1 uptake into the liver in humans after oral dosing	(37)
24. Rosuvastatin	Cyclosporine	↓ 90.6%* (V _{areal} /F)	↓ 80.1%* (CL/F)	↓ 52.7%	Biliary >> Metabolism	Inhibition of OATP1B1 uptake into the liver in humans after oral dosing; t _{1/2} is normally reported as lower than it is in this study's baseline; decrease in t _{1/2} might be artifactual	(38)
25. Rosuvastatin	Genfibrozil	↓ 27.5%* (V _{areal} /F)	↓ 46.8%* (CL/F)	↑ 36.3%	Biliary >> Metabolism	Inhibition of OATP1B1 uptake into the liver in humans after oral dosing	(39)
26. Rosuvastatin	OATP1B1 reduced function allele	↓ 50.9%* (V _{areal} /F)	↓ 38.3%* (CL/F)	↔	Biliary >> Metabolism	Reduced OATP1B1 uptake into the liver in humans after oral dosing	(11)
27. Sotalol	Cimetidine	↔ (V _{ss})	↓ 26.7%	↔	Renal	Inhibition of OCT-mediated uptake in rats after iv dosing, likely OCT1, OCT2	(40, 41)
28. Tacrolimus	mdr1a ^{-/-} mice	↔ (V _{ss})	↓ 65.4%	↑ 99.4%	Metabolism >> Biliary > Renal	Lack of P-gp mediated biliary clearance in mice after iv dosing	(42)

Table I. (continued)

Drug	Interaction	V	CL	t _{1/2}	Mechanism of clearance in reference organism	Related Transporter Comments	Reference
29. Telmisartan	Nisoldipine	↓ 62.2% (V _{area} /F)	↓ 51.2% (CL/F)	↔	Metabolism	Inhibition of P-gp efflux of metabolites from the liver in humans after oral dosing; the majority of the effect is likely a bioavailability consideration	(43)
30. Tetracycline	Diclofenac	↔ (V _{ss})	↓ 60.7% (CL); ↓ 61% (CL _r)	↑ 88.2%	Renal	Inhibition of OAT-mediated uptake in rats after iv dosing, likely OAT1, OAT2, OAT4	(15, 44, 45)
31. Tetracycline	Naproxen	↔ (V _{ss})	↓ 75.0% (CL); ↓ 72.0% (CL _r)	↑ 400%	Renal	Inhibition of OAT-mediated uptake in rats after iv dosing, likely OAT1, OAT2, OAT4	(15, 44, 45)
32. Tezosentan	Cyclosporine	↓ 65.2% (V _{ss})	↓ 74.8%	↔	Biliary	Likely inhibition of P-gp efflux in humans after iv dosing	(46)
33. Topotecan	Probenecid	↔ (V _{ss})	↔ (CL); ↓ 29.6% (CL _r)	↔	Renal > Biliary >> Metabolism	Inhibition of OAT-mediated uptake in mice after iv dosing	(47)
34. Topotecan	Novobiocin	↑ 25.4% (V _{ss})	↑ 33.7%	↑ 341%	Renal > Biliary >> Metabolism	Inhibition of Berp efflux in rats after iv dosing	(48)
35. Ulfloxacin	Cyclosporine	↓ 31.4% (V _{ss})	↓ 32.1% (CL); ↓ 66.5% (CL _b)	↔	Biliary ~ Renal	Inhibition of Oat/Oatp-mediated uptake into the liver in rats after iv dosing	(49-51)
36. Ulfloxacin	EHBR rats	↓ 34.1% (V _{ss})	↔	↓ 35.0%	Biliary ~ Renal	Lack of Mrp2 decreases biliary excretion of glucuronide; no change in clearance or percent of dose eliminated in the bile or urine after iv dosing	(49-51)
37. Valsartan	EHBR rats	↓ 35.8% (V ₁)	↓ 94.3% (CL); ↓ 97.7% (CL _b)	↑ 1030%*	Biliary >> Metabolism > Renal	Lack of Mrp2 decreases biliary clearance, leading to increased plasma and liver concentrations after iv dosing	(52)

* indicates calculated parameter, not reported in reference

CL_r: renal clearance, CL_b: biliary clearance; EHBR: Eisai hyperbilirubinemic rats

A few further explanations for this disparity are possible. For one, if the kidney transporters were unique to the renal epithelium, while liver transporters were also expressed at other tissues in the body, inhibition of liver transporters would cause a more significant pharmacokinetic change. However, it seems like the opposite may be true: Table IV shows the tissue distribution of the transporters studied in this analysis. A second possibility is that the transporters that are relatively uniquely expressed at the liver are also more specific for their substrates, while the renal transporters act on a wider range of substrates. In that case, inhibition of a renal transporter would not have much of an effect because another transporter could restore the activity of the dysfunctional transporter. In support of this is the fact that many of the studies focused on the kidney report only that the inhibited transporter is a member of the OAT or OCT family. Further, Sweet *et al.* (56) report that the OAT transporters, which predominantly mediate clearance at the renal tubules, have significant substrate overlap. An inhibitor would then also inhibit multiple transporters. On the other hand, liver studies often report a specific transporter that is affected. Alternatively, the renal epithelium may be a “looser” barrier than the hepatocyte membranes, implying the transporters may simply have less importance at the renal epithelium. In support of this is the fact that many drugs have different permeability characteristics at the enterocytes of the intestine and at the hepatocytes; certain drugs, such as atorvastatin, may diffuse passively into the intestine, but require an uptake transporter at the liver. Thus, some drugs may also have different permeability characteristics at the renal epithelium and hepatocyte membranes. However, the measured changes in clearance contradict these possibilities: if a single transporter were less important in the kidneys, clearance, in addition to volume, would be unaffected.

Three interactions at the renal tubules presented in Table III do lead to a decrease in volume. For the interaction between procainamide and cimetidine (36), V_{area} was calculated (as indicated by the asterisk) from the reported data assuming the reported half life was the terminal half life, which may be an incorrect assumption. Moreover, as discussed, the change in V_{area} is often more extensive than the change in the other volume parameters. However, the decrease in penicillin G V_{ss} in Oat3-/- knockout mice (31) and in famotidine V₁ in healthy human volunteers upon co-administration with probenecid (26) seem to be exceptions to the trend.

Interactions with efflux transporters at the liver generally lead to a decrease in volume of distribution, while those at the renal tubules lead to an increase in volume of distribution.

Efflux transporters serve a protective purpose preventing drug distribution at some of the most sensitive tissue sites, such as the brain, lungs, and heart. They are also expressed at the liver canalicular membrane and renal epithelia to facilitate clearance. An increase in distribution volume would be expected after inhibiting an efflux transporter, by increasing penetration to tissues protected by the transporters. Table V highlights the interactions attributed to efflux transporters. Of these thirteen interactions, five lead to an increase in volume, and they are all interactions at the renal tubules. The remaining eight that do not cause a change or lead to a decrease in volume are interactions at the liver.

Table II. Effect of change in transporter function on the distribution volume parameters

	(ml/kg)	CONTROL	+RIFAMPIN	% DECREASE
DIGOXIN (Oatp1a4)	V_1	933	454	51.2
Reference (24)	V_{ss}	7140	1640	77.1
	V_{area}	7360	1790	75.7
	(L/kg)	CONTROL	+PANTOPRAZOLE	% DECREASE
METHOTREXATE (Bcrp)	V_1	417	315	24.4
Reference (30)	V_{ss}	933	651	30.3
	V_{area}	1010	689	31.9
	(ml/kg)	CONTROL	+CYCLOSPORINE	% DECREASE
ULIFLOXACIN (Oat/Oatp)	V_1	831	702	15.6
Reference (49)	V_{ss}	4450	3070	31.0
	V_{area}	4880	3320	31.9
	(ml/kg)	CONTROL	in EHBR rats	% DECREASE
VALSARTAN (Mrp2)	V_1	51.0	44.7	12.4
Reference (52)	V_{ss}	258	111	57.2
	V_{area}	420	118	71.9

The plasma concentration-time data from four studies were extracted and reanalyzed using WinNonlin version 2.1 (Pharsight Corporation, Mountain View, CA). For each study, the three measures of volume were recalculated under both control and decreased transporter functionality conditions. These calculated parameters (not those in the reference) are presented.

EHBR: Eisai hyperbilirubinemic rats

Methotrexate and topotecan are both substrates of the efflux transporter Bcrp, distributed through the blood-brain barrier, liver, and kidneys, among other tissues. When methotrexate was dosed with the Bcrp inhibitor pantoprazole in mice, V_{area} decreased by 21.6%*, clearance decreased by 45.7%, and the half life increased by 44.4%. Methotrexate is primarily cleared via the bile, where Bcrp has a modulating role (30). On the other hand, when topotecan was dosed with the Bcrp inhibitor novobiocin in rats, V_{ss} increased by 254%, clearance decreased by 33.7%, and half life increased by 341%. Topotecan is primarily eliminated unchanged in the urine, again mediated by Bcrp. In this case, the authors note that increased brain concentrations of topotecan could lead to the increased volume of distribution (48). While increased peripheral tissue distribution is likely in both cases, the effect is not apparent in the liver interaction.

Similarly, the anti-cancer agents daunomycin and adriamycin are both substrates of P-glycoprotein. As daunomycin is eliminated predominantly in the liver by metabolism (23), when dosed with the P-gp inhibitor verapamil in rats, V_{area} decreased by 63.7%*, clearance decreased by 89.1%, and half life increased by 232% (22). On the other hand, adriamycin is eliminated both through the liver and urine. In rats, Tavoloni and Guarino (9) found that urinary elimination of adriamycin is saturable, while biliary excretion is not. This indicates that P-gp may play a more important role in the kidney than the liver. Upon co-administration with verapamil in humans, V_{ss} increased by 31.2%, clearance decreased by 32.6%, and half life increased by 37.7%. Further, while the values were not reported, the authors do note that the volume of the central compartment decreased, and the volume of the peripheral compartments increased after P-gp inhibition (8). Because V_{ss} is the sum of the volumes of all the compartments, the change in central compartment volume, which most likely includes the kidneys, must be minor

compared to the increase in volume of the peripheral compartments.

Therefore, it seems that efflux transporter inhibition leads to a decrease in distribution volume for the central compartment and an increase in distribution volume for the peripheral tissue compartments. The magnitude of the increase in peripheral distribution is greater than the magnitude of the decrease in central compartment volume for a renal interaction, but it is less than the magnitude of the decrease in central compartment volume for a hepatic interaction. So, an increase in total distribution volume is evident for a kidney interaction, but a decrease in total distribution volume is evident for a liver interaction. This conclusion follows the above analysis on the difference in volume changes following uptake transporter interactions in the kidney and liver: the liver is again a greater contributor to distribution volume than the kidneys. Because peripheral distribution does not seem to be a factor in uptake interactions, it is also clear that efflux transporters play a larger role than uptake transporters outside the liver and kidney, despite the fact that uptake transporters are expressed at these other tissues.

A mechanism for a decrease in central compartment volume, however, is not immediately clear. As mentioned in the Introduction, it is possible that when the efflux transporter is inhibited, the eliminating organ is no longer quickly equilibrating with the plasma. Effectually, the plasma concentrations do not reflect the amount of drug at the elimination site because drug is now so highly sequestered in the hepatocytes. As Yates and Arundel (7) derived for a two compartment model, the steady state volume is under-predicted by the value of $V_1 \cdot \frac{k_{10}}{k_{21}}$, when elimination is actually from the peripheral compartment, where k_{10} and k_{21} are defined for the central compartment elimination model such that k_{10} is the rate constant of elimination from the central compartment and k_{21} is the rate constant for flux from the peripheral compartment back in to the central compartment.

Table III. Summary of uptake transporter-based interactions at the liver and renal tubules

Drug	Interaction	V	Reference	Drug	Interaction	V	Reference
Hepatic Interactions				Renal Interactions			
Atorvastatin	Rifampin	↓ 94.3% (V_{ss}/F)	(10)	Cefazolin	Probenecid	↔ (V_1)	(12-15)
Atorvastatin	OATP1B1 reduced function allele	↓ 57.6%* (V_{area}/F)	(11)	Ciprofloxacin	Probenecid	↔ (V_{ss})	(19)
Cerivastatin	Cyclosporine	↓ 66.7% (V_1/F)	(16, 17)	Famotidine	Probenecid	↓ 39.5% (V_1/F)	(26, 27)
Digoxin	Rifampin	↓ 70.8% (V_{ss})	(24)	Penicillin G	oat3-/- mice	↓ 33.5% (V_{ss})	(31)
Glyburide	Rifampin	↓ 67.4% (V_{ss}/F)	(28)	Plicamide	Cimetidine	↔ (V_1/F)	(18, 32)
Metformin	OCT1 reduced function allele	↓ 53.9% (V_{area}/F)	(29)	Procainamide	Cimetidine	↓ 12.4%* (V_{area}/F)	(35, 36)
Pitavastatin	OATP1B1 reduced function allele	↓ 27.6% (V_{area}/F)	(33, 34)	Procainamide	Ciprofloxacin	↔ (V_{area} and V_{ss})	(35, 36)
Repaglinide	Cyclosporine	↓ 59.0%* (V_{area}/F)	(37)	Procainamide	Levofloxacin	↔ (V_{area} and V_{ss})	(35, 36)
Rosuvastatin	Cyclosporine	↓ 90.6%* (V_{area}/F)	(38)	Sotalol	Cimetidine	↔ (V_{ss})	(40, 41)
Rosuvastatin	Gemfibrozil	↓ 27.5%* (V_{area}/F)	(39)	Tetracycline	Diclofenac	↔ (V_{ss})	(15, 44, 45)
Rosuvastatin	OATP1B1 reduced function allele	↓ 50.9%* (V_{area}/F)	(11)	Tetracycline	Naproxen	↔ (V_{ss})	(15, 44, 45)
Ulfloxacin	Cyclosporine	↓ 31.4% (V_{ss})	(49-51)	Topotecan	Probenecid	↔ (V_{ss})	(48)

For further detail, see Table I.
 * indicates calculated parameter, not reported in reference

Thus, the decrease in steady state volume might be a consequence of the pharmacokinetic calculations and may not reflect a “real” volume change. It remains to be elucidated in which cases the central compartment elimination model does not hold, as it is foreseeable that uptake transporter dysfunction will also change the equilibration properties of the eliminating organs.

The primary location of the interaction (liver or kidneys) is a more important determinant of the change in distribution volume than the secondary change in tissue distribution is, as evidenced by interactions that affect the integrity of the blood-brain barrier.

While transporters function at almost all the major tissues in the body, including the heart, lungs, and muscle, they have been most studied, beside the liver and kidney, at the blood-brain barrier. Here, efflux transporters dominate, where they serve to protect the brain from xenobiotic penetration. Table VI highlights interactions that are associated with an increased distribution of drug to the brain. These five interactions involve P-gp and BCRP, the two transporters most highly implicated in maintaining the integrity of the blood-brain barrier. Despite the increase in brain concentrations in these five studies, there is no common increase in volume of distribution. Instead, it appears the trends discussed above for efflux transporters, that interactions attributed to the renal transporters lead to an increased volume, while interactions at hepatic transporters lead to either a decrease or no change in volume of distribution, generally hold true.

At the kidney tubules, as discussed, when the Bcrp substrate topotecan was dosed with novobiocin in rats, volume of distribution increased 254%, with increased distribution to the brain (48). At the liver, tacrolimus, a P-gp substrate, was dosed to wild-type and *mdr1*-/- (P-gp knockout) mice. In these mice, there was no significant change in volume, clearance decreased 65.4%, and half life increased 99.4% as compared to wild-type mice. Knockout mice also exhibited a 33-fold increase in brain concentrations of tacrolimus. Minor increases in liver concentrations were also evident. In mice, tacrolimus is predominantly excreted in the bile (42). Finally, tezoseptan, also eliminated into the bile, was also dosed with cyclosporine for inhibition of P-gp in humans. In this study, volume of distribution decreased 65.2%, clearance decreased 74.8%, and half life did not change. The authors note that an increased incidence of adverse events, including headache, hot flushes, and nausea, may have been caused by increased brain distribution of the drug (46).

Thus, while brain distribution may change, even dramatically as in the case of tacrolimus in P-gp knockout mice, these changes do not necessarily manifest in a total body volume of distribution change. It is possible, however, that changes at the other tissues expressing transporters might offer a different conclusion.

It is possible to predict the direction of the change in pharmacological effect given the mechanisms of action of the drug and the location of the interaction.

Glyburide, metformin, and atorvastatin are substrates for uptake transporters in the liver. Following uptake inhibition either via polymorphism or concomitant medication, subjects in the three studies exhibit significantly reduced distribution volumes. However, the direction of the resulting change in pharmacological effect is different.

Table IV. Tissue distribution of transporters for which volume modulation has been investigated

Transporter	Species	Tissue Expression	Reference
A. Efflux			
P-gp	Human	adrenal, sweat glands, blood vessels, liver, kidney, lung > muscle, mammary glands, spleen, gall bladder, heart	(57)
P-gp (mdr1a/b)	Rat	brain > kidney > lung > liver	(3)
P-gp (mdr1a)	Mouse	adrenal > placenta > kidney, heart > liver, uterus, muscle, spleen, brain, lung	(58)
Mrp2	Rat	liver > kidney, brain	(4)
Bcrp	Rat	kidney > liver > gonads > brain > thymus, spleen	(59)
Bcrp	Mouse	kidney > liver > gonads > brain > spleen, muscle, lung	(59)
B. OATPs			
OATP1B1	Human	liver	(54)
OATP2B1	Human	liver, placenta, ciliary body	(54)
Oatp1a4	Rat	liver, brain; eye	(45, 54)
C. OATs			
OAT1	Human	kidney > brain	(45)
OAT2	Human	liver > kidney	(60)
OAT3	Human	kidney > brain,	(45)
OAT4	Human	kidney; placenta	(45, 61)
Oat1	Rat	kidney > brain,	(45)
Oat2	Rat	liver > kidney	(45)
Oat3	Rat	liver ~ kidney, brain; eye	(45, 56, 61)
Oat4	Rat	kidney, likely placenta	(62)
Oat1	Mouse	kidney > brain	(45)
Oat2	Mouse	kidney > liver (female > male)	(63)
Oat3	Mouse	kidney > brain	(56)
Oat4	Mouse	kidney, placenta	(62)
D. OCTs			
OCT1	Human	liver > kidney	(45)
OCT2	Human	kidney > brain	(45)
OCT3	Human	liver > kidney > brain	(45)
Oct1	Rat	kidney, liver > brain	(45)
Oct2	Rat	kidney	(45)
Oct3	Rat	kidney, brain	(45)

Glyburide is a hypoglycemic agent indicated for patients with type 2 diabetes. Its main effect is at the pancreatic beta cells, where it stimulates insulin secretion. It is primarily eliminated via metabolism by CYP2C9 and, to a lesser degree, by CYP3A4 in the liver. It is a substrate for uptake mediated by OATP2B1 at the hepatocytes, and subjects show a decrease in

steady state volume of 67.4%, a decrease in clearance of 54.6%, without a change in half life, following uptake inhibition via concomitant dosing with rifampin. As would be predicted, inhibition of liver uptake decreases elimination, increasing plasma concentrations. This increases pancreatic beta cells' access to the drug, increasing the pharmacologic

Table V. Summary of efflux transporter-based interactions at the liver and renal tubules

Drug	Interaction	V	Reference	Drug	Interaction	V	Reference
Hepatic Interactions				Renal Interactions			
Colchicine	SDZ PSC 833	↔ (V_{area})	(20, 21)	Adriamycin	Verapamil	↑ 31.2% (V_{ss})	(8, 9)
Daunomycin	Verapamil	↓ 63.7%* (V_{area})	(22, 23)	Cetirizine	Pilsicainide	↑ 102%* (V_{ss}/F)	(18)
Methotrexate	Pantoprazole	↓ 21.6%* (V_{area})	(30)	Digoxin	Ritonavir	↑ 76.7% (V_{ss})	(25)
Tacrolimus	mdr1a-/- mice	↔ (V_{ss})	(42)	Pilsicainide	Cetirizine	↑ 106%* (V_{ss}/F)	(18)
Telmisartan	Nisoldipine	↓ 62.2% (V_{area}/F)	(43)	Topotecan	Novobiocin	↑ 254% (V_{ss})	(48)
Tezosentan	Cyclosporine	↓ 65.2% (V_{ss})	(46)				
Ulfloxacin	EHBR rats (Mrp2-/-)	↓ 34.1% (V_{ss})	(49-51)				
Valsartan	EHBR rats (Mrp2-/-)	↓ 35.8% (V_1)	(52)				

For further detail, see Table I.

* indicates calculated parameters, not reported in reference

EHBR: Eisai hyperbilirubinemic rats

Table VI. Summary of transporter-based interactions at the blood-brain barrier

Drug	Interaction	V	Reference
Colchicine	SDZ PSC 833	↔ (V_{area})	(20, 21)
Rhodamine-123	Cyclosporine	↔ (V_1)	(64, 65) [‡]
Tacrolimus	mdr1a-/- mice	↔ (V_{ss})	(42)
Tezosentan	Cyclosporine	↓ 65.2% (V_{ss})	(46)
Topotecan	Novobiocin	↑ 254.5% (V_{ss})	(47)

For further detail, see Table I.

[‡]Not included in Table I. Interaction resulted in increased distribution to the brain due to P-gp inhibition in rats, without significant effect on volume or clearance after iv dosing. Because the interaction did not result in any pharmacokinetic change, it has not been included in Table I.

effect. Following a single dose of glyburide and a single dose of rifampin, subjects exhibited significantly decreased blood glucose AUCs over a twelve hour period (28).

Similarly, metformin is the first line therapy for patients with type 2 diabetes. Its pharmacological effect is in the liver hepatocytes, where it prevents gluconeogenesis, effectively decreasing blood glucose levels. It is primarily eliminated via excretion at the renal tubules, a process mediated by OCT2. However, at the liver, it is a substrate for uptake by OCT1. In this unique case, the transporter interaction is not at the primary site of elimination, but because the drug is a substrate for hepatic uptake and it is highly distributed to the liver, the interaction still causes marked pharmacokinetic changes. This further attests to the importance of the liver in the determination of distribution volume in consideration of transporter dysfunction. In patients with polymorphisms in one of their OCT1 alleles, volume of distribution is decreased by 53.9% and clearance is reduced by 37.5%, without a change in half life (29). In this case, a reduced function OCT1 allele decreases hepatocyte access to the drug, decreasing the pharmacologic effect.

Along the same lines, Pasanen *et al.* (11) and Tachibana-Iimori *et al.* (66) both studied a single nucleotide polymorphism (SNP) at position 521 in the SLCO1B1 (OATP1B1) gene. Both groups looked at atorvastatin, among other statins, to measure the effect of this polymorphism. Atorvastatin is primarily metabolized by CYP3A4 and by CYP2C9 to a lesser extent, and it is a substrate for OATP1B1 uptake. The drug and its active metabolites are less than 1% excreted in the urine (10). The statins, HMG-CoA reductase inhibitors,

decrease cholesterol levels by preventing cholesterol synthesis and increasing clearance of LDL, or “bad”, cholesterol at the hepatocytes. From the pharmacokinetic perspective, Pasanen *et al.* (11) showed a decrease in V_{area} of 57.6%* and a decrease in clearance of 59.2%*, without a change in half life, between patients homozygous for either the wild-type or mutant alleles. From a pharmacodynamic perspective, Tachibana-Iimori *et al.* (66), showed the same patterns held for patients beginning atorvastatin, pravastatin, or simvastatin therapy, all OATP1B1 substrates. Analysis of patients on any of these three drugs found that patients homozygous for the wild-type alleles showed a decrease in total cholesterol of 22.3%, while patients heterozygous for the wild-type and polymorphic allele showed a decrease in total cholesterol of 16.5%, indicating a decreased pharmacological benefit. The difference between wild-type and polymorphic patients is likely to be greater for patients homozygous for the mutant alleles.

In this regard, while the pharmacokinetic consequences of an interaction are important for the clinician to understand, the pharmacodynamic change is also critical to consider before changes to the dosing regimen are made. Within these three examples, although the direction of pharmacokinetic change is the same, a glyburide-rifampin interaction would require a decreased dosing rate to maintain the same pharmacological effect, while patients with polymorphisms in OCT1 or OATP1B1 would require an increased dosing rate of metformin or atorvastatin, respectively, to maintain effect. The potential for toxicity when higher dosing rates are required complicates this issue, and may lead to alternative therapies for patients with such pharmacogenetic variation.

EXPERIMENTAL CONSIDERATIONS

As with any pharmacokinetic study, it is important to understand the experimental conditions and variability that complicate the conclusions that are drawn from transporter interaction studies.

First, there are wide interspecies differences in drugs' elimination pathways, the expression of transporters, and transporter substrate profiles. For instance, as noted above, digoxin is almost completely metabolized in rats, where it is a substrate for uptake mediated by Oatp1a4 at the liver (24). In humans, however, digoxin is predominantly eliminated in the urine. Similarly, as shown in Table IV, in humans OAT3 is expressed primarily at the kidney, and, to a lesser degree, in the brain, while in rats Oat3 is highly expressed in the liver in addition to the kidneys and brain. For substrates common to

Table VII. V_{ss} values from *ex situ* studies in rat

Interaction	Transporter	Organ	V_{ss} Change	Prediction from Trends	Reference
Digoxin+Rifampin	Oatp1a4 uptake	Liver	↓ 17.9%	↓	(68)
Quinapril+PAH	Oat uptake	Kidney	↓ 13.7%*	↔, (↓)	(69)
<i>Digoxin+Quinidine</i>	P-gp efflux	Liver	↑ 95.9%	↓	(70)
<i>Tacrolimus+GG918</i>	P-gp efflux	Liver	↑ 30.1%	↓	(70)
<i>Talinolol+GG918</i>	P-gp efflux	Liver	↑ 74.2%	↓	(70)
<i>Doxorubicin+GG918</i>	P-gp efflux	Liver	↑ 70.2%*	↓	(71)

Italicized interactions are those where the data does not match the prediction from the present analysis.

* indicates calculated parameters, not reported in reference

PAH: p-aminohippurate, GG918: GF120918 (Elacridar)

	Kidney	Liver
Uptake	↔, (↓)	↓
Efflux	↑	↓, ↔

Fig. 1. Summary of trends for predicting effects of transporter inhibition/dysfunction on volume of distribution, depending on whether it is an uptake or efflux transporter and the primary location of the interaction

both rat and human Oat3/OAT3, this will most likely lead to different tissue distributions and volume calculations between the two species, and indicates that results from an Oat3 focused pharmacokinetic study conducted in rats may not scale to humans. Finally, the interaction between famotidine and probenecid (26) resulting from the inhibition of OAT3 transport at the renal tubules in humans is not reproducible in rats (67). This is likely due to the increased expression of Oct1 in the rat kidney: because famotidine is also a substrate of Oct1 and probenecid does not inhibit Oct1, the transporter serves as an alternate, compensatory route for renal clearance in rats concomitantly dosed with famotidine and probenecid (13). Briefly, these few examples attest to the importance of considering interspecies differences before clinical extrapolations are made from animal data.

Further, while clearance is relatively easily extrapolated from *in vitro* data to *in vivo* relevance, the same is not true of volume of distribution. Because volume is focused on the entire body, even *ex situ* techniques, such as the isolated perfused rat liver (IPRL) or isolated perfused rat kidney, can lead to incorrect approximations of the direction of volume changes. Table VII highlights these discrepancies. Although the published data is sparse, it appears the *ex situ* results for inhibited uptake transporters in the liver and kidney follow the analysis above (68, 69). However, the IPRL data for inhibited efflux transporters, in particular P-glycoprotein, show an increase in steady-state volume of distribution (70, 71), while the *in vivo* trend predicts either a decrease or no change in this parameter. It is of interest to note the *ex situ* data follow what would be generally predicted for uptake and efflux transporter inhibition before the conclusions of the present analysis. A mechanism for this discrepancy remains to be elucidated.

CONCLUSIONS

Through the above analysis, we show that active drug transporters that modulate tissue distribution act as modifiers of distribution volume. Because transporters can be significantly affected by drug-drug interactions or genetic polymorphisms, changes in drug transporter activity as they affect distribution volume require attention. The above analysis indicates that it is the primary location of the interaction, at the kidneys or the liver, that serves as the major predictor of change in distribution volume. Figure 1 summarizes the trends in effects of transporter dysfunction on distribution

volume as discussed above. As knowledge pertaining to the location and function of drug transporters and the substrate status of drugs for these transporters becomes more available, the present analysis provides a framework for understanding future pharmacokinetic interactions rooted in active drug transporters.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grants GM61390 and GM75900, as well as by an unrestricted grant from Amgen, Inc.

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