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Impact of Hepatic Uptake Transporters on Pharmacokinetics and Drug−Drug Interactions: Use of Assays and Models for Decision Making in the Pharmaceutical Industry

Mathew G. Soars, Peter J. H. Webborn, and Robert J. Riley*

Department of Discovery DMPK, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, Leicestershire LE11 5RH, U.K.

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Abstract: The ability to predict hepatic metabolic clearance is a key component in the design and selection of small molecule drug candidates within the pharmaceutical industry. The recognition that metabolism−transporter interplay can influence hepatic metabolic clearance has presented new challenges, both in terms of the creation of experimental systems suitable for an industry setting and also in developing an understanding of the pharmacokinetic concepts that underpin them. This paper reviews the pharmacokinetic principles that govern the kinetics of uptake transporter substrates. In addition, new data are presented from a range of test systems for assessing hepatic drug clearance and the impact of drug−drug interactions (DDIs).

Keywords: Transporter; hepatic; prediction; uptake; OATP1B1; modeling; review; hepatocytes

1. Introduction

The liver is known to play a key role in drug elimination through both metabolism and the transporter-mediated secretion of drugs and/or metabolites into bile. An additional level of complexity was introduced when it was established that transporter-mediated uptake of drug from plasma could influence the overall rate of elimination.[1,2] This was most clearly observed for pravastatin, a compound eliminated predominantly via biliary secretion, for which hepatic uptake was shown to be the rate-determining step in its elimination in the rat.[3] Clearly there is interplay between these processes, with transporters either delivering compounds to, or removing compounds from, the site of metabolism[4] or biliary secretion.

One measure of the level of understanding of complex biological processes is the ability to accurately predict in vivo behavior from in vitro data. Understanding is encapsulated in the mathematical models used to integrate the in vitro parameters and link them quantitatively to their in vivo counterparts. Potentially a more important reflection of this understanding is the selection of appropriate in vitro experimental test systems and description of their capabilities and limitations.

Prediction of hepatic clearance from a variety of in vitro systems has formed a cornerstone of the design and selection of small molecule drug candidates within the pharmaceutical industry for more than a decade. For compounds whose clearance is predominantly via hepatic metabolism the approaches taken have been relatively successful,[5−9] although not without their failures.[9,10] The realization that hepatic transporters may add additional layers of complexity

* Corresponding author: Dr R J Riley, Department of Discovery DMPK, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, Leicestershire LE11 5RH, U.K. E-mail: rob.riley@astrazeneca.com. Phone: +44 (0)1509 64 4039. Fax: +44 (0)1509 64 5576.

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has added to the challenge in terms of both the modeling required[11,12] and the in vitro assays utilized.[10] This has allowed the opportunity to reassess compounds for which clearance was poorly predicted via more conventional methods.

The aim of this publication is to review the basic concepts and approaches for modeling hepatic metabolism involving transporters contrasted with the simpler case, where there is no transporter involvement. Although efflux transporters may also be pivotal in the hepatobiliary disposition of some compounds, this review focuses on recent breakthroughs in the hepatic uptake area. Reference will also be made to the different formats of assays available, with particular emphasis on the use of suspended hepatocytes. These assays will also be discussed in terms of their practical application within a screening strategy for the pharmaceutical industry.

2. The Role of Metabolism and Transporter Interplay in the Prediction of Hepatic Clearance

2.1. Basic Concepts Underlying the Prediction of Hepatic Clearance from in Vitro Data. The complexities of modeling metabolism−transporter interplay are best understood by comparison with standard models for predicting hepatic metabolic clearance, without the involvement of transporters. All models of drug elimination are based on the concept of drug clearance. Clearance is the parameter that relates the drug concentration to the rate of elimination, and is the best measure of an eliminating process (as opposed to the half-life, for example). Plasma clearance (CL_p) relates the plasma concentration to the rate of elimination from the body. Clearance has units of flow (e.g. mL/min) and can be contextualized by expressing it as a fraction of the blood flow to the eliminating organ, i.e. the extraction ratio. A more fundamental parameter is the intrinsic clearance (CL_{int}), which relates the rate of elimination to the free drug concentration at the site of elimination. Intrinsic clearance estimates form the basis of all in vitro−in vivo clearance predictions.

Classically, CL_{int} is determined from estimation of the rate of appearance of a metabolite using either the relationship

$$CL_{int} = \frac{V_{max}}{K_m + [S]_{free}} \quad (1)$$

or

$$CL_{int} = \frac{V_{max}}{K_m} \quad (2)$$

if $K_m \gg [S]_{free}$, or alternatively

$$CL_{int} = \frac{v}{[S]_{free}} \quad (3)$$

again, if $K_m \gg [S]_{free}$.

In drug discovery is has become accepted that the overall CL_{int} for all metabolic processes can be obtained by monitoring loss of the starting material predominantly using human liver microsomes[13] or hepatocytes.[7] Using this approach CL_{int} may also be obtained from the half-life of the starting material, using the relationship between clearance, half-life and volume.

$$CL_{int} = \frac{0.693V}{T_{1/2}} \quad (4)$$

It has become commonplace to use the term CL_{int}, for in vitro systems, without correction to obtain the value based on unbound drug and the term $CL_{int,ub}$ for corrected data. More recently, Paine et al.[14] have proposed the term "clearance from an incubation" (CL_{inc}) for in vitro data uncorrected for incubational binding, with CL_{int} used for the binding corrected data. For complex systems that may involve multiple processes e.g. uptake and metabolism, it is also necessary to specify the clearance process, i.e. $CL_{int,uptake}$ and $CL_{int,met}$, respectively (see section 2.5).

In order to convert a CL_{int} measured in vitro into an in vivo plasma clearance, a mathematical model of the liver is required that incorporates the intrinsic clearance, plasma protein binding and hepatic blood flow. A variety of liver models are available (e.g. well-stirred, parallel tube, dispersion)[15] all of which have two common features. First they

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are constructed to reflect the fact that the clearance is ultimately limited by the liver blood flow. Second, if a compound is eliminated by the liver, there is a concentration gradient within the liver. The simplest model is the "well-stirred" model, and in this model it is assumed that blood entering the liver instantly equilibrates with the venous blood. The other models incorporate mechanisms for introducing a concentration gradient within the liver. The effect of this is that higher concentrations and therefore higher plasma clearances are achieved using these more complex models, although this effect is only significant for high extraction compounds. The parallel tube model has the advantage of using the same number of parameters as the well-stirred model; the dispersion model is more complex. More physiological models incorporating aspects of hepatic anatomy may be used[16] but require an extra level of parameter estimation. In this publication the well-stirred model is used to illustrate the principles involved in understanding metabolism−transporter interplay.

The key difference between a simple model where the rate of metabolism is the determinant of the clearance and more complex models that can incorporate uptake transporters is whether it can be safely assumed that permeation is so rapid that the free intracellular concentration is equivalent to that in plasma at all times. The majority of oral drugs, because they require reasonable absorption from the gastrointestinal tract, are at least moderately lipophilic and permeable to cell membranes.[17] For this reason, the assumption is often valid for oral drugs and a simplified version of the well-stirred model can be applied.[18] In pharmacokinetic terms such liver models are termed "perfusion limited", as opposed to "diffusion limited". The key distinction is that in the perfusion limited model the rate of permeation through the hepatocyte cell membrane is high enough that, irrespective of the rate of metabolism, the free intracellular concentration does not fall below that in plasma, and the free plasma concentration is therefore an accurate reflection of the free concentration within hepatocytes. In diffusion limited models a degree of impermeability is assumed and models can describe events at the cell membrane (see section 2.5).[16]

The essence of "interplay" between hepatic metabolism and uptake with respect to drug elimination is evidenced when the net effect is to elevate the free intracellular concentration significantly above that in plasma. In simple terms, if the overall effect of the processes removing compounds from the hepatocytes (metabolism and diffusion out of the cell) and the processes adding to the cell content (active uptake and passive diffusion) is to double the free intracellular concentration ("seen" by the enzyme), relative to that in plasma, then the overall rate of metabolism will be doubled (see section 2.5.1, eq 9).

2.2. Application of Models for Prediction of Hepatic Metabolic Clearance. Methods for predicting the in vivo clearance of new chemical entities (NCEs) have progressed considerably since the seminal work of Rane et al.,[19] over thirty years ago. However, it was almost twenty years after the work of Rane that a detailed strategy was proposed for incorporating in vitro data generated using either liver microsomes or hepatocytes into liver models to obtain a prediction of in vivo clearance.[18]

While early reports[18,20] produced relatively successful predictions of in vivo hepatic metabolic clearance, the data sets were composed of predominantly neutral and basic drugs with clearance processes mediated by the cytochrome P450 (CYP) family. In addition, early studies advocated the omission of plasma protein binding in such predictions.[21] Subsequent studies have investigated drugs with more diverse physicochemical properties, highlighting the importance of plasma protein binding in clearance predictions.[13] However the use of plasma protein binding alone has been shown to underpredict the hepatic metabolic clearance of acidic drugs significantly.[22] Further investigation into the impact of incubational binding on clearance predictions has illustrated the critical role of this parameter.[23−26] Each refinement of the models used for clearance prediction has reflected an increased mechanistic understanding of this complex elimination process. This has resulted in several recent studies highlighting accurate predictions of human hepatic metabolic clearance for large, diverse data sets of drugs using a unified

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approach.[8,9,27] In addition to improving methodology, these studies have correlated data as unbound in vitro and in vivo CL_{int} measurements rather than predicted and observed CL estimates producing a more sensitive analysis of a linear relationship (recognizing the errors where clearance approaches blood flow, Q_h).

While the majority of studies have focused on drugs metabolized by phase I processes, several laboratories have also reported the successful use of hepatocytes in predicting the in vivo clearance of drugs primarily cleared by the key phase II enzyme family, uridine diphosphate glucuronosyltransferases (UGTs).[5,28] By contrast, microsomal studies have produced a significant underprediction of drug glucuronidation in vivo.[5,29,30] Interestingly, recent investigations have suggested that microsomal preparations contain long-chain fatty acids which inhibit the activity of UGT1A9 and UGT2B7.[31,32] Serum albumin has been used to sequester these inhibitory factors and in turn enhance the clearance by UGT1A9 and UGT2B7 by reducing the unbound K_m. The application of this technique to predict successfully the in vivo glucuronidation rates of a limited set of drugs has been published recently.[33]

Undoubtedly, the techniques highlighted above have enabled the pharmaceutical industry to produce accurate predictions of human hepatic metabolic clearance of drugs, to the extent that certain investigators have even suggested that significant improvements to current methods are unlikely and that a more prudent approach would be to now expedite the first dose to humans. However, Figure 1A shows the prediction of rat in vivo $CL_{int,ub}$ data over the last two years in the authors' laboratory. While ~80% of the NCEs tested predicted in vivo $CL_{int,ub}$ well using conventional rat hepatocyte assays, up to 20% significantly underpredicted rat in vivo $CL_{int,ub}$. A more detailed analysis of the outliers showed the NCEs to be organic anions or zwitterions (log $D_{7.4}$ range −0.2 to 3.5) from three distinct projects. It was therefore hypothesized that these compounds were substrates for hepatic uptake transporters. Work was subsequently initiated to develop a high-throughput hepatocyte assay to screen these compounds (see section 2.4.1).[10]

2.3. Hepatic Uptake Transporters and Key Substrates. The role that hepatic uptake transporters play in drug disposition has received considerable attention recently.[1,2,34−36] The majority of hepatic drug uptake is mediated by the solute carrier superfamily comprising the sodium-dependent taurocholate cotransporting polypeptide (NTCP), the organic anion transporting polypeptides (OATPs), the organic anion transporters (OATs) and the organic cation transporters (OCTs/OCTNs).

While NTCP plays a pivotal role in the sodium-dependent transport of both conjugated and unconjugated bile acids,[37,38] this transporter also catalyzes the transport of several nonbile acid substrates such as thyroxine[39] and estrone-3-sulfate.[38] However, the relative importance of NCTP in the transport of drugs is considered more limited.[40−42]

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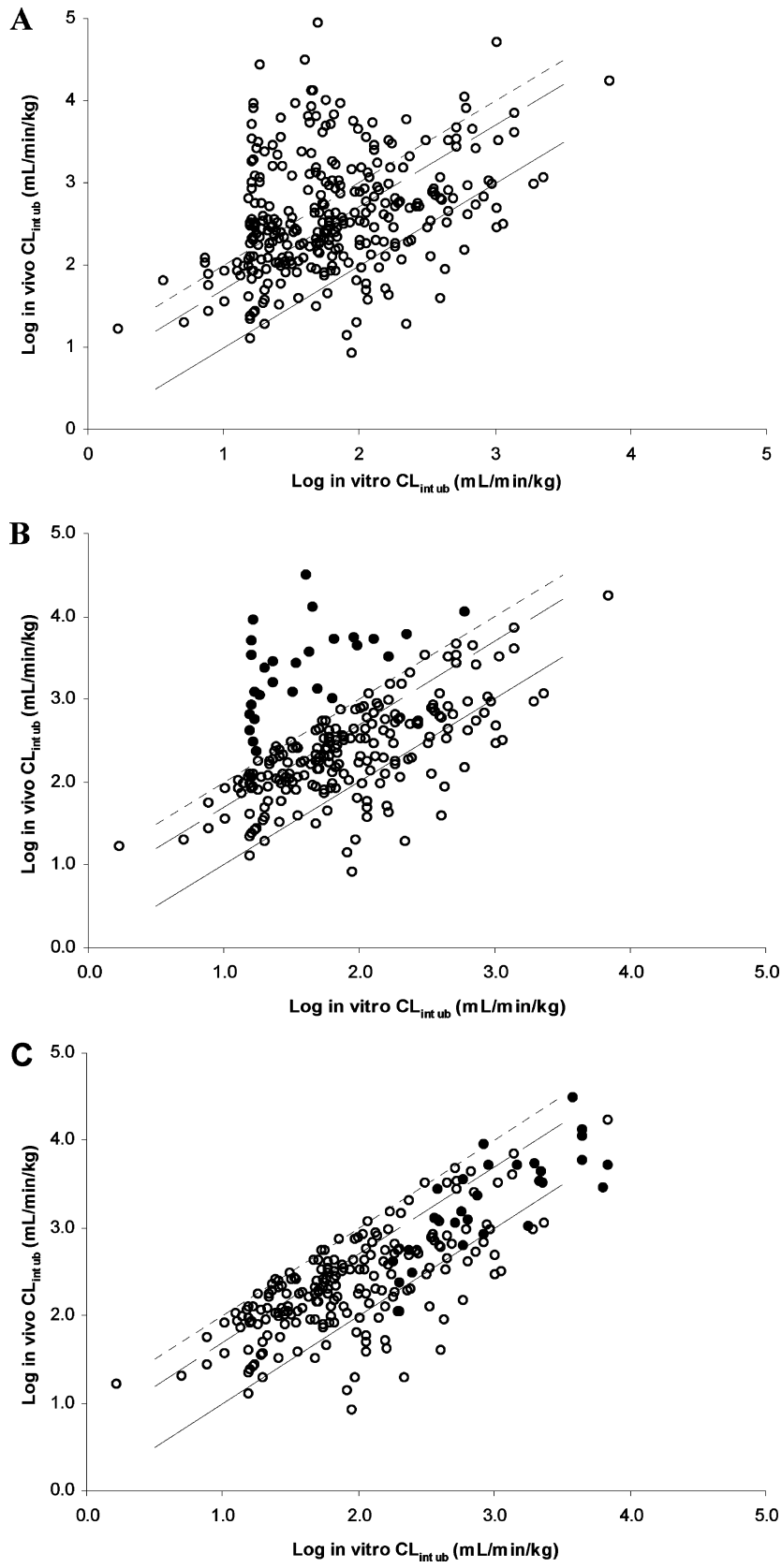

Figure 1. Prediction of in vivo $CL_{int,ub}$ for 200 AZ compounds using (A) conventional rat hepatocyte assay, (B) with subset of outliers highlighted (closed circles) and (C) analysis of outliers (closed circles) using media-loss assay. Solid lines represent line of unity and feint, dotted lines = 5- and 10-fold errors.

The structure and function of the members for the OATP family have been elucidated recently.[43] To date there have been 11 functional human OATP transporters identified,[43] however only 3 isoforms play a significant role in hepatic drug uptake (OATP1B1, OATP1B3 and OATP2B1). These key isoforms transport drugs from a wide range of therapeutic classes including the 3-hydroxymethylglutaryl-CoA reductase inhibitors (e.g. atorvastatin and pitavastatin), angiotensin II receptor antagonists (olmesartan and valsartan), angiotensin-coverting enzyme inhibitors (enalapril and temocaprilat), the H_1-receptor antagonist fexofenadine and the endothelin receptor antagonist, bosentan.[2,44]

The OAT family also facilitates the hepatic transport of anionic drugs. Although six functional human OAT transporters have been identified, OAT1, OAT3 and OAT4 play a primary role in renal transport with only OAT2 and OAT7 expressed significantly in the liver.[45−47] While OAT7 has a narrow substrate specificity mainly encompassing sulfate conjugates,[48] OAT2 has been shown to transport several drugs including methotrexate and salicylate.[47]

The transport of cationic drugs is predominantly catalyzed by the OCTs. Thus far, three human OCT isoforms have been extensively characterized (OCT1−3).[49] OCT1 is mainly expressed in the liver and transports drugs including the antidepressant desimipramine and antivirals such as acyclovir.[50] OCT2 is highly expressed in the kidney whereas OCT3 has a broad expression profile including skeletal muscle, liver, kidney and heart.[49,50] Both OCT2 and OCT3 have overlapping substrate specificities with OCT1 however Hayer-Zillgen et al.[51] have suggested "selective" inhibitors to discriminate the functional importance of the OCT isoforms.

The substrate specificity of the major members of the solute carrier superfamily described above has been generated using a host of experimental systems ranging from *Xenopus laevis* oocytes injected with complementary RNA[48,52,53] to stable transfection of the relevant transporters in mammalian cells lines.[54−56] These data have been useful in highlighting "selective" substrates/inhibitors for the main OATP, OAT and OCT members.[44,47,49,51] Subsequently, a relative activity factor (RAF) approach, used so successfully for CYPs,[57−59] has been adapted for Ntcp/Oat/OATPs/OCTs.[55,60−62] In particular the use of estrone-3-sulfate as a probe for OATP1B1 and CCK-8 for OATP1B3 has been invaluable in assessing the relative roles of these isoforms in the hepatic

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(62) Umehara, K.-I.; Iwatsubo, T.; Noguchi, K.; Kamimura, H. Functional involvement of organic cation transporter 1 (OCT1/oct1) in the hepatic uptake of organic cations in humans and rats. *Xenobiotica.* **2007**, *37*, 818–831.

uptake of pitavastatin,[55] fexofenadine,[63] and rosuvastatin.[56] Although estrone-3-sulfate is selective for OATP1B1 at low substrate concentrations, other transporters (eg OATP1B3)[64] contribute to its uptake which could result in an inaccurate assessment. Use of the RAF approach with transporter data merits further investigation, including evaluation of selective probes, although ultimately this may be superseded by methods based on yet to be determined transporter expression levels.[65]

Studies with the complex, specialized sandwich culture model may be used to estimate and compare in vivo biliary clearance of compounds and to characterize transport proteins responsible for their hepatic uptake and excretion.[66] The impact of the mechanistic interplay between uptake and efflux transporters and metabolism has also been investigated in studies using suspended hepatocytes.[67] Consequently, isolated hepatocyte suspensions may well provide the easiest and most direct system for studying the importance of incorporating hepatic uptake into assessments of drug clearance.[65] To date, the majority of studies using hepatocytes have determined drug uptake by monitoring the appearance of radiolabeled substrates in cells following a centrifugation step through silicone oil.[55,56,63] While this method is suitable for detailed, mechanistic studies with "probes" or advanced (early development) compounds, it is clearly not amenable for routine use within early drug discovery where higher-throughput to drive structure−activity relationships (SAR) is required and radiolabeled compound is often not available. To this end, we have developed assays that monitor either the rapid disappearance of compound from the media or appearance in cells via an LC/MS/MS end point.[10,14] These two formats enable simultaneous fitting of media and cell data over a longer time course to describe the full disposition of compounds in the system. The application of the different hepatocyte uptake assays within drug discovery and development will be discussed herein (see section 2.4).

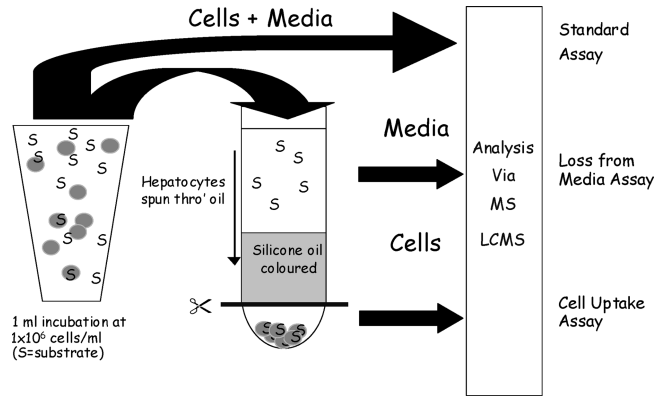

Figure 2. Illustration of assay formats based on suspended hepatocytes.

2.4. Hepatocyte Based Uptake Transporter Assays. *2.4.1. "Media-Loss" Assay.* While hepatocytes have been used to successfully generate intrinsic clearance (CL_{int}) estimates for the subsequent prediction of in vivo metabolic drug clearance (see section 2.2), the use of this in vitro system for hepatic uptake studies has been more limited (see section 2.3). The significant underprediction of in vivo $CL_{int,ub}$ obtained for a subset of compounds using conventional hepatocyte incubations (see Figure 1B, closed circles) and their physicochemical properties (anionic and zwitterionic chemistry) suggested that hepatic uptake may be involved in their clearance. To this end, the media-loss assay was developed.[10]

For conventional hepatocyte studies, homogeneous samples of the hepatocyte/drug incubation are taken over time and the concentration of drug is determined often by LC/MS/MS (see Figure 2). This concentration−time data can then be used to calculate the CL_{int} (see eq 4). Media-loss assays are conducted exactly as conventional hepatocyte assays with the exception that, once a homogeneous sample has been taken from the incubation, it is immediately centrifuged and the resultant supernatant quenched (see Figure 2). The decreasing concentration of drug in the media over time (0−6 min) driven by the uptake of drug into the cell can then be determined and an estimate of $CL_{int,uptake}$ calculated. The application of the media-loss assay is highlighted in Figures 1B and 1C. $CL_{int,uptake}$ can be used to predict unbound plasma clearance (or its in vitro equivalent $CL_{int,ub}$) when passive permeability is low (see section 2.5). While in vitro $CL_{int,ub}$ estimates obtained from conventional hepatocyte assays significantly underpredicted in vivo $CL_{int,ub}$ for several anionic and zwitterionic series of compounds (see Figure 1B closed circles), in vitro $CL_{int,ub}$ estimates determined using the media-loss assay for this subset were more consistent with in vivo data (see Figure 1C closed circles). Therefore a combination of conventional and media-loss hepatocyte assays can provide accurate predictions of in vivo clearance in both preclinical species and humans for the majority of chemistry encountered in early drug discovery (see Figure

(63) Shimizu, M.; Fuse, K.; Okudaira, K.; Nishigaki, R.; Maeda, K.; Kusuhara, H.; Sugiyama, Y. Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab. Dispos.* **2005**, *33*, 1477–1481.

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(65) Soars, M. G.; McGinnity, D. F.; Grime, K.; Riley, R. J. The pivotal role of hepatocytes in drug discovery. *Chem. Biol. Interact.* **2007**, *168*, 2–15.

(66) Abe, K.; Bridges, A. S.; Yue, W.; Brouwer, K. L. In vitro biliary clearance of angiotensin II receptor blockers and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in sandwich-cultured rat hepatocytes: comparison with in vivo biliary clearance. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 983–990.

(67) Lam, J. L.; Okochi, H.; Huang, Y.; Benet, L. Z. *In vitro* and *in vivo* correlation of hepatic transporter effects on erythromycin metabolism: characterizing the importance of transporter-enzyme interplay. *Drug Metab. Dispos.* **2006**, *34*, 1336–1344.

Table 1. Use of Media-Loss and Oil-Spin Methodology To Assess Bosentan Uptake with Varying BSA Concentrations in Rat Hepatocytes

	$CL_{int,uptake}$[a] (μL/min/10^6cells)	
[BSA] %	media loss	oil spin
0.05	96 ± 41	73 ± 13
0.1	61 ± 43	67 ± 16
0.5	53 ± 13	58 ± 21
1	45 ± 22	47 ± 10
3	29 ± 22	28 ± 1

[a] Values represent mean ± SD of three individual experiments.

1C).[10] A clear understanding of mechanism of clearance (uptake vs metabolism) in addition to an accurate assessment of in vivo clearance in preclinical species will add significant confidence when predicting human in vivo clearance.[27]

2.4.2. Oil-Spin Assay. While the media-loss assay provides a rapid initial assessment of hepatic uptake suitable for early drug discovery, the oil-spin assay also provides a valuable tool for the pharmaceutical industry (Figure 2). The original oil-spin method developed by Petzinger and Fuckel[68] has been adapted to utilize non-radiolabeled substrates.[14] Concentrated sodium hydroxide was traditionally used to release radiolabeled substrate that had accumulated in the hepatocyte, whereas the more recent approach uses cesium chloride to collect the centrifuged cells and extract the non-radiolabeled compound using methanol.[14] The amount of accumulated compound can then be determined using LC/MS/MS with a standard curve used for quantification. The development of this method is clearly beneficial for the assessment of hepatic uptake of non-radioactive NCEs in higher throughput format. The potential application of the oil-spin assay is highlighted in Table 1. Table 1 shows bosentan $CL_{int,uptake}$ estimates determined using both media-loss and oil-spin methods from incubations containing varying bovine serum albumin (BSA) concentrations. While the actual $CL_{int,uptake}$ values obtained at each BSA concentration are similar using each of the two methods, the error in the $CL_{int,uptake}$ estimate (as assessed by the standard deviation) is much smaller using the oil-spin method compared to the media-loss assay. This is particularly apparent at higher BSA concentrations where the decrease in free fraction of bosentan in the incubation leads to lower $CL_{int,uptake}$ estimates (see Table 1). These data help to illustrate possible applications of these two uptake assays.

The media-loss assay is suitable as a first line, higher throughput assay to assess the impact of hepatic uptake in clearance predictions for NCEs (where uptake rates are likely to be high due to significant underprediction of in vivo clearance using conventional assays). In essence this assay can be viewed as a rapid, easy to use method of obtaining uptake rates. This assumption is validated by the similar $CL_{int,uptake}$ estimates obtained via the media-loss and oil-spin methods (see Table 1) and the mass balance obtained when simultaneous media-loss and oil-spin methods are conducted on the same incubation.[14] The greater accuracy observed in $CL_{int,uptake}$ estimates determined using the oil-spin assay, albeit at a lower throughput, suggests that this method would be most appropriate for follow-up studies particularly for substrates with lower rates of hepatic uptake. The more definitive data obtained from the oil-spin assay would also make this the method of choice for uptake studies in drug development. Further, the oil-spin assay would also be most suitable for mechanistic studies such as future investigations into the effect of serum on hepatic uptake, or for the utilization of hepatocytes to assess potential drug−drug interactions in uptake.

2.4.3. The Effect of Cryopreservation on Uptake Transporters. Although the availability and quality of fresh human hepatocytes initially limited their use within drug discovery, recent advances in cryopreservation technology[69] have allowed an increasing use of this in vitro tool over the past decade.[65] Several studies have confirmed that cryopreserved hepatocytes retain phase I and phase II enzyme activity at levels akin to those observed in freshly prepared cells.[5,7,69] Since the use of hepatocytes for uptake studies is becoming more commonplace (see section 2.3), it is critical that the effect of cryopreservation on the major hepatic transporters is assessed. Shitara et al.[70] have studied the uptake of taurocholate and 17β-estradiol-glucuronide in ten preparations of freshly isolated human hepatocytes and again in subsequently cryopreserved cells. Taurocholate uptake in the cryopreserved cells varied between 10 and 200% of that observed in freshly prepared cells whereas the uptake of 17β-estradiol-glucuronide varied between 38 and 200%. It was concluded that these data suggest that at least part of the NTCP and OATP activity is retained in cryopreserved human hepatocytes.[70] To further investigate the effect of cryopreservation on OATP activity the uptake of estrone-3-sulfate, 17β-estradiol-glucuronide, atorvastatin and 5 AZ compounds (anions previously shown to be uptake substrates) was determined in hepatocytes from five human hepatocyte donors (both freshly isolated and following cryopreservation). Figure 3 shows that no statistically significant difference in the uptake of eight OATP substrates was observed between fresh and cryopreserved hepatocytes from five donors. These data suggest that OATP activity in cryopreserved human hepatocytes can be retained at a level comparable to that observed in freshly prepared cells. Therefore cryopreserved

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(70) Shitara, Y.; Li, A. P.; Kato, Y.; Lu, C.; Ito, K.; Itoh, T.; Sugiyama, Y. Function of uptake transporters for taurocholate and estradiol-17β-glucuronide in cryopreserved human hepatocytes. *Drug Metab. Pharmacokinet.* **2003**, *18*, 33–41.

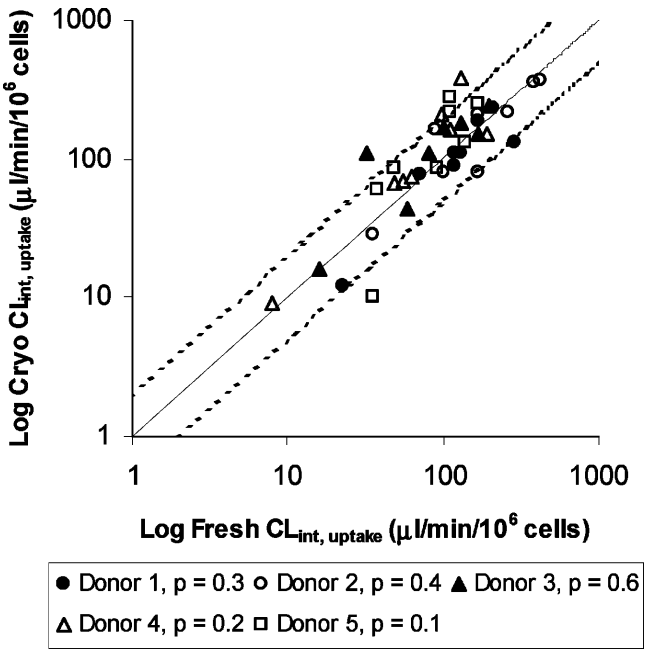

Figure 3. A comparison of $CL_{int,uptake}$ determined in five freshly isolated human hepatocyte donors and cryopreserved cells for 8 OATP substrates. Solid line represents line of unity and feint, dotted lines = 2-fold errors; *p* values are from two tailed Student's *t* test.

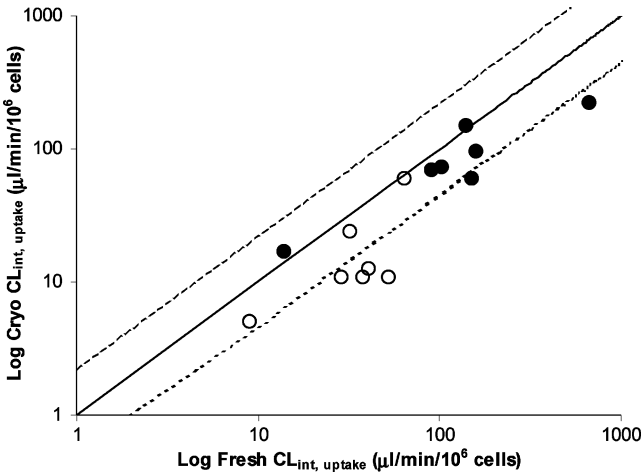

Figure 4. A comparison of $CL_{int,uptake}$ determined in two freshly isolated human hepatocyte donors and cryopreserved cells for 7 OCT substrates. Solid line represents line of unity and feint, dashed lines = 2-fold error; *p* values are from two tailed Student's *t* test (donor 1, ○, $p = 0.01$; donor 2, ●, $p = 0.2$).

human hepatocytes are a suitable in vitro system to assess the hepatic uptake of organic anions in humans.

The effect of cryopreservation on OCT1 activity was also assessed by investigating the uptake of seven known OCT1 substrates between fresh and cryopreserved hepatocytes from two donors (Figure 4). While OCT1 uptake was significantly lower in cryopreserved hepatocytes from one donor (donor 1, $p = 0.01$), there was no statistical difference in activity in another donor (donor 2, $p = 0.2$). Umehara et al.,[62] have also observed the uptake of the OCT1 substrates tetraethylammonium, cimetidine and 1-methyl-4-phenylpyridinium with cryopreserved human hepatocytes. Although OCT1 activity is retained after cryopreservation, freshly prepared human hepatocytes should be used for clearance predictions when the involvement of OCT1 is suspected in the clearance mechanism to guard against the potential for underpredicting in vivo clearance.

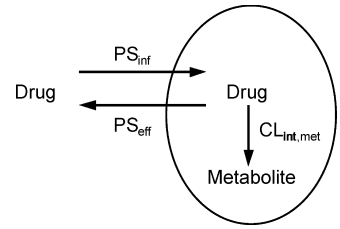

Figure 5. A simple model of diffusion limited kinetics, incorporating barrier events.

2.5. Modeling Hepatic Uptake Transporters. *2.5.1. Modeling Hepatic Clearance Incorporating Transporter Activity.* The distinction between perfusion and diffusion limited kinetics is described in the original publications of the well-stirred liver model[71−73] and has been developed further with the increased recognition of the role of uptake and efflux transporters.[12,74] The role and modeling of hepatic transporters in clearance and drug−drug interactions have recently been reviewed[75,76] and extended.[14] A general expression for the overall apparent intrinsic clearance ($CL_{int,app}$) that incorporates the potential for diffusion limitation[75] is

$$CL_{int,app} = CL_{int,met}\frac{PS_{inf}}{PS_{eff} + CL_{int}} \quad (5)$$

where PS_{inf} and PS_{eff} are the sinusoidal membrane permeabilities and $CL_{int,met}$ is the intrinsic metabolic clearance (Figure 5). In this model no distinction is made between active and passive permeation. Although the use of the term $CL_{int,app}$ may not be formally correct, as it does not relate free drug concentration at the enzyme to the rate of elimination, it is a useful concept as it relates free drug

(71) Gillette, J. R. Factors affecting drug metabolism. *Ann. N.Y. Acad. Sci.* **1971**, *179*, 43–66.
(72) Rowland, M.; Benet, L. Z.; Graham, G. G. Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Pharmacodyn.* **1973**, *1*, 123–136.
(73) Wilkinson, G. R.; Shand, D. G. Commentary: a physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.* **1975**, *18*, 377–390.
(74) De Lannoy, I. A. M.; Pang, K. S. Effect of diffusional barriers on drug and metabolite kinetics. *Drug Metab. Dispos.* **1987**, *33*, 51–58.
(75) Shitara, Y.; Sato, H.; Sugiyama, Y. Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 689–723.
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concentration in plasma to elimination rate and can be used directly in the well-stirred liver model to estimate hepatic clearance (see section 2.4.1):

$$\mathrm{CL} = \frac{Q_h f_{ub} \mathrm{CL}_{int,app}}{Q_h + f_{ub} \mathrm{CL}_{int,app}} \quad (6)$$

What is not obvious from eq 5, but is clear from its derivation,[77,78] is that $\mathrm{CL}_{int,app}$ is the product of the intrinsic clearance for the eliminating process (e.g. metabolism or biliary secretion) and the ratio of the intracellular and extracellular free concentrations. Uptake transporters increase clearance by elevating the concentration at the site of the eliminating enzymes. This is a key point in the understanding of the role of uptake transporters in hepatic disposition. This is apparent from considering the overall rate of change of amount of drug in the system (dx/dt) in terms of the true $\mathrm{CL}_{int,met}$ and $\mathrm{CL}_{int,app}$,

$$\frac{dx}{dt} = \mathrm{CL}_{int,met}[\mathrm{C_u}]_{liver} \quad (7)$$

and also,

$$\frac{dx}{dt} = \mathrm{CL}_{int,app}[\mathrm{C_u}]_{liver} \quad (8)$$

Then

$$\mathrm{CL}_{int,app} = \mathrm{CL}_{int,met}\frac{[\mathrm{C_u}]_{liver}}{[\mathrm{C_u}]_{plasma}} \quad (9)$$

Importantly, the ratio of the free drug concentration in liver and plasma is simply the ratio of the sum of the input and output terms for the liver (eq 5), and if the individual uptake and removal terms can be estimated, we have a mechanism for obtaining the impact of a metabolism−transporter interplay.

For hepatocyte based systems, Paine et al.[14] extended this model to avoid the use of the term $\mathrm{CL}_{int,app}$ and uses an analogous clearance term for drug removal from the media of a suspended hepatocyte experiment (CL_{med}). As CL_{med} is a composite of intrinsic clearance terms, it is more appropriate than an apparent intrinsic clearance.

$$\frac{dX}{dt} = \mathrm{CL}_{med}\mathrm{fu}_{med}C_{med} \quad (10)$$

where fu_{med} is fraction unbound in the media and C_{med} is concentration in the media.

Paine et al.[14] defined clearance from the medium by considering the overall rate of change of amount of drug in the system at steady state:

$$\frac{dX}{dt} = \mathrm{CL}_{int,met}\mathrm{fu}_{cell}C_{cell} \quad (11)$$

The subscripts cell and med refer to the cell (excluding the membrane) and medium compartments, respectively. X is the amount of drug, C is the total drug concentration and fu is the free fraction in the corresponding compartment. If albumin is not added to the medium, fu_{med} can be assumed to be 1.

Hence,

$$\mathrm{CL}_{med} = \mathrm{CL}_{int,met}\frac{\mathrm{fu}_{cell}C_{cell}}{\mathrm{fu}_{med}C_{med}} = \mathrm{CL}_{int,met}\Psi \quad (12)$$

where Ψ represents the ratio of the free concentration inside the cell to the free concentration in the medium.

The simplest model with which to explore metabolism−uptake transporter interplay incorporates active uptake, metabolism, and sinusoidal bidirectional passive permeation. In this case, the passive components of influx and efflux are parametrized as a distribution clearances ($\mathrm{CL}_{int,pass}$) rather than as influx and efflux permeability−surface area products and are therefore assumed to be equivalent in the model. In this case clearance from the media is expressed as

$$\mathrm{CL}_{med} = \mathrm{CL}_{int,met}\frac{\mathrm{CL}_{int,pass} + \mathrm{CL}_{int,uptake}}{\mathrm{CL}_{int,pass} + \mathrm{CL}_{int,met}} \quad (13)$$

or

$$\Psi = \frac{\mathrm{CL}_{int,pass} + \mathrm{CL}_{int,uptake}}{\mathrm{CL}_{int,pass} + \mathrm{CL}_{int,met}} \quad (14)$$

The inclusion of a term for the passive permeation into and from hepatocytes is a key component of the model and allows exploration of the effect of changes in passive diffusion on metabolism−transporter interplay.[76]

For highly permeable compounds, $\mathrm{CL}_{int,pass} \gg \mathrm{CL}_{int,uptake}$ and $\mathrm{CL}_{int,pass} \gg \mathrm{CL}_{int,met}$ and therefore

$$\mathrm{CL}_{med} = \mathrm{CL}_{int,met}$$

as would be expected in a perfusion-limited model. The implication of this analysis is that hepatic uptake is unlikely to have a significant impact on plasma clearance, if compounds are highly permeable (see Figure 6). This interpretation is supported by the fact that the vast majority of drugs that are uptake substrates are ionized at physiological pH and tend to have low lipophilicities. Indeed, one could question the rationale or evolutionary drive underlying uptake transporters for endogenous or exogenous compounds that are readily permeable to cell membranes as recently suggested by some authors.[79]

For poorly permeable compounds that are uptake substrates, $\mathrm{CL}_{int,uptake} \gg \mathrm{CL}_{int,pass}$, $\mathrm{CL}_{int,met} \gg \mathrm{CL}_{int,pass}$ and CL_{med}

(77) Iwatsubo, T.; Suzuki, H.; Sugiyama, Y. Determination of the rate-limiting step in the hepatic elimination of YM796 by isolated rat hepatocytes. *Pharm. Res.* **1999**, *16*, 110–116.

(78) Miyauchi, S.; Sugiyama, Y.; Sawada, Y.; Morita, K.; Iga, T.; Hanano, M. Kinetics of hepatic transport of 4-methylumbelliferone in rats. Analysis by multiple indicator dilution method. *J. Pharmacokinet. Pharmacodyn.* **1987**, *15*, 25–38.

(79) Dobson, P. D.; Kell, D. B. Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule. *Nat. Rev. Drug Discovery* **2008**, *7*, 205–220.

$= CL_{int,uptake}$, indicating that, for poorly permeable compounds, uptake can become the primary determinant of clearance, as described by Yamazaki et al.[3] for the elimination of the polar acid, pravastatin (log $D_{7.4} = -0.5$).

2.5.2. Modeling Binding and Intracellular Free Drug Concentrations. The importance of the free intracellular concentration as a determinant of elimination from hepatocytes is expressed in eq 15,

$$\frac{dX}{dt} = CL_{int,met} fu_{cell} C_{cell} \quad (15)$$

but clearly this is also the critical concentration driving the pharmacological effect at intracellular targets and CYP based drug−drug interactions. Understanding and interpreting clearance and potency data is complex because the intracellular free concentration cannot be determined directly. As stated earlier, for systems with no transporter involvement, and rapid permeation through the cell membrane, the intracellular free concentration can be assumed to be equal to the media concentration, and all that is required is an estimate of the binding in the experimental system as a whole. To this end, the concept of the fraction unbound in the incubation (fu_{inc}) has been used for several years to obtain more accurate CL_{int} estimates from both microsomes and hepatocytes (see section 2.2), following equilibrium dialysis of the biological matrix, in a manner similar to that established for determining the free fraction in plasma. When there is no transporter involvement, $C_{med} = Cu_{cell}$, and $fu_{cell} = C_{med}/C_{cell}$, and eq 15 therefore simplifies to

$$\frac{dX}{dt} = CL_{int,met} C_{med} \quad (16)$$

and as $C_{med} = C_{inc} \times fu_{inc}$,

$$\frac{dX}{dt} = CL_{int,met} C_{inc} fu_{inc} \quad (17)$$

For compounds with significant binding to the in vitro system the intrinsic clearance is underestimated because the substrate concentration at the enzyme is underestimated:

$$CL_{int,met} = \frac{CL_{inc}}{fu_{inc}} \quad (18)$$

and

$$CL_{med} = \frac{CL_{inc}}{fu_{inc}} \quad (19)$$

For hepatic uptake substrates the situation is more complex, as the free concentrations inside and outside of the cells are different. As the binding to the internal and external components of the cell is driven by different concentrations, two binding terms are required in any model. Reinoso et al.[80] established that there is a rapid equilibrium of drug in the media with that in the outer cell membrane (K_{mem}), and a second binding component in which free drug inside the cell equilibrates with intracellular components. These binding terms have been included in a model that incorporates uptake transporters and metabolism by Paine et al.,[14] who derived an expression for the unbound fraction

$$CL_{med} = CL_{int,met} \frac{CL_{int,uptake} + CL_{int,pass}}{CL_{int,met} + CL_{int,pass}}$$

$$E_H = \frac{CL_{med}}{Q + CL_{med}}$$

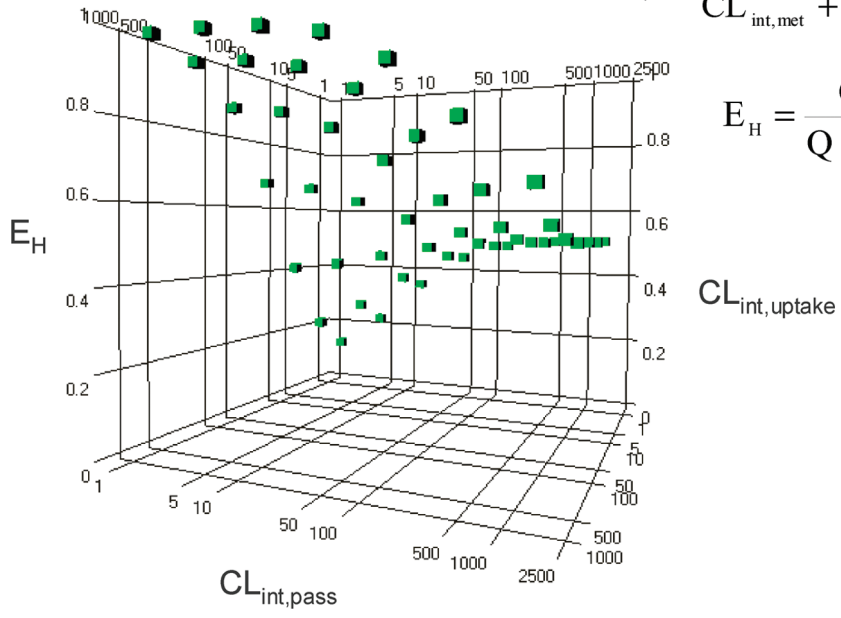

Figure 6. Simulation of the effect of changes in passive diffusion on hepatic extraction (E_h), for a given uptake clearance. $CL_{int,met}$ was fixed to give a hepatic extraction of 0.5. $CL_{int,uptake}$ was varied from 1 to 1000 mL/min. $f_{ub} = 1.0$, $Q_h = 15$ mL/min.

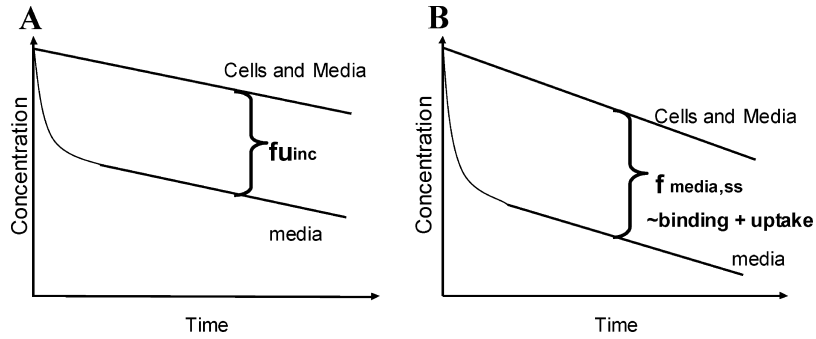

Figure 7. Illustration of the relationship between media concentration and "cells + media" concentration for (A) non-uptake substrates and (B) uptake substrates.

in a cell (fu_{cell}), using an experimentally derived fu_{inc} (by dialysis in the absence of uptake) corrected for the exterior K_{mem}.

$$fu_{cell} = \frac{C_{med}}{C_{cell}} = \frac{V_{cell}}{\frac{V_{inc}}{fu_{inc}} - V_{med} - k_{mem}} \quad (20)$$

The concept fu_{cell} is an important one. It is a measure of the binding in cells, and unlike fu_{inc} is independent of the content or volume of the media. It is also the key link between binding in in vitro and in vivo systems and allows estimation of the fraction in the media at steady state ($f_{med,ss}$). $f_{med,ss}$ is a key parameter as it allows appropriate correction of the observed clearance from an incubation for uptake substrates and thereby prediction of in vivo clearance.

The analogous expression to eq 19, in cases where there is uptake and metabolism, is (see Figure 7)

$$CL_{med} = \frac{CL_{inc}}{f_{med,ss}} \quad (21)$$

Likewise, enabling estimation of the media concentration allows determination of DDI risk, in systems where the media concentration deviates from that in the system as a whole (see section 3.2). An expression for the $f_{med,ss}$ has been derived by Paine et al.[14] It incorporates terms for both the media concentration driven binding (k_{mem}) and intracellular concentration driven binding (fu_{cell} and ψ).

$$f_{med,ss} = \frac{1}{\frac{k_{mem}}{V_{med}} + \frac{V_{cell}\Psi}{fu_{cell}V_{med}} + 1} = \frac{V_{med}}{Vss_{med}} \quad (22)$$

2.5.3. Case Study. Paine et al.[14] have modeled the disposition of atorvastatin, cerivastatin and indomethacin, using simultaneous fitting of cell and media data, and a model of binding and clearance as described above. The key findings were that plasma clearance was more accurately predicted than by the standard metabolism assay, high passive permeability negated the high uptake rate of indomethacin and that the overall binding in the system as expressed by $f_{media,ss}$ was significantly greater than that obtained by dialysis of the cells (fu_{inc}), when the uptake transporters are not functional. This finding has important implications for the use of hepatocytes for CYP based DDIs (see section 3.3). In the case of atorvastatin, fu_{inc} was estimated as 0.69, whereas $f_{media,ss}$ was 5-fold lower (0.14). $f_{media,ss}$ is a reasonable measure of binding as the free drug in the cells contributed to <1% of the compound in the system at steady state.

3. The Role of Metabolism and Transporter Interplay in the Prediction of DDIs

3.1. CYP Based DDIs. It has been estimated that up to 2.8% of hospital admissions may be the result of drug−drug interactions.[81] Since CYPs are involved in the metabolism of around 60% of marketed drugs, the majority of work in this area has focused on inhibition and induction of this enzyme family.[82,83] Perhaps the most frequently cited, clinically relevant drug−drug interaction is the inhibition of CYP3A-mediated metabolism of terfenadine by ketoconazole. This interaction can lead to a 35-fold increase in drug exposure,[84] which results in cardiac arrhythmias and even death.[85] The magnitude of interaction potentially caused by the competitive inhibition of CYPs has necessitated a thorough understanding of the inhibition potential of NCEs against the major hepatic CYPs (CYP1A2, CYP2C9,

(80) Reinoso, R. F.; Telfer, B. A.; Brennan, B. S.; Rowland, M. Uptake of teicoplanin by isolated rat hepatocytes: comparison with in vivo hepatic distribution. *Drug Metab. Dispos.* **2001**, *29*, 453–459.

(81) Jankel, C.; Fitterman, L. K. Epidemiology of drug-drug interactions as a cause of hospital admissions. *Drug Safety* **1993**, *9*, 51–59.

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CYP2C19, CYP2D6 and CYP3A4) early within drug discovery.[86,87]

IC_{50} values have been determined for NCEs using a range of in vitro systems including recombinant CYPs, liver microsomes and more recently hepatocytes.[87−90] This in vitro data has subsequently been used to produce quantitative predictions of potential in vivo DDIs.[27,91] While the $IC_{50,unbound}$ values generated via recombinant CYPs and hepatocytes agree for the majority of drugs investigated, there are certain cases where discrepancies have been observed.[90,92] These reports have led to speculation that active transport processes in hepatocytes may be responsible for these differences[65,90] and that the use of hepatocytes as an in vitro system to assess inhibitory potential of CYPs should be further evaluated (see section 3.3).

3.2. Use of Hepatocytes To Determine Potency of Uptake Substrates at Intracellular Targets. It would seem advantageous to use hepatocytes to determine the potency of uptake substrates at intracellular targets such as HMG CoA reductase, or at CYPs to assess the increased DDI risk, as any accumulation can be accounted for. However, such an experimental system is not without its pitfalls, and there are very few literature reports of the use of hepatocytes to determine CYP inhibition based DDI risk (see section 3.1). The complexity arises because there are at least three concentrations to which the observed inhibition can be related: the initial concentration achieved in the incubation (C_{inc}), the free intracellular concentration (Cu_{cell}) and the free media concentration (C_{med}).

For reasons of efficiency, it is most common to construct potency assays in formats that do not require explicit determination of the free concentration of compound in the assay. To achieve this, the applied concentration must be reflective of the free concentration in the environment of the target. Assays are therefore commonly performed using low protein concentrations, to avoid high nonspecific binding and the concomitant reduction in free drug levels. Use of excessive, uncorrected, microsomal protein concentrations can lead to inaccurate reporting of CYP DDI risk.[93] The principle also applies to excessive reduction of free drug levels through target binding, and the ability to assess the most potent ligands is ultimately limited by this factor. The same principles apply to cell based assays, and are even more complex when saturable uptake processes are involved.[94]

If a cellular potency assay was combined with determination of Cu_{cell}, the resultant IC_{50} would be a measure of the true IC_{50}, but would be similar to that obtained in a cell free system. In this case nothing would have been learned about the contribution of the active uptake to the likely in vivo effect.

C_{inc} can be used, but is susceptible to error, if the free media concentration deviates significantly from C_{inc} (C_{inc} may be corrected by fu_{inc}, but this may not be appropriate for uptake substrates). When the deviation is small, the IC_{50} determined using C_{inc} reflects both the intrinsic potency at the target and the contribution of uptake to elevating the intracellular concentration, and can be directly compared to the free plasma concentration to assess DDI risk. The parameter that governs this error is the fraction in the media at steady state ($f_{med,ss}$; see section 2.5.2). $f_{med,ss}$ is a function of the degree of nonspecific binding (which in turn is a function of the cell concentration in the assay and the affinity for cellular constituents) and the degree to which uptake elevates the intracellular concentration.

This point was illustrated by Paine et al.,[14] who showed that, in an incubation of atorvastatin at 1 μM with 1×10^6 cells per mL, a steady-state concentration of 200 μM in cells was achieved, within 10 min fu_{cell} was estimated as 0.01, and Cu_{cell} as 2 μM indicating that the C_{inc} was within a factor of 2 of the concentration at the target (Cu_{cell}). If, for example, the 1 μM incubation produced 50% inhibition of turnover of a CYP probe substrate, use of C_{inc} to estimate the potency would indicate that there was little evidence of enhanced potency due to uptake (as $C_{inc} \approx Cu_{cell}$). However, the appropriate reference point is $C_{media,ss}$ which was significantly lower than Cu_{cell} (0.1 μM vs 2 μM), indicating that uptake does indeed increase the DDI risk of the compound (by 20-fold).

What is apparent from simulation (Figure 8) is that for typical uptake substrates (e.g. statins) with relatively low hepatocyte binding (due to their acidic nature and low log $D_{7.4}$), used at typical cell concentrations for metabolism

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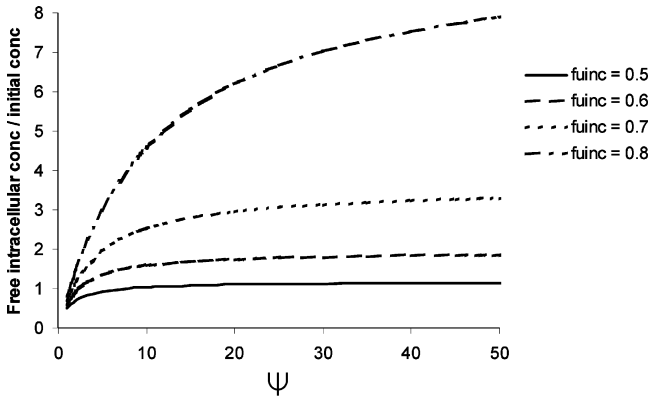

Figure 8. Simulation used to demonstrate relationships between ratio of free intracellular concentration to applied concentration against ψ (ratio of free concentrations, cell:medium), eq 22.

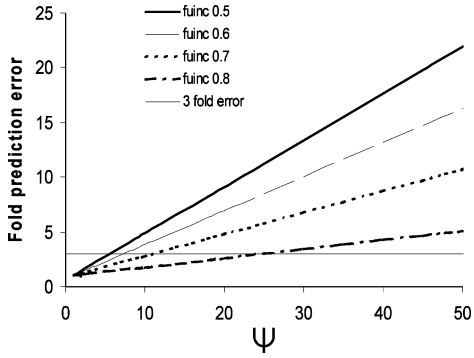

Figure 9. Dependence of CYP inhibition prediction accuracy on degree of nonspecific binding of uptake substrates, if the nominal incubation concentration is only corrected by fu_{inc} (eq 22). (Simulation: $K_{vitro} = 0.15$ mL, cells $= 1 \times 10^6$ per mL, cell volume $= 4$ μL per 1×10^6 cells[14].)

studies (1×10^6 cells per mL), Cu_{cell} cannot achieve more than $3 \times C_{inc}$, even if the ratio of the free concentration inside the cell to the free concentration in the medium (Ψ) approached 50. This is because as Ψ gets larger, $f_{med,ss}$ becomes small and the amount of drug bound intracellularly becomes high (eq 22). For such compounds, the effect of the active uptake on DDI risk is masked, unless inhibition potency is calculated from the media concentration. For compounds with very low affinities for cells, and for assay systems sensitive enough to give signals at low cell concentrations, C_{inc} approximates to C_{med}, and estimates based on C_{inc} give an appropriate indication of potency (see Figures 9 and 10). For moderately bound compounds in assay systems with a high cell number, the true DDI risk is only obtained by estimation of the media concentration, as demonstrated by Grime et al.[94] This publication also highlighted one additional complexity, demonstrating that if the IC_{50} at the target is similar to the K_m for the transporter, complex inhibition curves are generated. The method was shown to have value only if the affinity for the uptake transporter was significantly less than that at the target CYP.

These studies indicate that hepatocyte assays can be of value for assessment of the CYP based DDI risk of uptake substrates,

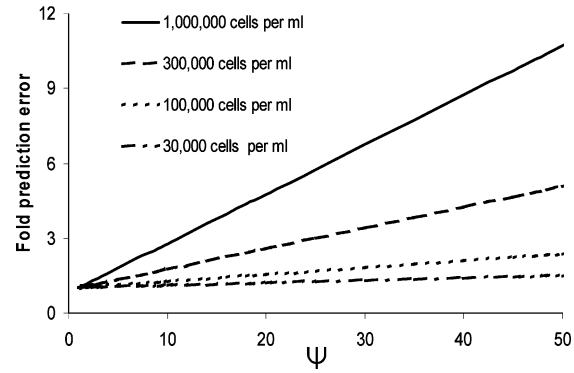

Figure 10. Dependence of CYP inhibition prediction accuracy on cell concentration for uptake substrates, if the nominal incubation concentration used in IC_{50} determination (eq 22). (Simulation: $K_{vitro} = 0.15$ mL, cell volume $= 4$ μL per 1×10^6 cells[14].)

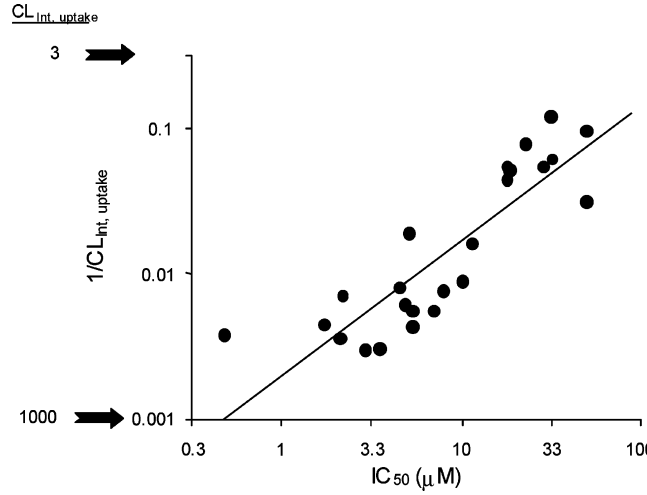

Figure 11. Relationship between inhibition of OATP1B1 and $CL_{int,uptake}$ determined in human hepatocytes for a series for congeneric AZ compounds. Arrows on *y* axis are $CL_{int,uptake}$ values provided for clarity. Solid line represents the line of best fit ($r^2 = 0.75$).

but that key constraints apply. In many cases it may be preferable to determine the CYP IC_{50} in a recombinant system and to model DDI risk utilizing the anticipated free plasma concentrations and experimentally determined Ψ.

3.3. Potential DDIs Mediated through the Inhibition of Hepatic Uptake Transporters. While hepatic transporters have been shown to modulate DDIs through an effect on CYPs (see section 3.3), the inhibition of hepatic transporters per se can also elicit DDIs. Since the OAT and OCT families play a minor role in the uptake of drugs into the liver (via OAT2 and OCT1), studies on these enzyme families have focused on their involvement in DDIs in the kidney.[35] By contrast, there have been many studies focusing on the role of OATPs in hepatic DDIs.[16,35] Perhaps the most notable example of a significant clinical DDI involving the inhibition of uptake via OATPs is that observed between cerivastatin and gemfibrozil which resulted in the withdrawal from the

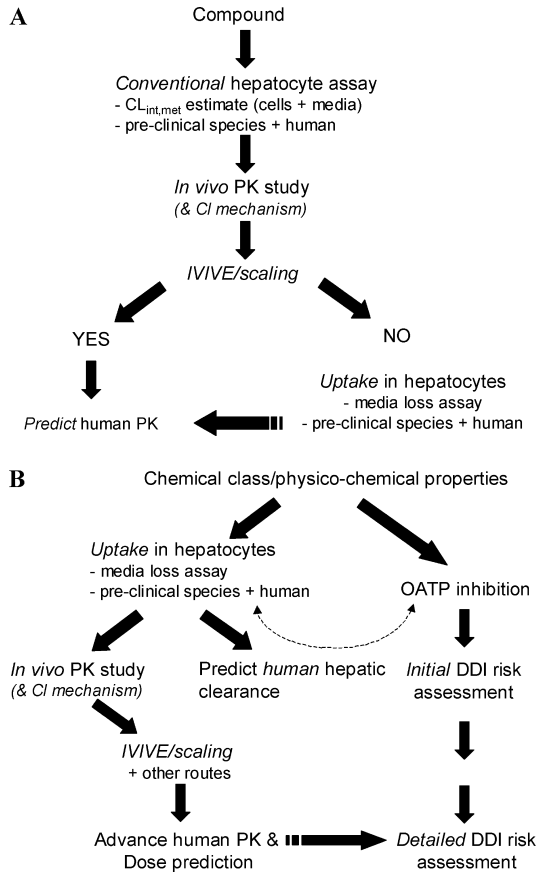

Figure 12. Schematic of the process for estimating clearance from in vitro data using (A) conventional methodology and (B) applying recent advances in hepatic uptake understanding.

market of cerivastatin.[95] Recent mechanistic studies have attributed the 4−6-fold increase in cerivastatin AUC observed on comedication with gemfibrozil, to the inhibition of both CYP2C8 and OATP1B1 by gemfibrozil glucuronide.[96] Further examples of uptake-mediated DDIs have been observed with the comedication of fibrates or cyclosporin with a range of statins including atorvastain, pravastatin and simvastatin[16,35] often resulting in myopathy and rhabdomyolysis.[97]

The significant clinical implications of inhibiting hepatic uptake via OATPs has highlighted to the pharmaceutical industry the importance of assessing this potential liability for NCEs within early drug discovery, particularly when working within the cardiovascular disease area. Although a number of tools are available to assess the inhibition of hepatic uptake (see section 2.3), perhaps the most applicable for use within early drug discovery are mammalian cells lines stably expressing the main hepatic drug uptake transporters.[98] This has been illustrated recently by Hirano et al.,[98] who investigated the inhibition potential of 34 drugs on OATP1B1-mediated pitavastatin uptake. A consideration of the unbound plasma levels of each of the drugs was then used in combination with the in vitro K_i values to determine the likelihood of a clinical DDI via the inhibition of OATP1B1. A further application of the OATP1B1 inhibition assay within the authors' laboratory is shown in Figure 11. While the determination of OATP1B1 inhibition (in combination with predicted unbound plasma levels) allows an assessment of DDI potential, the excellent correlation obtained with hepatic uptake assessed using human hepatocytes ($r^2 = 0.75$; see Figure 11) suggests that for discrete series of compounds this assay may also act as a prescreen for more costly and labor intensive hepatocyte assays. Furthermore, these data suggest that the observed differences in uptake are driven by K_m rather than V_{max}.

4. Conclusions

In recent years, studies with drugs such as pravastatin have clearly demonstrated an additional level of complexity in understanding and predicting hepatic clearance and pharmacokinetics. Such data helped to establish that transporter-mediated uptake of drug from plasma could influence the overall rate of elimination for a range of compounds, most notably the 3-hydroxymethylglutaryl-CoA reductase inhibitors, angiotensin II receptor antagonists and angiotensin-coverting enzyme inhibitors.

The net effect of the interplay between the rates of metabolism and hepatic uptake is often to elevate the free intracellular concentration significantly above that in plasma and is most pronounced for poorly permeable compounds. It has been proposed that marketed drugs may be categorized (qualitatively) with respect to their interaction with transporters based on chemical properties in a similar manner to that used in the Biopharmaceutics Classification System.[99]

One measure of the level of understanding of these complex biological processes is the ability to predict accurately in vivo behavior from in vitro data. Incorporation of fu_{inc} into in vitro−in vivo extrapolations (IVIVEs) has been instrumental in facilitating the optimization of metabolic clearance within drug discovery.

However, application of such principles to a wider range of chemical classes has necessitated an evaluation of both

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conventional assay methodology and the mathematical models used in IVIVE approaches. Modeling and simulation have highlighted the pivotal role for $f_{med,ss}$ and fu_{cell} in controlling the concentration driving the pharmacological effect at intracellular targets and CYP based DDIs.

Our laboratory has developed an enhanced throughput "media-loss" assay to evaluate the uptake of NCEs into hepatocytes. The volume of data afforded by this assay has proven invaluable in understanding species differences in hepatic disposition and predicting human DDIs. Further, more detailed analysis and consolidation has been provided via modifications to an existing oil-spin method. Examination of cryopreserved human hepatocytes has confirmed their suitability for studying OATP-mediated uptake but has cautioned their routine use for OCT substrates.

Clearly, our limited appreciation of the impact resulting from hepatic uptake and enzyme interplay has confounded our ability to project in vivo effects with confidence. In turn, this has undoubtedly contributed to recent late-phase attrition in terms of human PK, target organ toxicity and DDI risk mitigation. However, as with drug metabolism,[27] a clear mechanistic understanding of these inter-related processes and in vivo validation in relevant preclinical species will provide confidence when predicting human in vivo clearance and DDIs. A suggested paradigm for such investigations is outlined in Figure 12.

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