Viewpoint

A Novel Approach to Predicting Organic Anion Transporting Polypeptide Function in Human Hepatic Drug Disposition and Biliary Clearance

The transport of drugs into and out of the liver plays a critical role in determining drug disposition and therapeutic efficacy. Competition between drugs or inhibition of transporter function also makes membrane transporters an important site for drug-drug-interactions that can impact therapeutic efficacy and the likelihood of adverse effects. Among the numerous plasma membrane carrier proteins found in the liver, the organic anion transporting polypeptide (OATP; \textit{SLCO} gene family) transporters are among the most important for many anionic drugs and amphiphilic substrates, including cancer chemotherapeutic agents (Kalliokoski and Niemi, 2009; Tu et al., 2013; van de Steeg et al., 2013). The OATP or \textit{SLCO} gene family of transporters comprises 11 individual carrier proteins in humans (https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=238). These carriers are further divided into 6 families and 10 subfamilies based on amino acid sequence. While they all have in common the properties of 12 transmembrane domains with intracellular termini, multiple glycosylation sites, and the mediation of sodium-independent uptake of various amphiphilic substrates, the different carriers have distinct tissue localizations. There are six OATP proteins that are expressed in the liver, including SLCO1A2, SLCO2A1, and SLCO2B1 (ubiquitously expressed), SLCO1B1 and SLCO1B3 (selectively expressed in the liver), and SLCO4A1 (expressed in the liver as well as the heart, placent, and lung).

The importance of hepatic OATP transporters in drug design and development is highlighted by the recommendations from the U.S. Food and Drug Administration (Food and Drug Administration Center for Drug Evaluation and Research, 2020), European Medicines Agency (European Medicines Agency Committee for Human Medicinal Products, 2012), and Japan Ministry of Health, Labor and Welfare (Ministry of Health, Labour and Welfare Pharmaceuticals and Medical Device Agency, 2018) to include assessments of OATP transport function in the preclinical stages of drug development. The prominence of OATP carriers in hepatic drug disposition and their relationship with other carriers is highlighted in Fig. 1. Uptake of amphiphilic drugs from blood into liver is mediated predominantly by the four OATP carriers expressed on the sinusoidal plasma membrane. Hepatic disposition of these drugs is then balanced by intracellular metabolism and efflux into bile or back into blood via carriers such as various multidrug resistant carriers (e.g., P-glycoprotein) and ATP-binding cassette carriers for efflux into bile and multidrug resistance-associated protein carriers for efflux into blood.

A major issue in the inclusion of OATP assessments is what preclinical model to use to determine OATP transporter function and the potential for drug-drug-interactions that will be relevant to humans. The paper by Miyake and colleagues (Miyake et al., 2023) from this issue of \textit{JPET} that is highlighted in this viewpoint directly addresses this critical issue by presenting and validating a novel humanized or chimeric mouse model to evaluate OATP-mediated transport and biliary clearance of a broad range of drugs and transporter substrates.

Before highlighting the novel findings of this study, however, it is important to understand the need for better models that can be used to predict drug disposition in human liver. Hepatic OATP transporters exhibit both species-dependent (Chu et al., 2013; Grime and Paine, 2013; Wang et al., 2015) and interindividual differences among human subjects (König et al., 2006; Badée et al., 2015; Wang et al., 2015; Taniguchi...

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ABBREVIATIONS: $\text{CL}_{\text{bile}}$, biliary clearance; Fah, fumarylacetoacetate hydrolase; Hu-FRG mouse, Fah$^{-/-}$/Rag2$^{-/-}$/Il2rg$^{-/-}$/Frag mouse transplanted with human hepatocytes; Il2rg, interleukin 2 receptor subunit gamma; Mu-FRG, native murine triple knockout mouse; OATP, organic anion transporting polypeptide; RAG2, recombination activating gene 2; RMSE, root mean square error.
et al., 2020). Based on these differences, the question arises as to how accurately data from species such as rats and mice can be used to make predictions for humans. Figure 1 also shows the effect of humanization of the mouse liver with respect to the expression of specific OATP/Oatp proteins. Further, considering the existence of genetic polymorphisms in OATP gene expression, the question then arises about how predictions on drug disposition derived from a given model system can be interpreted to account for these interindividual differences.

Experimental model systems to assess OATP function in hepatic drug transport include in vivo rodent models and in vitro cell lines derived from rodent or human liver. Comparisons between these models regarding how well they reflect events that occur in the in vivo human liver suggest that in vitro to in vivo extrapolation approaches can provide some degree of predictability, although some uncertainties still exist (Izumi et al., 2017). One way that has become increasingly used to circumvent species differences has been the development of humanized mice (Fujiwara, 2018). Mice are engrafted with functional human cells or tissues so that the same array of enzymes or membrane transporters can be studied in vivo but with a small animal model. Of relevance to the paper being highlighted in this Viewpoint, humanized mouse livers have been primarily applied to study drug metabolism, among other physiologic processes (Chow et al., 2016). While these humanized mouse liver models have been used mostly for metabolism studies, membrane transport processes have not been as extensively studied to date.

The highlighted article by Miyake et al. (2023) is the first study to comprehensively demonstrate that human OATP-mediated clearance and drug-drug-interactions can be quantitatively predicted using a human liver chimeric mouse model. Further, these authors evaluated the accuracy of measuring biliary clearance of a large array of drugs that are OATP substrates in these mice for predicting results in human liver. Miyake and colleagues used the Fah⁺/⁻/Rag2⁺/⁻/Il2rg⁻/⁻ [FRG] transplanted with human hepatocytes (Hu-FRG mouse), which was originally developed by Azuma et al. (2007). This humanized mouse is a triple knockout of the fumarylacetoacetate hydrolase (Fah), recombination activating gene 2 (RAG2), and interleukin 2 receptor subunit gamma (Il2rg) genes on a nonobese diabetic mouse strain background. The Fah gene disrupts the tyrosine catabolic pathway to result in accumulation of the toxic metabolite fumarylacetoacetate, leading to cell death. The absence of this gene, together with RAG2, which is responsible for producing proteins in the RAG complex in lymphocytes, and Il2rg⁻/⁻, which encodes a subunit of the IL2 receptor important for immune function, result in disruption of the immune system and allows for repopulation with foreign hepatocytes.

The rationale for focusing on OATP-mediated disposition as the initial prediction target was based on OATP-mediated drug-drug interactions being the most frequent hepatic transporter-mediated problem in clinical settings and that such adverse interactions can easily be detected by measuring plasma concentrations.
of drugs. The authors then used predictions of biliary clearance \( (\text{CL}_{\text{bile}}) \) as an output that combines hepatic uptake, passive diffusion, metabolism, and biliary excretion.

Pharmacokinetic studies were conducted for the purposes of predicting OATP-mediated disposition and \( \text{CL}_{\text{bile}} \). For the former purpose, six established OATP substrates were used and included atorvastatin, fexofenadine, glibenclamide, pitavastatin, pravastatin, and rosuvastatin. These are all clinically used drugs that are frequently prescribed. For the latter purpose, 20 substrates were administered to the Hu-FRG mice in what is termed two cassette doses of 10 drugs each. The first group included cyclosporine A, epirubicin, erythromycin, fexofenadine, indocyanine green, paclitaxel, pitavastatin, pravastatin, rosuvastatin, and valsartan. The second group included cefazolin, cefixime, cefoperazone, cefotetan, cefpiramide, ceftriaxone, ciprofloxacin, diclofenac, irinotecan, and ranitidine. Again, as with the disposition studies, the compounds used in the \( \text{CL}_{\text{bile}} \) studies are diverse and included numerous commonly used clinical drugs that represent antibiotics, analgesics, anticancer agents, statins, and other agents.

Comparisons were then made between pharmacokinetic data obtained in Hu-FRG and native murine triple knockout (Mu-FRG) mice and those from humans. The results for both the drug disposition studies and the \( \text{CL}_{\text{bile}} \) studies clearly demonstrated a better correspondence of human data with data from Hu-FRG mice than with data from Mu-FRG mice. Several of the observed kinetic properties were more like those from humans with the Hu-FRG mice, including inhibition of OATP transporter function with rifampicin. The closer correspondence of human data with those from Hu-FRG mice versus Mu-FRG mice is obvious from the analysis of \( \text{CL}_{\text{bile}} \), where the correlation coefficient \( (R^2) \) and the root mean square error \( (\text{RMSE}) \) for Hu-FRG mice versus humans \( (R^2 = 0.563, \text{RMSE} = 0.887) \) were markedly better than those values for Mu-FRG mice versus humans \( (R^2 = 0.027, \text{RMSE} = 2.741) \). The closer the \( R^2 \) value is to 1.0, the better the correspondence of the data in the two groups. In contrast, the RMSE value is a measure of the differences between values predicted by a model or an estimator and the values observed; the lower this value, the closer the correspondence between the two sets of values. These data are consistent with the relatively poor conservation of OATP family transporters among various mammalian species and humans and the fact that, for some carriers, no orthologs are known (Pacyniak et al., 2011; Chu et al., 2013).

In conclusion, the paper by Miyake et al. (2023) provides strong data that support the use of a humanized mouse liver model for characterization of the role of OATP transporters in determining drug disposition and the potential for drug-drug interactions in humans. Clear strengths of the work include the use of a large number of clinically important drugs whose liver transport is largely mediated by OATPs, the methodological approaches taken to present the calculations, and a careful consideration of variability factors and potential issues with the humanized mouse model.

Although the authors do provide a fairly robust discussion of the care needed in selecting donor hepatocytes and sex dependence of processes involving OATPs, future studies could be aimed at better modeling the impact of genetic polymorphisms or other factors (e.g., nutritional differences or coexisting diseases) that could impact OATP function. A limitation of the current study was that donor hepatocytes came from Caucasians. Future studies could include donor hepatocytes from more diverse populations to uncover potential differences among those populations. Further, although the correlation between data from Hu-FRG mice and humans are much better than those between Mu-FRG mice and humans, it could be further improved. For example, other processes are likely important in the \( \text{CL}_{\text{bile}} \) of many of the drugs studied here. Examples of these processes include other plasma membrane carriers such as P-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 and drug-metabolism enzymes such as cytochrome P450 3A4 and cytochrome P450 2C9. Further manipulations of the humanized mouse model and additional characterization and validation of responses can help to further improve predictions for normal human liver.

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References


