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PERSPECTIVE

Role of the Murine Organic Anion-Transporting Polypeptide 1b2 (Oatp1b2) in Drug Disposition and Hepatotoxicity

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ABSTRACT

Several members of the organic anion transporting polypeptide (OATP/Oatp) family of uptake transporters are expressed in the hepatocyte sinusoidal membrane in humans and preclinical species. The mouse liver specific Oatp is Oatp1b2, and the human homologs most closely related are OATP1B1 and 1B3. The substrate specificity of these transporters is broad, and the widely accepted view is that they play an important role in drug disposition. However, direct evidence that OATP/Oatps are important for drug disposition in vivo has been lacking thus far. In this issue of *Molecular Pharmacology*, Zaher et al. (p. 320), along with Lu et al. (*Toxicol Sci* **103:**35–45, 2008), report on the characterization of mice with a targeted disruption of the organic anion transporting polypeptide Oatp1b2. The Oatp1b2(−/−) mice were viable and fertile and did not demonstrate obvious phenotypic abnormalities. Zaher et al. performed a pharmacokinetic analysis with the human OATP1B1 and -1B3 substrates rifampicin and pravastatin and demonstrated a reduced liver-to-plasma ratio for these drugs in knockout compared with control mice, providing strong evidence that Oatp1b2 played an important role in the disposition of these drugs. Lu et al. found that the Oatp1b2(−/−) mice were completely resistant to hepatoxicity induced by phalloidin and microcystin-LR. Taken together, these data illustrate that Oatp1b2(−/−) mice are an important new model to investigate the role of this transporter in drug disposition and hepatotoxicity.

Members of the organic anion transporter (human OATP/SLCO, rodent Oatp/Slco) family function as uptake transporters of a wide variety of drugs, xenobiotics, and endogenous substances. In humans and mice, 11 and 15 family members, respectively, have thus far been identified. OATPs/Oatps are widely accepted to play an important role in transporting substrates from the blood into tissues. For drugs mainly eliminated by hepatobiliary excretion, polymorphic variants of OATPs with altered transport characteristics or inhibition of OATP-mediated transport could therefore potentially affect drug efficacy or toxicity and increase the propensity for drug-drug interactions with coadministered drugs.

There is considerable current interest, both in academia and in the pharmaceutical industry, in OATPs expressed in the sinusoidal (basolateral) membrane of hepatocytes, because the identification of substrates for these transporters could be used, for instance, as a strategy to increase the intrahepatic concentration of drugs targeting the liver. Important questions related to this approach are how to quantitatively predict the potential for drug-drug interactions for OATP substrates and how to extrapolate liver-to-plasma ratios measured in preclinical species to humans. A good understanding of the contribution of OATP family members to drug disposition is therefore important. In this issue of *Molecular Pharmacology*, Zaher et al. (2008) increase our knowledge of the in vivo role of the mouse Oatp1b2 by characterizing Oatp1b2(−/−) mice and studying the disposition of prototypical substrate drugs. This article nicely complements a very recently published article by Lu et al. (2008) in *Toxicological Sciences*, who independently generated Oatp1b1(−/−) mice and studied the pharmacokinetics and toxicity of several established liver toxins. In this *Perspective*, we provide a brief overview on the current knowledge of Oatps and discuss how the articles by Zaher et al. (2008) and Lu et al. (2008) add to our understanding of Oatp1b2.

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ABBREVIATIONS: Oatp/OATP, organic anion-transporting polypeptide; ABC, ATP-binding cassette; MRP, multidrug resistance protein; BCRP, breast cancer resistance protein.

From the Oatps in mouse, Oatp1a1, -1a4, -1b2, and -2b1 are expressed at relatively high levels in liver, with Oatp1b2 detectable only in liver (Cheng et al., 2005). OATPs expressed at high levels in human liver are OATP1B1, -1B3, and -2B1 (Fig. 1). Of these, expression of the first two is largely restricted to hepatocytes. Human transporters most closely related to mouse Oatp1b2 are OATP1B1 and -1B3 and together they are considered as the Oatp1b2 ortholog (Hagenbuch and Meier, 2003).

The substrate specificity of OATP1B1 and -1B3 is broad. Whereas most examples of substrates for OATP1B1 or -1B3 are anionic (e.g., statins like pravastatin, pitavastatin, and rosuvastatin), others are zwitterionic (e.g., rifampicin), or neutral and lipophilic (e.g., paclitaxel). For a comprehensive list of substrates of human OATP family members, see König et al. (2006) and Niemi (2007). In addition, a structurally diverse range of drugs has been identified that are inhibitors of OATP1B1 or -1B3 (e.g., rifampicin and cyclosporin A) (Smith et al., 2005; Gui et al., 2008). Coadministration of OATP substrates or inhibitors could therefore result in drug-drug interactions, and polymorphic forms of these transporters with altered transport characteristics could cause inter-individual variation in pharmacokinetics. Several excellent reviews have appeared on this topic (Ho and Kim, 2006; König et al., 2006; Niemi, 2007). For example, it has been demonstrated that coadministration of atorvastatin with an intravenous dose of rifampicin in healthy subjects does result in a substantially increased systemic exposure to both the acid and lactone form of atorvastatin, which is probably due to the inhibition of atorvastatin uptake into hepatocytes by rifampicin (Lau et al., 2007).

The working mechanism of OATP/Oatp family members is currently not clear. Transport is independent of sodium, chloride, and potassium gradients, membrane potential, and ATP levels (Mahagita et al., 2007, and references therein). A general model has been proposed for all members of the OATPs/Oatps, whereby substrates would be transported through a central, positively charged pore in a rocker-switch type of mechanism (Meier-Abt et al., 2005). By expressing rat Oatp1a1 in *Xenopus laevis* oocytes, a coupled exchange with GSH could be demonstrated that was bidirectional (Li et al., 1998). However, for other OATP/Oatp family members, a counter-ion–driven transport has not been identified. Recent data would suggest that OATP1B1 and -1B3 most probably function as bidirectional diffusion transporters and that GSH is not a substrate or activator of their transport activity (Mahagita et al., 2007). It should be noted that most experiments to elucidate the mechanism of action of OATPs/Oatps have been conducted in cRNA-injected *X. laevis* oocytes and that it cannot be excluded entirely that these transporters behave differently in the context of a mammalian cell membrane. Another open question is why pravastatin, a substrate for Oatp1b2, demonstrates highly concentrative uptake in rat liver and isolated hepatocytes (Ishigami et al., 1995), if Oatp1b2 is a simple bidirectional diffusion carrier.

After uptake into hepatocytes, drugs will be metabolized by phase I or II drug-metabolizing enzymes or directly transported into bile as parent drugs. Drug efflux transporters in the canalicular membrane of the hepatocyte are members of the ATP-binding cassette (ABC) transporter family, which consists of primary active transporters that can transport against a concentration gradient. The main ABC transporters relevant to drug transport in the canalicular membrane are MDR1 P-glycoprotein (ABCB1), the multidrug resistance protein 2 (MRP2; ABCC2), the bile salt export pump (BSEP; ABCB11), and the breast cancer resistance protein (BCRP; ABCG2) (Leslie et al., 2005; Fig. 1). It should be noted that one ABC transporter, MRP3/Mrp3, is present at high levels in the mouse hepatocyte sinusoidal membrane but is barely detectable in hepatocytes of rats or humans under normal conditions (Kruh et al., 2007). The currently available in vitro model systems, such as hepatocytes or polarized cell monolayers expressing OATP/Oatp family members in the basolateral membrane and ABC transporters in the apical membrane (Kopplow et al., 2005; Ishiguro et al., 2008), are valuable model systems to elucidate the transporters potentially involved in hepatic elimination of a drug. However, it is difficult to predict the relative contribution in vivo of the various uptake and efflux transporters and metabolism based on in vitro data generated with these systems, and therefore the development of representative in vivo model systems is desirable.

The establishment of the Oatp1b2(−/−) mice by Zaher et al. (2008) and Li et al. (2008) is an important step to study the relative importance of Oatp1b2 in the uptake of substrates into the liver. Because there is potential for the up-regulation of compensatory genes in knockouts, the mRNA of a number of uptake and efflux transporters was assessed in knockout and control mice. Of the genes analyzed, a higher expression was observed for Oatp1a4 (~2-fold) and Oatp2b1 (~1.6-fold) in female knockout compared with control mice but not in male mice. In the Oatp1b2(−/−) characterized by

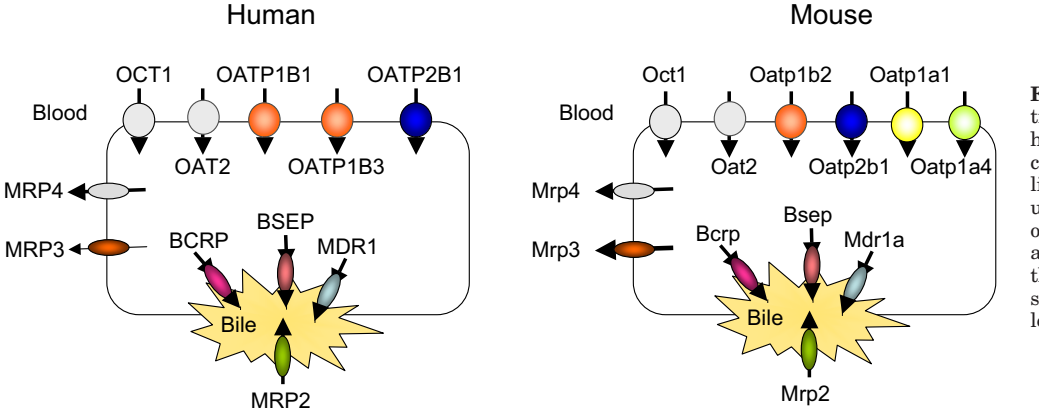

Fig. 1. Expression of the major drug transporters in human and mouse hepatocytes. The transporters discussed in this *Perspective* are highlighted in color. The same color is used for transporters considered to be orthologs in mouse and human. The arrow in human MRP3 is thinner than in mouse Mrp3 to illustrate the significant difference in expression level.

Lu et al. (2008), a similar increase in Oatp1a4 was detected, but the increase was found in both male and female mice. No change in expression of Oatp2b1 was detected. It's likely that the differences found by the two groups are explained by the different mouse strains used or by differences in diet and housing conditions.

Blood chemistry was in general comparable between knockout and control mice. In both Oatp1b2(−/−) mouse strains, an increased plasma level of conjugated bilirubin was reported. By analyzing both genders, Lu et al. (2008) found that a moderate increase in serum levels of conjugated bilirubin was found in Oatp1b2(−/−) female mice, whereas serum levels of unconjugated bilirubin tended to be higher in knockout male mice compared with control mice. It was speculated that the differences in (conjugated) bilirubin levels between male and female knockout mice could be explained by gender differences in the expression levels of glucuronosyltransferase 1a1, Mrp3, and Oatp1a1.

Lu et al. (2008) demonstrate that the Oatp1b2(−/−) mice are an excellent model to study the contribution of Oatp1b2 to the uptake of toxic molecules into the liver. Several hepatotoxins such as phalloidin, microcystin-LR, and α-amanitin accumulate in liver, resulting in cholestasis and elevated levels of alanine aminotransferase and aspartate aminotransferase. Oatp1b2 plays a critical role in liver uptake of phalloidin and microcystin-LR in that hepatoxicity was observed in control but not in knockout mice treated with these compounds. A similar susceptibility was observed in knockout compared with control mice treated with α-amanitin, however, indicating that mechanisms other than uptake via Oatp1b2 are important for α-amanitin to cause hepatotoxicity.

In humans and rats, pravastatin is primarily eliminated as parent drug, and the main transporters involved in its hepatic excretion are probably OATP1B1/Oatp1b2 and MRP2/Mrp2 (Nishizato et al., 2003; Sasaki et al., 2004). In the Oatp1b2(−/−) mice, Zaher et al. (2008) found that the liver-to-plasma ratio for pravastatin was 4-fold lower than in control mice at steady state, indicating that Oatp1b1 contributed, at least in part, to uptake into the liver. After a subcutaneous infusion of rifampicin, another substrate for human OATP1B1 and -1B3, an ~8-fold reduction in the liver-to-plasma ratio was observed at steady state in the knockout compared with wild-type mice. The plasma clearance in the knockout was 43% lower than in control mice after an intravenous bolus injection of rifampicin, indicating that Oatp1b2 played an important role in the disposition of this drug.

Many current examples are available illustrating the importance of knockout mice to understand the in vivo role of drug transporters (Klaassen and Lu, 2008). Interpretation of data obtained in transporter knockouts can be difficult in some cases (for instance, as a result of the induction of compensatory mechanisms). In addition, quantitative extrapolation of transporter data between species is difficult because of the limited understanding of species differences in substrate specificity and differences in the relative expression levels of drug transporters. Oatps are no exception, especially because there is no highly conserved human ortholog for mouse Oatp1b2, and mouse liver contains two Oatps not detected in human liver. The findings by Zaher et al. (2008) and Lu et al. (2008) illustrate, however, that the Oatp1b2(−/−) mice are a valuable new model to investigate the relative contribution of Oatp1b2 to the disposition of drugs and toxins. As a next step, the other Oatp family members expressed in mouse liver should be knocked out, and humanized mice expressing OATP1B1 and -1B3 should be generated.

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