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Perspective

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

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Running Title: Targeted disruption of Oatp1b2 in mice

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Oatp, Organic anion-transporting polypeptide; GSH, reduced glutathione; DDI, drug-drug interaction; AST, alanine aminotransferase; ALT, aspartate aminotransferase

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.* (Pages xxx-xxx), and in a very recent issue of *Toxicological Sciences* Lu *et al*. 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 to control mice, providing strong evidence that Oatp1b2 played an important role in the disposition of these drugs. In the accompanying paper, 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.

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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, currently 11 and 15 family members have been identified, respectively. 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 (DDIs) with co-administered drugs.

Currently, there is a considerable interest both in academia and the pharmaceutical industry in OATPs expressed in the sinusoidal (basolateral) membrane of hepatocytes as the identification of substrates for these transporters could for instance be used 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 DDIs 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 therefore is important. In this issue of *Molecular Pharmacology*, Zaher and colleagues 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 paper complements nicely a very recently published manuscript by Lu and colleagues 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 papers by Zaher and Lu add to our understanding of Oatp1b2.

From the Oatps in mouse, Oatp1a1, 1a4, 1b2 and 2b1 are expressed at relatively high levels in liver, with Oatp1b2 only detectable in liver (Cheng *et al*., 2005). OATPs expressed at high levels in human liver are OATP1B1, 1B3 and 2B1 (Figure 1). From these, expression of the former two is largely restricted to hepatocytes. Human

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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 Konig *et al*. (2006), and Niemi (2007). In addition, a structurally diverse range of drugs has been identified which are inhibitors of OATP1B1 or 1B3, like for instance rifampicin and cyclosporine A (Smith *et al*., 2005; Gui *et al*., 2008). Co-administration 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; Niemi, 2007; Konig *et al*. 2006). As an example, it has been demonstrated that co-administration of atorvastatin with an intravenous dose of rifampicin in healthy subjects does result in a substantially increased systemic exposure of both the acid and lactone form of atorvastatin, which is likely due to the inhibition of atorvastatin uptake into hepatocytes by rifampicin (Lau *et al*., 2007).

The working mechanism of OATP/Oatp family members currently is not clear. Transport is independent of sodium, chloride, and potassium gradients, membrane potential, and ATP levels (See 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 oocytes, a coupled exchange with reduced glutathione (GSH) could be demonstrated, which 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 likely 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

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in cRNA injected Xenopus 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 would be a simple bidirectional diffusion carrier.

After uptake into hepatocytes, drugs will be metabolized by either Phase I or Phase 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 are primary active transporters which 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 ressitance protein 2 (MRP2; ABCC2), the bile salt export pump (BSEP; ABCB11), and the breast cancer resistance protein (BCRP; ABCG2) (Leslie *et al*., 2005; Figure 1). It should be noted that one ABC-transporter, MRP3/Mrp3, is present at high levels in the mouse hepatocyte sinusoidal membrane, but is hardly detectable in hepatocytes of rats or humans under normal conditions (Kruh *et al*., 2007). Currently, the 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.* and Li *et al.* is an important step to study the relative importance of Oatp1b2 in the uptake of substrates into the liver. As there is potential for the upregulation 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

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Oatp2b1 (~1.6-fold) in female knockout compared to control mice, but not in male mice. In the Oatp1b2$^{-/-}$ characterized by Lu *et al.*, 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. Likely, the differences found by the two groups are explained by the different mouse strains used, or 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.* found that a moderate increase in serum levels of conjugated bilirubin was found in Oatp1b2$^{-/-}$ females, whereas serum levels of unconjugated bilirubin tended to be higher in knockout males compared to 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.* 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 (ALT) and aspartate aminotransferase (AST). Oatp1b2 plays a critical role in liver uptake of phalloidin and microcystin-LR as hepatoxicity was observed in control but not in knockout mice treated with these compounds. A similar susceptibility was observed in knockout compared to 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 (Sasaki *et al*., 2004; Nishizato *et al*., 2003). In the Oatp1b2$^{-/-}$ mice, Zaher *et al*. found that the liver-to-plasma ratio for pravastatin was 4-fold lower than in control mice at steady state, indicating that Oatp1b1, at least in part, contributed to uptake into

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the liver. After a subcutaneous infusion of rifampicin, another substrate for human OATP1B1 and OATP1B3, an ~8-fold reduction in the liver-to-plasma ratio was observed at steady state in the knockout compared to 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.

Currently, many 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, however, for instance due to the induction of compensatory mechanisms. In addition, quantitative extrapolation of transporter data between species is difficult due to a limited understanding of species differences in substrate specificity and differences in the relative expression levels of drug transporters. Oatps are no exception, especially since 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.* and Lu *et al.* 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, it should now be considered to knockout the other Oatp family members expressed in mouse liver and to generate humanized mice expressing OATP1B1 and 1B3.

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Figure legend

Figure 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.

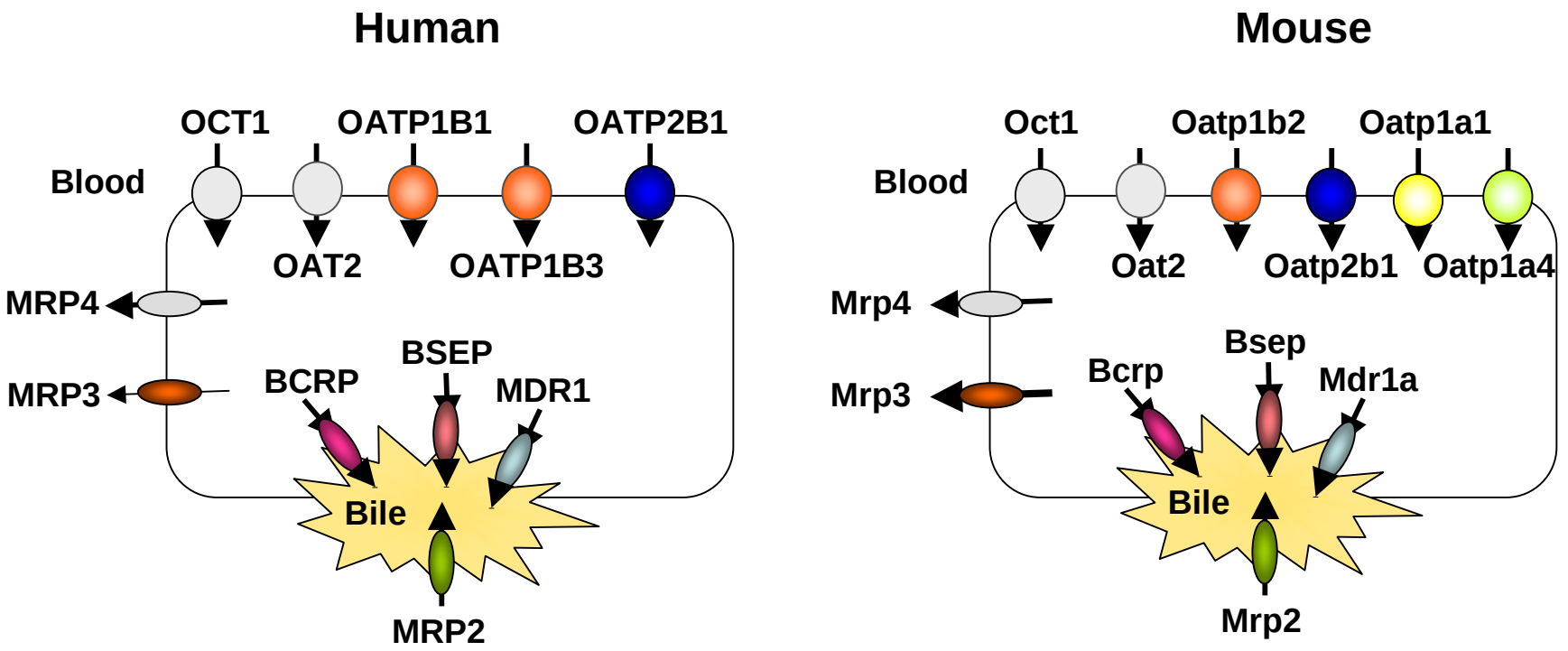

Figure 1