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The impact of Organic Anion-Transporting Polypeptides (OATPs) on disposition and toxicity of antitumor drugs: Insights from knockout and humanized mice

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ABSTRACT

It is now widely accepted that organic anion-transporting polypeptides (OATPs), especially members of the OATP1A/1B family, can have a major impact on the disposition and elimination of a variety of endogenous molecules and drugs. Owing to their prominent expression in the sinusoidal plasma membrane of hepatocytes, OATP1B1 and OATP1B3 play key roles in the hepatic uptake and plasma clearance of a multitude of structurally diverse anti-cancer and other drugs. Here, we present a thorough assessment of the currently available OATP1A and OATP1B knockout and transgenic mouse models as key tools to study OATP functions *in vivo*. We discuss recent studies using these models demonstrating the importance of OATPs, primarily in the plasma and hepatic clearance of anticancer drugs such as taxanes, irinotecan/SN-38, methotrexate, doxorubicin, and platinum compounds. We further discuss recent work on OATP-mediated drug–drug interactions in these mouse models, as well as on the role of OATP1A/1B proteins in the phenomenon of hepatocyte hopping, an efficient and flexible way of liver detoxification for both endogenous and exogenous substrates. Interestingly, glucuronide conjugates of both the heme breakdown product bilirubin and the protein tyrosine kinase-targeted anticancer drug sorafenib are strongly affected by this process. The clinical relevance of variation in OATP1A/1B activity in patients has been previously revealed by the effects of polymorphic variants and drug–drug interactions on drug toxicity. The development of *in vivo* tools to study OATP1A/1B functions has greatly advanced our mechanistic understanding of their functional role in drug pharmacokinetics, and their implications for therapeutic efficacy and toxic side effects of anticancer and other drug treatments.

1. Introduction to OATP1A/1B transporters and genetically modified mouse models to study their functions

1.1. Properties of OATP1A/1B transporters

Organic anion-transporting polypeptide (OATP) uptake transporters can play a major role in the uptake of numerous compounds, including many anticancer drugs, into cells and organs. Positioned in the plasma membrane, these multispanning transmembrane proteins can mediate the uptake of a structurally highly diverse range of substrates into the cell, by as yet incompletely resolved mechanisms. As a consequence, they can have a major impact on the pharmacokinetic disposition of transported drugs, determining their oral availability and plasma clearance, as well as their distribution to liver and other organs, and the main route(s) of elimination (for recent reviews see: Gong and Kim, 2013; Konig et al., 2013; Niemi et al., 2011; Shitara et al., 2013; Stieger and Hagenbuch, 2014). OATPs can therefore have a strong effect on the therapeutic efficacy, but also the toxic side effects of substrate drugs. Moreover, several OATPs are variably expressed in a range of human cancers. As this may obviously influence the effective intracellular exposure of the cancer cells to OATP substrate anticancer drugs, this can directly affect the therapy susceptibility of these cancers (for recent reviews see: Nakanishi and Tamai, 2014; Obaidat et al., 2012; Sissung et al., 2012; Thakkar et al., 2015). The activity of the human

Abbreviations: ABC, ATP-binding cassette; AUC, area under the concentration-time curve; DDI, drug–drug interactions; E2G, estradiol 17β-D-glucuronide; i.v., intravenous; OATP, organic anion-transporting polypeptide; TKI, tyrosine kinase inhibitor; UGT1A1, UDP-glucuronosyltransferase 1A1.

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OATPs that are thought to be most important for the general pharmacokinetic behavior of drugs, OATP1A2, OATP1B1, and OATP1B3 (as well as possibly OATP2B1, but see below), can further vary dramatically because of genetic polymorphisms and mutations that affect drug transport, but also because of drug–drug interactions with a variety of co-administered drugs (e.g. Durmus et al., 2015; Franke et al., 2009; Gong and Kim, 2013; Konig et al., 2013; Niemi et al., 2011; Obaidat et al., 2012; Shitara et al., 2013; Stieger and Hagenbuch, 2014; van de Steeg et al., 2012).

Given their obvious medical importance, it is crucial to obtain clear insight into the *in vivo* pharmacological, toxicological, and physiological functions of the OATP proteins, especially those of the OATP1A/1B family. One way to achieve this goal is to generate and study mouse strains that have the mouse Oatp1a and Oatp1b genes knocked out, or that have replaced the mouse Oatp1a/1b genes with one or more of their human analogues (although the formal gene name for the OATP-encoding genes is SLCO (for Solute Carrier of Organic Anions), for simplicity we will mostly use the OATP/Oatp nomenclature in this review). These mouse models can then be used to investigate the impact of the genetic modifications on the behavior of, amongst others, anticancer drugs. This review focuses on recent studies on such mouse strains and the insights obtained for a number of anticancer drugs. As some aspects have already been extensively reviewed previously (Iusuf et al., 2012b,c; Sprowl and Sparreboom, 2014; Tang et al., 2013), we will only briefly touch upon those.

1.2. OATP1A/1B knockout and transgenic mouse strains characterized to date

To date, most characterized knockout and transgenic mouse models concern members of the OATP1A and OATP1B subfamilies, as these are thought to be most relevant for overall pharmacokinetics in man. Some initial studies suggested that human OATP1A2 was expressed in the intestinal epithelium, which would potentially indicate an important role in drug absorption (Glaeser et al., 2007). However, many independent later studies could not corroborate these findings, and it is now probably safe to conclude that normally there is no substantial level of OATP1A2 present in the small or large intestine of humans (e.g. Drozdzik et al., 2014). On current data, OATP1A2 is substantially expressed in cholangiocytes lining the bile ducts in the liver, in the human blood-brain barrier, in apical membranes of kidney tubules, and in a variety of human tumors (van de Steeg et al., 2013 and references therein). In contrast to OATP1A2, human OATP1B1 and OATP1B3 are highly and primarily expressed in the basolateral (sinusoidal) membrane of human hepatocytes, where they can mediate the hepatic uptake of numerous substrate compounds (e.g. Nakanishi and Tamai, 2012). As these genes are also known to be substantially affected by genetic polymorphisms and mutations in humans (e.g. Niemi et al., 2011; van de Steeg et al., 2012), they have attracted most attention. The functionally related OATP2B1 protein is also a broad-specificity multidrug-uptake transporter, especially at lower pH, and highly expressed in both intestine and the sinusoidal membrane of hepatocytes. It has therefore been suggested that it might also have considerable pharmacokinetic impact (Nakanishi and Tamai, 2012). However, to date no OATP2B1 mouse models have been published, so we will not further cover this transporter here.

A complication in studying mouse models for the OATP1A/1B transporters is that there are no straightforward orthologues between the individual mouse and human Oatp1a/1b and OATP1A/1B genes. As indicated in Fig. 1, there are no less than 4 different Oatp1a genes in the mouse, Oatp1a1, Oatp1a4, Oatp1a5 and Oatp1a6, in addition to 2 Oatp1a-like elements that may be pseudo-genes. This compares with the single OATP1A2 gene in humans. On the other hand, the mouse has only one Oatp1b2 gene, contrasting with the two human OATP1B1 and OATP1B3 genes (Fig. 1). Although the mouse and human OATP1A proteins are obviously more similar to each other than to the mouse and human OATP1B proteins, and vice versa, the amino acid divergence within each subfamily is still considerable (as low as 67% amino acid identity within the OATP1A subfamily, and 65% within the OATP1B subfamily). Consequently, with these broad-specificity multidrug transporters, no reliable statements can be made on overlapping substrates just based on amino acid similarity. As the tissue distribution is also not conserved between members of one subfamily (for instance, mouse Oatp1a1 and Oatp1a4 are present in the sinusoidal membrane of hepatocytes, whereas human OATP1A2 is not) it is clear that one cannot use single-gene mouse Oatp1a or Oatp1b knockout strains to make reliable predictions on the *in vivo* behavior of human OATP1A2, OATP1B1, or OATP1B3. This was an important motivation to generate a complete Oatp1a/1b knockout strain, and use it to specifically express human OATP1A2, OATP1B1, and OATP1B3 in this knockout background (van de Steeg et al., 2012, 2013, 2010).

Table 1
Oatp1a/b knockout and transgenic mouse models described to date.

Mouse models	Genetic background	Primary references
Oatp1a1 knockout	C57Bl/6 N	(Gong et al., 2011)
Oatp1a4 knockout	C57Bl/6 N	(Gong et al., 2011; Ose et al., 2010)
Oatp1b2 knockout (variant 1)	C57BL/6	(Lu et al., 2008)
Oatp1b2 knockout (variant 2)	DBA1/lacJ	(Zaher et al., 2008)
Oatp1a/1b knockout	FVB	(van de Steeg et al., 2010)
Humanized hepatic OATP1A2 transgenic	FVB	(van de Steeg et al., 2013)
Humanized hepatic OATP1B1 transgenic	FVB	(van de Steeg et al., 2009)
Humanized hepatic OATP1B3 transgenic	FVB	(van de Steeg et al., 2013)
Humanized hepatic OATP1B1 and OATP1B3 transgenic	FVB	(Salphati et al., 2014)

Table 1 lists the various Oatp1a and Oatp1b knockout strains, and OATP1A/1B humanized strains that have been described so far. Oatp1a1 and Oatp1a4 knockout strains were described by Ose et al. (2010) and Gong et al. (2011). These mouse strains, originally made in 129/Ola ES cells by Deltagen, were backcrossed 10 times to a C57BL/6 background, and subsequent characterization of these lines was mainly done in this genetic background. A few independent Oatb1b2 knockout strains were generated. Lu et al. (2008) described the generation and initial characterization of an Oatp1b2 knockout strain generated in 129S1 ES cells, which was backcrossed for 7 generations to a C57BL/6 background. Independently, Zaher et al. (2008) generated Oatp1b2 knockout mice using DBA1/lacJ ES cells, which were further kept and characterized in a DBA1/lacJ genetic background. Combined Oatp1a/1b knockout mice, covering all the mouse Oatp1a and Oatp1b genes, were generated in 129/Ola ES cells. Initial characterization of Oatp1a/1b$^{-/-}$ mice was done in a mixed (~50%) 129/Ola and FVB genetic background (van de Steeg et al., 2010), but this strain was subsequently backcrossed for at least 7 generations to an FVB background, in which further characterization took place (van de Steeg et al., 2012). These FVB background Oatp1a/1b$^{-/-}$ mice were then used to generate three different humanized mouse strains with predominant expression of human OATP1A2, OATP1B1, or OATP1B3 cDNA, respectively, in the liver parenchyme cells, again all in FVB background (van de Steeg et al., 2012, 2013). Moreover, a combined humanized OATP1B1/OATP1B3 strain was created by crossing the separate transgenic strains (Salphati et al., 2014).