Inhibition of OATP1B1 by tyrosine kinase inhibitors: \textit{in vitro–in vivo} correlations

S Hu\textsuperscript{1}, R H J Mathijssen\textsuperscript{2}, P de Bruijn\textsuperscript{2}, S D Baker\textsuperscript{1} and A Sparreboom*\textsuperscript{1,2}

\textsuperscript{1}Department of Pharmaceutical Sciences, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA and \textsuperscript{2}Department of Medical Oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

**Background:** Several tyrosine kinase inhibitors (TKIs) can decrease docetaxel clearance in patients by an unknown mechanism. We hypothesised that these interactions are mediated by the hepatic uptake transporter OATP1B1.

**Methods:** The influence of 16 approved TKIs on transport was studied \textit{in vitro} using HEK293 cells expressing OATP1B1 or its mouse equivalent Oatp1b2. Pharmacokinetic studies were performed with Oatp1b2-knockout and OATP1B1-transgenic mice.

**Results:** All docetaxel-interacting TKIs, including sorafenib, were identified as potent inhibitors of OATP1B1 \textit{in vitro}. Although Oatp1b2 deficiency \textit{in vivo} was associated with increased docetaxel exposure, single- or multiple-dose sorafenib did not influence docetaxel pharmacokinetics.

**Conclusion:** These findings highlight the importance of identifying proper preclinical models for verifying and predicting TKI–chemotherapy interactions involving transporters.

Docetaxel is widely used for the treatment of multiple solid tumours, including cancers of the breast, lung, head and neck, stomach, and prostate. The interindividual pharmacokinetic variability seen with docetaxel treatment remains high, and this phenomenon may have important ramifications for the agent’s clinical activity and toxicity (Baker et al., 2006). Docetaxel is mainly metabolised by the hepatic enzyme CYP3A4, and the importance of this pathway has been confirmed in mice with a deletion of the Cyp3a gene cluster (Van Herwaarden et al., 2007). We previously reported that differential expression of organic anion-transporting polypeptides of the OATP1B family in the human liver regulates the initial step in the elimination of docetaxel, before metabolism (De Graan et al., 2012). In view of the relevance of these uptake transporters in the pharmacokinetics of docetaxel, instances of idiosyncratic hypersensitivity to docetaxel could possibly be the result of currently unrecognised drug–drug interactions at the level of hepatocellular uptake mechanisms involving OATP1B1, the main OATP1B-family member expressed in the human liver (Konig et al., 2013).

In this context, it is worth noting that several tyrosine kinase inhibitors (TKIs) evaluated in combination regimens with docetaxel, including axitinib (Martin et al., 2012), pazopanib (Hamberg et al., 2012), and sorafenib (Awada et al., 2012), can increase the systemic exposure to docetaxel in cancer patients by a mechanism that is currently not understood (Table 1). In the current study, we tested the hypothesis that these TKIs can inhibit the function of OATP1B1 and its murine equivalent Oatp1b2 \textit{in vitro}, and evaluated the contribution of this process to an interaction with docetaxel \textit{in vivo} using mice that are knocked out for Oatp1b2 or knocked in for OATP1B1.

**MATERIALS AND METHODS**

Crizotinib, lapatinib, nilotinib, regorafenib, ruxolitinib, sorafenib and vemurafenib were purchased from Chemie Tek (Indianapolis, IN, USA); dasatinib, erlotinib, imatinib, pazopanib, and vandetanib from LC laboratories (Woburn, MA, USA); gefitinib and sunitinib from Toronto Research Chemicals (Toronto, ON, Canada); axitinib from Selleckchem (Houston, TX, USA); and bosutinib from Pfizer (New York, NY, USA). \[^3\text{H}\]Docetaxel (specific activity, 60 Ci mmol\(^{-1}\); radiochemical purity, 99.0%) and \[^3\text{H}\]estradiol-17\text{\beta}-\text{D}-glucuronide (specific activity, 50.1 Ci mmol\(^{-1}\); radiochemical purity, 99.0%) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Dimethyl sulfoxide (Sigma Aldrich, St. Louis, MO, USA) was used as a solvent for all TKIs, and ethanol for docetaxel.

*Correspondence: Dr A Sparreboom; E-mail: alex.sparreboom@stjude.org

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Plasma from each mouse was collected at 5, 15, 30, 60, 120, and 240 min after docetaxel administration, and samples were analysed by a validated method based on liquid chromatography with tandem mass-spectrometric detection (De Graan et al., 2012). Pharmacokinetic parameters were calculated using non-compartmental methods in the WinNonlin 6.2 software (Pharsight, St. Louis, MO, USA). All data are presented as mean ± s.d. Statistical analyses were based on a two-tailed, non-parametric t-test (GraphPad Prism v5.0, La Jolla, CA, USA), and P < 0.05 was considered statistically significant.

RESULTS

Inhibition of OATP1B1 by TKIs in vitro. We initially determined whether FDA-approved TKIs can inhibit OATP1B1 function in mammalian cells that overexpress the transporter, using estradiol-17β-β-glucuronide as a prototypical substrate (Konig et al., 2000). Of the 16 TKIs evaluated, axitinib, nilotinib, pazopanib, and sorafenib were identified as potent inhibitors of OATP1B1 (>90% inhibition; Figure 1A). As a representative of this class of TKIs, sorafenib was further evaluated and found to also potently inhibit the OATP1B1-mediated transport of docetaxel with a half-inhibitory maximum concentration of 6.96 nM (Figure 1B), and almost completely inhibit the function of human OATP1B1 (Figure 1C) and mouse Oatp1b2 (Figure 1D) at 10 μM, a concentration achievable in humans and mice (Hu et al., 2011).

Pharmacokinetic studies in vivo. To test whether sorafenib inhibits OATP1B-type transporters in vivo, we determined the pharmacokinetic profile of docetaxel in a DBA/1LacJ strain of mice deficient in Oatp1b2 (Oatp1b2(-/-/-) mice). In the absence of the TKI, Oatp1b2 deficiency was associated with a significantly increased exposure to docetaxel, as measured by peak plasma concentration (P = 0.00033; Figure 2A) and area under the curve (AUC) (P < 0.0001; Figure 2B). Unexpectedly, coadministration of a single oral dose of sorafenib did not result in a significantly altered AUC of docetaxel in either wild-type (P = 0.97) or Oatp1b2(-/-/-) mice (P = 0.75). The lack of a pharmacokinetic interaction was also noted when sorafenib was given twice daily for 4 consecutive days before docetaxel administration to wild-type (P = 0.14) or Oatp1b2(-/-/-) mice (P = 0.29; Figure 2B).

As hepatocytes of the Oatp1b2(-/-/-) mice express multiple members of Oatp1a, a related subfamily of transporters that can potentially provide compensatory restoration of function when Oatp1b2 is inhibited (Iusuf et al., 2012), we next determined the pharmacokinetics of docetaxel in mice on an FVB strain deficient in both the Oatp1a and Oatp1b gene loci (Oatp1a1b(-/-/-) mice), either with or without liver-specific expression of OATP1B1 (OATP1B1(tg)). The AUC of docetaxel was similar in Oatp1a1b(-/-/-) and Oatp1b(-/-/-) mice (P = 0.73; Figure 2B), and was significantly reduced in OATP1B1(tg) mice (P = 0.0052), supporting a direct role of OATP1B1 in the elimination of this agent. However, despite the ability of sorafenib to inhibit the OATP1B1-mediated transport of docetaxel in vitro, sorafenib did not influence the AUC of docetaxel in this mouse model (P = 0.15; Figure 2B).

DISCUSSION

In this study, we demonstrate that several TKIs, including axitinib, pazopanib, nilotinib, and sorafenib, can inhibit the activity of the human OATP1B1 transporter by more than 90%. The results for pazopanib are consistent with a previous report using a similar model (Xu et al., 2010). Interestingly, among TKIs that have been evaluated clinically in combination with docetaxel, only those

| Table 1. Evaluation of pharmacokinetic interactions between TKIs and docetaxel in patients |
|-----------------|-----------------|---------------------|
| TKI             | Docetaxel dose (mg m⁻²) | Observation | Reference |
| Axitinib        | 100             | AUC ~55% increased | Martin et al., 2012 |
| Bosutinib       | NA              | NA                  | NA         |
| Crizotinib      | NA              | NA                  | NA         |
| Dasatinib       | 75              | No change           | Araujo et al., 2012 |
| Erlotinib       | 25              | No change           | Chiorean et al., 2008 |
| Gefitinib       | 75              | No change           | Manegold et al., 2005 |
| Imatinib        | 20–25           | No change           | Connolly et al., 2011 |
| Lapatinib       | 75              | No change           | Lofusso et al., 2008 |
| Nilotinib       | NA              | NA                  | NA         |
| Pazopanib       | 50–60           | AUC ~57% increased | Hamberg et al., 2012 |
| Regorafenib     | NA              | NA                  | NA         |
| Ruxolitinib     | NA              | NA                  | NA         |
| Sorafenib       | 75–100          | AUC ~36–80% increased | Awada et al., 2012 |
| Sunitinib       | 75              | No change           | Bergh et al., 2012 |
| Vandetanib      | NA              | NA                  | NA         |
| Vemurafenib     | NA              | NA                  | NA         |

Abbreviation: NA = no data available.
found here to be potent OATP1B1 inhibitors cause clinical drug–drug interactions, resulting in increases in the systemic exposure to docetaxel of up to 80%. Of these TKIs, pazopanib is a known weak inhibitor of CYP3A4 and can moderately increase exposure to other CYP3A4 substrates such as midazolam (Goh et al., 2010) and paclitaxel (Tan et al., 2010). This suggests that the mechanism by
which pazopanib affects the pharmacokinetic profile of docetaxel may involve both metabolism and transport. However, unlike pazopanib, axitinib does not inhibit CYP3A4 (Chen et al, 2013), and although sorafenib competitively inhibits recombinant CYP3A4 in vitro (Sugiyama et al, 2011) it has no influence on the pharmacokinetics of midazolam (Flaherty et al, 2011) or paclitaxel (Flaherty et al, 2008; Okamoto et al, 2010). The previous demonstration that axitinib (Reyner et al, 2013) and sorafenib (Zimmerman et al, 2013) are themselves substrates of OATP1B1 supports the possibility that the reported pharmacokinetic interactions of these TKIs with docetaxel in patients are the result of a competitive inhibitory mechanism at the level of docetaxel entry into hepatocytes mediated by OATP1B1. This would be consistent with the notion that, unlike for docetaxel, the pharmacokinetics of neither midazolam (Ziesenitz et al, 2013) nor paclitaxel (Van De Steeg et al, 2013) is affected by OATP1B1.

The human and rodent OATP1B-type transporters share a high degree of sequence homology, similarity in basolateral membrane localisation, and have largely overlapping substrate and inhibitor specificity (Roth et al, 2012). Our current in vitro data are in line with that prior knowledge in that sorafenib was found to be an inhibitor for both human OATP1B1 and mouse Oatp1b2. Moreover, our in vivo studies confirmed the significant influence of Oatp1b2 deficiency in mice on the pharmacokinetics of docetaxel (De Graan et al, 2012), and demonstrated that this defect can be fully restored by introducing OATP1B1 in the hepatocytes of these animals without involvement of the related Oatp1a-type transporters.

Surprisingly, the reported clinical pharmacokinetic interaction between sorafenib and docetaxel (Awada et al, 2012) could not be replicated in mice. We previously demonstrated that Oatp1b2 deficiency in mice is not associated with any pronounced compensatory alterations in expression of hepatic transporters that can explain these findings (Lancaster et al, 2012). Moreover, there are no changes in the functional expression of Cyp3a isoforms, the key enzymes associated with docetaxel metabolism in these transporter knockout mice (Lancaster et al, 2012). In our current study, we found no substantial differences in pharmacokinetic parameters of docetaxel when comparing results in Oatp1b2(−/−) mice to those in Oatp1a/1b(+/−) mice. This eliminates the possibility that the lack of a change in docetaxel plasma levels in the presence of sorafenib was due to compensatory effects involving Oatp1a-type transporters. The discrepancy between the clinical observations and those observed here in mice supports the possibility that additional uptake transporters for docetaxel may exist in mice that are insensitive to inhibition by sorafenib. Such demonstration of inherent interspecies differences in drug–drug interactions is not unprecedented, although this phenomenon is usually associated with differential affinity of inhibitors for human compared with rodent transporters (Shirasaka et al, 2010). Studies are ongoing to evaluate the influence of human uptake transporting proteins of inhibitors for human compared with rodent transporters (Shirasaka et al, 2010).

Overall, our findings support a direct contribution of OATP1B1 in previously recorded pharmacokinetic interactions between TKIs and docetaxel, which can be predicted from a simple in vitro experiment. Although the present investigation involved in vivo studies with only one TKI, our failure to reproduce an established interaction of sorafenib with docetaxel in mice suggests that caution is warranted when attempting to extrapolate in vivo findings to a clinical scenario.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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