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Inhibition of OATP-1B1 and OATP-1B3 by tyrosine kinase inhibitors

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Abstract

Background—The potential of tyrosine kinase inhibitors (TKIs) interacting with other therapeutics through hepatic uptake transporter inhibition has not been fully delineated in drug-drug interactions (DDIs). This study was designed to estimate the half-maximal inhibitory concentration (IC₅₀) values of five small-molecule TKIs (pazopanib, nilotinib, vandetanib, canertinib and erlotinib) interacting with organic anion-transporting polypeptides (OATPs): OATP-1B1 and -1B3.

Methods—The IC₅₀ values of TKIs and rifampicin (positive control) were determined by concentration-dependent inhibition of TKIs on cellular accumulation of radiolabeled probe substrates [³H]estrone sulfate and [³H]cholecystokinin octapeptide. Chinese hamster ovary cells transfected with humanized OATP-1B1 and OATP-1B3 transporter proteins, respectively, were utilized to carry out these studies.

Results—Pazopanib and nilotinib show inhibitory activity on OATP-1B1 transporter protein. IC₅₀ values for rifampicin, pazopanib and nilotinib were 10.46 ± 1.15 , 3.89 ± 1.21 and 2.78 ± 1.13 μ M, respectively, for OATP-1B1 transporter. Vandetanib, canertinib and erlotinib did not exhibit any inhibitory potency toward OATP-1B1 transporter protein. Only vandetanib expressed inhibitory potential toward OATP-1B3 transporter protein out of the five selected TKIs. IC₅₀ values for rifampicin and vandetanib for OATP-1B3 transporter inhibition were 3.67 ± 1.20 and

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18.13 ± 1.21 µM, respectively. No significant inhibition in the presence of increasing concentrations of pazopanib, nilotinib, canertinib and erlotinib were observed for OATP-1B3 transporter.

Conclusion—Because selected TKIs are inhibitors of OATP-1B1 and -1B3 expressed in hepatic tissue, these compounds can be regarded as molecular targets for transporter-mediated DDIs. These findings provide the basis for further preclinical and clinical studies investigating the transporter-based DDI potential of TKIs.

Keywords

drug-drug interactions; hepatic transporters; IC₅₀ values; nilotinib; pazopanib; vandetanib

Introduction

The role of membrane transporters in drug disposition, safety and efficacy, i.e., particularly concerning drug-drug interactions (DDIs) has been extensively investigated [1]. Giacomini et al. recently emphasized that drug development information on *in vitro* studies of drug-transporter interactions can be extrapolated to clinical studies of transporter-based DDIs. Such transporter-mediated DDIs can occur by (i) inhibition of membrane transporter resulting in potential DDI, and/or (ii) interacting drug may be a substrate for the transporter. Attention has been drawn toward various approaches and algorithms for predicting transporter-mediated DDIs. *In vitro* and preclinical transport studies are prerequisites for drug development. Recent progress in clinical translation of these results may impact on regulatory matters for delineation of transport-mediated DDIs [2]. In order to predict whether a potential DDI may occur, *in vitro* studies were performed to compare the concentration of an inhibitor (I, the maximum unbound plasma concentration) and its half-maximal inhibitory concentration (IC₅₀) for a transporter. Lower IC₅₀ of the drug relative to its unbound plasma concentration is a strong indicator of a potential clinical DDI. An I/IC₅₀ value > 0.1 has been advocated as a measure to evaluate clinical transporter-based DDIs [2].

Tyrosine kinase inhibitors (TKIs) are the new class of anticancer drugs that specifically target tyrosine kinases that are fused, mutated and overexpressed in cancer [1, 3]. Many of these compounds have been associated with low patient response along with unwanted toxicity, which is unexpected and also largely unexplained. Even though TKIs offer theoretical advantages (selectively target/kill the cancer precursor cells and protect normal tissues) over traditional anticancer agents, these agents are still associated with unpredictable toxicity [4, 5]. Many TKIs exhibit limited efficacy with a high degree of unexpected and unexplained toxicity [6]. The most common side effects associated with TKIs are diarrhea, hypertension, nausea, anorexia and vomiting. The most common treatment-emergent laboratory abnormalities noticed were elevation of total bilirubin, liver transaminases and alanine aminotransferases. Hepatotoxicity is the most frequently reported toxicity among the TKIs with mandatory black box warnings [7]. There is a possibility that treatment-associated elevation in liver enzymes with TKIs reveals overlapping on-target and off-target class effects; however, the exact mechanism needs to be clarified [1, 8, 9]. These hepatic abnormalities associated with TKIs may lead to treatment interruption, compromising the potential treatment benefit to the patient. A clear understanding of the

exact mechanism responsible for hepatic abnormalities will give a better chance to interpret and manage these adverse effects that will ultimately benefit patients from continued chemotherapeutic treatment [8].

Despite their frequent use as a chemotherapeutic agent, limited studies have been performed to examine the interactions of these TKIs with hepatic uptake transporters such as organic anion-transporting polypeptides (OATPs). Most studies examining the interaction of TKIs with these transporters have focused on substrate specificity instead of inhibition interactions [1, 5, 10–14]. Also, several TKIs have higher molecular weight, polar surface area and lipophilicity, which are essential for OATP inhibition and therefore have the potential to inhibit OATPs including OATP-1B1 and OATP-1B3 [15]. Several *in vitro* and *in vivo* studies have indicated that drugs inhibiting these OATPs are responsible for clinically relevant DDIs. In such cases, inhibition of OATPs can lead to unexpected toxicity, causing marked increase in plasma concentration and area under the plasma concentration time curve (AUC) for compounds that are substrates of these hepatic transporters. DDIs caused by the inhibition of these transporters represent a large number of drugs that act as substrates or inhibitors of OATP-1B1 and/or -1B3 [16]. Hence, it is of utmost importance to estimate the inhibitory potential of TKIs on OATP-1B1 and -1B3. In the present study, we have evaluated the interaction of TKIs (pazopanib, erlotinib, canertinib, nilotinib and vandetanib) with human OATPs expressed on the sinusoidal membrane of the liver by employing an *in vitro* model system with transfected Chinese hamster ovary (CHO) cells. *In vitro* studies were designed to compare the inhibitory potential of TKIs on the transport of [³H]estrone sulfate ([³H]ES, substrate for OATP-1B1) and cholecystokinin octapeptide (CCK-8, substrate for OATP-1B3) in OATP-1B1 and -1B3-transfected CHO cells.

Materials and methods

Chemicals

Pazopanib, erlotinib, canertinib, nilotinib and vandetanib were purchased from LC Laboratories (Woburn, MA, UDS). Rifampicin was purchased from TCI America, PA, USA. [³H]ES (specific activity 40–60 Ci/mmol) and [³H]cholecystokinin octapeptide ([³H]CCK-8, specific activity 60–100 Ci/mmol) were procured from Perkin Elmer (Boston, MA, USA). All other chemicals used were of high-performance liquid chromatography grade and were obtained from either Sigma Aldrich (MO, USA) or Fisher Scientific (NH, USA). Cell culture medium and other ingredients were purchased from Life Sciences. Fetal bovine serum (FBS) was received from Atlanta Biologicals (GA, USA).

In vitro studies

Cell lines—CHO cells (passage numbers 17–50) were selected for all *in vitro* experiments. OATP-1B1 and -1B3 CHO transfected cells were obtained as a gift from Dr. Bruno Stieger (Department of Clinical Pharmacology and Toxicology, University Hospital Zürich, Switzerland). Cells were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% heat-inactivated FBS, 1-proline (50 µg/mL), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), penicillin (100 µg/mL), streptomycin (100 µg/mL)

and geneticin (100 µg/mL). Cell cultures were maintained at 37°C with 5% CO₂ under humidifying conditions.

***In vitro* cellular accumulation studies**—Confluent CHO OATP-1B1 and -1B3 cells were utilized for uptake experiments. Following medium removal, cells were rinsed three times for 5 min each with 1–2 mL of Dulbecco's phosphate-buffered saline (DPBS) containing 130 mM NaCl, 0.03 mM KCl, 7.5 mM Na₂ HPO₄, 1.5 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgSO₄, 20 mM HEPES and 5 mM glucose maintained at pH 7.4. Uptake studies were initiated by adding 250 µL of solution containing 0.25 µCi/mL of [³H]ES (for OATP-1B1) or [³H]CCK-8 (for OATP-1B3) in the presence of two different concentrations (25 and 50 µM) of TKIs and rifampicin (positive control). Following incubation, the solution was removed and uptake was terminated with 2 mL of ice-cold stop solution containing 200 mM KCl and 2 mM HEPES. The cell monolayer was washed three times for 5 min each, and 1 mL of lysis buffer (0.1% Triton X solution in 0.3% NaOH) was added to each well; plates were stored overnight at room temperature. Subsequently, cell lysate (400 µL) from each well was transferred to scintillation vials containing 3 mL of scintillation cocktail (Universal ES from MP Bio-medicals). Samples were analyzed by measuring the radioactivity in a liquid scintillation counter (model LS-6500, Beckman Instruments Inc., Fullerton, CA, USA). The protein content of each sample was estimated with a BioRad Protein Estimation Kit (BioRad, Hercules, CA, USA).

Estimation of IC₅₀—To determine the IC₅₀ of TKIs for the differential uptake of OATP-1B1 and -1B3 transporter proteins, intracellular accumulation of the probe substrates (ES and CCK-8) in the presence of increasing concentrations (0.1–100 µM) of TKIs was measured. Using a concentrated stock solution of the TKIs, several working concentrations were prepared ranging from 0.1 to 100 µM in fresh DPBS buffer spiked with [³H]ES (0.25 µCi/mL or 5.0 nM) or [³H]CCK-8 (0.25 µCi/mL or 3.1 nM). Uptake was carried out at different concentrations of TKIs in OATP-1B1 and -1B3 transfected CHO cells. The data were fitted to Eq. (1), and the IC₅₀ values were calculated according to a nonlinear least squares regression analysis program, GraphPad Prism version 5.

Data analysis

IC₅₀ values of TKIs on intracellular accumulation of the probe substrate uptake via hepatic OATP-1B1 and -1B3 were calculated with GraphPad Prism version 5. The data were plotted and fitted to Eq. (1) and the IC₅₀ values were calculated.

$$Y = \min + \frac{\max - \min}{1 + 10^{(\log \text{IC}_{50} - x) \times H}} \quad (1)$$

where x denotes the log concentration of the inhibitors, Y is the cellular accumulation of the probe substrate (ES or CCK-8), IC₅₀ represents the TKI concentration, where the influx of the substrate is inhibited by 50%, and H is the Hill constant. Y starts at a minimum value and then plateaus at a maximum value, resulting in a sigmoidal plot.

Cytotoxicity studies

Cell Titer 96® Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega, Madison, WI, USA) was employed to carry out cytotoxicity assay. OATP-1B1 and -1B3 transfected CHO cells were cultured in a 96-well plate. Sterile drug solutions of the highest concentration (100 μ M) of TKIs were prepared in the culture medium using 0.22- μ m sterile nylon membrane filters. Aliquots of TKIs having a volume of 100 μ L (previously made in culture medium) were added to each well and incubated for 24 h. Cell proliferation in the presence of TKIs was measured and compared with a negative control (medium without TKIs) and a positive control (Triton X). Twenty microliters of dye solution was added to each well after 24 h of incubation with TKIs. Cells were then incubated for 4 h in order to complete the reaction with dye. UV absorbance of purple formazan product was measured at a wavelength of 590 nm with a 96-well microtiter plate reader (SpectraFluor Plus, Tecan, Maennedorf, Switzerland). The toxicity of TKIs in OATP-1B type transfected CHO cells was estimated by the amount of formazan formed, which is directly proportional to the number of viable cells.

Results

In vitro inhibitory activity of TKIs

Initial *in vitro* uptake experiments were carried out to determine the inhibitory activity of TKIs for OATP-1B1 and -1B3 transfected CHO cells utilizing radiolabeled substrates. [³H]ES and [³H]CCK-8 were employed as radiolabeled probe substrates for OATP-1B1 and -1B3 transporter proteins, respectively. Cellular accumulation of [³H]ES was measured by exposing OATP-1B1 transfected CHO cells to two different concentrations (25 and 50 μ M) of TKIs and rifampicin (positive control). In previously reported results, higher concentration ranges of various drugs have shown inhibitory activity toward OATP-1B1 and -1B3 mediated transport. We performed our studies within these concentration ranges and also at a concentration that was well within our detection limit. Also, the tested concentration of TKIs is achievable in humans and mice [17, 18]. Hence, on the basis of these considerations, we chose 25 and 50 μ M as our concentration ranges. Of the selected TKIs, pazopanib and nilotinib showed significant inhibition on cellular accumulation of [³H]ES in OATP-1B1-transfected cells. The remaining three TKIs (canertinib, vandetanib and erlotinib) did not show any significant inhibitory activity on cellular accumulation of [³H]ES in OATP-1B1 transfected cells. Pazopanib and nilotinib (25 and 50 μ M) diminished the uptake of [³H]ES by ~90% ($p < 0.05$) in OATP-1B1 transfected cells. Rifampicin reduced uptake of [³H]ES by -50% and 70% at 25 and 50 μ M, respectively (Figure 1).

It has been reported previously that OATP-1B3 shares 80% amino acid homology with OATP-1B1. Also, both the ATP isoforms share multiple overlapping substrates, such as rifampicin pravastatin, pitvastatin and docetaxel [19, 20]. In this study, we have also determined the inhibitory activity of TKIs and rifampicin (positive control) at two different concentrations (25 and 50 μ M) on cellular accumulation of [³H]CCK-8 in OATP-1B3 transfected cells. Only vandetanib showed significant inhibition on cellular accumulation of [³H]CCK-8 in OATP-1B3 transfected cells. Pazopanib, nilotinib, canertinib and erlotinib at both concentrations did not show any significant effect on the cellular accumulation of

[³H]CCK-8 in OATP-1B3 transfected cells. Reduced uptake of [³H]CCK-8 was also observed in the presence of rifampicin in OATP-1B3 transfected cells. Uptake of [³H]CCK-8 in OATP-1B3 transfected cells was reduced to ~10% to 20% and ~5% to 10% in the presence of vandetanib and rifampicin (25 and 50 μ M), respectively (Figure 2). Pazopanib and nilotinib showed inhibitory activity toward OATP-1B1, whereas vandetanib only indicated inhibitory potential toward OATP-1B3.

Estimation of IC₅₀—To determine the IC₅₀ concentrations of TKIs that inhibit OATP-1B1 and -1B3 functional activity, cellular accumulation experiments were conducted using probe substrates, ([³H] ES for OATP-1B1 and [³H]CCK-8 for OATP-1B3) in the presence of increasing concentrations (0.1–100 μ M) of TKIs and rifampicin. Previous studies have shown time-dependent uptake on similar cell lines to be linear up to 15 min [21–24]. We incubated cells for 10 min because it remains within the linear range of uptake as well as gives us concentrations well within the detectable range. A modified log[dose]-response curve was applied to fit the data [Eq. (1)] in order to obtain IC₅₀ values. Diminished net uptake rate of probe substrate ([³H]ES) in the presence of increasing concentrations of the TKIs was observed in OATP-1B1 cells. IC₅₀ values for rifampicin, pazopanib and nilotinib toward OATP-1B1 transporter inhibition were 10.46 ± 1.15 , 3.89 ± 1.21 and 2.78 ± 1.13 μ M, respectively (Figure 3A–C and Table 1). Nilotinib appeared to be a more potent inhibitor of OATP-1B1 than pazopanib. Vandetanib, canertinib and erlotinib did not cause any concentration-dependent inhibition on cellular accumulation of probe substrate ([³H]ES) via OATP-1B1 transporter (Figure 3D–F). Also, reduced intracellular accumulation of [³H]CCK-8 was observed in OATP-1B3 transfected cells in a concentration-dependent manner in the presence of vandetanib. IC₅₀ values for rifampicin and vandetanib for OATP-1B3 transporter inhibition were 3.67 ± 1.20 and 18.13 ± 1.21 μ M, respectively (Figure 4A and B and Table 1). Likewise, no significant inhibition in net uptake rate of probe substrate ([³H]CCK-8) was observed in OATP-1B3 cells in the presence of pazopanib, nilotinib, canertinib and erlotinib (Figure 4C–F).

Cytotoxicity studies

To evaluate the cytotoxic effect of the selected TKIs, a cell proliferation assay was performed on cell monolayers of transfected CHO cells for a period of 24 h. No cytotoxic effects of pazopanib, erlotinib, canertinib, vandetanib and nilotinib at a concentration of 100 μ M were observed in OATP-1B1 and -1B3 transfected cells relative to positive control (Triton X). The findings from this study clearly demonstrate that the selected TKIs are noncytotoxic even at a concentration of 100 μ M (Figure 5).

Discussion

In the present study, we investigated the inhibitory potential of several TKIs on OATP family transporters. This study also estimated the IC₅₀ of TKIs for OATP-1B1 and -1B3. CHO cells were originally selected for the transfection because of the lack of expression of this family of proteins in the parent cell line, resulting in minimal background activity. Also, these cells can be maintained in culture for sustained periods and can be ready for use in a specific experiment within a few days. Our findings are in partial agreement with the study

done by Hu et al. [24], where the authors reported the inhibitory potency of nilotinib and pazopanib (only at 10 μ M) in Flp-In T-Rex293 cells expressing OATP-1B1. Similar results were observed in studies evaluating the *in vitro* inhibitory activity of TKIs, demonstrating inhibition of OATP-1B1 transporter protein by nilotinib and pazopanib. Hu et al. [24] and Zimmerman et al. [6]; Minematsu and Giacomini [1] also reported the inhibitory activity of erlotinib and vandetanib in Flp-In T-Rex293 cells expressing OATP-1B1 at a concentration of 10 μ M. Conversely, our findings demonstrate that there is no active involvement of erlotinib and vandetanib in the inhibition of OATP-1B1 transporter protein. These contrasting results suggest that utilization of different radiolabeled probe substrates and *in vitro* model-based systems may affect the inhibitory action of TKIs on cellular accumulation of probe substrate via OATP and can lead to misinterpretation of the role of TKIs causing inhibition of OATPs.

Expression of OATP-1B1 and -1B3 has been exclusively reported on the liver, suggesting their vital role in hepatic uptake of many drugs. Moreover, expression of OATP-1B3 has also been reported on cancer tissues [16, 25–29]. Several substrates of OATP like statins, paclitaxel, and docetaxel are taken and metabolized by the liver. The rate of hepatic transporter-mediated uptake is considered as one of the important parameters of total metabolic rate [16, 30–33]. Hence, plasma concentration of drugs that undergo hepatic metabolism may be altered by inhibition of hepatic uptake transporters (OATPs). Cyclosporin A (CsA) is a well-known inhibitor of OATP-1B1, CYP3A4 and MDR1. It is a more potent inhibitor of OATP-1B1 (IC_{50} 0.2 μ M) than CYP3A4 (IC_{50} > 0.3 μ M). Coadministration of pravastatin, rosuvastatin and atorvastatin together with CsA resulted in increased AUC by 9.93-, 7.08- and 8.69fold, respectively [16, 34, 35]. Considering this result, concentration of CsA in systemic circulation was not high enough from oral administration to inhibit hepatic CYP3A4 function. Thus, increase in AUC of statins on coadministering CsA was due to inhibition of OATP-1B1. Similarly, gemfibrozil is also a more potent inhibitor of OATP-1B1 than metabolic enzymes, i.e., CYP1A2, 2C8, 2C9 and 2C19. Coadministration of pravastatin, rosuvastatin and atorvastatin together with CsA resulted in higher AUC by 2.01-, 1.88- and 1.35-fold, respectively [16, 36–38]. Because these statins are primarily excreted from the liver in an unchanged form, the magnitude of increase can be attributed to inhibition of OATP-1B1. Hence, inhibition of hepatic uptake transporters (OATPs) can result in many clinically relevant DDIs. Unlike statins, information on the inhibitory potency of TKIs on hepatic uptake transporters (OATP-1B1 and -1B3) is very sparse. Thus, it is of utmost importance to understand clinically relevant DDIs that may arise due to the inhibitory action of TKIs on OATP-1B1 and -1B3.

The results obtained from our studies evaluating the *in vitro* inhibitory activity of TKIs reveals inhibition of OATP-1B1 and/or -1B3 by selected TKIs. In this article, we have reported the inhibitory potential of TKIs for OATP-1B1 and/or -1B3 by estimating IC_{50} . Our findings suggest that nilotinib is a more potent inhibitor of OATP-1B1 than pazopanib. No significant inhibition in uptake of radiolabeled probe substrates were observed in OATP-1B1 transfected cells with vandetanib, canertinib and erlotinib, indicating that all tested TKIs do not act as inhibitors for OATP-1B1. Similarly for OATP-1B3 transporter protein, whereas vandetanib showed its inhibitory action on the intracellular accumulation

of radiolabeled probe substrate, pazopanib, nilotinib, canertinib and erlotinib did not produce any inhibitory effect on OATP-1B3. Hence, OATP-1B1 and/or -1B3 can be considered as important factors in determining the pharmacokinetics or DDIs of pazopanib, nilotinib and vandetanib. These results, though, act mainly as a proof of concept, and the actual inhibitory potency/activity in humans may vary based on various physiological and pathological conditions resulting in altered expression of these transporters in the liver.

Currently, OATP-1B1 and -1B3 related DDIs involving clinical as well as preclinical interactions have been published in many reports. These DDIs are considered as vital components in the discovery and development of drugs with safer profiles as these DDIs may lead to elevated risk of drug-induced adverse effects, even resulting in withdrawal from the market. Many therapeutic agents are substrates or inhibitors of OATP-1B1 and/or -1B3. Alteration in the hepatic uptake of these compounds via OATPs may result in clinically relevant DDIs. Expression and involvement of these OATP-1B type isoforms in the liver need to be delineated for better understanding of the factors governing absorption, distribution, metabolism and elimination (ADME) of therapeutic agents. Any change in the activity of OATP-1B type transporter proteins will result in suboptimal treatment or high toxicity. Thus, it is necessary to investigate the role of these transporters in order to avoid DDIs [16].

Previously published articles have focused on TKIs as substrates and not on the inhibition potential of these agents with OATPs [1, 5, 10–13]. In this study, we have observed concentration-dependent inhibition of OATP-1B1 transporter by pazopanib and nilotinib in an *in vitro* model system and also concentration-dependent inhibition of OATP-1B3 in the presence of vandetanib.

OATP-1B1 and/or -1B3 are responsible for regulating the initial step of hepatic elimination of therapeutic agents (substrates of OATPs) by carrying out the uptake of selected agents into the hepatic tissue, exposing the molecules to CYP enzyme-mediated metabolism followed by elimination via biliary secretion. These OATPs expressed on the basolateral membrane of hepatocytes may induce drug uptake and can be regarded as one of the determinants of overall metabolic rate in liver [39]. An efficient directional movement of therapeutic agents across hepatic tissues requires synchronized activity of hepatic uptake, metabolizing enzymes and efflux transporters [40]. Tan et al. reported that coadministration of pazopanib (weak CYP3A4 and CYP2C8 inhibitor) with paclitaxel resulted in 14% lower paclitaxel clearance and a 31% higher concentration [41]. We have shown that pazopanib inhibits OATP-1B1 transporter, which is responsible for hepatic uptake of paclitaxel. Therefore, 31% higher plasma concentration of paclitaxel may be due not only to the inhibition of metabolizing enzymes but also to the inhibition of OATP-1B1 transporter. This hypothesis of OATP inhibition by TKIs resulting in increased plasma taxol concentration (docetaxel) has also been reported by Hu et al. [24]. OATP-1B1 plays a vital role in hepatic uptake of paclitaxel, making it vulnerable to metabolism by CYP3A4 and ultimately accelerating elimination by biliary secretion via P-glycoprotein (P-gp) [42, 43].

Coadministration of vandetanib has been warranted with digoxin. Vandetanib is a weak inhibitor of the efflux pump, P-gp. Coadministration of vandetanib and digoxin (substrate of

P-gp) may result in increased plasma concentrations of digoxin (caprelsa, product monograph). Also, digoxin is a well-known substrate of OATP-1B3 [2]. We have shown that vandetanib inhibits OATP-1B3 transporter, which is responsible for hepatic uptake of digoxin. Hence, enhanced plasma concentration of digoxin will not only be due to inhibition of P-gp but also to inhibition of OATP-1B3 transporter by vandetanib. Inhibition of OATPs (localized on the basolateral membrane of hepatic tissues), metabolizing enzymes and efflux transporters (expressed on bile canalicular membrane) may be responsible for enhanced plasma concentration of OATP substrates.

OATP-mediated DDIs have the potential to influence drug efficacy and toxicity. Therefore, coadministration of pazopanib, nilotinib and vandetanib (OATP-1B1 and/or -1B3 inhibitors) along with other hepatic OATP substrates (paclitaxel, docetaxel, cyclosporine, protease inhibitors, rifampicin, statins, telmisartan, valsartan, mTOR inhibitors, antibiotics, etc.) may result in altered pharmacokinetics and pharmacodynamics of OATP substrates. Inhibition of hepatic uptake of OATP substrates by coadministration of OATP inhibitor is a plausible explanation for several clinically observed DDIs. Inhibition of metabolizing enzymes and/or efflux transporter may not be the only cause of DDI-induced effects. Drug-induced alteration of OATP-1B1 and -1B3 transporter function is an essential auxiliary mechanism underlying DDIs [44].

Several TKIs are associated with drug-induced hepatotoxicity. Rise in serum transaminase and bilirubin levels was the common adverse effect associated with TKI therapeutic regimen. Xu et al. [8] reported that higher bilirubin levels in plasma or hyperbilirubinemia is associated with the inhibition of both OATP-1B1 and the enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). OATP-1B1 is responsible for hepatic uptake of bilirubin, whereas UGT1A1 is responsible for bilirubin metabolism prior to its elimination. Hence, pazopanib-induced hyperbilirubinemia may result due to inhibition of both OATP-1B1 and UGT1A1. Here, we have also reported the inhibitory potency of pazopanib for OATP-1B1. This result is consistent with the observation that pazopanib-induced inhibition of OATP-1B1 may cause diminished hepatic uptake of bilirubin, resulting in hyperbilirubinemia. Similarly, we have reported the inhibitory activity of nilotinib toward OATP-1B1 transporter protein. Singer et al. [45] reported that inhibition of UGT1A1 activity by nilotinib and genetic polymorphism could be the cause of the increased rate of hyperbilirubinemia. On the basis of our findings along with published reports, we postulate that nilotinib-induced hyperbilirubinemia may be the result of inhibition of both OATP-1B1 and UGT1A1.

TKIs do not exhibit any cytotoxicity at their highest tested concentration (100 μ M). This result shows that the inhibitory potential of TKIs toward OATP-1B1 and -1B3 is not due to their cytotoxic activity toward OATP-transfected cells.

In conclusion, we have shown that selected TKIs may cause inhibition of OATP-1B1 and OATP-1B3. Pazopanib and nilotinib exhibit concentration-dependent inhibitory activity against OATP-1B1, whereas vandetanib generates inhibitory action with OATP-1B3. These findings delineate the involvement of TKIs in inhibiting hepatic uptake of OATP-1B1 and -1B3 substrate. These findings also confirm that inhibitory activity of TKIs toward hepatic

uptake transporters can be utilized as a vital determinant of the pharmacokinetic profile of coadministered therapeutic agents. As coadministration of TKIs with other drugs is fairly common in multidrug therapy, hepatic uptake transporters OATP-1B1 and -1B3 can be regarded as important molecular targets for potential DDIs. Thus, hepatic uptake mediated by OATP-1B1 and -1B3 for selected TKIs should be dynamically scrutinized in order to circumvent DDIs. These transporters, in conjunction with the metabolizing enzymes and efflux proteins, may eventually decide on the overall flux/loss of the therapeutic agents within the hepatic tissue. These studies act as a proof of concept substantiating the need for further clinical studies investigating the OATP-based DDI potential of TKIs. Further *in vivo* studies are required for better understanding of the contribution of OATP-1B1 and/or -1B3 transporter proteins in the hepatic disposition of drugs coadministered with TKIs and for predicting any adverse drug reactions associated with these hepatic transporter-mediated DDIs.

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Figure 1. Effect of five different TKIs and rifampicin at two different concentrations (25 and 50 μM) on the activity of OATP-1B1, expressed in CHO cells, as determined by the intracellular accumulation of [^{3} H]ES (10-min incubation). Data are shown as mean $\pm$ standard derivation (SD). n = 4. *p < 0.05.

Figure 2. Effect of five different TKIs and rifampicin at two different concentrations (25 and 50 μM) on the activity of OATP-1B3, expressed in CHO cells, as determined by the intracellular accumulation of [^3H]CCK-8 (10-min incubation). Data are shown as mean $\pm$ SD. n = 4. *p < 0.05.

Figure 3.

(A) Inhibitory potency of rifampicin toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^{3} H]ES in the presence of increasing concentrations of rifampicin (0.1–100 μ M). Data are shown as mean $\pm$ SD. n = 4. (B) Inhibitory potency of pazopanib toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^{3} H]ES in the presence of increasing concentrations of pazopanib (0.1–100 μ M). Data are shown as mean $\pm$ SD. n = 4. (C) Inhibitory potency of nilotinib toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^{3} H]ES in the presence of increasing concentrations of nilotinib (0.1–100 μ M). Data are shown as mean $\pm$ SD. n = 4. (D) Inhibitory potency of vandetanib toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^{3} H]ES in the presence of increasing concentrations of vandetanib (0.1–100 μ M). Data are shown as mean $\pm$ SD. n = 4. (E) Inhibitory potency of canertinib toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^{3} H]ES in the presence of increasing concentrations of

canertinib (0.1–100 μM). Data are shown as mean $\pm$ SD. n = 4. (F) Inhibitory potency of erlotinib toward OATP-1B1. Intracellular accumulation of OATP-1B1 substrate [^3H]ES in the presence of increasing concentrations of erlotinib (0.1–100 μM). Data are shown as mean $\pm$ SD. n = 4.

Figure 4.

(A) Inhibitory potency of rifampicin toward OATP-1B3. Intracellular accumulation of OATP-1B3 substrate [³H]CCK-8 in the presence of increasing concentrations of rifampicin (0.1–100 μM). Data are shown as mean ± SD. n = 4. (B) Inhibitory potency of vandetanib toward OATP-1B3. Intracellular accumulation of OATP-1B3 substrate [³H]CCK-8 in the presence of increasing concentrations of vandetanib (0.1–100 μM). Data are shown as mean ± SD. n = 4. (C) Inhibitory potency of pazopanib toward OATP-1B3. Intracellular accumulation of OATP-1B3 substrate [³H]CCK-8 in the presence of increasing concentrations of pazopanib (0.1–100 μM). Data are shown as mean ± SD. n = 4. (D) Inhibitory potency of nilotinib toward OATP-1B3. Intracellular accumulation of OATP-1B3 substrate [³H]CCK-8 in the presence of increasing concentrations of nilotinib (0.1–100 μM). Data are shown as mean ± SD. n = 4. (E) Inhibitory potency of canertinib toward OATP-1B3. Intracellular accumulation of OATP-1B3 substrate [³H]CCK-8 in the presence of increasing concentrations of canertinib (0.1–100 μM). Data are shown as mean ± SD. n = 4. (F) Inhibitory potency of erlotinib toward OATP-1B3. Intracellular accumulation of

OATP-1B3 substrate [^3H]CCK-8 in the presence of increasing concentrations of erlotinib (0.1–100 μM). Data are shown as mean $\pm$ SD. n = 4.

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Figure 5. Cytotoxicity in the presence of TKIs at the highest studied concentration (100 μ M) on OATP-1B1- and -1B3-transfected cells. Data represent the mean $\pm$ SD. n = 4.

Table 1

IC₅₀ of tested compounds toward OATP-1B1 and -1B3 transporter proteins.

TKIs	OATP-1B1 IC ₅₀ , μ M	OATP-1B3 IC ₅₀ , μ M
Rifampicin	10.46 $\pm$ 1.15	3.67 $\pm$ 1.20
Pazopanib	3.89 $\pm$ 1.21	–
Nilotinib	2.78 $\pm$ 1.13	–
Vandetanib	–	18.13 $\pm$ 1.21
Canertinib	–	–
Erlotinib	–	–