Substrate-Dependent Drug-Drug Interactions between Gemfibrozil, Fluvastatin and Other Organic Anion-Transporting Peptide (OATP) Substrates on OATP1B1, OATP2B1, and OATP1B3

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ABSTRACT:

Hepatic uptake carriers of the organic anion-transporting peptide (OATP) family of solute carriers are more and more recognized as being involved in hepatic elimination of many drugs and potentially associated drug-drug interactions. The gemfibrozil-statin interaction was studied at the level of active hepatic uptake as a model for such drug-drug interactions. Active, temperature-dependent uptake of fluvastatin into primary human hepatocytes was shown. Multiple transporters are involved in this uptake as Chinese hamster ovary or HEK293 cells expressing either OATP1B1 (K_m = 1.4–3.5 μM), OATP2B1 (K_m = 0.7–0.8 μM), or OATP1B3 showed significant fluvastatin uptake relative to control cells. For OATP1B1 the inhibition by gemfibrozil was substrate-dependent as the transport of fluvastatin (IC_{50} of 63 μM) was not affected. The OATP1B1- but not OATP2B1-mediated transport of estrone-3-sulfate displayed biphasic saturation kinetics, with two distinct affinity components for estrone-3-sulfate (0.23 and 45 μM). Only the high-affinity component was inhibited by gemfibrozil. Recombinant OATP1B1-, OATP2B1-, and OATP1B3-mediated fluvastatin transport was inhibited to 97, 70, and 62% by gemfibrozil (200 μM), respectively, whereas only a small inhibitory effect by gemfibrozil (200 μM) on fluvastatin uptake into primary human hepatocytes was observed (27% inhibition). The results indicate that the in vitro engineered systems can not always predict the behavior in more complex systems such as freshly isolated primary hepatocytes. Therefore, selection of substrate, substrate concentration, and in vitro transport system are critical for the conduct of in vitro interaction studies involving individual liver OATP carriers.

After oral administration, drugs are absorbed and distributed throughout the body, often by passive diffusion processes driven by physicochemical compound properties. For many drugs and xenobiotics, active membrane transporters limit or facilitate these processes in an active, energy-dependent way. Also the renal and hepatic elimination of xenobiotics and many different drugs is mediated by such membrane transporters in a close interplay with drug metabolic processes (Schuetz and Schinkel, 1999). For liver, the uptake of anionic compounds is mediated by organic anion transporting polypeptides (OATPs), transmembrane proteins expressed in the basolateral membrane of hepatocytes (Hagenbuch and Meier, 2004; Mikkaichi et al., 2004) and other organs. These OATP transporters can play an essential role in drug clearance from the body in addition to the well-established metabolic processes catalyzed by several cytochrome P450 isoenzymes. In humans, three members of this transporter family, OATP1B1, OATP1B3, and OATP2B1, are expressed in liver (Hagenbuch and Meier, 2003), and more and more drugs are recognized as substrates of one or several of these solute carriers (van Montfoort et al., 2003).

In the last few years, statins, HMG-coenzyme A reductase inhibitors, became an important class of drugs, widely used in clinics to treat dyslipidemia. These drugs target the rate-limiting enzymatic step in the de novo synthesis of cholesterol in the liver. Active uptake of many statins, such as cerivastatin, fluvastatin, pravastatin, pitavastatin, and rosuvastatin, by organic anion transporting polypeptides into the liver has been demonstrated in many recent publications (Hsiang et al., 1999; Nezasa et al., 2003; Hirano et al., 2004; Kopplow et al., 2005; Ho et al., 2006). OATP1B1, OATP1B3, and OATP2B1 have been shown to be involved in the transport of these statins. The carrier-mediated hepatic uptake process not only represents the first step of hepatic drug elimination but is also an active drug delivery system for many statins to the liver as a target organ (Sai and Tsuji, 2004). As an additional step in the hepatic elimination, many statins are metabolized by cytochrome P450 isoenzymes of the 3A or 2C subfamilies. Either of these active transport or metabolism processes can be subject to drug-drug interactions with different consequences for the pharmacokinetics of the drugs involved (Poirier et al., 2007). To treat patients with dyslipidemias resistant to diet or single-agent pharmacotherapy (Spence et al., 1995) or transplant patients who develop hypercholesterolemia after organ transplantation (Simonson et al., 2004), combination therapies of statins with other drugs such as fibrates or cyclosporin A are widely used. Such combination therapies are prone to drug-drug interactions (DDIs), which can lead to altered
pharmacokinetic profiles of either drug, an effect observed for many statins in combination with fibrates. An increase in the plasma concentration of many of the currently used statins can cause severe side effects such as muscle toxicity or even rhabdomyolysis. Cerivastatin has been withdrawn from the market, after combinations of cerivastatin with gemfibrozil (Ozdemir et al., 2000; Lai et al., 2001; Shirata et al., 2004) or cyclosporin A in kidney transplant patients led to marked increases in cerivastatin exposure (Mück et al., 1999) associated with severe muscle toxicity. As the mechanism leading to this increase in systemic cerivastatin exposure, an inhibition of the CYP2C8-mediated cerivastatin metabolism by gemfibrozil (Wang et al., 2002; Shirata et al., 2004) or an inhibition of the OATP-mediated hepatic statin uptake into the liver by the fibrate was proposed (Shirata et al., 2004). Moreover, the high total plasma concentrations of gemfibrozil, which are in the range of 120 to 240 μM after usual daily treatments (600 mg b.i.d.) have to be considered in the assessment of its in vitro inhibition potential (Wen et al., 2001).

Statins are widely used in clinics; thus, the likelihood of new drugs to be coadministered with statins is high. Therefore, the DDI potential with statins is of great importance for the clinical safety of new drug candidates. In addition to cytochrome P450 enzymes, active drug transporters of the OATP family are more and more considered in addressing the DDI potential with this class of therapeutic agents. Therefore, predictive in vitro strategies are needed to characterize and quantify possible DDI risks at organic anion transporters in addition to the established tools addressing cytochrome P450-mediated DDIs.

In the present study, we explored the in vitro inhibition potential of gemfibrozil on the active hepatic uptake mediated by major liver OATPs as a potential mechanism responsible for the DDI of gemfibrozil with statins observed in patients. The effects of gemfibrozil on the transport of fluvastatin into primary human hepatocytes and into cells overexpressing OATP1B1, OATP2B1, or OATP1B3 were studied. Gemfibrozil showed a substrate-dependent inhibition of OATP1B1, interfering with statin transport but not with the transport of the prototypic model substrate estrone-3-sulfate used at a high concentration, indicative of multiple substrate binding sites of OATP1B1.

Materials and Methods

Materials. [3H]Fluvastatin and [3H]pravastatin were made by Hartmann Analytic GmbH (Braunschweig, Germany) and [3H]simvastatin was made by RC Tritec Ltd. (Teufen, Switzerland). [3H]Estrone-3-sulfate was from PerkinElmer Life and Analytical Sciences (Boston, MA) and [14C]-troglitazone sulfate was prepared using [14C]-troglitazone as outlined previously (Funk et al., 2001).

Fluvastatin was obtained from APIN Chemicals Limited (Abingdon, Oxon, UK), pravastatin was from Calbiochem (San Diego, CA), and simvastatin was obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). Gemfibrozil was from Sigma (Buchs, Switzerland). All cell culture media and reagents were purchased from Invitrogen (Carlsbad, CA), and standard tissue culture flasks and 24-well plates were from Falcon (Cowley, UK).

Methods. OATP-expressing cell lines. Chinese hamster ovary (CHO) cells expressing OATP1B1 were transfected with pRES neo2-OATP1B1 construct. Single clones were selected based on functional activity and characterized. The CHO cells expressing OATP1B3 were obtained from the laboratory of Peter Meier-Abt, University of Zürich, Zürich, Switzerland. CHO cells were grown in Ham’s F-12K medium, 2.5 g/l sodium bicarbonate, supplemented with 10% fetal calf serum, a penicillin-streptomycin solution, and geneticin (0.5 mg/ml). Cells were cultured at 37°C in a humidified 5% CO₂ cell culture incubator. For the transport assay, cells were split as follows: cells from a confluent 75 cm² flask (detached with trypsin-EDTA) were uniformly resuspended in the desired volume of Ham’s F-12K medium. One milliliter of the uniformly resuspended cells (2 × 10⁶ cells/ml) was added to each well of a 24-well plate (collagen-coated for HEK293 cells). The cells were used for transport assays 38 to 42 h later, when they were 80 to 90% confluent.

Plating of primary human hepatocytes. Freshly isolated human hepatocytes were purchased from Cytonet GmbH & Co. KG (Weinheim Germany). They were resuspended in medium A (Cytonet GmbH & Co. KG) at a cell number of 3 × 10⁶ viable cells/ml, and 1 ml of the uniformly resuspended cells was added to each well of a collagen coated 24-well plate and evenly distributed. Plated human hepatocytes were kept in the incubator (37°C, 5% CO₂) for 1 to 2 h, until they were attached to the plate. Uptake studies were performed as described for the cell lines.

Uptake studies. Recombinant cells or hepatocytes were prepared as outlined above. Assays were run using three wells as one set. The medium was removed from the wells by aspiration. The wells were washed once with 1 ml of Hank’s balanced salt solution (HBSS) (Invitrogen/Gibco, no. 14175-053) for cell lines or HBSS containing calcium and magnesium for hepatocytes at 37°C. The uptake experiment was started by aspiration of wash buffer and addition of 150 μl of a prewarmed 37°C HBSS solution containing the substrate of interest. In the case of IC₅₀ measurements, the 150 μl also contained a selected inhibitor, ensuring that the entire cell surface area was covered (T = 0). The plate was transferred on a 37°C heating block (Eppendorf-5 Prime, Inc., Boulder, CO). After the incubation time, the plate was removed from the heating block, and immediately 1 ml of ice-cold phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) was added to stop the transport activity, with the ice-cold buffer and the large volume of PBS effectively diluting the compound. The solution was removed by aspiration. BSA was also included in the subsequent washing buffer to minimize background due to unspecific binding of radioactive compound to the external surface of the cells. The wells were washed twice with approximately 2 ml of ice-cold PBS (0.2% BSA) each and finally with ice-cold PBS without BSA (approximately 3 ml total, to remove added BSA protein). Then 0.5 ml of 0.1 N NaOH/0.1% SDS was added to solubilize the cells. After incubation for 15 min at 60°C on a shaking heat block, 0.25 ml of this solubilized cell mix was added to 5 ml of scintillation fluid, and radioactivity was determined. Protein content was determined for each well using the Pierce BCA assay (Pierce Chemical, Rockford, IL) with BSA as standard.

Results

Fluvastatin Uptake by Freshly Isolated Human Hepatocytes and Human OATP Carriers Expressed in CHO or HEK293 Cells. Uptake of 1 μM [3H]Fluvastatin by freshly isolated human hepatocytes was time- and temperature-dependent (Fig. 1A). The initial fluvastatin uptake rate at 37°C was 71 pmol/min/mg protein compared with 4.9 pmol/min/mg protein at 4°C. The initial uptake was linear for up to approximately 1 min.

The time-dependent fluvastatin (1 μM) uptake into stably transfected CHO cells expressing OATP1B1 or OATP1B3 and HEK293 cells stably expressing OATP2B1 is shown in Fig. 1B. Fluvastatin uptake into control CHO or HEK293 cells was low. Transport of fluvastatin into the cells expressing OATP1B1 was higher (69.6 pmol/min/mg) compared with the cells expressing OATP2B1 (47.9 pmol/min/mg). OATP1B3-expressing cells showed only very limited fluvastatin transport (24.9 pmol/min/mg), close to the unspecific binding to control CHO cells (14.6 pmol/min/mg). The initial uptake for both transporters OATP1B1 and OATP2B1 was linear up to approximately 1 min.

Kinetics of OATP1B1-Dependent Transport of Estrone-3-Sulfate and Fluvastatin. The kinetics of the OATP1B1-dependent transport of estrone-3-sulfate as a model substrate of this transporter and of fluvastatin was studied in CHO cells expressing OATP1B1 (Fig. 2). For estrone-3-sulfate the OATP1B1-dependent transport was studied...
in a concentration range of 20 nM to 50 μM and the net transport data are shown (Fig. 2A). Analyzing the linearized data according to Eadie-Hoffstee (inset), a clear biphasic kinetics was observed, suggesting the presence of two distinct affinity components for estrone-3-sulfate on OATP1B1. The $K_m$ value for the low-affinity component was determined to be 45 μM and 0.23 μM for the high-affinity component. This biphasic transport kinetics has been reported previously using estrone-3-sulfate and OATP1B1 (Tamai et al., 2001a).

Furthermore, the kinetics for fluvastatin transport by the same transporter, OATP1B1 stably expressed in CHO cells, was determined (Fig. 2B). Initial net uptake rates (45 s incubation) of fluvastatin by OATP1B1 exhibited clear saturability with increasing substrate concentrations. The apparent $K_m$ values (3.5 and 1.4 μM) and the $V_{max}$ values (432 and 292 pmol/mg/min) were calculated by nonlinear fitting for OATP1B1-mediated fluvastatin transport from two individual experiments. One representative experiment is shown in Fig. 2B. The Eadie-Hoffstee analysis of the same data set indicated monophasic kinetics for the OATP1B1-dependent fluvastatin transport. The apparent $K_m$ value for fluvastatin was comparable with that recently published from OATP2B1 expressed in Madin-Darby canine kidney II cells (Kopplow et al., 2005).

Substrate-Dependent Inhibition of OATP1B1 by Gemfibrozil. A preliminary in vitro inhibition experiment using the two OATP1B1 substrates estrone-3-sulfate and fluvastatin, indicated that gemfibrozil interfered only with fluvastatin transport but not with estrone-3-sulfate transport by OATP1B1 at the conditions used. Therefore, we selected taurocholate, troglitazone sulfate, fluvastatin, pravastatin, and simvastatin, as known OATP1B1 substrates, in addition to estrone-3-
sulfate, as the prototypical substrate for OATP1B1, for inhibition studies with gemfibrozil. Gemfibrozil did not inhibit the uptake of estrone-3-sulfate or troglitazone sulfate by OATP1B1 at either concentration tested (100 or 200 μM) (Fig. 4). In contrast, uptake of all three statins into OATP1B1-expressing CHO cells was strongly inhibited in a concentration-dependent manner by gemfibrozil. The rank order of the inhibitory effect of gemfibrozil (200 μM) was pravastatin>fluvastatin>simvastatin, with an uptake inhibition of approximately 100, 90, and 70%, respectively for the three statins, relative to the uptake by OATP1B1 in absence of inhibitor (Fig. 4). Taurocholate, a substrate often used as model substrate for OATP-mediated transport, behaved similarly to the three statins studied. A strong inhibition of the OATP1B1-mediated taurocholate transport was observed in the presence of gemfibrozil.

**Differential Inhibition of Two OATP1B1 Affinity Components by Gemfibrozil and Fluvastatin.** Based on the unexpected OATP1B1 inhibitory effects of gemfibrozil based on the different substrates used, the interaction of gemfibrozil with estrone-3-sulfate transport was further studied. The concentration-dependent inhibition of OATP1B1 by gemfibrozil was measured, using two different estrone-3-sulfate substrate concentrations (10 nM and 3 μM). At the high concentration, the OATP1B1-mediated estrone-3-sulfate transport was not inhibited by gemfibrozil used in a concentration range of up to 250 μM (Fig. 5A). However, at the low estrone-3-sulfate concentration (10 nM), uptake by OATP1B1 was inhibited by gemfibrozil in a concentration-dependent manner (Fig. 5A).

To further explore the interaction of gemfibrozil with the two affinity components of OATP1B1, we studied the kinetics of estrone-3-sulfate transport in the absence and presence of a fixed inhibitor concentration (200 μM gemfibrozil). Estrone-3-sulfate was used in a concentration range of 20 nM to 50 μM in CHO cells expressing OATP1B1 (Fig. 5B). Only the high-affinity component of the biphasic estrone-3-sulfate kinetics was affected. The apparent high-affinity $K_m$ value was increased by a factor of approximately 3 in the presence of gemfibrozil, whereas the respective $V_{max}$ value did not change significantly, suggesting a competitive interaction of the two compounds.

**Inhibitory Effect of Gemfibrozil on Fluvastatin-Mediated Transport.** To elucidate the relative contribution of the different OATP transporters mainly involved in fluvastatin transport, OATP1B1, OATP2B1 and OATP1B3, the effect of gemfibrozil on fluvastatin transport in hepatocytes compared with that in cell lines was explored. A strong, concentration-dependent inhibition of fluvastatin uptake into OATP1B1-expressing CHO cells was observed at 100 and 200 μM gemfibrozil concentrations (Fig. 6). OATP1B1-mediated fluvastatin transport was inhibited by approximately 60 and 90% at 100 and 200 μM inhibitor concentrations, respectively, compared with transport in absence of inhibitor. The inhibitory effect of gemfibrozil on OATP2B1-mediated fluvastatin uptake was somewhat smaller. OATP2B1-mediated fluvastatin transport was only inhibited by approximately 40 and 70% at the two gemfibrozil concentrations relative to transport in absence of inhibitor. The inhibitory effect of gemfibrozil on OATP2B1-mediated fluvastatin uptake was somewhat smaller. OATP2B1-mediated fluvastatin transport was only inhibited by approximately 40 and 70% at the two gemfibrozil concentrations relative to transport in absence of inhibitor. The OATP1B3-mediated transport of fluvastatin was even less affected by gemfibrozil; whereas no inhibition was observed at 100 μM gemfibrozil, the transport activity was reduced by approximately 60% at 200 μM gemfibrozil. On the other hand, the inhibitory effect of gemfibrozil on fluvastatin transport into human hepatocytes was small; fluvastatin uptake was inhibited only by approximately 17% and 25% at 100 and 200 μM inhibitor, respectively (Fig. 6).
Discussion

Today, combination therapies are widely used for drugs of many different therapeutic classes. Statins are often comedicated with other drugs such as immunosuppressants, antidiabetic drugs, or fibrates to treat patients in clinics. This comedication increases the risk for significant DDIs among these drugs (Backman et al., 2002; Hodel, 2002; Williams and Feely, 2002; Campbell et al., 2004). Therefore, it is important to address possible DDI risks relevant for the respective patient population by using appropriate in vitro tools to guide tailor-made clinical interaction studies in drug development. In addition to the well established cytochrome P450 interaction studies, DDIs can also occur at the level of active drug transport, involving OATP and OAT carriers and/or different ABC transporters. In the context of DDIs involving statins, members of the OATP solute carrier family were found to be involved in addition to cytochrome P450 enzymes. Recombinant cell lines expressing human OATP carriers are often used to study the in vitro DDI potential at the level of these carriers (Hirano et al., 2006).

Fluvastatin uptake into human hepatocytes was temperature- and time-dependent, probably mediated by active hepatic uptake carriers (Fig. 1A). Using recombinant cell lines expressing either OATP1B1, OATP2B1, or OATP1B3, we have shown that fluvastatin is a substrate of all three hepatic uptake carriers (Fig. 1B), consistent with published data (Hsiang et al., 1999; Nezasa et al., 2003; Hirano et al., 2004; Kopplow et al., 2005; Ho et al., 2006). Similar apparent $K_m$ values (1–3 $\mu$M) and monophasic kinetics were observed for the uptake of fluvastatin mediated by either OATP1B1 (Fig. 2B) or OATP2B1 (Fig. 3B).

Estrone-3-sulfate is a widely used prototypical model substrate to demonstrate OATP1B1- or OATP2B1-mediated transport (Tamai et al., 2000), as well as interactions of other compounds with either transporter (Shimizu et al., 2005; Fuchikami et al., 2006). Estrone-3-sulfate has been shown previously to be transported by OATP1B1 with a biphasic kinetic behavior, and the presence of two binding sites with different affinities for this substrate was suggested (Tamai et al., 2001a). For the estrone-3-sulfate transport by OATP1B1, the biphasic kinetics could be confirmed in this study. High- and Low-affinity components were evident from the kinetic data, with apparent $K_m$ values of 0.23 and 45 $\mu$M, respectively (Fig. 2A) in good agreement with published results (Tamai et al., 2001a).

We observed that the OATP1B1 inhibitor gemfibrozil did not interfere with the transport of estrone-3-sulfate in a standard experimental setup, whereas the transport of fluvastatin was potently inhibited by gemfibrozil. By extending the set of OATP1B1 substrates, we explored this unexpected strong substrate dependence of the gemfibrozil inhibitory effect. Taurocholic acid, troglitazone sulfate, fluvastatin, pravastatin, and simvastatin, as known OATP1B1 substrates, were used in addition to estrone-3-sulfate as the prototypical substrate for OATP1B1. The transport of all three statins used as substrates of OATP1B1 was strongly inhibited by gemfibrozil, whereas the transport of estrone-3-sulfate and troglitazone sulfate was not inhibited by gemfibrozil at the tested micromolar substrate concentrations (Fig. 4).

The effect of gemfibrozil on the OATP1B1-mediated transport of estrone-3-sulfate was assessed at two substrate concentrations. An inhibition by gemfibrozil was apparent only at the low (10 nM) but not at the high (3 $\mu$M) estrone-3-sulfate concentration (Fig. 5A). This result was indicative of an interference of gemfibrozil with the high-affinity component of OATP1B1 only. These data are compatible with the potential presence of two different binding sites for OATP1B1.
with different affinities for estrone-3-sulfate. A similar conclusion was proposed before (Tamai et al., 2001b).

The OATP1B1-mediated transport of fluvastatin followed a monophasic kinetic behavior, suggesting that it only binds to one of the two putative binding sites of OATP1B1, the estrone-3-sulfate high-affinity binding site. As the transport of all three statins (fluvastatin, pravastatin, and simvastatin) tested was found to be potently inhibited by gemfibrozil (Fig. 4), it is very likely that these three statins will interact with the same putative binding site of OATP1B1, the estrone-3-sulfate-high-affinity binding site, to which also gemfibrozil binds.

With use of estradiol-17β-glucuronide (E17βG) or estrone-3-sulfate as substrates of OATP1B1 and gemfibrozil or fexofenadine as inhibitors, it has been reported that E17βG transport was sensitive to gemfibrozil (Yamazaki et al., 2005) and fexofenadine (Shimizu et al., 2005), whereas estrone-3-sulfate transport was only weakly inhibited by fexofenadine (Shimizu et al., 2005). Therefore, most substrates and inhibitors studied, including E17βG, taurocholate, gemfibrozil, fexofenadine, fluvastatin, pravastatin, and simvastatin, apparently bind with preference to the putative estrone-3-sulfate high-affinity binding site of OATP1B1, whereas troglitazone sulfate was the only other substrate in addition to estrone-3-sulfate itself that we identified as binding to the putative estrone-3-sulfate low-affinity binding site of OATP1B1. Therefore, interactions of different substrates and inhibitors of solute carriers, in particular OATP1B1 might be complex, explainable by the presence of at least two binding sites. Under the conditions of standard in vitro studies with the OATP1B1 model substrate estrone-3-sulfate at a concentration of 3 μM, interactions with inhibitors binding to the putative high-affinity binding site, including statins, cannot be predicted correctly. The apparent OATP1B1 inhibition may be unique for a given substrate /inhibitor pair, regardless of the interaction of the inhibitor with other substrates for the same transporter. Therefore, it is of importance to test multiple substrate/inhibitor combinations covering the different binding sites of OATP1B1 to predict the interaction potential for this transporter.

For OATP2B1, on the other hand, monophasic kinetics were found for transport of both estrone-3-sulfate and fluvastatin (Fig. 3) and the transport of both substrates was inhibited by gemfibrozil. At least for these two substrates only one binding site appears to exist in this OATP2B1 transporter, consistent with literature in which no biphasic transport kinetics has been reported so far.

The in vitro interaction of gemfibrozil at the level of hepatic fluvastatin uptake was further investigated, as in clinical interaction studies no interference of the two compounds was observed upon their coadministration (Spence et al., 1995). Fluvastatin transport by OATP1B1 was strongly inhibited by gemfibrozil at 200 μM, whereas the effects on the OATP2B1 and OATP1B3-mediated fluvastatin transport by gemfibrozil were considerably weaker (Fig. 6). This inhibitory effect of gemfibrozil on active fluvastatin uptake was much weaker when freshly isolated human hepatocytes were used. Less than 30% inhibition was seen with 200 μM gemfibrozil, whereas the individual expressed transporters were 60 to 90% inhibited by the same gemfibrozol concentration. Multiple transport pathways including other solute carriers also operational in the primary human hepatocytes used might compensate for individual transporters particularly sensitive to inhibition by gemfibrozil. To further explore the quantitative contribution of individual transporters to the hepatic uptake of fluvastatin, selective inhibitors would be required. The gemfibrozol concentration was within the range of total plasma peak concentrations (120–240 μM) found after the usual daily treatments (600 mg b.i.d.) (Wen et al., 2001). However, the protein binding of gemfibrozol (~95%), which was not considered under the in vitro assay conditions as no protein was added to the incubation medium, might further reduce the inhibitory effect of gemfibrozol in vivo. No significant effect on the area under the curve, maximal plasma concentration, and time-to-maximum concentration, was reported for either compound comparing the combination of fluvastatin and gemfibrozil with the administration of each individual drug alone (Spence et al., 1995). The in vitro results on the fluvastatin-gemfibrozil interaction obtained with overexpressed OATP carriers might therefore be misleading, whereas the inhibition of fluvastatin transport in human hepatocyte better reflects the in vivo results. Therefore, it is important to study the effect of an inhibitor on all transporters significantly involved in the uptake of a given substrate to prevent misleading results based on the inhibition of a single transporter.

In conclusion, our results indicate that in vitro engineered systems expressing individual drug transporters cannot always predict the behavior of substrate/inhibitor combinations in more complex systems, such as human hepatocytes or even in clinical in vivo studies. Results from these in vitro engineered systems are very valuable as they provide a mechanistic insight in what might be expected, but they should always be complemented with experiments in more complex systems, such as primary human hepatocytes, either freshly isolated or cryopreserved, with functional activities of multiple transporters to confirm or contradict the findings. Additionally, different affinity components for certain substrates or inhibitors to transporters, as was demonstrated for estrone-3-sulfate transport by OATP1B1 should be considered. Only such a stepwise mechanistic approach, addressing different, parallel transport pathways and multiple substrates, can ultimately provide in vitro data, predictive of the potential for clinically relevant drug-drug interactions for given substrate/inhibitor combinations.

References


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