Drug-Drug Interactions between Rosuvastatin and Oral Antidiabetic Drugs Occurring at the Level of OATP1B1


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ABSTRACT

Organic anion-transporting polypeptide 1B1 (OATP1B1) is an important hepatic uptake transporter, of which the polymorphic variant OATP1B1*15 (Asn130Asp and Val174Ala) has been associated with decreased transport activity. Rosuvastatin is an OATP1B1 substrate and often concomitantly prescribed with oral antidiabetics in the clinic. The aim of this study was to investigate possible drug-drug interactions between these drugs at the level of OATP1B1 and OATP1B1*15. We generated human embryonic kidney (HEK)293 cells stably overexpressing OATP1B1 or OATP1B1*15 that showed similar protein expression levels of OATP1B1 and OATP1B1*15 at the cell membrane as measured by liquid chromatography-tandem mass spectrometry. In HEK-OATP1B1*15 cells, the V_max for OATP1B1-mediated transport of E2,17β-G (estradiol 17β-o-glucuronide) was decreased >60%, whereas K_m values (Michaelis constant) were comparable. Uptake of rosuvastatin in HEK-OATP1B1 cells (K_m 13.1 ± 0.43 μM) was nearly absent in HEK-OATP1B1*15 cells. Interestingly, several oral antidiabetics (glyburide, glimepiride, troglitazone, pioglitazone, glipizide, gliclazide, and tolbutamide), but not metformin, were identified as significant inhibitors of the OATP1B1-mediated transport of rosuvastatin. The IC₅₀ values for inhibition of E2,17β-G uptake were similar between OATP1B1 and OATP1B1*15. In conclusion, these studies indicate that several oral antidiabetic drugs affect the OATP1B1-mediated uptake of rosuvastatin in vitro. The next step will be to translate these data to the clinical situation, as it remains to be established whether the studied oral antidiabetics indeed affect the clinical pharmacokinetic profile of rosuvastatin in patients.

Introduction

The prevalence of diabetes is overwhelming nowadays, with more than 345 million people suffering from diabetes worldwide (http://www.who.int/mediacentre/factsheets/fs312/en/; World Health Organization, 2012). Type 2 diabetes comprises 90% of people with diabetes around the world and is largely the result of excess body weight and physical inactivity (World Health Organization, 2012). Although lifestyle interventions, such as dietary adjustments and increased exercise, are initially very effective (and with low costs), long-term adherence is in general poor. Therefore, most type 2 diabetic patients often concomitantly receive therapeutic lifestyle interventions, such as dietary adjustments and increased physical activity (World Health Organization, 2012). Type 2 diabetes comprises 90% of people with diabetes around the world and is largely the result of excess body weight and physical inactivity (World Health Organization, 2012). Although lifestyle interventions, such as dietary adjustments and increased exercise, are initially very effective (and with low costs), long-term adherence is in general poor. Therefore, most type 2 diabetic patients will inevitably need treatment with one or more antidiabetic drugs to manage their hyperglycemia. This treatment mainly includes drugs from the class of oral antidiabetics such as metformin, sulfonylureas, and thiazolidinediones (Kalliokoski et al., 2010). The American Diabetes Association and the European Association for the Study of Diabetes recently published a consensus statement providing guidance for treatment of hyperglycemia in type 2 diabetic patients (Nathan et al., 2009). According to this guideline, treatment of type 2 diabetes with metformin or sulfonylureas belongs to the class 1 category of well-validated core therapies, as it has been in clinical use for over 50 years, whereas treatment with thiazolidinediones is less well-validated and has been associated with serious cardiovascular side effects (Nathan et al., 2009; Aquilante, 2010). Notably, type 2 diabetic patients often concomitantly receive therapeutic drugs directed against other coincident features, such as dyslipidemia and hypertension, and it is therefore important to investigate possible drug-drug interactions.

Many oral drugs (including oral antidiabetics and the lipid-lowering statins) are extracted from the portal blood by the liver before they reach their target, which can either be intrahepatic (e.g., statins, metformin) or extrahepatic (e.g., pancreatic cells in case of sulfonylureas, or adipocytes in case of thiazolidinediones). In this study we focus on the organic anion-transporting polypeptide 1B1 (OATP1B1; gene SLCO1B1) as one of the main hepatic uptake transporters (Hagenbuch and Meier, 2004). OATP1B1 can transport a wide variety of drugs, including many statins (e.g., rosuvastatin, pravastatin, pitavastatin) and some oral antidiabetics (repaglinide, troglitazone).

ABBREVIATIONS: E2,17β-G, estradiol 17β-o-glucuronide; HBSS, Hanks’ balanced salt solution; HEK, human embryonic kidney; K_m, Michaelis constant; OATP, organic anion-transporting polypeptide.
The clinical importance of OATP1B1 in the pharmacokinetics of drugs has been confirmed by several studies that focused on the effect of commonly occurring single nucleotide polymorphisms in OATP1B1. In particular, the OATP1B1*15 variant (Asn130Asp and Val174 Ala), with an average haplotype frequency of 16–24% in Europe and America (Pasanen et al., 2008), is generally known to have a strongly reduced transport activity and has been associated with markedly increased plasma levels of certain OATP1B1 substrates. This might be advantageous for some drugs that do not have a target in the liver, resulting in an increased pharmacological response. However, for drugs that have a target inside the liver, decreased hepatic uptake by OATP1B1*15 might result in decreased pharmacological response and give rise to unforeseen side effects. Indeed, it has been demonstrated that patients carrying the OATP1B1*15 variant show markedly increased plasma levels, decreased pharmacological response, and even increased extrahepatic toxicity, for instance, after pravastatin, pitavastatin, or rosuvastatin treatment (Niemi et al., 2010; Chung et al., 2005; Igel et al., 2006; Choi et al., 2008; Fahrmayr et al., 2010; Niemi et al., 2011).

Although glyburide, troglitazone, and pioglitazone have previously been reported as inhibitors of OATP1B1 (Nozawa et al., 2004; Hirano et al., 2006; Gui et al., 2009; Bednarczyk, 2010), the effects of a complete set of oral antidiabetic drugs on both OATP1B1 and OATP1B1*15 have not been systematically studied before. In this study we therefore aimed to reveal the possible drug-drug interactions between several oral antidiabetic drugs belonging to the class of sulfonylureas and thiazolidinediones and the lipid-lowering drug rosuvastatin at the level of OATP1B1. We also included studies that explore the influence of the *15 haplotype on this interaction.

### Materials and Methods

#### Chemicals and Reagents

- [3H]-estradiol 17β-a-glucuronide ([3H]-E217β; 1.85 TBq/mmol) was purchased from PerkinElmer Life and Analytical Chemistry (Madison, WI).
- [3H]-E217β-G; 0.95 TBq/mmol) was purchased from PerkinElmer Life and Analytical Chemistry (Madison, WI).
- OATP1B1*15 cDNA into the pIRESpuro vector was performed by GeneArt (London, UK); rosuvastatin was from Sequoia Research Products (Pangbourne, UK).
- Glimepiride, tolbutamide, pioglitazone, troglitazone, metformin, E217β-G; 0.3TBq/mmol) and pIRESpuro-OATP1B1 or -OATP1B1*15 (2.7

#### Membrane Isolations

To determine the active transporter protein expression levels in transfected cells, the outer cellular membranes were isolated using a sucrose density gradient centrifugation protocol. After trypsinization (~5 × 10^5 cells), cell pellets were resuspended in hypotonic buffer (0.5 mM sodium phosphate, 0.1 mM EDTA, and a cocktail of protease inhibitors containing 2 mM phenylmethylsulfonylfluoride, aprotinin, leupeptin, and pepstatin). After 1 hour of incubation at 4°C, the homogenized cells were centrifuged at 100,000g for 30 minutes at 4°C using a LE-80k centrifuge with SW28 rotor (Beckman Coulter, Brea, CA). The pellet was resuspended in hypotonic buffer following the addition of 2 volumes of isotonic buffer [10 mM Tris-Hepes and 250 mM sucrose (pH 7.4)]. The extract was again homogenized using a Potter-Elvehjem homogenizer and centrifuged at 12,000g for 10 minutes at 4°C. The supernatant was collected and centrifuged at 100,000g for 30 minutes at 4°C. The pellet was resuspended in isotonic buffer and was layered on top of a 38% sucrose solution and centrifuged at 100,000g for 90 minutes at 4°C. The turbid layer at the interface, containing the plasma membranes, was recovered, diluted with isotonic buffer, and centrifuged at 100,000g for 40 minutes at 4°C. The plasma membrane fraction was obtained from the resulting pellet, which was resuspended in 50 μl isotonic buffer.

#### Protein Digestion

Isolated plasma membrane extracts (~50 μg) from individual cell lines were diluted with 2 volumes of 90% methanol. The proteins were subsequently reduced with 0.1 M dithiothreitol at 37°C for 60 minutes and alkylated with 0.04 M iodoacetamide for 20 minutes at room temperature in the dark. Digestion was performed after addition of CatCl2 (final concentration 1 mM) and 0.5 μg trypsin (Promega, Sunnyvale, CA) in 17% methanol by diluting the solution with 50 μl NH4HCO3. After overnight incubation the samples were incubated for another 2 hours with 0.5 μg trypsin. The efficiency of the tryptic digestion was checked using SDS-PAGE followed by silver stain. Finally the protein digests were evaporated by vacuum centrifugation (Scanvac, Ballerup, Denmark) and dissolved in 100 μl 15% acetonitrile containing 0.1% formic acid (Merck, Darmstadt, Germany) and 5 ng/ml internal standard (AQUA peptide mix, see below).

#### Liquid Chromatography–Tandem Mass Spectrometry Analysis

The peptide analysis was performed on a ultra-performance liquid chromatograph coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA). The extract (7.5 μl) was injected on an Acquity C18 BEH ultra-performance liquid chromatograph column (2.1 × 100 mm, 1.7 μm) and was separated using gradient elution with a stable flow of 600 μl/min. The gradient started with 95% A (MilliQ water with 0.1% formic acid) and 5% B (acetonitrile with 0.1% formic acid) followed by a linear increase to 45% B, which was achieved at 5.0 minutes. Subsequently, the gradient was linearly increased within 0.1 minutes to 100% B and maintained for 2 minutes. The system subsequently switched to 5% B, which was achieved at 7.1 minutes, after which the column equilibrated at 95% A for approximately 3 minutes. The column was maintained at 50°C during analysis, and the samples were kept at 10°C. Blank injections were run after each sample and the needle and tubings were washed with strong wash 0.1% formic acid in methanol/H2O (8:2) and weak wash 0.25% trifluoroacetic acid in acetonitrile/H2O (4:6). The mass spectrometer was operating in selective reaction mode using electrospray ionization in positive ion mode, with a capillary voltage of 3.3 kV, a source temperature of 150°C, and a desolvation temperature of 600°C. Cone voltage and collision energy were optimized for each compound individually. The multiple-reaction–monitoring transitions were determined from tandem mass spectra obtained by direct infusion of 0.5
μg/ml peptide solution. For each peptide four transitions were chosen (Q3-1, Q3-2, Q3-3, and Q3-4) for quantitation and confirmation. The transitions for OATP1B1 are listed in Table 1. A peptide labeled with ¹⁵N and ¹³C (AQUA peptide) was synthesized (Sigma-Aldrich) and used as an internal standard for quantification. Peak identification and quantification was performed using MassLynx software version 4.1 (Waters).

Transport and Inhibition Assays. Transport and inhibition assays were performed at different laboratories, with minor differences. At TNO, cells were seeded at a density of 4 × 10⁵ cells/well on Poly-D-Lysine-coated 24-well plates (BD Biosciences, San Jose, CA) and grown to confluence for 48 hours. All incubations were performed at 37°C. For initial characterization of the cell lines and for inhibition of [¹³C]-rosuvastatin uptake by selected oral antidiabetic drugs, we used the following method. Prior to the start of the experiment cells were preincubated for 15–30 minutes with prewarmed incubation medium (145 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM d-glucose, 5 mM HEPES; pH 7.4), containing appropriate concentrations of the inhibitor where applicable. The uptake was initiated after removal of the preincubation medium and addition of incubation medium containing substrate (and inhibitor, if applicable) concentrations as described, spiked with [¹³C]-labeled substrate (~10 kBq/μl). The uptake was terminated by washing the cells twice with 1 ml ice-cold incubation medium, followed by the addition of 0.5 ml 0.5 M NaOH, of which 0.4 ml of the cell lysate was transferred to a 20-ml plastic scintillation vial for radioactivity measurement by liquid scintillation counting using a Tri-Carb 3100 TR from PerkinElmer (Shelton, CT).

For the inhibition of [¹³C]-E₂₁-seven inhibitor uptake by selected oral antidiabetic drugs, cells were seeded at a density of 4 × 10⁵ cells/well on Poly-D-Lysine-coated 24-well plate (BD Biosciences) and grown to confluence for 48 hours. Prior to the start of the experiment cells were washed twice with prewarmed Hanks’ balanced salt solution (HBSS)/HEPES (pH 7.4) buffer, followed by the addition of HBSS/HEPES containing substrate (and inhibitor) concentrations as described, spiked with [¹³C]-labeled substrate (~10 kBq/μl), final concentration 1 μM. The uptake was terminated by washing the cells with 0.4 ml ice-cold HBSS/HEPES + 0.5% bovine serum albumin and twice with 0.4 ml ice-cold HBSS/HEPES, followed by the addition of 0.2 ml 0.5% Triton X-100, of which 0.15 ml of the cell lysate was transferred to a 5-ml plastic scintillation vial for radioactivity measurement by liquid scintillation counting using a MultiCarb 3100 TR from PerkinElmer (Shelton, CT).

Experiments performed in the laboratory of Janssen Pharmaceuticals, Inc., of Johnson & Johnson (J&J) were carried out according to the above mentioned protocol, with three minor differences: Cells were seeded at a density of 4 × 10⁵ cells/well on collagen-coated 24-well plates (BD Biosciences) and grown to confluence for 24 hours; the uptake was terminated by washing the cells with 1.5 ml ice-cold HBSS/HEPES, followed by the addition of 0.2 ml mPER-lysis (Thermo Scientific).

Data Analysis. Uptake into mock-transfected cells served as a control in all experiments to correct for uptake that was not related to OATP1B1-mediated transport. Concentration-dependent uptake of compounds is expressed as pmol/mg protein per minute Inhibition of OATP1B1-mediated uptake is expressed as the percent of uptake into HEK-OATP1B1 cells incubated with vehicle and plotted against the nominal inhibitor concentration. To determine KM and Vmax values for radiolabeled substrates, the Michaelis-Menten model was fitted to the corrected data. To estimate IC₅₀ values, a one-site binding model was fitted to the data, assuming a Hill slope of ~1. To investigate whether inhibitory potency was related to compound lipophilicity, logP values of sulfonylurea-type oral antidiabetics were calculated according to the algorithms described by Cheng et al. (2011) and were obtained from the PubChem database (National Center for Biotechnology Information, Bethesda, MD). The calculated logP values were as follows [drug (xlogP)]; glyburide (4.8), glipizide (1.9), gliclazide (1.5), glimepiride (3.9), and tolbutamide (2.3).

The two-sided unpaired Student’s t test was used to assess the statistical significance of differences between two sets of data. Results are presented as the means ± S.D. Differences were considered to be statistically significant when P < 0.05.

Results

Cellular Localization and Absolute Protein Expression of Recombinant OATP1B1 in HEK-OATP1B1 and HEK-OATP1B1*15 Cells. Subcellular localization of the human OATP1B1 protein in transfected HEK-OATP1B1 and HEK-OATP1B1*15 cells was investigated by immunocytochemical staining and fluorescence microscopy. Results clearly indicate that in the HEK-OATP1B1 and HEK-OATP1B1*15 cells the transfected protein is mainly expressed at the cell membrane (Fig. 1, A and B). No staining was observed in the vector-transfected control cells (HEK-MOCK; Fig. 1C). The absolute protein expression of OATP1B1 at the cell membrane of both cell lines was comparable (9.8 ± 2.3 and 8.5 ± 1.9 pmol OATP1B1/mg membrane protein, respectively; Fig. 2), justifying direct comparison of these two cell lines without further corrections.

Functional Characterization of the HEK-OATP1B1 and HEK- OATP1B1*15 Cells. The uptake of the control substrate [¹³C]-E₂₁-seven-G by HEK293 cells stably expressing OATP1B1 or OATP1B1*15 was determined in a time- and concentration-dependent manner. Uptake of [¹³C]-E₂₁-seven-G was significantly higher in the transfected cell lines compared with the control cells, and was linear up to 2 minutes (unpublished data). Analyses of the kinetic parameters are shown in Fig. 3, A and B, and revealed similar KM values for the transport of [¹³C]-E₂₁-seven-G by OATP1B1 and OATP1B1*15 (7.0 ± 0.3 and 10.0 ± 0.6 μM, respectively), whereas the Vmax value for OATP1B1*15 was decreased by more than 60% (798 ± 11 and 311 ± 7.2 pmol/mg protein per minute for OATP1B1 and OATP1B1*15, respectively). These results are consistent with a previous report of Iwai et al. (2004).

Uptake of [¹³C]-rosuvastatin was significantly higher in HEK- OATP1B1 cells compared with HEK-MOCK, but was only minimal in HEK-OATP1B1*15 cells (Fig. 3C), as published before (Ho et al., 2006; Choi et al., 2011). Due to the small difference in rosuvastatin uptake between HEK-MOCK and HEK-OATP1B1*15 cells, the KM and Vmax of OATP1B1*15-mediated rosuvastatin transport could not be determined. The KM for uptake of [¹³C]-rosuvastatin by OATP1B1 was 13.1 ± 0.43 μM with a Vmax of 202 ± 2.1 pmol/mg protein per minute (Fig. 3D).

Our results were independently verified by experiments performed at J&J laboratories using the same cell lines. Despite minor protocol

### TABLE 1

<table>
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<tr>
<th>Protein</th>
<th>Peptide Sequence</th>
<th>MW</th>
<th>Q1</th>
<th>Q3-1</th>
<th>Q3-2</th>
<th>Q3-3</th>
<th>Q3-4</th>
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<tr>
<td>OATP1B1</td>
<td>LNTVGIAK</td>
<td>814.7</td>
<td>408.5</td>
<td>588.3</td>
<td>487.3</td>
<td>702.4</td>
<td>388.3</td>
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<tr>
<td>OATP1B1-AQUA</td>
<td>LNTVGI[^15N]AK</td>
<td>822.0</td>
<td>411.8</td>
<td>595.4</td>
<td>494.3</td>
<td>709.4</td>
<td>395.3</td>
</tr>
</tbody>
</table>

AQUA, absolute quantification; MW, molecular weight; OATP, organic anion-transporting polypeptide.
differences (see Materials and Methods), the $K_m$ and $V_{\text{max}}$ values for the OATP1B1- and OATP1B1*15-mediated transport of $[\text{H}]$-E217β-G and $[\text{H}]$-rosuvastatin were highly comparable between the two independent laboratories (results are summarized in Table 2), demonstrating the stability and robustness of the transfected cell lines.

Interestingly, inhibition of E217β-G transport by known OATP1B1 inhibitors was comparable between HEK-OATP1B1 and HEK-OATP1B1*15 cells, with IC$_{50}$ values for cyclosporine A (1.64 and 1.25 μM) and rifampicin (3.49 and 2.19) for OATP1B1 and OATP1B1*15, respectively (Supplemental Fig. 1). The uptake of the lipid-lowering drug $[\text{H}]$-rosuvastatin was also inhibited by cyclosporine A, with an IC$_{50}$ value of 0.89 μM (Supplemental Fig. 1).

Inhibition of OATP1B1 and OATP1B1*15 by Several Oral Antidiabetic Drugs. In a preliminary in vitro inhibition screen, we investigated the inhibitory effects of a library of 640 FDA-approved drugs on the transport of $[\text{H}]$-E217β-G by OATP1B1 (unpublished data), and identified a set of oral antidiabetic drugs as (novel) inhibitors of this uptake transporter. We therefore selected these drugs and determined their inhibitory effect on the uptake of $[\text{H}]$-E217β-G and $[\text{H}]$-rosuvastatin by OATP1B1 and OATP1B1*15 (Figs. 4 and 5). The oral antidiabetic drugs can be grouped into three classes and the IC$_{50}$ values obtained are summarized in Table 3. OATP1B1-mediated transport of $[\text{H}]$-E217β-G was significantly inhibited by glyburide (IC$_{50}$ 2.99 μM), glimepiride (IC$_{50}$ 3.55 μM), troglitazone (IC$_{50}$ 2.50 μM), pioglitazone (IC$_{50}$ 5.09 μM), and glipizide (estimated IC$_{50}$ ~45.3 μM, with high 95% confidence interval). These compounds were also identified as significant inhibitors of OATP1B1-mediated transport of $[\text{H}]$-rosuvastatin: glyburide (IC$_{50}$ 1.77 μM), glimepiride (IC$_{50}$ 3.62 μM), troglitazone (IC$_{50}$ 2.84 μM), pioglitazone (IC$_{50}$ 32.2 μM), and glipizide (IC$_{50}$ 110 μM). Gliclazide, tolbutamide, and metformin did not significantly affect the uptake of $[\text{H}]$-E217β-G by OATP1B1 or OATP1B1*15 up to the highest concentration tested (100 μM). The inhibitory effect of these three compounds on wild type OATP1B1-mediated rosuvastatin uptake was tested up to higher concentrations (1000 μM). Also in these studies, metformin did not display any effect, whereas gliclazide and tobutamide were only weak inhibitors (IC$_{50}$ of 580 and 368 μM, respectively). Interestingly, the IC$_{50}$ values for inhibition of $[\text{H}]$-E217β-G uptake by the several oral antidiabetic drugs were similar for OATP1B1 and its polymorphic variant OATP1B1*15 (Fig. 4; Table 2). Notably, at low concentrations, gliclazide (<1 μM) appeared to stimulate OATP1B1*15-mediated transport of $[\text{H}]$-E217β-G. Drug-induced stimulation of OATP1B1 (and several other transporters) in vitro has been reported several times (Kindla et al., 2011; Roth et al., 2011); however the underlying molecular mechanism and clinical relevance has remained unclear.

Within the class of sulfonylureas it appeared that increased lipophilicity of these antidiabetic drugs was associated with a more potent inhibition of OATP1B1-mediated transport of rosuvastatin (Fig. 6).

**Discussion**

Rosuvastatin belongs to the class of statin drugs, which are widely prescribed and act by inhibiting hydroxymethylglutaryl-CoA reductase, resulting in reduced plasma concentrations of low-density lipoprotein cholesterol (McKenney et al., 2003). After oral dosage, the rather hydrophilic rosuvastatin is efficiently and rapidly taken up from the portal vein into hepatocytes, which is predominantly mediated by the uptake transporter OATP1B1 (Ho et al., 2006; Kitamura et al., 2008; Choi et al., 2011). Since statins lower cholesterol levels by inhibiting HMG-CoA reductase within hepatocytes, and the transport processes in hepatocytes are key drivers of the clearance of statins as well, it is clear that the process of hepatic uptake is crucial for both drug efficacy and toxicity. It has been estimated that hepatic elimination of rosuvastatin in humans accounts for approximately 70% of its total clearance (Martin et al., 2003). The clinical importance of OATP1B1 in the pharmacokinetics of rosuvastatin has been demonstrated by several studies showing that subjects carrying the polymorphic variant OATP1B1*15 (Asn130Asp and Val174Ala), which has generally been associated with decreased transport activity, have more than twofold higher plasma levels of rosuvastatin (Lee et al., 2005; Pasanen et al., 2007; Choi et al., 2008). Due to its wide
prescription, but also due to the combined prevalence of hypercholesterolemia and type 2 diabetes (e.g., in overweight/obese patients), rosuvastatin is often concomitantly prescribed with oral antidiabetic drugs. In this study we therefore investigated the possible drug-drug interaction between rosuvastatin and several oral antidiabetics in vitro by generating and characterizing HEK293 cells stably overexpressing OATP1B1 or its polymorphic variant OATP1B1*15.

**Fig. 3.** OATP1B1- and OATP1B1*15-mediated uptake of [3H]-E217β-G and [3H]-rosuvastatin. (A and B) Concentration-dependent uptake of [3H]-E217β-G into HEK-MOCK, HEK-OATP1B1, and HEK-OATP1B1*15 cells after 2 minutes incubation. (C) Uptake of 1 μM [3H]-rosuvastatin into HEK-MOCK, HEK-OATP1B1 and HEK-OATP1B1*15 cells after 1 minute incubation. (D) Concentration-dependent uptake of [3H]-rosuvastatin in HEK-MOCK and HEK-OATP1B1 cells after 1 minute incubation. The dotted line represents the nonlinear fit of the uptake into the transfected cells corrected for the uptake in the mock-transfected cells. The dotted line therefore represents the OATP1B1- or OATP1B1*15-mediated uptake of [3H]-E217β-G or [3H]-rosuvastatin. Data are presented as mean ± S.D. (n = 3, *P < 0.05, ***P < 0.001, when compared with HEK-MOCK).

**TABLE 2**

<table>
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<tr>
<th>Substrate</th>
<th>Cell Line</th>
<th>Experiments Performed by TNO</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (pmol/mg protein per minute)</th>
<th>Experiments Performed by J&amp;J</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (pmol/mg protein per minute)</th>
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</thead>
<tbody>
<tr>
<td>[3H]-E217β-G</td>
<td>HEK-OATP1B1</td>
<td>7.0 ± 0.3</td>
<td>798 ± 11</td>
<td>8.8 ± 1.2</td>
<td>795 ± 39</td>
</tr>
<tr>
<td>[3H]-E217β-G</td>
<td>HEK-OATP1B1*15</td>
<td>10 ± 0.6</td>
<td>311 ± 7.2 (61% ↓)</td>
<td>10 ± 1.5</td>
<td>203 ± 11 (74% ↓)</td>
</tr>
<tr>
<td>[3H]-Rosuvastatin</td>
<td>HEK-OATP1B1</td>
<td>13 ± 0.4</td>
<td>202 ± 2.1</td>
<td>12 ± 6.3</td>
<td>206 ± 34</td>
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</table>

E217β-G, estradiol 17β-β-glucuronide; K<sub>m</sub>, Michaelis constant; OATP, organic anion-transporting polypeptide.
Inhibition of $[^{3}H]$-E217β-G uptake (1 μM) in HEK-OATP1B1 (black closed circles) and HEK-OATP1B1*15 (gray open circles) cells by (A) glyburide, (B) glipizide, (C) gliclazide, (D) glimepiride, (E) tolbutamide, (F) troglitazone, (G) pioglitazone, and (H) metformin. Results are presented as mean ± S.E.M. of three independent experiments ($n = 2$).

Fig. 4.
was previously reported (Iwai et al., 2004). The absolute protein expression levels of OATP1B1 in the outer cell membrane were determined by liquid chromatography-tandem mass spectrometry. The importance of determining the absolute expression levels of transfected transport proteins in different cell lines has been demonstrated before by Iwai et al. (2004), who found that in their generated HEK293 transfected cell lines the protein expression of OATP1B1*15 at the cell membrane was 11-times higher than the expression of wild type OATP1B1 as measured by Western blotting. Without correction for this increased expression of OATP1B1*15, the $V_{\text{max}}$ of $E_217\beta$-G

![Graphs](image-url)

**Fig. 5.** Inhibition of $[^3H]$-rosuvastatin (1 μM) uptake in HEK-OATP1B1 cells by (A) glyburide, (B) glipizide, (C) gliclazide, (D) glimepiride, (E) tolbutamide, (F) troglitazone, (G) pioglitazone, and (H) metformin. Results are presented as mean ± S.D. ($n = 3$).
uptake by OATP1B1*15 was comparable to that of OATP1B1, whereas after correction for the increased expression, the \( V_{\text{max}} \) of OATP1B1*15 was only 10% of that of wild type OATP1B1. Importantly, in the current study similar absolute protein expression levels of OATP1B1 and OATP1B1*15 at the cell surface membrane in both cell lines were measured, justifying direct comparison of these two cell lines. This direct comparison prevents overcorrection for increased expression levels, and we observed that the \( V_{\text{max}} \) of \( \text{E}_2\text{17}'/\text{G} \) uptake by OATP1B1*15 was \(<40\%\) of its wild type variant, while the \( K_m \) values were comparable. Together, our results demonstrate that the reduced transport of \( \text{E}_2\text{17}'/\text{G} \) by OATP1B1*15 is most likely caused by a reduction in translocation ability, instead of a reduced intrinsic affinity or reduced protein expression in the cell membrane.

To assess whether the established \( IC_{50} \) values in the current study are of clinical relevance, we compared the obtained \( IC_{50} \) values with reported \( C_{\text{max}} \) values of the studied oral antidiabetics after the maximum dose in patients. Since the concentration of orally taken drugs is the highest in the portal vein (resulting from intestinal absorption), we estimated the drug concentration in portal venous blood by the method described by Ito et al. (1998). The \( C_{\text{max}} \) in patients taking glyburide (1.25 mg), glipizide (5 mg), glimepiride (5 mg), or troglitazone (600 mg) has been reported at 0.77, 1.04, 0.73, and 6.4 \( \mu \text{M} \) (Spencer and Markham, 1997; Zheng et al., 2009; Brunton et al., 2011), respectively, with predicted portal vein concentrations of 0.95, 1.88, 1.15, and 100 \( \mu \text{M} \). These concentrations are within the same (low) micromolar range as some of the \( IC_{50} \) values for OATP1B1-mediated uptake of rosuvastatin as measured in this study (glyburide: 1.77 \( \mu \text{M} \); glimepiride: 3.62 \( \mu \text{M} \)) and even 35-times higher than the \( IC_{50} \) values for troglitazone (2.84 \( \mu \text{M} \)). This might suggest that these oral antidiabetic drugs can cause clinically relevant drug-drug interactions when concomitantly taken with rosuvastatin, or other OATP1B1 drug substrates. However, due the high plasma protein binding of these oral antidiabetics (>98%) (Spencer and Markham, 1997; Zheng et al., 2009; Brunton et al., 2011), the unbound concentration is expected to be only a fraction of the total plasma concentration and are mainly below the \( IC_{50} \) values observed in this study. Similar conclusions were drawn by Hirano et al. (2006), who stated that although OATP1B1-mediated pitavastatin uptake was inhibited by glyburide in vitro, it was unlikely that—due to high plasma protein binding of glyburide—a drug-drug interaction occurs in the clinical stage. Importantly however, the plasma protein binding of the well-known OATP1B1 inhibitor cyclosporine A is high as well, with a predicted unbound portal vein concentration of \(<0.5 \mu \text{M} \) (\( C_{\text{max}} \) 1900 ng/ml at 400-mg dose with 98.6% plasma protein binding) (Akhlaghi et al., 1997; Falcè et al., 2008). Whereas this value is also below the determined \( IC_{50} \) value for inhibition of rosuvastatin uptake into HEK-OATP1B1 cells (1.64 \( \mu \text{M} \)), coadministration of rosuvastatin with cyclosporine A in the clinic resulted in 5- to 10-fold higher systemic exposure to rosuvastatin, which could mainly be explained by inhibition of OATP1B1-mediated hepatic uptake of rosuvastatin (Asberg, 2003; Simonson et al., 2004; Neuvonen et al., 2006). This indicates that a straightforward comparison of the unbound maximum plasma concentration of a drug with in vitro measured \( IC_{50} \) parameters alone might not always be predictive for potential drug-drug interactions in vivo. The clinical relevance of the drug-drug interactions between rosuvastatin and oral antidiabetic drugs therefore needs to be established, as there are no clinical trials present in the literature today. Results of the present study can well be used to set up an intelligent drug development strategy to predict the clinical relevance of drug-drug interactions, as the combination with physiologically based pharmacokinetic models enables a prediction of pharmacokinetic profiles of the (unbound) drug over time and whether drug-drug interactions are to be expected in patients. In addition to in vitro studies and physiologically based pharmacokinetic modeling, preclinical animal studies (such as humanized mice) could also contribute to an efficient preclinical phase in drug development leading to an optimized clinical trial.

![Fig. 6. Relation between inhibition potency of selected oral antidiabetic drugs for OATP1B1-mediated rosuvastatin uptake (LogIC_{50}) and the lipophilicity of these compounds (xlogP). \( r^2 = 0.91; P < 0.05 \).](image-url)

### Table 3

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Inhibition of [(^3)H]-E(_2)17β-G Uptake</th>
<th>Inhibition of [(^3)H]-Rosuvastatin Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC(_{50}) ( \mu \text{M} ) 95% CI</td>
<td>IC(_{50}) ( \mu \text{M} ) 95% CI</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>Glyburide</td>
<td>2.99 1.88–7.56</td>
<td>1.77 1.50–2.10</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>45.3 0.2–2845*</td>
<td>110 54.4–224</td>
</tr>
<tr>
<td></td>
<td>Glimepiride</td>
<td>&gt;100 na</td>
<td>3.62 2.79–4.69</td>
</tr>
<tr>
<td></td>
<td>Tolbutamide</td>
<td>&gt;100 na</td>
<td>368 168–806</td>
</tr>
<tr>
<td></td>
<td>Gliclazide</td>
<td>&gt;100 na</td>
<td>2.84 2.23–3.63</td>
</tr>
<tr>
<td></td>
<td>Pioglitazone</td>
<td>5.09 1.89–13.7</td>
<td>32.2 17.4–59.8</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>&gt;100 na</td>
<td>&gt;1000 na</td>
</tr>
<tr>
<td>Thiazolidinedione</td>
<td>Troglitazone</td>
<td>2.50 1.3–4.70</td>
<td>2.84 2.23–3.63</td>
</tr>
<tr>
<td></td>
<td>Pioglitazone</td>
<td>5.09 1.89–13.7</td>
<td>32.2 17.4–59.8</td>
</tr>
<tr>
<td>Biguanide</td>
<td></td>
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</tr>
</tbody>
</table>

IC\(_{50}\), confidence interval; E\(_2\)17β-G, estradiol 17β-glucuronide; na, not applicable; OATP, organic anion-transporting polypeptide.

*95% confidence interval is very wide due to incomplete and nonsigmoidal fitting of the curve; IC\(_{50}\) value is estimated (although >50% inhibition is observed at highest measured concentration of 100 \( \mu \text{M} \)).
The frequency of the low-activity OATP1B1*15 differs between various geographical populations, ranging from only 2 and 9% in Sub-Saharan African and South/Central Asian population, respectively, to 15–24% in populations from North Africa, Europe, Middle East, and America (Passanen et al., 2008). This rather high frequency of the OATP1B1*15 haplotype in selected populations demonstrates the importance of studying its effect on the disposition of drugs. Importantly, the effect of the *15 variation on drug-drug interaction at the level of OATP1B1 has never been systematically studied in vitro before. We found similar IC₅₀ values for inhibition of E₂₁₇G uptake into HEK-OATP1B1 and HEK-OATP1B1*15 cells for the different oral antidiabetics studied and the known inhibitors cyclosporine A, rifampicin, and pravastatin. This indicates that the intrinsic affinity of OATP1B1 for these inhibitors is not affected by the amino acid changes in the *15 variant. Further studies are needed to explore whether this is a general finding, or whether it is dependent on the substrate/inhibitor combination.

It has previously been reported that the lipophilicity of compounds can determine their inhibition capacity, as was reported for the metabolic enzyme CYP3A4 (Regev-Shoshani et al., 2004; Lewis et al., 2006). We therefore explored whether this is also the case for inhibition of the transport protein OATP1B1 and indeed found that within the class of sulfonylureas an increased lipophilicity of the antidiabetic drugs was associated with an increased potency to inhibit OATP1B1-mediated transport of rosuvastatin.

In summary, we generated and fully characterized HEK-OATP1B1 and HEK-OATP1B1*15 cells and provide evidence for potentially important drug-drug interactions between rosuvastatin and selected sulfonylureas and thiazolidinediones. It remains to be established, however, whether the studied oral antidiabetics affect the clinical pharmacokinetic profile of rosuvastatin in patients. The in vitro studies as described can be considered as an important step in an intelligent preclinical test strategy to detect potential drug-drug interactions which need to be confirmed in subsequent (preclinical and/or clinical) studies to provide a decisive answer.

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Authorship Contributions

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Conducted experiments: van de Steeg, Schreurs, Nooijen, Verhoeckx, Hanemaaijer, Ripken.

Performed data analysis: van de Steeg, Schreurs, Nooijen, Verhoeckx.

Wrote or contributed to the writing of the manuscript: van de Steeg, Greupink, Verwey, Russel, Huisman, Wortelboer.

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