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# Predicting carrier-mediated hepatic disposition of rosuvastatin in man by scaling from individual transfected cell-lines *in vitro* using absolute transporter protein quantification and PBPK modeling

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ABSTRACT

In contrast to primary hepatocytes, estimating carrier-mediated hepatic disposition by using a panel of single transfected cell-lines provides direct information on the contribution of the individual transporters to the net disposition. The most direct way to correct for differences in transporter abundance between cell-lines and tissue is by using absolute protein quantification. In the present study, the performance of this strategy to predict human hepatic uptake transport was investigated and compared with traditional scaling from primary human hepatocytes. Rosuvastatin was used as a model compound. The uptake activity was measured in HEK293 cell-lines stably overexpressing OATP1B1*1a, OATP1B3 or OATP2B1, the major transporters involved in human hepatic uptake of rosuvastatin, or expressing OATP1B1*15, associated with reduced hepatic uptake of rosuvastatin. The abundance of these transporter proteins in the outer membranes of HEK293-cells, in human primary hepatocytes and in human liver tissue was determined by LC–MS/MS. The measured activity, corrected for protein abundance and scaled to the whole liver, gave a very accurate prediction of the hepatic intrinsic clearance observed *in vivo*. Embedded in a PBPK model describing the hepatic disposition and enterohepatic circulation, the collective *in vitro* data resulted in a good explanation of the observed oral and intravenous pharmacokinetic profiles of rosuvastatin. The model allowed simulation of the effect of polymorphic variants of OATP1B1 on rosuvastatin pharmacokinetics. These results encourage a larger scale validation. This approach may facilitate prediction of drug–drug interactions, scaling of transporter processes across subpopulations (children, diseased patients), and may be extended to tissues for which primary cells may be more difficult to obtain.



## 1. Introduction

Membrane embedded influx and efflux transporter proteins play an important role in the hepatic disposition and biliary excretion of a wide variety of drugs (Borst and Elferink, 2002; Schinkel and Jonker, 2003; Hagenbuch and Stieger, 2013). Differences in drug transporter expression and polymorphisms that alter transporter functionality and their allele frequency differences may cause interindividual variations in drug disposition and differences in kinetics between populations (Pasanen et al., 2008; Fahrmayr et al., 2010). In addition, inhibition of drug transporters has been shown to cause clinically relevant drug–drug interactions (DDI) (Asberg, 2003; Simonson et al., 2004). Thus, the ability to predict the impact of drug transporters on drug disposition is important in various stages of drug development.

Primary hepatocytes *in vitro* are commonly used to investigate the hepatic uptake of compounds. The uptake in these cells can be scaled to *in vivo* directly by multiplying by number of hepatocytes in the liver. Scaled transporter data can be used to derive

*Abbreviations:* AUC, area under the plasma concentration-time curve; DDI, drug–drug interaction; $CL_{int}$, intrinsic clearance; BCRP, Breast Cancer Resistance Protein; HEK293, Human Embryonic Kidney 293 (cell-line); MRP, Multidrug resistance-associated protein; NTCP, Sodium-taurocholate cotransporting polypeptide; OATP, Organic Anion Transporting Polypeptide; PBPK, physiology-based pharmacokinetic (model).

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*in vivo* pharmacokinetic parameters of interest by non-compartmental methods (Ito and Houston, 2004; Liu and Pang, 2005; Watanabe et al., 2009a), or integrated with other pharmacokinetic parameters in a physiology-based pharmacokinetic (PBPK) model to simulate their influence on the overall pharmacokinetic profile (Liu and Pang, 2006; Zamek-Gliszczynski et al., 2013). Such PBPK models have been successfully used for early prediction of plasma profiles and target tissue concentrations, animal-to-human translation and simulation of target subpopulations, and prediction of DDI (Watanabe et al., 2009b; Poirier et al., 2009a; Jones et al., 2012; Jamei et al., 2014).

However, human primary cells may not be as easy to obtain from transporter-expressing organs other than the liver, making it difficult to extend this primary cell-based strategy to other tissues. Moreover, they do not readily reveal the relative contributions of individual transporters to the overall hepatic disposition, which is needed for simulation of specific target populations and DDI (Shitara et al., 2006). Attempts have been made to address the latter issue by using specific transporter inhibitors (e.g. Ishiguro et al., 2005; Shitara et al., 2002, 2006) or specific transporter model substrates in primary hepatocytes (e.g. Hirano et al., 2004; Shitara et al., 2006). Alternatively, the activity of each transporter can be quantified separately in cell-lines transfected with individual human transporter genes (Hirano et al., 2004; Kitamura et al., 2008; Poirier et al., 2009a). Several methods have been proposed to correct for differences in transporter expression between cell-lines and hepatocytes. Kitamura et al. (2008) measured the ratio of uptake of specific transporter model substrates in transfected cell-lines and primary hepatocytes as a factor to estimate the contribution of OATP1B1 and OATP1B3 to the uptake of rosuvastatin. A similar approach was used by Poirier et al. (2009a) to predict the pharmacokinetics of valsartan from transfected cell-lines. Clearly, the success of such approaches depends on whether the inhibitors or model substrates available are truly transporter-specific. Lippert et al. (2012) used transporter gene expression data to estimate the relative role of OATP1B1 in the hepatic uptake of simvastatin and pravastatin. Gene expression, however, may not be a strong predictor of actual transporter protein levels (Ohtsuki et al., 2012).

The most direct way to scale up the intrinsic uptake activity to the whole organ level would be by absolute quantification of transporter protein abundance in individual transfected cell-lines and in human tissue. Hirano et al. (2004) used Western blotting to estimate the expression of OATP1B1 and OATP1B3 in transfected cells and human hepatocytes to scale the measured uptake of pitavastatin. We recently described an approach to quantify absolute membrane embedded transporter protein levels at the outer cell membranes of transfected Human Embryonic Kidney 293 (HEK293) cell-lines, human hepatocytes and human liver samples by LC–MS/MS (Van de Steeg et al., 2013). A similar strategy was recently used by Vildhede et al. (2014) to elucidate the role of OATPs and Sodium-taurocholate cotransporting polypeptide (NTCP) in the hepatic uptake of atorvastatin.

In the present paper, we demonstrate the use of these LC–MS/MS derived transporter data to predict hepatic disposition *in vivo* from uptake transport measured in cell-lines overexpressing single transporters. We illustrate this approach by revisiting the example of rosuvastatin, addressed previously by Jones et al. (2012) and Jamei et al. (2014) based on primary hepatocytes. We measured rosuvastatin uptake into HEK293 cell-lines stably overexpressing OATP1B1*1a, OATP1B3 or OATP2B1, the major transporters involved in human hepatic uptake of rosuvastatin (Ho et al., 2006; Kitamura et al., 2008), as well as HEK293 cells stably overexpressing the polymorphic variant OATP1B1*15, which is associated with reduced hepatic uptake of rosuvastatin (Pasanen et al., 2007; Choi et al., 2008; Fan et al., 2008). The absolute levels of these proteins in the outer membranes of the HEK293-cells, human primary hepatocytes and human liver tissue were determined by LC–MS/MS. The measured uptake activity was corrected for protein abundance, scaled to whole liver, and compared with the hepatic intrinsic clearance observed *in vivo*. Uptake measurement in primary human hepatocytes and scaling to whole liver was also included in this study to compare the accuracy of prediction between these two strategies. Further, we demonstrated that a PBPK model in which the scaled uptake transporter activities were embedded, and with its remaining parameter values also based as much as feasible on nonclinical data, was able to reproduce the observed pharmacokinetic profile of rosuvastatin. Finally, this PBPK model was used to simulate the effect of different haplotypes of OATP1B1 on the systemic exposure to rosuvastatin.

## 2. Materials and methods

### 2.1. Chemicals

[$^3$H]-Rosuvastatin (rosuvastatin[2]-calcium; 40.7 GBq/mmol; analytical grade) was custom synthesized by Moravek Biochemicals (Brea, CA) and purified prior to use (purity > 90%). [$^3$H]-estradiol 17β-D-glucuronide ([$^3$H]-$E_2$17β-G; 1.85 TBq/mmol) and [$^3$H]-estrone-sulphate ([$^3$H]-$E_3$S; 1.85 TBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). Rosuvastatin was obtained from Sequoia Research Products (Pangbourne, UK); $E_2$17β-G, $E_3$S, cyclosporin A and bromosulphthalein (BSP) were obtained from Sigma–Aldrich (St Louis, MO).

### 2.2. Construction of cell-lines and culture conditions

Generation of HEK293 cells overexpressing OATP1B1*1a (NM_006446.4 referring to wild-type; hereafter named OATP1B1) or OATP1B1*15 was described before by our group (Van de Steeg et al., 2013). HEK293 cells stably overexpressing OATP1B3 (NM_019844.3) or OATP2B1 (NM_007256.4) were generated in a comparable way. In brief, HEK293 cells (ATTC, Wesel, Germany; lot# 57954093) were seeded in 6-wells plates at a density of $9 \cdot 10^5$ cells/well and grown for 24 h, followed by co-transfection with pcDNA3.1-Ukcol (0.3 μg/well), pIRESpuro-OATP1B3 and pIRESpuro-OATP2B1 (2.7 μg/well) using 6 equivalents of Exgen 500 (ThermoScientific, Waltham, MA). After 24 h, the transfected cells were cultured in medium containing puromycin (1 μg/mL), and after 3–4 weeks several colonies were selected and grown into 75 cm$^2$ tissue culture flasks. Excretion of UKcol into the medium of cells of the different colonies was used to select colonies for further analysis. For culturing, all cells were grown in 75 cm$^2$ tissue culture flasks containing culture medium consisting of Dulbecco's modified Eagle medium (DMEM) with L-GlutaMax (4.5 g of glucose per liter), supplemented with heat-inactivated fetal calf serum (10% v/v; Lonza), 100 U/mL penicillin (Invitrogen) and 100 μg/mL streptomycin (Invitrogen) at approximately 37 °C in approximately 95% air/5% $CO_2$. Near confluent cell cultures were harvested by trypsinisation, resuspended in culture medium and the process was repeated once or twice weekly to provide sufficient amounts of cells for further use.

### 2.3. Transporter activity in transfected HEK293 cells

To confirm and validate functionality of the generated HEK293-OATP1B1, HEK293-OATP1B1*15, HEK293-OATP1B3 and HEK293-OATP2B1 cells, the uptake of [$^3$H]$E_2$17β-G (OATP1B1, OATP1B1*15 and OATP1B3) or [$^3$H]$E_3$S (OATP2B1) were used as positive controls. Cyclosporin A (10 μM) (OATP1B1, OATP1B1*15 and OATP1B3) or BSP (20 μM) (OATP2B1) were used as control inhibitors. For