Effect of Organic Anion Transporting Polypeptides on the Hepatic Elimination of Fluvastatin and Rosuvastatin

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Purpose

The Biopharmaceutical Drug Distribution Classification System (BDDCS) can predict drug-drug interactions (DDIs) mediated through drug metabolizing enzymes and drug uptake and/or efflux transporters. BDDCS suggests that for Class I (highly metabolized) drugs, DDIs caused by drug transporters are not clinically relevant. A recent publication suggests that the pharmacokinetics of fluvastatin (FLST) in humans may be affected by cyclosporine and fluconazole via drug transporter inhibition, although it is categorized as BDDCS class I. However, we believe that the increases of FLST AUCs by these drugs are more likely due to inhibition of drug metabolizing enzymes, not transporter inhibition. Moreover, it is reported that the mutation on the \textit{SLCO1B1} gene, especially in c.521 position, did not affect FLST pharmacokinetics in healthy human volunteers. Here we investigate whether FLST pharmacokinetics can be influenced by transporters, such as organic anion transporting polypeptide (OATP) as compared to rosuvastatin (ROST, BDDCS class III) using in \textit{in vitro} and \textit{ex situ} rat models.

Methods

\textbf{Substrate drugs.} To compare the effect of uptake transporters on drug distribution in the liver, FLST (BDDCS class I) and ROST (BDDCS class III) were used. \textit{In vitro drug transport study using rat hepatocytes.} FLST or ROST was incubated with freshly isolated 2x10^6 Sprague Dawley (SD) rat hepatocytes (viability >94%) at concentrations of 0.001, 0.01, 0.1, 1, 10, 100, 200 and 400 μM (including 0.1 μCi tritiated drug) for 1 min at 37°C. To evaluate the contribution of uptake transporters, 20 μM of rifamycin SV (R-SV) was co-incubated. Hepatocytes were collected by the oil-filtration technique and intracellular drug measured by liquid-scintillation counter. \textit{In vitro drug transport study using rat uptake transporter overexpressing system.} Rodent OATPs (rOatp1a1, rOatp1a4 and rOatp1b2) over-expressing HEK293 cell lines were grown on 12-well plates until confluence, then incubated with FLST or ROST at concentrations of 0.1, 1 and 10 μM for 2 min with or without the OATP inhibitor R-SV. Cells were collected and drug concentrations measured by HPLC coupled with tandem MS/MS. \textit{Ex situ isolated perfused rat liver (IPRL) study.} Livers from SD rats were isolated and perfused \textit{via} the cannulated portal vein and inferior vena cava with oxygenated Krebs-Henseleit buffer supplemented with 3.5% BSA, HEPES and NEAA at 37°C. After a 20 min incubation, FLST or ROST (1 μM initial concentration) perfusions were started. The OATP inhibitor, R-SV (20 μM), was added 5 min before each drug. Aliquots of perfusate were sampled at 0, 3, 5, 10, 15, 20, 30, 45 and 60 min, and stored at -20°C. Concentrations of FLST and ROST in perfusate were quantified by the HPLC-MS/MS system.

Results

The \textit{in vitro} drug uptake studies showed that both FLST and ROST were substrates of rOatp1a4 and rOatp1b2, and their uptake levels were strongly inhibited by R-SV. Hepatocyte uptake studies also suggested that both FLST and ROST were substrates of uptake transporters based on inhibition by R-SV. Between 1 nM to 1 μM, R-SV inhibited approximately 50% of FLST uptake into the hepatocytes. Uptake of ROST was inhibited more than 65% over the concentration range. \(K_m\) and \(V_{\text{max}}\) values for FLST from the hepatocyte studies were 16.6 μM and 614 pmole/min/10^6 cells, respectively. Calculated intrinsic clearance (\(V_{\text{max}}/K_m\)) was 0.0454 /min/10^6 cells and was comparable with the passive diffusion coefficient (0.0421 /min/10^6 cells). The passive diffusion coefficient of FLST following R-SV co-administration was 0.0388 /min/10^6 cells, a value was close to the control. In the \textit{ex situ} IPRL studies, the AUC of FLST in perfusate was increased approximately 15% by co-administration of R-SV, however it was not statistically significant. Whereas, the AUC of ROST was elevated significantly, approximately 69%, by R-SV. The maximum increase in perfusate drug concentrations by R-SV was 50% for FLST versus 386% for ROST. Unbound fractions of FLST and ROST in perfusate were 0.825% and 10.1%, respectively, and were not changed in the presence of R-SV.

Conclusion

FLST and ROST were substrates of rOatps and there distribution into suspended hepatocytes was decreased by OATP inhibitor R-SV. Since the passive diffusion and intrinsic clearance of FLST were close, lower FLST concentrations (at least around \(K_m\) value 16 μM) were suspected to be affected by uptake transporter function. However, the \textit{ex situ} liver perfusion study revealed that there was no significant alteration of FLST pharmacokinetics in the presence of OATP inhibitor R-SV at clinically relevant concentrations. These results in rats yield the expected findings for a BDDCS class I drug. That is the drug may be a substrate of transporters in cellular systems, but clinically relevant effects of the transporter will not be observed in an organ perfusion study.