The role of Organic Anion Transporting Polypeptides (OATPs) and their common genetic variants in mycophenolic acid pharmacokinetics

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Supplementary Information

Cloning of OATP1B3 and uptake experiments with radiolabeled model substrates

Cloning of OATP1B3 variants

The cDNA coding human OATP1B3 (GenBank accession number NM_019844) was PCR-amplified from human total liver cDNA using the Pfx DNA polymerase (Invitrogen, Carlsbad, CA). Primers used were 5'-ATGCCTTAAGATGGACCAACATCAACATTTG-3' (forward) and 5'-ATGCCTCGAGTTAGTTGGCAGCAGCATTGTCTTG-3' (reverse). The resulting PCR product was ligated into pcDNA5/FRT (Invitrogen). Two nucleotide exchanges corresponding to the *SLCO1B3* T334G (rs4149117) and *SLCO1B3* G699A (rs7311358) polymorphisms were introduced into the *SLCO1B3* plasmid using the Quick change Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) and the following primers: T334G, 5'-GGAACTGGAAGTATTTTGACATCTTTACCACATTTCTTCATGG-3' (forward) and 5'-CCATGAAGAAATGTGGTAAAGATGTCAAAATACTTCCAGTTCC-3' (reverse); G699A, 5'-CACTGGGATCTCTGTTTGCTAAAATGTACGTGGATATTGGA-3' (forward) and 5'-TCCAATATCCACGTACATTTTAGCAAACAGAGATCCCAGTG-3' (reverse). *SLCO1B3* plasmid sequences were verified by sequencing.

Control uptake experiments

Intra-cellular accumulation of radiolabeled model substrates (^{3}H-estrone sulfate for OATP1B1 or OATP1A2 and ^{3}H-Cholecystokinin for OATP1B3) were analyzed as controls for transfected cell lines in parallel of cold-uptake experiments. Uptake was initiated by adding Hank's Buffer Salt Solution

(HBSS, Invitrogen) containing radiolabeled substrates after cells had been washed twice and pre-incubated with HBSS at 37°C for 15 min. After 2 to 10 minutes, the cells were washed twice with ice-cold uptake buffer and lysed in 800µl of NaOH 0.1N/0.1% sodium dodecyl sulfate. 750µl of the lysate was added to 3ml Ecolite scintillation fluid (ICN Biomedicals, Costa Mesa, CA) and intracellular radioactivity was determined by scintillation counting. Results were normalized to per-well protein content as measured using the bicinchoninic acid protein assay (Pierce, Rockford, IL).

OATP1B1 and 1A2 expression in HEK293T cells resulted in a significant increase of ^{3}[H]-estrone sulfate uptake after 2 min (33.2±12.0 fold; $p<0.0001$ and 6.5±4.8 fold; $p=0.0011$, respectively), while the expression of OATP1B3 significantly enhanced ^{3}[H]-cholecystokinin (3.7±0.6 fold; $p=0.0001$) uptake.