Reduced Renal Clearance of Cefotaxime in Asians with a Low-Frequency Polymorphism of OAT3 (SLC22A8)

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ABSTRACT: Organic anion transporter 3 (OAT3, *SLC22A8*), a transporter expressed on the basolateral membrane of the proximal tubule, plays a critical role in the renal excretion of organic anions including many therapeutic drugs. The goal of this study was to evaluate the *in vivo* effects of the OAT3-Ile305Phe variant (rs11568482), present at 3.5% allele frequency in Asians, on drug disposition with a focus on cefotaxime, a cephalosporin antibiotic. In HEK293-Flp-In cells, the OAT3-Ile305Phe variant had a lower maximum cefotaxime transport activity, V_{max}, [159 ± 3 nmol*(mg protein)$^{-1}$/min (mean ± SD)] compared with the reference OAT3 [305 ± 28 nmol*(mg protein)$^{-1}$/min, (mean ± SD), $p < 0.01$], whereas the Michaelis-Menten constant values (K_m) did not differ. In healthy volunteers, we found volunteers that were heterozygous for the Ile305Phe variant and had a significantly lower cefotaxime renal clearance (CL_R; mean ± SD: 84.8 ± 32.1 mL/min, $n = 5$) compared with volunteers that were homozygous for the reference allele (158 ± 44.1 mL/min, $n = 10$; $p = 0.006$). Furthermore, the net secretory component of cefotaxime renal clearance (CL_{sec}) was reduced in volunteers heterozygous for the variant allele [33.3 ± 31.8 mL/min (mean ± SD)] compared with volunteers homozygous for the OAT3 reference allele [97.0 ± 42.2 mL/min (mean ± SD), $p = 0.01$]. In summary, our study suggests that a low-frequency reduced-function polymorphism of OAT3 associates with reduced cefotaxime CL_R and CL_{sec}. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3451–3457, 2013

Keywords: organic anion transporter; cefotaxime; tubular secretion; OAT3; polymorphisms; pharmacogenomic; pharmacokinetics; renal clearance

INTRODUCTION

OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*) are well-studied organic anion transporters known to reabsorb and secrete endogenous organic anions (e.g., pyruvate, α-ketoglutarate, oxalate, urate, and estrone sulfate) and exogenous anions (e.g., methotrexate, ciprofloxacin, acyclovir, captopril, and thiazides) across the basolateral membrane of the proximal tubule.[1–4] *In vivo* studies in *Oat3* knockout mice demonstrate the importance of OAT3 in the renal elimination of drugs. The area under the plasma concentration–time curve (AUC) is increased, and the renal clearance (CL_R) of drugs such as ciprofloxacin,[5] methotrexate,[6] and penicillin[7] is reduced in *Oat3* knockout mice compared with wild-type mice. Importantly, organic-anion-transporter-mediated drug—drug interactions in the kidney have been described in several clinical studies. In particular, administration of inhibitors of OATs, such as probenecid, may result in increased plasma levels and reduced clearances of drugs including furosemide and ciprofloxacin.[8,9] Despite the importance of OAT3 in the pharmacokinetics of many drugs, limited data on the functional

Additional Supporting Information may be found in the online version of this article. Supporting Information

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impact of genetic polymorphisms in human *SLC22A8* (OAT3) exist. Nonsynonymous variants in *SLC22A8* are uncommon,[10,11] whereas variants are more common in the promoter regions of the gene encoding OAT3.[12,13] To date, pharmacogenomic studies have examined the effect of *SLC22A8* variants on drug disposition and response, such as the pharmacokinetics of pravastatin,[14] CL_R of torsemide,[15] and blood pressure response to hydrochlorothiazide.[16] However, none of these studies have demonstrated significant associations of synonymous or intronic variants in *SLC22A8* with drug response phenotypes. Studies conducted previously in our laboratory have revealed five less common *SLC22A8* variants with allele frequencies of less than 5% in a particular ethnic group, which result in loss of uptake transport.[10] Four of them were singletons for which only one individual in the cohort carries the variant. A nonsynonymous variant, Ile305Phe (3.5% frequency in Asian-Americans), exhibited functional changes in *in vitro* assays.[10] This variant was also reported in the 1000 Genome Project, exclusively in individuals of Asian ancestry (e.g., allele frequencies 3.6%–6.0% among Japanese and Chinese).[17] Based on the 1000 Genome Project and our discovery cohort (http://pharmacogenetics.ucsf.edu/), other reduced-function variants with allele frequencies of greater than 3% have not been described. Hence, the current study focused on the effect of the OAT3 Ile305Phe variant on drug disposition.

Cefotaxime is a third-generation cephalosporin antibacterial drug commonly used to treat upper respiratory tract infections and bacterial meningitis.[18] Approximately 40%–60% of cefotaxime is excreted unchanged in the urine.[19,20] Cefotaxime belongs to class 3 of the Biopharmaceutics Drug Disposition Classification System (BDDCS), which are characterized by high water solubility, poor passive permeability, and poor metabolism (<70% metabolism).[21] For example, these drugs generally require transporters to permeate plasma membranes. The CL_R of cefotaxime exceeds creatinine clearance (CL_{CR}), suggesting active tubular secretion.[22] Cefotaxime is known to inhibit the uptake of estrone sulfate ($K_i = 290\ \mu M$) in OAT3 overexpressing cells, and the inhibition is more selective for OAT3 compared with OAT1 and OAT4.[23] The goals of this study were twofold: to determine the effect of OAT3 and the variant Ile305Phe on the uptake of cefotaxime in HEK293-Flp-in stable cells and to determine the effect of the OAT3 variant on the pharmacokinetics of cefotaxime in healthy Asian-Americans.

RESULTS AND DISCUSSION

In vitro cefotaxime transport was measured using HEK293-Flp-in cells stably transfected with empty vector (EV), reference *SLC22A8* cDNA or with OAT3-Ile305Phe variant cDNA, reference *SLC22A6* (OAT1), *SLC22A7* (OAT2), and *SLC22A11* (OAT4) cDNA subcloned into the mammalian expression vector pcDNA5/FRT. These cell lines, which were previously generated in our laboratory,[10,24–26] took up radiolabeled model substrates of organic anion transporters (Fig. S1). Cefotaxime uptake studies were performed at 37°C for 5 min, which was in the linear portion of the uptake versus time curve (data not shown). Results showed that cefotaxime had a greater uptake (5–10-fold) in HEK293-Flp-in cells transfected with OAT3 compared with EV (Fig. 1). Interestingly, HEK293-Flp-in cells transfected with OAT2 and OAT4 showed weak fold uptake (approx. twofold) of cefotaxime compared with EV-transfected cells, whereas OAT1 expressing cells did not take up cefotaxime significantly (Fig. 1a). OAT3-Ile305Phe expressing cells had lower fold uptake of cefotaxime compared with OAT3 reference cells (Fig. 1b, $p < 0.01$), and probenecid (100 μM), an inhibitor of OAT3 and other organic anion transporters, significantly inhibited uptake. Kinetic studies revealed that (1) the rate of cefotaxime uptake by both reference and variant of OAT3 was saturable; and (2) the mean ± SD of the K_m and maximum cefotaxime transport activity (V_{max}) for OAT3 reference were 717 ± 141 μM and 305 ± 28 nmol/mg protein/min, respectively. For OAT3-Ile305Phe, the K_m and V_{max} were 549 ± 21 μM and 159 ± 3 nmol/mg protein/min, respectively (Fig. 2). The V_{max}, but not the K_m, of the variant was significantly lower than that of the reference ($p < 0.01$). Previous studies of chimeric GFP-tagged transporters suggested that both OAT3 and OAT3-Ile305Phe had similar localization patterns.[10] However, because of limitations in our previous studies, which used confocal microscopy in transiently transfected cells, we conducted cell surface biotinylation studies in stably transfected cells (see Supplementary Information). The studies showed that HEK293-Flp-In cells stably expressing the OAT3 variant Ile305Phe had lower cell surface protein levels (36% lower) compared with cells expressing OAT3 reference (Fig. S2a). The *SLC22A8* mRNA levels in these two cell lines were similar (Fig. S2b). Consistent with the lower surface expression, the cells with OAT3-Ile305Phe variant showed lower uptake of four OAT3 substrates (Figs. S1 and S3). The methods used in the *in vitro* uptake studies and analytical method to measure cefotaxime levels in HEK293-Flp-in cells using liquid chromatography–tandem mass spectrometry (LC–MS/MS) are available in the Supplementary Information.

The collective information from the *in vitro* studies suggested that OAT3-Ile305Phe might affect the disposition of cefotaxime in humans. Our Pharmacogenomics of Membrane Transporters (PMT) group