Short Communication

Kidney Cortical Transporter Expression across Species Using Quantitative Proteomics

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ABSTRACT

Limited understanding of species differences in kidney transporters is a critical knowledge gap for prediction of drug-induced acute kidney injury, drug interaction, and pharmacokinetics in humans. Here, we report protein abundance data of 19 transporters in the kidney cortex across five species (human, monkey, dog, rat, and mouse). In general, the abundance of all of the 19 membrane transporters was higher in preclinical species compared with human except for multidrug resistance protein 1 (MDR1), organic cation transporter (OCT) 3, and OCTN1. In nonhuman primate, the total abundance of 12 transporters for which absolute data were available was 2.1-fold higher (P = 0.025) relative to human but the percentage distribution of these transporters was identical in both species.

Objectives

Despite advances in experimental and computational discovery of transporters, there remains limited understanding of species differences in kidney transporters since a fraction of serum creatinine (sCr) is secreted by active transporters, cross-species and sex-dependent protein abundance data are important for animal to human scaling of drug clearance as well as for mechanistic understanding of kidney physiology and derisking of kidney toxicity for new therapeutic candidates in drug development.

Materials and Methods

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Chemicals and Reagents. Liquid chromatography mass spectrometry (MS) grade acetonitrile, methanol, chloroform, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ) and formic acid was purchased from Sigma-Aldrich (St. Louis, MO). The ProteoExtract native membrane protein extraction kit was procured from Pierce Biotechnology (Rockford, IL). The protein quantification bicinechonic acid kit and in-solution trypsin digestion kit were purchased from Pierce Biotechnology. Iodoacetamide, dithiothreitol, and pierce trypsin protease (MS grade) were purchased from Thermo Fisher Scientific and Pierce Biotechnology. Iodoacetamide, dithiothreitol, and pierce trypsin protease (MS grade) were purchased from Thermo Fisher Scientific.

Introduction

In the early toxicity assessment of candidate therapeutics in drug development, the data obtained from animal models need to be interpreted with caution for predicting human pharmacokinetics (PK) (Tang and Meyersohn, 2011) and risk of organ injury as well as drug-drug interactions (Fisel et al., 2014). Clinical and preclinical species differences in the mRNA expression, protein abundance, and activity of transporters in organs relevant to drug disposition (i.e., intestine, liver, and kidney) remain a major reason for the poor allometric scaling (Wang et al., 2015). Although significant progress has been made toward the understanding of species and sex differences of drug transporters in the liver (Wang et al., 2015), limited data exist in the kidney and intestine. Particularly, kidney transporters can affect systemic drug clearance by regulating drug secretion and/or reabsorption and contribute to kidney toxicity by affecting intracellular drug concentration (Filipski et al., 2009). For example, kidney toxicity of tenofovir, methotrexate, cisplatin, ifosfamide, ciprofloxacin, and sitagliptin is associated with solute carrier transporters such as organic anion transporters (OATs) and organic cation transporters (OCTs) (Fisel et al., 2014) (Fig. 1). Allometric scaling methods are used for extrapolation of preclinical renal disposition data to human (Zou et al., 2012); however, these methods are not always successful, particularly when a drug undergoes kidney transport and metabolism. For example, tenofovir is taken up by proximal tubules mainly by OAT1 and effluxed into the urine by multidrug resistance-associated protein (MRP) 4, and both of these transporters are shown to be associated with tenofovir kidney toxicity (Kohler et al., 2011). Furthermore, examples exist where kidney toxicity is different in males and females, e.g., cisplatin, an OCT2 substrate, is more toxic to male rats (Nematbakhsh et al., 2013). In addition, rise in serum creatinine (sCr) is used as a surrogate of kidney function. However, since a fraction of sCr is secreted by active transporters, cross-species and sex-dependent variability in sCr transport could lead to false positive or negative conclusions regarding kidney function. In particular, a rise in sCr could be a result of inhibition of renal transporters without direct kidney injury (Chu et al., 2016).

Therefore, characterization of the cross-species and sex-dependent differences in protein abundances of kidney transporters is important for scaling and better prediction of renal reabsorption, and toxicity in humans. Accordingly, we hypothesized that mapping interspecies and sex differences in the abundance of kidney cortical transporters would enable development of physiologically based PK (PBPK) models that will improve the prediction of PK, kidney toxicity, and the potential risk of drug-drug interactions.

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ABBREVIATIONS: CL, clearance; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MDR1, multidrug resistance protein 1; MRP, multidrug resistance-associated protein; MS, mass spectrometry; OAT, organic anion transporter; OCT, organic cation transporter; PBPK, physiologically based pharmacokinetics; PK, pharmacokinetics; sCr, serum creatinine.
Scientific (Rockford, IL). Ammonium bicarbonate buffer (98% purity) was purchased from Acros Organics (Geel, Belgium). Human serum albumin and bovine serum albumin were obtained from Calbiochem (Billerica, MA) and Thermo Fisher Scientific, respectively. Surrogate light and heavy peptides were obtained from New England Peptides (Boston, MA) and Thermo Fisher Scientific, respectively. OAT1 antibody was procured from Abcam (Cambridge, MA), anti-mouse IgG horseradish peroxidase–linked secondary antibody was purchased from Cell Signaling Technologies and SDS gel (Mini-PROTEAN TGX) was obtained from Bio-Rad (Hercules, CA).

**Procurement of Normal Kidney Cortices from Experimental Animals.**

Normal kidney cortical tissue (approximately 100–150 mg) was collected at autopsy from the same kidney region from the preclinical species, i.e., cynomolgus monkey (n = 11; five males and six females), beagle dog (n = 12; six males and six females), Wistar Han rat (n = 20; 10 males and 10 females), and CD-1 mouse (n = 18; eight males and 10 females). The autopsy was carried out at the Pfizer Worldwide R&D facility (Cambridge, MA), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. After collection, kidney tissue was flash frozen and stored at −80°C before shipping to the University of Washington. The study was conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf) and in compliance with the Animal Welfare Act and its implementing regulations, under Institutional Animal Care and Use Committee approved protocols.

**Membrane Protein Isolation from Kidney Tissue.**

Total membrane protein was extracted from the kidney cortex (~30–60 mg) of preclinical species using a previously described protocol (Xu et al., 2018). In brief, 30–60 mg of kidney cortical tissue was homogenized using a handheld rotary homogenizer with plastic probes. The homogenate was centrifuged at 16,000g for 30 minutes at 4°C. The supernatant (cytosolic fraction) was transferred to a new tube and the remaining pellet (membrane fraction) was resuspended with 600 μl of solubilization buffer (Pierce Biotechnology) and incubated at 4°C for 30 minutes with continuous mixing. The membrane fraction was used for transporter quantification.

**Peptide Selection and Liquid Chromatography–Tandem Mass Spectrometry Protein Quantification of Kidney Drug Transporters.**

We applied an optimized liquid chromatography–tandem mass spectrometry (LC-MS/MS) methodology, which relies on selective quantification of surrogate peptides of drug transporters. Whenever applicable, conserved peptides across species were selected for precise comparison. When conserved peptides were not available, a novel matrix approach (Supplemental Fig. 1) was used.

Peptide selection for individual drug transporters (Supplemental Table 1) in kidney across species was performed using a previously discussed in silico approach (Bhatt and Prasad, 2018). Total protein in kidney samples was quantified using a bicinchoninic acid assay kit (Pierce Biotechnology) and incubated at 4°C for 30 minutes with continuous mixing. The peptide fraction was used for transporter quantification.

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such as OCT2, OCT3, and OAT2 (Zhang et al., 2015) and efflux sCr were attributed to interference with kidney uptake transporters (e.g., Cystatin C) or glomerular filtration rate, but rather the rises in Medicines Agency qualified nonclinical kidney toxicity biomarkers revealed no changes in Food and Drug Administration/European 2015). A follow-up dedicated kidney investigative clinical study resulted in rises in sCr in a healthy volunteer's investigational Janus kinase inhibitor, INCB039110 (itacitinib), injury) or indirect inhibition of kidney transporters. For example, an could be due to direct tubular damage (i.e., drug-induced kidney injury) or indirect inhibition of kidney transporters. For example, an investigational Janus kinase inhibitor, INCB039110 (itacitinib), resulted in rises in sCr in a healthy volunteer's study (Zhang et al., 2015). A follow-up dedicated kidney investigative clinical study revealed no changes in Food and Drug Administration/European Medicines Agency qualified nonclinical kidney toxicity biomarkers (e.g., Cystatin C) or glomerular filtration rate, but rather the rises in sCr were attributed to interference with kidney uptake transporters such as OCT2, OCT3, and OAT2 (Zhang et al., 2015) and efflux transporters such as MATE1 and MATE2K (Lepist et al., 2014; Zhang et al., 2015). Thus, understanding which transporters are potentially involved in uptake or efflux of the therapeutic candidate and knowing the relative abundance of the transporter across nonclinical species and humans will allow better interpretation of clinical data.

This data set for species and sex-specific abundances for kidney transporters is also useful to the biomedical community and drug developers in predicting the drug clearance and safety of new molecule entities. In agreement with our data, OCT1 and breast cancer resistance protein were not detected in human and nonhuman primate but were abundant in rodents (Bleasby et al., 2006; Prasad et al., 2016). The relatively low abundance of MDR1 in mouse kidney compared with human and rat is consistent with the mRNA data (Bleasby et al., 2006). Similarly, both nonhuman primate and rat have been shown to be associated with kidney toxicity with repeated dosing of tenofovir; however, such toxicity was not observed in the mouse at a similar dose (Ng et al., 2015; Ustianowski and Arends, 2015). The high correlation between OCT1 versus MRP2 and MRP4 indicates that the latter are important for the apical efflux of organic anions. Similarly, the high correlation of cation transporters OCT2 and MATE1 in human and nonhuman primate perhaps suggests that these transporters are coregulated. We also observed that OAT2 (a sCr secreting transporter) is a highly abundant anion transporter in mouse, whereas OAT1 is predominantly expressed in other species. Therefore, results of individual OAT knockout mouse versus rat are expected to be significantly different. Differential sex abundance in mouse and rat (Table 1) was supported by negative correlations between transporters (Fig. 3). In particular, in the mouse the basolateral uptake transporters such as OATs and OCT2 were higher in male but the apical transporters (primarily efflux) were higher in females. In the rat, sex-dependent regulation of the basolateral uptake transporters was consistent with mouse; however, OCTN2, breast cancer resistance protein, and P-glycoprotein were also higher in males. Although the mechanisms of sex-dependent expression of these proteins are unknown, these transporters are important in secretion of conjugated sex hormones (Bush et al., 2017), and therefore can be regulated by the latter.

Discussion

Here, we report a comparison of 19 clinically relevant kidney drug transporters between human and four common preclinical animal models used in drug development. The presented data are the total membrane expression because it is technically difficult to reproducibly extract purified plasma membrane from frozen tissues (Kumar et al., 2015). While plasma membrane isolation involves multiple steps, we have previously demonstrated that total membrane extraction from different parts of the kidney is highly reproducible using the total membrane extraction kit (Prasad et al., 2016).

The quantitative information on cross-species transporter abundance is useful in interpreting kidney safety data in humans. In particular, drug-induced modest rises (20%–30%) in sCr in preclinical models could be due to direct tubular damage (i.e., drug-induced kidney injury) or indirect inhibition of kidney transporters. For example, an investigational Janus kinase inhibitor, INCB039110 (itacitinib), resulted in rises in sCr in a healthy volunteer’s study (Zhang et al., 2015). A follow-up dedicated kidney investigative clinical study revealed no changes in Food and Drug Administration/European Medicines Agency qualified nonclinical kidney toxicity biomarkers (e.g., Cystatin C) or glomerular filtration rate, but rather the rises in sCr were attributed to interference with kidney uptake transporters such as OCT2, OCT3, and OAT2 (Zhang et al., 2015) and efflux transporters such as MATE1 and MATE2K (Lepist et al., 2014; Zhang et al., 2015).

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$P < 0.05$) kidney. OCTN2 abundance was 1.3- and 1.4-fold higher in female mouse and male rats, respectively. SGLT2 was only 1.2-fold higher ($P < 0.05$) in female rats.
Consistent with our data, the abundance and activity of some OATs and OCTs have been shown to be sex dependent (Cerrutti et al., 2002; Groves et al., 2006; Breljak et al., 2010; da Silva Faria et al., 2015). Good correlation of OAT1 and OAT3 abundance is supported by the fact that these are transcriptionally coregulated (Prasad et al., 2016). Because most of the coregulated proteins work in tandem...
OCT vs. multidrug and toxin extrusion protein and OAT vs. MRP) in the vectorial transport process, substrate-mediated regulation could be tested as a potential mechanism of correlations.

Regarding the limitations of this study, it does not allow comparison of all transporters because of the lack of MS-quantifiable or conserved peptides across species (e.g., dog). Nevertheless, our novel LC-MS/MS proteomics method allowed quantification of a majority of kidney transporters across species, which is not currently feasible using conventional immunoquantification. We also demonstrated that the quality of the LC-MS/MS proteomics data is superior to western blot data using OAT1 as an example (Supplemental Fig. 4). In addition, the protein abundance data (in the absence of in vitro functional data) are a critical piece of information (i.e., a prerequisite), which constitute key physiologic parameters for PBPK modeling (Harwood et al., 2013). In particular, these data can be integrated with transport kinetics data (e.g., $K_m$ in eq. 1) of a drug or new chemical entity to

**Fig. 3.** (A) Correlation plot for kidney transporter proteins across all five species. Values in the cell represent the coefficient of correlation ($r^2$) between two transporter proteins. (B) Positive correlation between the transporter proteins with $r^2 > 0.70$. The number of samples from individual species used in this study (with few exceptions) was human ($n = 34$), monkey ($n = 11$), dog ($n = 12$), rat ($n = 20$), and mouse ($n = 18$). Not conserved (NC) indicates that a conserved peptide was not found for that particular species. BLQ denotes below limit of quantification but the peptide was conserved. The BLQ values were derived by extrapolation of the signal-to-noise ratio to 3. No significant (NS) correlation ($r^2 < 0.70$) was found between transporter proteins.
predict in vivo transporter activity in animal versus human models (eq. 2). Furthermore, sex-dependent quantitative differences in the transporter abundance can be directly integrated into a PBPK model to extrapolate drug PK or drug toxicity (eqs. 1 and 3); where $K_m$ can be assumed similar between male versus female in a single species:

$$CL = \frac{V_{\text{max}}}{K_m + [S]} = \frac{[\text{Abundance} \times K_{\text{cat}}]}{K_m + [S]}$$

Thus,

$$CL_{\text{human}} = CL_{\text{male}} \times \frac{[\text{Abundance}_{\text{human}}]}{[\text{Abundance}_{\text{animal}}]}$$

$$CL_{\text{female}} = CL_{\text{male}} \times \frac{[\text{Abundance}_{\text{human}}]}{[\text{Abundance}_{\text{animal}}]}$$

where $CL$ is the intrinsic clearance (picomoles per minute per milligram protein or picomoles per minute per gram of kidney); $V_{\text{max}}$ is the maximum transport activity; $K_m$ is substrate affinity to a transporter; $K_{\text{cat}}$ is the turnover number; and $[S]$ is the substrate concentration. Here, $V_{\text{max}}$ depends on the transporter abundance, whereas $K_m$ and $K_{\text{cat}}$ are independent of protein levels (Bhatt and Prasad, 2018). It is also noteworthy that the data obtained from the absolute peptide approach should not be considered as absolute molar protein abundance data because complete trypsin digestion may not be confirmed. However, these data (absolute peptide levels) can be used in allometric scaling of animal-to-human transporter-mediated clearances using eqs. 1–3. Because the scaling factor is derived by dividing the peptide abundance values, it does not matter whether absolute protein or absolute peptide values are used in the scaling as long as the trypsin digestion and the sample extraction are reproducible and consistent across species/genders. We have previously described this scaling approach (Bhatt and Prasad, 2018).

In summary, our data on cross-species kidney transporter abundances, particularly in human, monkey, and rodents, provide useful quantitative information, which can be leveraged by: 1) allowing for preclinical-to-clinical translation of transporter-mediated secretary clearance using PBPK modeling, 2) distinguishing direct drug-induced kidney toxicity from indirect impact on kidney transporters (e.g., transporter-mediated creatinine clearance), and 3) utilizing cross-species transporter levels in conjunction with kidney injury biomarkers to better understand kidney safety signals and human safety risk assessment.

**Acknowledgments**

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**Authorship Contributions**

Participated in research design: Basit, Radi, Vaidya, Prasad. Conducted experiments: Basit, Karasu. Performed data analysis: Basit, Prasad. Wrote or contributed to the writing of the manuscript: Basit, Radi, Vaidya, Prasad.

**References**


**TABLE 1**

Sex differences in protein abundance of transporters across species

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ABC, ATP-binding cassette subfamily A; ABCC, ATP-binding cassette subfamily C; ABCG, ATP-binding cassette superfamily G; BCRP, breast cancer resistance protein; BLQ, below limit of quantification; F, female; M, male; NC, peptide not conserved; Na+/K+-ATPase, sodium/potassium-transporting ATPase; ND, sex difference was not determined, data were obtained with pooled quality control samples; ↔, no significant difference; *, fold difference.


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