OPEN

Evaluation of Renal Anionic Secretion Following Living-donor and Deceased-donor Renal Transplantation: A Clinical Pharmacokinetic Study of Cefoxitin Microdosing

Hari V. Kalluri, PharmD, PhD,[1] Puneet Sood, MD,[2,3] Wenchen Zhao, BS,[1] Parmjeet S. Randhawa, MD,[3,4] Amit D. Tevar, MD,[3,5] Sundaram Hariharan, MD,[2,3] Abhinav Humar, MD,[3,5] and Raman Venkataramanan, PhD[1,4]

Background. Renal transplantation is the treatment of choice for patients with end-stage renal disease. Because kidneys are the primary excretory organs for various drugs/drug metabolites, changes in renal graft function would significantly alter the clearance and exposure of renally secreted drugs. Renal allografts from living and deceased donors normally undergo numerous insults, including injuries associated with prolonged cold ischemic time, reperfusion, and nephrotoxicity due to calcineurin inhibitors. These physiologic and pharmacologic stresses can alter the expression and functional capacity of renal organic anionic transporters (OATs). **Methods.** The objectives of this study were to assess the longitudinal changes in renal anionic secretion in kidney transplant patients, to study the effect of prolonged cold ischemic time on OAT secretion in kidney transplant patients (living- versus deceased-donor recipients), and to compare OAT secretory capacity of renal transplant recipients with healthy volunteers. Cefoxitin was used as a probe drug to assess OAT secretion. Cefoxitin pharmacokinetics was studied in 15 de novo renal transplant recipients following intravenous administration of 200 mg cefoxitin within 14 d and beyond 90 d posttransplantation. **Results.** No longitudinal changes in real OAT secretion in early posttransplant period were observed, and there were no differences in renal OAT secretion between living- and deceased-donor renal transplant recipients. Overall, cefoxitin exposure was 2.6-fold higher and half-life increased by 2.2-fold in renal transplant recipients when compared with historical healthy controls. **Conclusions.** These results suggest that OAT system is functioning well, but renal transplant recipients would need significantly lower dosage of drugs that are primarily secreted via the OAT system compared with normal subjects.

(*Transplantation Direct* 2020;6: e561; doi: 10.1097/TXD.0000000000001001. Published online 28 May, 2020.)

Chronic kidney disease (CKD) is the ninth leading cause of deaths in the United States. An estimated 26 million adults, or 13% of the US population, are expected to have CKD.[1] About 500 000 CKD patients are classified as having end-stage renal disease with an estimated glomerular filtration rate of <15 ml/min/1.73 m^2.[2] Kidney transplantation is the treatment of choice for the patients diagnosed with end-stage renal disease. In the year 2018, 21 167 kidney transplantations were performed in the United States with 14 725 kidneys coming from deceased donors and 6442 kidneys coming from living donors (based on Organ Procurement and Transplantation Network data as of 31 March 2019).[3,4] Renal

Received 11 March 2020.

Accepted 19 March 2020.

[1] Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA.

[2] Renal and Electrolyte Division, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA.

[3] Thomas E Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA.

[4] Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA.

[5] Department of Surgery, University of Pittsburgh, Pittsburgh, PA.

The authors declare no funding or conflicts of interest.

R.V. is the principal investigator of this study. All authors have made substantial contributions to the conception, study design, acquisition of biologic specimen, and interpretation of data, and to summarize the results of the study to draft important intellectual content for the submitted work. H.V.K., W.Z., and R.V. were also involved in the bioanalysis of the blood and urine samples collected in this study. H.V.K. and R.V. were involved in the pharmacokinetic and statistical analysis of the generated data. All authors were also involved in drafting the final work, provided final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Correspondence: Raman Venkataramanan, PhD, Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, 4103 PTC Bldg, 700 Technology Dr, Pittsburgh, PA 15219. (rv@pitt.edu).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001001

allografts are subjected to a unique set of injurious conditions such as injury associated with prolonged cold ischemic time (CIT) before being transplanted into the recipient, warm reperfusion injury immediately after transplantation, exposure to nephrotoxic calcineurin inhibitor (CNI) based immunosuppression therapy, type of induction therapy, varying grades of allograft rejection, and bacterial/fungal/viral infections post-transplantation.[3,5–10] The cold ischemic injury and nephrotoxic CNI therapy that the renal transplant recipients receive have been shown to lead to progressive loss of renal function with a 5-y recipient survival of 82.1% for deceased-donor kidney transplantations when compared with 92.1% for living-donor kidney transplantations (based on 2008–2011 transplants).[11,12] The tubular damage caused by CIT and CNI could lead to alteration in the expression and activity of renal drug transporters, which primarily reside in renal tubular epithelial cells. This damage may eventually affect the clearance of drugs, drug metabolites, and various endogenous compounds that are predominantly cleared by renal secretion.

Drugs that are eliminated by tubular secretion primarily undergo active transport into the lumen of the proximal tubule. Renal organic anionic transporters (OATs) are specifically of interest in the context of renal transplant recipients because they are involved in the clearance of various medications prescribed to renal transplant recipients. Renal OAT1/3 uptake transporters are considered to be the most important renal OATs by the US Food and Drug Administration and European Medicines Agency for their role in drug disposition and drug–drug interactions.[13–16] For the disposition of various anti-infective medications, multidrug resistance-associated protein 2/4 are thought to be the efflux partners for OAT1/3 (Figure 1).[17–21] Drugs that are primarily eliminated by renal OAT1/3 secretory pathway include acyclovir, adefovir, cefaclor, cefoxitin, ceftizoxime, cidofovir, ciprofloxacin, famotidine, furosemide, ganciclovir, methotrexate, oseltamivir carboxylate, and penicillin G.[22,23]

The present study was conducted (1) to assess the longitudinal changes in renal anionic secretory capacity in kidney transplant patients on tacrolimus-based maintenance immunosuppression therapy; (2) to evaluate the effect of prolonged cold ischemia on renal anionic secretory capacity (a comparison of living- versus deceased-donor kidney transplant recipients); and (3) to compare renal anionic secretory capacity of renal transplant recipients with that of healthy volunteers, using cefoxitin as a surrogate marker of transport activity.

MATERIALS AND METHODS

Probe Drug Selection

Probenecid, a nonspecific potent OAT inhibitor, has been clinically used to successfully show the involvement of OATs in the renal secretion of several drugs.[24–33] A systematic literature search was performed to identify renally cleared drugs which have been shown to have altered clinical pharmacokinetics (PK) with the administration of probenecid in healthy volunteers. Table 1 summarizes the observed significant changes in the clinical PK parameters reported in literature.[24–33]

Among the drugs identified in Table 1, nephrotoxic agents and drugs that transplant clinicians were not comfortable administering to their patients for research purposes without a clinical need were excluded. Cefoxitin was selected as a probe drug because of its safety profile when given at low doses as an intravenous push, short half-life ($t_{1/2}$) and highest change in exposure when coadministered with probenecid

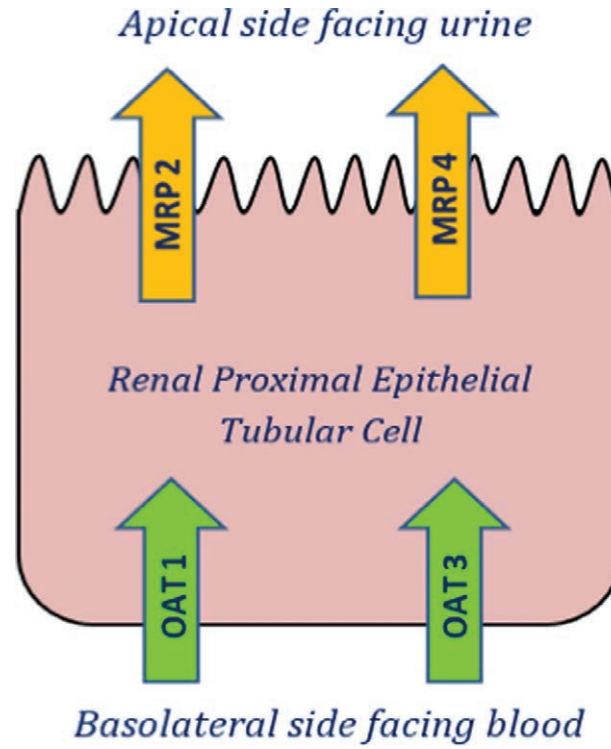

FIGURE 1. Orientation of organic anionic drug transporter 1 (OAT1) and organic anionic drug transporter 3 (OAT3) uptake transporters and multidrug resistance-associated protein 2 (MRP2) and multidrug resistance-associated protein 4 (MRP4) efflux transporters in renal proximal epithelial tubular cells.

when compared with cefoxitin administered alone (area under the curve [$AUC_{0-\infty}$] was 2.4-fold higher).[24,34] The PK properties of cefoxitin are summarized in Table 2.

Renal Transplant Recipients

This study was performed in adult living-donor renal transplant (LDRT) and deceased-donor renal transplant (DDRT) recipients who were transplanted and had their follow-up transplant care at the University of Pittsburgh Medical Center Montefiore hospital. The study protocol was approved by the Institutional Review Board of the University of Pittsburgh (IRB# PRO15010155), and written consent was obtained from all patients before participation in this study.

Key inclusion criteria included the following:

1. men and women between 18 and 65 y of age;
2. subjects who are scheduled to receive de novo kidney transplant; and
3. subjects treated in accordance with the standard care protocols currently in effect for LDRT and DDRT patients.

Key exclusion criteria included the following:

1. subjects receiving United Network for Organ Sharing extended criteria donor organs;
2. subjects who cannot undergo antithymocyte globulin-based induction therapy;
3. subjects allergic to tacrolimus or cefoxitin; and
4. subjects with unresolved delayed graft function by 14 d posttransplantation.

Screening procedures included subject's ability to understand the informed consent, provide consent to participate willingly in the study, medical history, medication allergy and dietary

TABLE 1.

Clinical drug–drug interaction with probenecid and anionic drugs

	Fold change in clinical PK parameters					
Affected drug	**$AUC_{0-\infty}$**	**C_{max}**	**CL_R**	**CL/F**	**$t_{1/2}$**	**References**
Acyclovir	1.4	—	0.7	—	—	Laskin et al[25]
Cefaclor	2.1	1.5	—	—	1.6	Welling et al[26]
Cefonicid	2.1	1.2	0.3	—	1.5	Pitkin et al[27]
Cefoxitin	2.4	—	0.4	—	2	Vlasses et al[24]
Cidofovir	—	—	0.5	0.6	—	Cundy et al[28] and Momper et al[29]
Ciprofloxacin	1.7	—	0.4	0.6	1.5	Jaehde et al[30]
Dicloxacillin	1.9	1.8	0.3	0.5	—	Beringer et al[31]
Famotidine	1.8	1.5	0.4	0.1	—	Inotsume et al[32]
Furosemide	2.7	1.5	0.3	0.4	1.7	Vree et al[33]

Summary of significant changes in clinical PK parameters ($P < 0.05$) of anionic drug substrates when probenecid is used to inhibit OAT-mediated secretory transport. Dashes indicate not significant or not reported.

AUC, area under the curve; C_{max}, maximum concentration; CL_R, renal clearance; CL/F, apparent clearance; OAT, organic anionic drug transporter; PK, pharmacokinetics; $t_{1/2}$, half-life.

TABLE 2.

Pharmacokinetic properties of cefoxitin in healthy volunteers[24,34]

Drug	Cefoxitin
Dosage form	Intravenous
Half-life (h)	0.8
Clearance (ml/min/1.73 m^2)	329
Renal clearance (ml/min/1.73 m^2)	280
Percentage unchanged in urine	85%
Protein binding	74%
AUC fold change with probenecid	2.4

AUC, area under the curve.

history, and baseline clinical laboratory measurements. These key eligibility criteria were selected to eliminate the effect of different induction therapies, maintenance immunosuppression therapies, multiple transplantations, or different posttransplant care on the expression or activity of renal secretion.

Historical Controls/Healthy Volunteers

Data from 6 healthy volunteers who participated in a cefoxitin PK study (2 g intravenous [IV] cefoxitin) conducted in the presence and the absence of orally administered probenecid (1 g) by Vlasses et al[24] were used as historic controls. Historic controls were used instead of prospective controls to minimize resource utilization and minimize unnecessary drug exposure in healthy volunteers.

Study Design

This was a prospective, longitudinal, single-center study performed in 2 phases in the LDRT as well as DDRT recipients who met the study criteria. Phase 1 was conducted approximately 1–2 wk posttransplantation, once the serum creatinine level stabilized, as determined by the transplant clinicians. Phase 2 was conducted approximately 3 mo following transplantation. In both phases, the PK parameters of cefoxitin were evaluated following administration of a single dose of 200 mg IV cefoxitin administered over 1–2 min (intravenous push). The intravenous line was flushed with 0.9% sodium chloride solution before and after cefoxitin administration and before each blood draw. The study design is outlined in Figure 2.

Blood and Urine Sampling

Whole blood was collected at approximately time 0, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, and 4 h postadministration of cefoxitin, and plasma was separated within 30 min of blood collection and frozen at −80°C until analysis. The total volume of urine voided by each subject in 0–1, 1–2, 2–4, and 4–8 h intervals was collected, measured, aliquoted, and stored at −80°C until analysis.

Analytic Methodology

Cefoxitin concentrations in plasma and urine were determined by the liquid chromatographic-mass spectrometric method with detection by a triple quadrupole mass spectrometer in negative electron spray ionization mode using multiple reaction monitoring with cefuroxime as the internal standard. The lower limit of quantification for the cefoxitin assays in plasma and urine was 50 ng/ml and 10 μg/ml, respectively.

Noncompartmental PK Analysis

Descriptive PK parameters for cefoxitin were estimated by noncompartmental analysis using Phoenix WinNonlin (Certara, St. Louis, MO). The terminal disposition rate constant (k) was obtained by linear regression of at least the last 3 data points, and half-life ($t_{1/2}$) was calculated by dividing 0.693 by k. The area under the plasma concentration–time profile from the time of dosing until infinity ($AUC_{0-\infty}$) was calculated by the log-linear trapezoidal method with extrapolation beyond the last measured concentration, according to the following:

$$AUC_{0-\infty} = AUC_{0-4} + C_4 / k$$

where C_4 is the concentration at 4 h.

Total body clearance (CL_{Total}) and the volume of distribution during terminal phase (V_z) were determined using the following equations:

$$CL_{Total} = Dose / AUC_{0-\infty}$$

$$V_z = Dose / ([AUC_{0-\infty}] \times k)$$

Urine cefoxitin concentration in samples collected following intravenous dose was multiplied with the volume of urine collected for that particular time interval to estimate the amount of cefoxitin renally eliminated in a given time depending on last urine collection time. Sum of the amount of cefoxitin eliminated during all urine collection periods was used to estimate the total amount of cefoxitin renally eliminated in 4

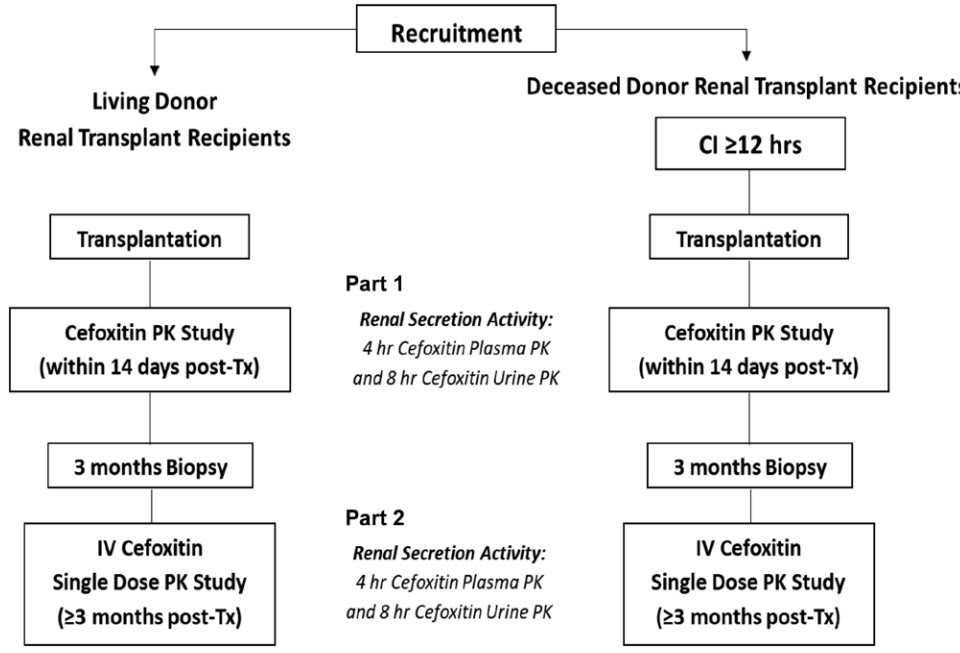

FIGURE 2. Schematic of cefoxitin pharmacokinetic study design. CI, cold ischemia; IV, intravenous; PK, pharmacokinetics; Tx, transplantation.

h ($A_{e[0–4]}$). Renal clearance (CL_{Renal}) was estimated using the following equation:

$$CL_{Renal} = \left(A_{e[0–4]}\right) / AUC_{0–4}$$

Cefoxitin tubular reabsorption was assumed to be negligible (0 ml/min), and cefoxitin filtration clearance ($CL_{Filtration}$) and tubular secretion clearance ($CL_{Secretion}$) were estimated using the following equations:

$$CL_{Filtration} = fu \times CL_{Cr}$$

$$CL_{Secretion} = CL_{Renal} - CL_{Filtration}$$

where fu is the fraction of cefoxitin unbound (0.26)[34] and CL_{Cr} is the creatinine clearance–based estimate of the glomerular filtration rate that is calculated using the Cockcroft–Gault equation:

$$CL_{Cr} = (140 - age)\,(weight\,kg)/(72 \times SrCr)$$

where SrCr is the serum creatinine.

Statistical Analysis

All data were expressed as mean ± SD. Student *t* tests were used to statistically compare patient demographic parameters and PK parameters between LDRT recipients and DDRT recipients at both time points. PK parameters within LDRT and DDRT recipients for both time points were compared using paired *t* test. Dose-normalized PK parameters were used when comparing PK results from renal transplant recipients and PK results from historical healthy controls. Data were analyzed using GraphPad Prism 7 statistical software for windows (GraphPad Software, La Jolla, CA). A *P* value of <0.05 was considered as statistically significant difference.

RESULTS

Patient Demographics

Patient characteristics for subjects who completed ≥1 PK study are provided in Table 3. Forty-seven renal transplant recipients who met the inclusion/exclusion criteria for the study were approached and 15 of them consented to participate in the study and underwent part 1 (PK study ≤14 d posttransplantation) and 9 of the 15 subjects who underwent part 1 also completed part 2 of the study (PK study ≥90 d posttransplantation).

Difficulty in obtaining intravenous access and scheduling conflict were the reasons for the 6 subjects to not complete part 2 of the study. On average, the study participants were 47.5 ± 12.7 y of age and weighed 86.6 ± 27.2 kg. Of the 15 study participants, 8 underwent LDRT and 7 underwent DDRT. Majority of LDRT recipients were Caucasian (7 of 8, 87.5%) and majority of DDRT recipients were African American (5 of 7, 71.4%). The average CIT experienced by allografts transplanted to LDRT recipients (1.3 ± 0.4 h) was significantly shorter compared with that of DDRT recipients (15.8 ± 4.8 h). Majority of the living donors were related to recipients (7 of 8, 87.5%), and the age was not significantly different between living donors (47.0 ± 17.5 y) and deceased donors (38.5 ± 12.9) ($P > 0.05$).

All subjects underwent rabbit antithymocyte globulin-based induction therapy and received tacrolimus and mycophenolic acid-based maintenance immunosuppression. Prophylactic anti-infective regimens taken by all patients included valganciclovir and sulfamethoxazole–trimethoprim. None of the patients were taking any other medications that were known to be renally eliminated by or known to modulate the expression of OAT/multidrug resistance-associated protein transporters.

Additional details on patient characteristics before starting phase 1 and phase 2 of the study are provided in Table 4. On average, all study subjects were 7.1 ± 2.3 d posttransplantation before starting phase 1 of the study and 115.6 ± 20.0 d before starting phase 2 of the study with 114.3 ± 21.0 d between both the PK studies. All patients had stable renal function during both the PK studies, and the tacrolimus trough levels were within the target therapeutic ranges of 8.0–10.0 ng/ml. Serum creatinine during phase 2 of the study for DDRT recipients (1.2 ± 0.1 mg/dl) was lower than that of LDRT recipients (1.4 ± 0.1 mg/dl); this was not clinically significant.

TABLE 3.

Patient characteristics

Patient characteristics	All subjects (n = 15)	LDRT recipient (n = 8)	DDRT recipient (n = 7)	P[a]
Age (y), mean ± SD	47.5 ± 12.7	50.3 ± 15.6	44.3 ± 8.6	0.39
Weight (kg), mean ± SD	86.6 ± 27.2	92.1 ± 25.9	80.3 ± 29.2	0.42
BSA (m^2), mean ± SD	1.9 ± 0.3	2.1 ± 0.3	1.8 ± 0.2	0.05
Sex	M = 6; F = 9	M = 4; F = 4	M = 2; F = 5	—
African American	6	1	5	—
Caucasian	9	7	2	—
CIT (h), mean ± SD	8.1 ± 8.1	1.3 ± 0.4	15.8 ± 4.8	<0.05
WIT (h), mean ± SD	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.4	0.95
Transplant reason				
IgA Nephropathy	5	2	3	—
Hypertension	5	3	2	—
DM-II/HTN	2	2	0	—
Other	3	1	2	—
Donor information				
Age (y), mean ± SD	43.4 ± 15.8	47.0 ± 17.5	38.5 ± 12.9	0.31
Deceased = 7; living related = 7; living unrelated = 1				

[a]Comparing LDRT vs DDRT.
BSA, body surface area; CIT, cold ischemic time; DDRT, deceased-donor renal transplant; DM-II, type-2 diabetes mellitus; HTN, hypertension; LDRT, living-donor renal transplant; WIT, warm ischemic time.

TABLE 4.

Patient characteristics comparing LDRT vs DDRT recipients and Part 1 vs Part 2

Patient characteristics comparing LDRT vs DDRT recipients			
	LDRT recipient (mean ± SD), N = 8	DDRT recipient (mean ± SD), N = 7	P[a]
Part 1: d since Tx	6.9 ± 1.8	7.3 ± 3.0	0.75
Part 2: d since Tx	112.8 ± 10.6	121.50 ± 27.1	0.41
Days between parts 1 and 2	110.8 ± 13.7	118.8 ± 29.7	0.51
SrCr (mg/dl)	1.4 ± 0.1	1.2 ± 0.08	<0.05
CrCL (ml/min)	55.9 ± 19.5	52.5 ± 8.4	0.68
Blood concentrations of FK (ng/ml)	8.2 ± 1.9	9.9 ± 3.2	0.21
Patient characteristics comparing all subjects at part 1 vs part 2			
	Part 1: ≤14 days post-Tx (mean ± SD), N = 15	Part 2: ≥90 days post-Tx (mean ± SD), N = 9	P[a]
Days since Tx	7.1 ± 2.3	115.6 ± 20.0	—
Days between parts 1 and 2	114.3 ± 21.0		—
SrCr (mg/dl)	1.5 ± 0.7	1.3 ± 0.1	0.30
CrCL (ml/min)	49.8 ±17.4	54.4 ± 14.8	0.52
Blood concentrations of FK (ng/ml)	8.8 ± 2.9	9.1 ± 2.1	0.78

[a]Comparing LDRT vs DDRT and part 1 vs part 2.
— indicates that this comparison was not done as this criteria defines the cohorts.
CrCL, creatinine clearance calculated by Cockcroft–Gault equation; DDRT, deceased-donor renal transplant; FK, tacrolimus trough level; LDRT, living-donor renal transplant; SrCr, serum creatinine; Tx, transplantation.

Cefoxitin CL_{Renal} was estimated in 21 of the 24 PK studies as 3 patients accidentally flushed down the urine samples. These 3 subjects were excluded from CL_{Renal} estimation as partial urine data were not sufficient. The amount of cefoxitin excreted unchanged into the urine for both study periods is presented in Table 5.

TABLE 5.

Cefoxitin urine data

Study grouping	Duration of urine collection (h)	Amount of drug excreted into urine, Ae (mg)	Percent of drug excreted into urine (%)
LDRT recipients	4.0 ± 0.3	164.5 ± 25.6	82.1 ± 12.6
DDRT recipients	5.0 ± 1.8	189.1 ± 13.6	94.1 ± 7.70
≤14 d post-Tx	4.5 ± 1.2	166.31 ± 24.3	83.2 ± 12.2
≥90 d post-Tx	4.5 ± 1.6	192.4 ± 11.5	95.4 ± 7.11

Ae, amount excreted; DDRT, deceased-donor renal transplant; LDRT, living-donor renal transplant; Tx, transplantation.

Safety and Tolerability

Cefoxitin given at a low dose of 200 mg as an intravenous push over 1–2 min was well tolerated. There were no injection site reactions in any of the patients, and none of the patients were allergic to cefoxitin. No changes were observed in biochemical indices of kidney or liver function after administration. Two patients experienced metallic taste following cefoxitin administration, and this was resolved within 5 min. The resolution of this effect is consistent with the observed rapid disposition of cefoxitin.

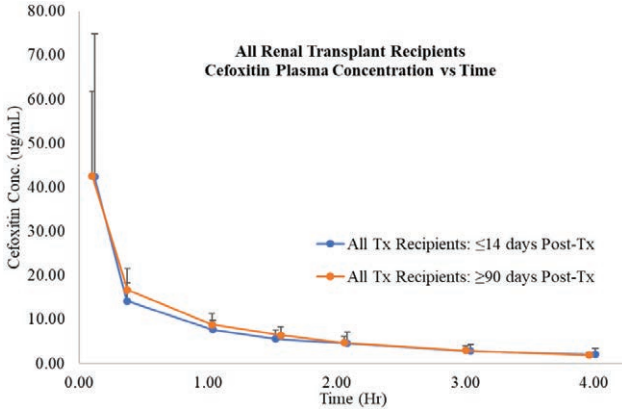

FIGURE 3. Concentration vs time plot of 200 mg cefoxitin given as intravenous push in renal transplant recipients at ≤14 d (blue) and ≥90 d (orange) posttransplantation. Tx, transplantation.

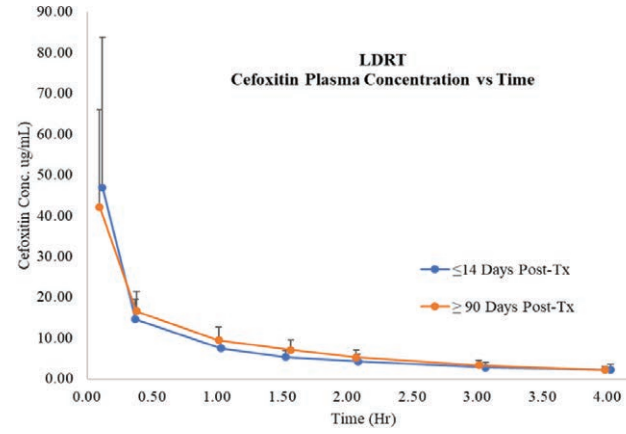

FIGURE 4. Concentration vs time plot of 200 mg cefoxitin given as intravenous push in living-donor renal transplant (LDRT) recipients at ≤14 d (blue) and ≥90 d (orange) posttransplantation. Tx, transplantation.

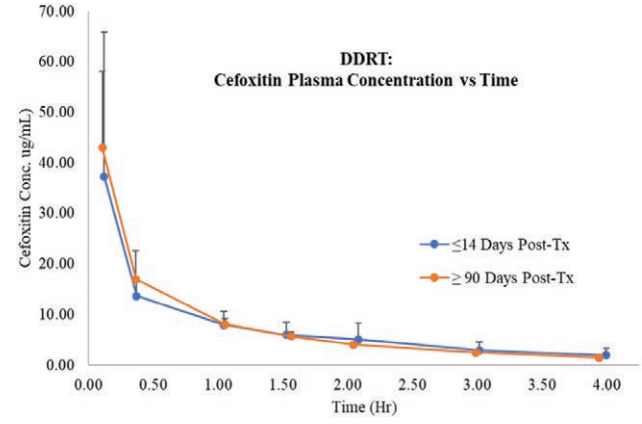

FIGURE 5. Concentration vs time plot of 200 mg cefoxitin given as intravenous push in deceased-donor renal transplant (DDRT) recipients at ≤14 d (blue) and ≥90 d (orange) posttransplantation. Tx, transplantation.

Assessment of Longitudinal Changes in Renal Anionic Secretory Capacity in Renal Transplant Recipients

Posttransplant changes in renal anionic secretory capacity among LDRT and DDRT recipients were evaluated by assessing cefoxitin PK at 2 early posttransplant time points (≤14 and ≥90 d posttransplantation). Linear plots of cefoxitin plasma concentration versus time at both time points are shown in Figure 3. The concentration–time curves were virtually superimposable, suggesting no difference in cefoxitin clearance in renal transplant recipients by ≥90 d posttransplantation when compared with early after transplantation. A summary of PK parameters for intravenous cefoxitin at these 2 time points is presented in Table 6.

Cefoxitin exposure ($AUC_{0-\infty}$), CL_{Total}, CL_{Renal}, $CL_{Filtration}$, and $CL_{Secretion}$ were statistically similar during phase 1 and phase 2 of the study. The majority of CL_{Total} was attributed to $CL_{Secretion}$ (≈71%). $t_{1/2}$ of cefoxitin in renal transplant patients is about 1.3 ± 0.6 h in both phases (Table 6).

Linear plots of cefoxitin plasma concentration versus time at both time points among LDRT and DDRT are shown in Figures 4 and 5, respectively. The concentration–time curves were virtually superimposable in LDRT and DDRT recipients, suggesting no difference in cefoxitin clearance in renal transplant recipients by ≥90 days posttransplantation when compared with immediately after transplantation in LDRT or DDRT recipients. Summaries of PK parameters for intravenous cefoxitin at these 2 time points in LDRT and DDRT recipients are presented in Tables 7 and 8, respectively.

Cefoxitin exposure ($AUC_{0-\infty}$), CL_{Total}, CL_{Renal}, $CL_{Filtration}$, and $CL_{Secretion}$ were statistically similar during phase 1 and phase 2 of the study for LDRT and DDRT recipients when compared

TABLE 6.

Summary of pharmacokinetic parameters of 200 mg cefoxitin in renal transplant recipients at ≤14 and ≥90 d posttransplantation

PK parameters	Part 1 ≤14 days post-Tx (N = 15), mean ± SD	Part 2 ≥90 days post-Tx (N = 9), mean ± SD	Combined (N = 15), mean ± SD	P[a]
$AUC_{0-\infty}$ (mg × h/L)	35.0 ± 13.1	35.6 ± 9.3	35.2 ± 11.6	0.91
$t_{1/2}$ (h)	1.4 ± 0.7	1.1 ± 0.2	1.3 ±0.6	0.25
V_z (L)	15.0 ± 4.6	12.1 ± 3.4	13.9 ± 4.4	0.10
CL_{Total} (ml/min)	108.1 ± 40.0	99.3 ± 24.7	104.8 ± 34.7	0.56
CL_{Renal} (ml/min)	90.2 ± 33.4	82.9 ± 20.6	87.5 ± 29.0	0.56
$CL_{Filtration}$ (ml/min)	13.0 ± 4.5	14.1 ± 3.4	13.4 ± 4.2	0.52
$CL_{Secretion}$ (ml/min)	77.3 ± 28.9	73.3 ± 25.1	74.1 ± 24.8	0.74

[a]Comparing part 1 and part 2.

$AUC_{0-\infty}$, area under the concentration–time curve from time dose administration to infinite time; $t_{1/2}$, half-life; V_z, terminal volume of distribution; CL_{Total}, cefoxitin total clearance; CL_{Renal}, cefoxitin renal clearance; $CL_{Filtration}$, cefoxitin filtration clearance; $CL_{Secretion}$, cefoxitin secretion clearance.

TABLE 7.

Summary of pharmacokinetic parameters of 200 mg cefoxitin in LDRT recipients at ≤14 and ≥90 d posttransplantation

PK parameters	Part 1 ≤14 d post-Tx (N = 8), mean ± SD	Part 2 ≥90 d post-Tx (N = 5), mean ± SD	Combined LDRT (N = 8), mean ± SD	P[a]
$AUC_{0-\infty}$ (mg × h/L)	36.3 ± 9.7	38.0 ± 11.3	37.0 ± 9.9	0.77
$t_{1/2}$ (h)	1.5 ± 0.8	1.2 ± 0.2	1.4 ± 0.7	0.47
V_z (L)	15.2 ± 4.70	12.3 ± 4.3	14.1 ± 4.60	0.28
CL_{Total} (ml/min)	97.4 ± 24.3	94.9 ± 30.5	96.5 ± 25.6	0.87
CL_{Renal} (ml/min)	81.4 ± 20.3	79.2 ± 25.5	80.6 ± 21.4	0.88
$CL_{Filtration}$ (ml/min)	13.2 ± 5.3	14.5 ± 5.1	13.7 ± 5.03	0.66
$CL_{Secretion}$ (ml/min)	68.2 ± 15.0	64.7 ± 20.4	66.8 ± 16.4	0.73

[a]Comparing part 1 and part 2.

$AUC_{0-\infty}$, area under the concentration–time curve from time dose administration to infinite time; $CL_{Filtration}$, cefoxitin filtration clearance; CL_{Renal}, cefoxitin renal clearance; $CL_{Secretion}$, cefoxitin secretion clearance; CL_{Total}, cefoxitin total clearance; LDRT, living-donor renal transplant; PK, pharmokinetics; $t_{1/2}$, half-life; Tx, transplantation; V_z, terminal volume of distribution.

TABLE 8.

Summary of pharmacokinetic parameters of 200 mg cefoxitin in DDRT recipients at ≤14 and ≥90 d posttransplantation

PK parameters	Part 1 ≤14 d post-Tx (N = 7), mean ± SD	Part 2 ≥90 d post-Tx (N = 4), mean ± SD	Combined DDRT (N = 7), mean ± SD	P[a]
$AUC_{0-\infty}$ (mg × h/L)	33.6 ± 16.9	32.6 ± 6.1	33.2 ± 13.6	0.92
$t_{1/2}$ (h)	1.3 ± 0.5	1.0 ± 0.2	1.2 ± 0.4	0.30
V_z (L)	14.8 ± 4.8	11.8 ± 2.6	13.7 ± 4.3	0.28
CL_{Total} (ml/min)	120.2 ± 52.2	104.7 ± 17.7	114.6 ± 42.3	0.60
CL_{Renal} (ml/min)	100.3 ± 43.6	87.5 ± 14.8	95.7 ± 35.3	0.58
$CL_{Filtration}$ (ml/min)	12.7 ± 3.8	13.6 ± 2.18	13.0 ± 3.2	0.66
$CL_{Secretion}$ (ml/min)	87.7 ± 39.7	73.8 ± 12.6	82.6 ± 32.1	0.52

[a]Comparing part 1 and part 2.

$AUC_{0-\infty}$, area under the concentration–time curve from time dose administration to infinite time; $CL_{Filtration}$, cefoxitin filtration clearance; CL_{Renal}, cefoxitin renal clearance; $CL_{Secretion}$, cefoxitin secretion clearance; CL_{Total}, cefoxitin total clearance; DDRT, deceased-donor renal transplant; PK, pharmokinetics; $t_{1/2}$, half-life; Tx, transplantation; V_z, terminal volume of distribution.

separately. Majority of CL_{Total} was attributed to its $CL_{Secretion}$ (≈72%). The average $t_{1/2}$ of cefoxitin in LDRT and DDRT was similar (1.4 ± 0.67 and 1.2 ± 0.41 h, respectively).

Effect of Prolonged CIT on Renal OAT Secretory Capacity in Kidney Transplant Recipients on Tacrolimus-based Maintenance Immunosuppression Therapy

In all recipients, comparisons of cefoxitin PK parameters were made between those with CIT ≥10 h and those with CIT <10 h at 2 time points posttransplantation (≤14 and ≥90 d posttransplantation). Those with CIT <10 h were predominantly LDRT recipients. The linear plots of cefoxitin plasma concentration versus time during part 1 (≤14 d posttransplantation) and part 2 (≥90 d posttransplantation) are shown in Figures 6 and 7, respectively.

Cefoxitin exposure ($AUC_{0-\infty}$), CL_{Total}, CL_{Renal}, $CL_{Filtration}$, and $CL_{Secretion}$ were statistically similar between renal transplant recipients with CIT ≥10 h and those with CIT <10 h during both parts of the study. There was no significant impact of prolonged cold ischemia (15.8 ± 4.8 versus 1.3 ± 0.4 h) on renal anionic secretion of cefoxitin immediately after transplantation and beyond 90 d posttransplantation (Tables 7 and 8).

Comparing Renal OAT Secretory Capacity of Renal Transplant Recipients With That of Healthy Volunteers

Summarized linear plots of dose-normalized cefoxitin concentration versus time in renal transplant patients (15 patients; 24 PK studies) and in historical healthy controls (6 patients) with and without probenecid treatment are shown in Figure 8. Cefoxitin exposure in renal transplant recipients was higher when compared with healthy volunteers (with 2 native kidneys) not treated with probenecid. However, when the healthy control group was given probenecid to block anionic secretion, no significant differences remained in the exposure of kidney transplant recipients and healthy volunteers (Table 9).

Renal transplant recipients had significantly higher dose-normalized exposures of cefoxitin when compared with healthy volunteers who were not administered probenecid (176.2 ± 58.0 versus 68.5 ± 8.10 mg × h/L/g). CL_{Total} and CL_{Renal} were significantly lower in renal transplant recipients when compared with healthy volunteers who were not administered probenecid. The CL_{Total} per functioning kidney was also numerically lower for renal transplant recipients compared with healthy volunteers (104.8 versus 123.1 ml/min). $CL_{secretion}$ in healthy controls was estimated to be about 117 ml/min by subtracting CL_{Renal} in probenecid-treated arm from CL_{Renal} in the control arm. For this estimate, probenecid was assumed to have blocked all the anionic secretion in healthy volunteers. Cefoxitin exposure, CL_{Total}, CL_{Renal}, and $t_{1/2}$ were statistically similar between renal transplant recipients and healthy volunteers who were administered 1 g of probenecid 1 h before cefoxitin administration.

DISCUSSION

Glomerular filtration, transporter-mediated active tubular secretion, and reabsorption are the main mechanisms involved in CL_{Renal} of many drugs. Following transplantation, renal transplant patients have only 1 functioning kidney that

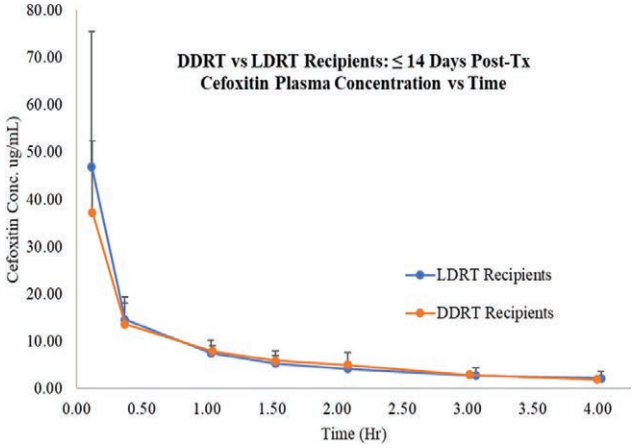

FIGURE 6. Concentration vs time plot of 200 mg cefoxitin given as intravenous push in living-donor renal transplant (LDRT; blue) and deceased-donor renal transplant (DDRT; orange) recipients at ≤14 d posttransplantation. Tx, transplantation.

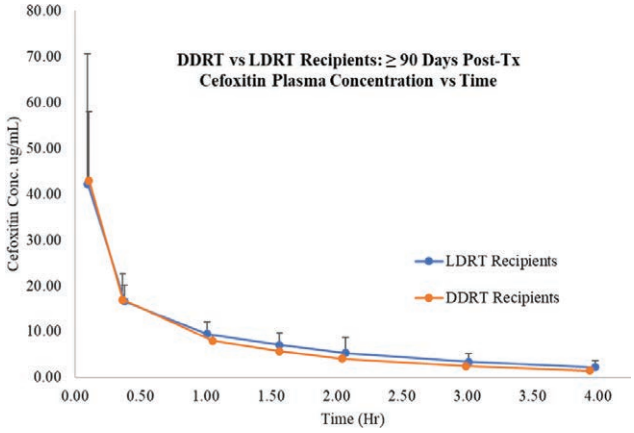

FIGURE 7. Concentration vs time plot of 200 mg cefoxitin given as intravenous push in living-donor renal transplant (LDRT; blue) and deceased-donor renal transplant (DDRT; orange) recipients at ≥90 d posttransplantation. Tx, transplantation.

is subjected to various insults such as prolonged CIT, CNI exposure, opportunistic infections, BK virus nephropathy (BKVN), and acute T-cell–mediated rejection. Clinicians routinely monitor changes in filtration capacity alone, to evaluate allograft function and adjust dose/frequency of renally cleared drugs, including those that are primarily secreted. A better understanding of changes in secretory capacity following renal transplantation is needed to optimize pharmacotherapy of renally secreted drugs.

This study is one of the first attempts to systematically assess renal anionic secretory capacity in kidney transplant recipients. For this, we studied longitudinal changes in cefoxitin exposure and renal secretory clearance in early posttransplant period in both living- and deceased-donor kidney transplant recipients. We also compared differences in cefoxitin exposure and renal secretory clearance between DDRT and LDRT recipients to assess the effect of prolonged CIT on renal anionic secretory capacity. Furthermore, a dose-normalized cefoxitin exposure and renal secretory clearance in renal transplant recipients were compared with that of historical healthy controls. Cefoxitin was chosen as a suitable probe drug to assess the renal anionic secretion in this study

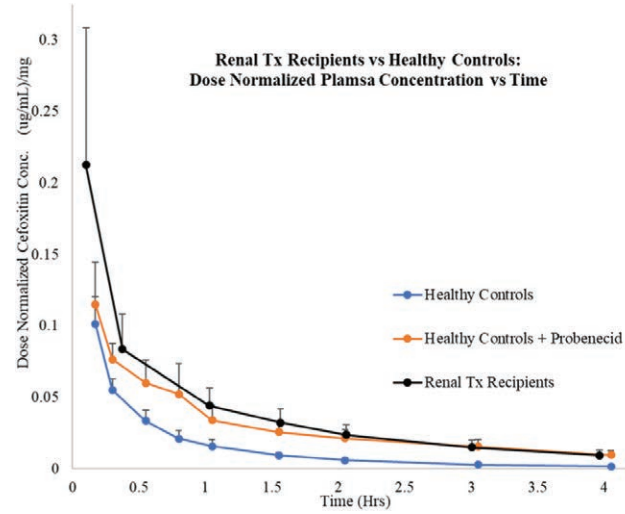

FIGURE 8. Dose-normalized concentration vs time plot following administration of intravenous cefoxitin in renal transplant recipients in early posttransplant period (black), historical healthy controls without probenecid treatment (blue), and historical healthy controls. Cefoxitin concentration–time data in healthy volunteers reported by Vlasses et al[24] were used as historical healthy controls. Tx, transplantation.

as majority of the drug is cleared by renal secretion as evidenced by a 2.4-fold increase in cefoxitin exposure in healthy volunteers after a pretreatment with probenecid (a potent OAT1/3 inhibitor).[24]

Overall, low-dose cefoxitin was well tolerated by study subjects with no adverse events. Four-hour PK study was sufficient to characterize cefoxitin secretion in this patient population. The PK results of this longitudinal study show that cefoxitin exposure and renal secretory clearance in renal transplant patients were similar at ≤14 and ≥90 d posttransplantation (Table 6). No significant difference in cefoxitin PK was observed when comparing DDRT recipients (CIT, ≥10 h; mean CIT, 15.8 h) and LDRT recipients (CIT, <10 h; mean CIT, 1.3 h) at ≤14 and ≥90 d posttransplantation (Tables 7 and 8). Although LDRT recipients had higher body surface area, their body weight was not significantly different from DDRT recipients at baseline and this did not result in different volume of distribution estimates.

Cefoxitin PK in renal transplant recipients in early posttransplant period was compared with that in historical healthy volunteers (with 2 native kidneys) with and without probenecid treatment to understand differences in renal anionic secretory capacity between these 2 populations. The results show that dose-normalized cefoxitin exposure in renal transplant recipients was significantly higher when compared with healthy controls (mean $AUC_{0-\infty}$/dose, 176.2 versus 68.5 mg × h/L/g). Based on this, we conclude that cefoxitin exposure was 2.6-fold higher and $t_{1/2}$ was 2.2-fold higher in renal transplant recipients when compared with healthy volunteers (2 kidneys and no probenecid treatment). When the healthy volunteers were pretreated with 1 g oral probenecid, which blocked OAT1/3 responsible for cefoxitin secretion, the difference in PK parameters between the transplant recipients and healthy volunteers became nonsignificant (Table 9).

Although the total cefoxitin clearance was lower in renal transplant recipients (104.8 ml/min) when compared with healthy volunteers (246.2 ml/min), on a per kidney basis, there was no significant difference in the ability to clear cefoxitin.

TABLE 9.

Comparison of dose-normalized cefoxitin PK parameters between healthy controls ± 1 g probenecid administered orally 1 h before cefoxitin administration and in renal transplant recipients

PK parameters	Historical healthy controls (mean ± SD)	Historical healthy controls + 1 g probenecid (mean ± SD)	Renal Tx recipients (mean ± SD)	*P*[a]
$AUC_{0-\infty}$/dose (mg × h/L)/g	68.5 ± 8.1	170.1 ± 43.9[b]	176.2 ± 58.0	0.0001
$t_{1/2}$ (h)	0.6 ± 0.1	1.5 ± 0.2[b]	1.3 ±0.6	0.0070
V_z (L)	17.5 ± 5.1	16.1 ± 5.2	13.9 ± 4.4	0.090
CL_{Total} (ml/min)	246.2 ± 29.8	105.8 ± 37.5[b]	104.8 ± 34.7	0.0001
CL_{Renal} (ml/min)	205.6 ± 24.9	88.3 ± 31.3[b]	87.5 ± 30.0	0.0001
$CL_{Secretion}$ (ml/min)	≈117	—	74.1 ± 24.8	—

[a]Comparing healthy controls and renal Tx recipients.
[b]Comparing healthy controls and healthy controls + probenecid.
— indicates that this comparison was not done as this criteria defines the cohorts.
Cefoxitin concentration–time data in healthy volunteers reported by Vlasses et al[24] were used as historical healthy controls.
$AUC_{0-\infty}$, area under the concentration–time curve from time dose administration to infinite time; CL_{Renal}, cefoxitin renal clearance; $CL_{Secretion}$, cefoxitin secretion clearance; CL_{Total}, cefoxitin total clearance; PK, pharmacokinetics; $t_{1/2}$, half-life; Tx, transplantation; V_z, terminal volume of distribution.

Furthermore, the contribution of secretory clearance when compared with CL_{Total} was considerably higher in renal transplant recipients (71% versus 48%) suggesting that renal secretion is an important clearance mechanism when filtration capacity is compromised posttransplantation with a reduced renal mass of a solitary kidney allograft.

Results of this study also suggest that overall cefoxitin clearance including cefoxitin secretion is lower in renal transplant recipients when compared with healthy subjects and renal transplant recipients would need considerably lower doses (43% of normal dose) for the same exposure as nontransplant subjects.

There are a few shortcomings of this study. Although 47 de novo renal transplant recipients were approached, only 15 consented to participate in the study. Of the 15 patients who participated in phase 1 of the study, only 9 (5 LDRT recipients and 4 DDRT recipients) completed part 2 of the study. Lower number of subjects could be one of the reasons for not having enough power to detect a potential difference between LDRT and DDRT subjects at a given time point or within these subjects at different time points posttransplantation. Difficulty in obtaining intravenous access and scheduling conflict were the reasons for the 6 subjects not to complete phase 2 of the study. Another limitation was that we used data from historic controls for comparison. All healthy volunteers were young male subjects between the ages of 21 and 35 y in contrast to the higher average age and mixed gender of the transplant recipients in our cohort. In this study, the investigators did not measure $CL_{Filtration}$ and so cefoxitin secretory clearance $CL_{secretion}$ in healthy controls was estimated by subtracting CL_{Renal} in probenecid-treated arm from CL_{Renal} in the control arm.

Moving forward, a comparative quantitation of transporter expression in renal allografts and healthy renal tissue, which at the current time is not available, will be useful. Currently published gene expression studies reported only relative expression of renal transporters using semiquantitative approaches (real-time quantitative polymerase chain reaction and Microarray).[23,35–37] Preliminary clinical observations in renal transplant recipients with BKVN involving cidofovir treatment in the presence and the absence of probenecid suggest that renal anionic secretory function is decreased in allografts with BKVN.[29] A larger prospective study evaluating longitudinal changes in renal anionic secretion in renal transplant recipients with common transplantation-associated complications (BKVN and rejection) and in healthy volunteers will help us better understand changes in renal OAT-mediated secretory capacity in this patient population. Additionally, PK studies with microdosing, limited PK sampling, and dried-blood-spot based sample collection methods should be explored to validate a minimally invasive sampling strategy to assess renal secretion in renal transplant recipients. Such studies would give clinicians the opportunity to optimize pharmacotherapy of renally secreted drugs.

Overall, this study shows that organic anionic secretory capacity is well preserved in clinically stable renal transplant recipients in the early posttransplantation period; however, renal transplant patients have significantly lower organic anionic secretory capacity compared with normal healthy adults. Current clinical practices of using doses and frequency of anionic drugs that are primarily renally secreted based only on patients' filtration capacity may result in significant over exposure of these drugs as evidenced by 43% lower need for cefoxitin dose although estimated glomerular filtration rate-based renal dosing schedule suggests no dose adjustment. This overexposure would increase the risk for drug-induced adverse events. The results of this study support development, validation, and the use of clinical monitoring of renal OAT function by transplant clinicians for optimal posttransplantation pharmacotherapy.

REFERENCES

1. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA.* 2007;298:2038–2047. doi:10.1001/jama.298.17.2038
2. Bayliss EA, Bhardwaja B, Ross C, et al. Multidisciplinary team care may slow the rate of decline in renal function. *Clin J Am Soc Nephrol.* 2011;6:704–710. doi:10.2215/CJN.06610810
3. DiPiro JT. *Pharmacotherapy: A Pathophysiologic Approach*. 8th ed. New York, NY: McGraw-Hill Medical; 2011.
4. Organ Procurement and Transplantation Network. Transplants by Donor Type. 2019. Available at https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#. Accessed March 31, 2019.
5. Abedini S, Holme I, März W, et al; ALERT Study Group. Inflammation in renal transplantation. *Clin J Am Soc Nephrol.* 2009;4:1246–1254. doi:10.2215/CJN.00930209
6. Andreoni KA, Brayman KL, Guidinger MK, et al. Kidney and pancreas transplantation in the United States, 1996-2005. *Am J Transplant*. 2007;7(5 Pt 2):1359–1375. doi:10.1111/j.1600-6143.2006.01781.x

7. Chapman JR. Chronic calcineurin inhibitor nephrotoxicity-lest we forget. *Am J Transplant.* 2011;11:693–697. doi:10.1111/j.1600-6143.2011.03504.x
8. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol.* 2009;4:481–508. doi:10.2215/CJN.04800908
9. Salahudeen AK. Cold ischemic injury of transplanted kidneys: new insights from experimental studies. *Am J Physiol Renal Physiol.* 2004;287:F181–F187. doi:10.1152/ajprenal.00098.2004
10. Waiser J, Budde K, Katalinic A, et al. Interleukin-6 expression after renal transplantation. *Nephrol Dial Transplant.* 1997;12:753–759. doi:10.1093/ndt/12.4.753
11. Organ Procurement and Transplantation Network. Kidney Kaplan-Meier Patient Survival Rates for Transplants Performed: 2008 - 2015. 2019. Available at https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#. Accessed March 31, 2019.
12. Lee SY, Chung BH, Piao SG, et al. Clinical significance of slow recovery of graft function in living donor kidney transplantation. *Transplantation.* 2010;90:38–43. doi:10.1097/TP.0b013e3181e065a2
13. Bonate PL, Howard DR, editors. *Pharmacokinetics in Drug Development: Advances and Applications.* Volume 3. Boston, MA: SpringerLink (Online service): Springer US; 2011.
14. Food and Drug Administration. Clinical drug interaction studies – study design, data analysis, and clinical implications guidance for industry. 2017. Available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf. Accessed May 1, 2019.
15. Food and Drug Administration. In vitro drug interaction studies – cytochrome P450 enzyme- and transporter-mediated drug interactions guidance for industry. 2020. Available at https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf. Accessed January 27, 2020.
16. European Medicines Agency. Guideline on the investigation of drug interactions. 2012. Available at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions_en.pdf. Accessed May 1, 2019.
17. Brown CD, Sayer R, Windass AS, et al. Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. *Toxicol Appl Pharmacol.* 2008;233:428–438. doi:10.1016/j.taap.2008.09.018
18. Hagos Y, Schley G, Schödel J, et al. α-Ketoglutarate-related inhibitors of HIF prolyl hydroxylases are substrates of renal organic anion transporters 1 (OAT1) and 4 (OAT4). *Pflugers Arch.* 2012;464:367–374. doi:10.1007/s00424-012-1140-9
19. Madejczyk MS, Aremu DA, Simmons-Willis TA, et al. Accelerated urinary excretion of methylmercury following administration of its antidote N-acetylcysteine requires Mrp2/Abcc2, the apical multidrug resistance-associated protein. *J Pharmacol Exp Ther.* 2007;322:378–384. doi:10.1124/jpet.107.122812
20. Robertson EE, Rankin GO. Human renal organic anion transporters: characteristics and contributions to drug and drug metabolite excretion. *Pharmacol Ther.* 2006;109:399–412. doi:10.1016/j.pharmthera.2005.07.005
21. Wong CC, Barron D, Orfila C, et al. Interaction of hydroxycinnamic acids and their conjugates with organic anion transporters and ATP-binding cassette transporters. *Mol Nutr Food Res.* 2011;55:979–988. doi:10.1002/mnfr.201000652
22. Food and Drug Administration. Drug development and drug interactions: table of substrates, inhibitors and inducers. 2017. Available at https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm. Accessed May 1, 2019.
23. Morrissey KM, Wen CC, Johns SJ, et al. The UCSF-FDA TransPortal: a public drug transporter database. *Clin Pharmacol Ther.* 2012;92:545–546. doi:10.1038/clpt.2012.44
24. Vlasses PH, Holbrook AM, Schrogie JJ, et al. Effect of orally administered probenecid on the pharmacokinetics of cefoxitin. *Antimicrob Agents Chemother.* 1980;17:847–855. doi:10.1128/aac.17.5.847
25. Laskin OL, de Miranda P, King DH, et al. Effects of probenecid on the pharmacokinetics and elimination of acyclovir in humans. *Antimicrob Agents Chemother.* 1982;21:804–807. doi:10.1128/aac.21.5.804
26. Welling PG, Dean S, Selen A, et al. Probenecid: an unexplained effect on cephalosporin pharmacology. *Br J Clin Pharmacol.* 1979;8:491–495. doi:10.1111/j.1365-2125.1979.tb01032.x
27. Pitkin D, Dubb J, Actor P, et al. Kinetics and renal handling of cefonicid. *Clin Pharmacol Ther.* 1981;30:587–593. doi:10.1038/clpt.1981.208
28. Cundy KC, Petty BG, Flaherty J, et al. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother*. 1995;39:1247–1252. doi:10.1128/aac.39.6.1247
29. Momper JD, Zhao Y, Shapiro R, et al. Pharmacokinetics of low-dose cidofovir in kidney transplant recipients with BK virus infection. *Transpl Infect Dis.* 2013;15:34–41. doi:10.1111/tid.12014
30. Jaehde U, Sörgel F, Reiter A, et al. Effect of probenecid on the distribution and elimination of ciprofloxacin in humans. *Clin Pharmacol Ther.* 1995;58:532–541. doi:10.1016/0009-9236(95)90173-6
31. Beringer PM, Kriengkauykiat J, Zhang X, et al. Lack of effect of P-glycoprotein inhibition on renal clearance of dicloxacillin in patients with cystic fibrosis. *Pharmacotherapy.* 2008;28:883–894. doi:10.1592/phco.28.7.883
32. Inotsume N, Nishimura M, Nakano M, et al. The inhibitory effect of probenecid on renal excretion of famotidine in young, healthy volunteers. *J Clin Pharmacol.* 1990;30:50–56. doi:10.1002/j.1552-4604.1990.tb03438.x
33. Vree TB, van den Biggelaar-Martea M, Verwey-van Wissen CP. Probenecid inhibits the renal clearance of furosemide and its acyl glucuronide. *Br J Clin Pharmacol.* 1995;39:692–695.
34. *Product Information: MEFOXIN(R) IV injection, cefoxitin IV injection*. Deerfield, IL: Merck & Co, Inc; 2004.
35. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab Pharmacokinet.* 2005;20:452–477. doi:10.2133/dmpk.20.452
36. Motohashi H, Sakurai Y, Saito H, et al. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J Am Soc Nephrol.* 2002;13:866–874.
37. Khosroshahi HT, Oskui R, Shoja MM, et al. Time-dependent variations in urine output after renal transplantation. *Transplant Proc*. 2007;39:932–933. doi:10.1016/j.transproceed.2007.04.006