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

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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 m².2 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 6,442 kidneys coming from living donors (based on Organ Procurement and Transplantation Network data as of 31 March 2019).3,4 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.
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 posttransplantation.15–19 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).15,16 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, cefotaxime, 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 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...

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**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.
Clinical drug–drug interaction with probenecid and anionic drugs

| Affected drug | Fold change in clinical PK parameters |
|---------------|--------------------------------------|
| Cefoxitin     | AUC<sub>0–∞</sub> 2.4              |
| Cefonicid     | AUC<sub>0–∞</sub> |                |
| Cefaclor      | AUC<sub>0–∞</sub> |                |
| Acyclovir     | AUC<sub>0–∞</sub> 1.4              |

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<sub>max</sub>, maximum concentration; CL<sub>tot</sub>, renal clearance; CL/F, apparent clearance; OAT, organic anionic drug transporter; PK, pharmacokinetics; $t_{1/2}$, half-life.

**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<sub>0–∞</sub>) was calculated by the log-linear trapezoidal method with extrapolation beyond the last measured concentration, according to the following:

$$AUC_{0–∞} = AUC_{0–t} + C_{t} / k$$

where $C_{t}$ is the concentration at 4 h.

Total body clearance (CL<sub>total</sub>) and the volume of distribution during terminal phase ($V_z$) were determined using the following equations:

$$CL_{total} = Dose / AUC_{0–∞}$$
$$V_z = Dose / ([AUC_{0–∞}] \times k)$$

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
Renal clearance ($CL_{\text{Renal}}$) was estimated using the following equation:

$$CL_{\text{Renal}} = \frac{A_e(t=0-4)}{\text{AUC}_{0-4}}$$

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

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

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

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

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

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.
Cefoxitin CL\textsubscript{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\textsubscript{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.

### 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.

### Table 3.
Patient characteristics

| Patient characteristics | All subjects (n = 15) | LDRT recipient (n = 8) | DDRT recipient (n = 7) | P* |
|-------------------------|----------------------|-----------------------|------------------------|----|
| 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 (m²), 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

*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* |
|----------------------------------------------------------|-----------------------------------|-----------------------------------|----|
| Part 1: d since Tx                                       | 6.9 ± 1.8                         | 7.3 ± 3.0                         | 0.75 |
| Part 2: d since Tx                                       | 112.8 ± 10.6                      | 121.5 ± 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 |                  |                | P* |
| Days since Tx                                            | 7.1 ± 2.3                         | 115.6 ± 20.0                      |    |
| Days between parts 1 and 2                               | 114.3 ± 21.0                      | 1.3 ± 0.1                         | 0.30 |
| SrCr (mg/dl)                                             | 1.5 ± 0.7                         | 54.4 ± 14.8                       | 0.52 |
| CrCl (mL/min)                                            | 49.8 ± 17.4                       | 8.8 ± 2.9                         | 0.78 |

*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.

### 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; SrCr, serum creatinine; 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 (AUC₀–∞), CLTotal, CLRenal, CLFiltration, and CLSecretion were statistically similar during phase 1 and phase 2 of the study. The majority of CLTotal was attributed to CLSecretion (=71%). t₁/₂ 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 (AUC₀–∞), CLTotal, CLRenal, CLFiltration, and CLSecretion 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<sup>+</sup> |
|---------------|---------------------------------|---------------------------------|-----------------|--------|
| AUC₀–∞ (mg × h/L) | 35.0 ± 13.1 | 35.6 ± 9.3 | 35.2 ± 11.6 | 0.91 |
| t₁/₂ (h) | 1.4 ± 0.7 | 1.1 ± 0.2 | 1.3 ± 0.6 | 0.25 |
| Vᵣ (L) | 15.0 ± 4.6 | 12.1 ± 3.4 | 13.9 ± 4.4 | 0.10 |
| CL₀ (ml/min) | 108.1 ± 40.0 | 99.3 ± 24.7 | 104.8 ± 34.7 | 0.56 |
| CL赇 (ml/min) | 90.2 ± 33.4 | 82.9 ± 20.6 | 87.5 ± 29.0 | 0.56 |
| CL trò (ml/min) | 13.0 ± 4.5 | 14.1 ± 3.4 | 13.4 ± 4.2 | 0.52 |
| CL trò (ml/min) | 77.3 ± 28.9 | 73.3 ± 25.1 | 74.1 ± 24.8 | 0.74 |

<sup>a</sup>Comparing part 1 and part 2.

AUC₀–∞, area under the concentration–time curve from time dose administration to infinite time; t₁/₂, half-life; Vᵣ, terminal volume of distribution; CL₀, cefoxitin total clearance; CL赇, cefoxitin renal clearance; CL trò, cefoxitin filtration clearance; CL trò, cefoxitin secretion clearance.

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**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.
Figures 6 and 7, respectively.

The linear plots of cefoxitin plasma concentration versus time during part 1 (≤14 d posttransplantation). Those with CIT <10 h were predominately 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 Figure 8.

### 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 = | Comparing part 1 and part 2. | Comparing part 1 and part 2. |
|---------------|-------------------------------------|-------------------------------------|----------------------------------|---|------------------|------------------|
| **AUC_{0–5} (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 | – | – |
| **Vz (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_{Secretion} (ml/min)** | 13.2 ± 5.3 | 14.5 ± 5.1 | 13.7 ± 5.03 | 0.66 | – | – |
| **CL_{Filtration} (ml/min)** | 68.2 ± 15.0 | 64.7 ± 20.4 | 66.8 ± 16.4 | 0.73 | – | – |

**<sup>a</sup>** Comparing part 1 and part 2.

**<sup>b</sup>** Comparing part 1 and part 2.

AUC_{0–5}, 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; Vz, 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 = | Comparing part 1 and part 2. | Comparing part 1 and part 2. |
|---------------|-------------------------------------|-------------------------------------|----------------------------------|---|------------------|------------------|
| **AUC_{0–5} (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 | – | – |
| **Vz (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_{Secretion} (ml/min)** | 12.7 ± 3.8 | 13.6 ± 2.18 | 13.0 ± 3.2 | 0.66 | – | – |
| **CL_{Filtration} (ml/min)** | 87.7 ± 39.7 | 73.8 ± 12.6 | 82.6 ± 32.1 | 0.52 | – | – |

**<sup>a</sup>** Comparing part 1 and part 2.

AUC_{0–5}, 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; Vz, 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_{0–5}), 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 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_{Total} 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
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 anion 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 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 AUC0–∞/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.
Furthermore, the contribution of secretory clearance when compared with CL\(_{\text{Tm}}\) 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 non-transplant 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\(_{\text{Tm}}\) and so cefoxitin secretory clearance CL\(_{\text{S}}\) in healthy controls was estimated by subtracting CL\(_{\text{Tm}}\) in probenecid-treated arm from CL\(_{\text{Tm}}\) 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).\(^{21,15-17}\) Preliminary clinical observations in renal transplant recipients with BKVN involving cidovudor 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 overexposure 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.

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### Table 9

| PK parameters | Historical healthy controls (mean ± SD) | Historical healthy controls + 1 g probenecid (mean ± SD) | Renal Tx recipients (mean ± SD) | \(P^*\) |
|---------------|----------------------------------------|----------------------------------------------------------|--------------------------------|--------|
| AUC\(_{0-\infty}\)/dose (mg × h/L)/g | 68.5 ± 8.1 | 170.1 ± 43.9 | 176.2 ± 58.0 | 0.0001 |
| \(t_{1/2}\) (h) | 0.6 ± 0.1 | 1.5 ± 0.2 | 1.3 ± 0.6 | 0.0070 |
| \(V_f\) (L) | 17.5 ± 5.1 | 16.1 ± 5.2 | 13.9 ± 4.4 | 0.090 |
| CL\(_{\text{Total}}\) (ml/min) | 246.2 ± 29.8 | 105.8 ± 37.5 | 104.8 ± 34.7 | 0.0001 |
| CL\(_{\text{Filtration}}\) (ml/min) | 206.5 ± 24.9 | 88.3 ± 31.3 | 87.5 ± 30.0 | 0.0001 |
| CL\(_{\text{Secretion}}\) (ml/min) | — | — | 74.1 ± 24.8 | — |

\(AUC_{0-\infty}\); area under the concentration–time curve from time dose administration to infinite time; CL\(_{\text{Tm}}\), cefoxitin renal clearance; CL\(_{\text{S}}\), cefoxitin secretion clearance; CL\(_{\text{Total}}\), cefoxitin total clearance; PK, pharmacokinetics; \(t_{1/2}\), half-life; Tx, transplantation; \(V_f\), terminal volume of distribution.

\(\text{AUC}_{0-\infty}\) area under the concentration–time curve from time dose administration to infinite time; CL\(_{\text{Tm}}\) cefoxitin renal clearance; CL\(_{\text{S}}\) cefoxitin secretion clearance; CL\(_{\text{Total}}\) cefoxitin total clearance; PK, pharmacokinetics; \(t_{1/2}\) half-life; Tx, transplantation; \(V_f\) terminal volume of distribution.
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