CHARACTERIZATION OF RENAL ANIONIC DRUG TRANSPORT CAPACITY IN KIDNEY TRANSPLANT RECIPIENTS WITH PERSISTENT BK VIREMIA: PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF CIDOFOVIR

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Jeremiah D. Momper, Pharm.D.

University of Pittsburgh, 2011

Kidney transplantation is the treatment of choice for end-stage renal disease (ESRD), and drug therapy plays a significant role in the management of transplant recipients. A variety of commonly used drugs are actively secreted by transporters in the kidney, yet the functional expression and activity of these proteins in transplant patients has not been investigated. The first objective of this research was to characterize anionic tubular secretion capacity in kidney transplant recipients by evaluating the pharmacokinetics of cidofovir, a prototypical organic anion transporter substrate frequently used to treat BK virus infections in this patient population. A sensitive and specific analytical technique was developed to measure cidofovir concentrations in human plasma. Pharmacokinetic analysis of cidofovir in adult kidney transplant recipients suggested reduced OAT1-mediated secretion in these patients. The mechanistic basis of this observation was evaluated in a syngeneic rat model of kidney transplantation, which established that the transplant process itself leads to a sustained reduction in the expression of anionic transport proteins localized to the basolateral membrane of the renal proximal tubule. Additionally, apical anionic transporters were differentially regulated in this model.

The next objective was to evaluate the pharmacodynamics of cidofovir in kidney transplant patients. This study demonstrated that cidofovir transiently reduced the degree of BK

viremia and viruria *in vivo*, though the effect was not sustained, and viral loads returned to baseline by the next sampling period. A retrospective analysis employed serum creatinine-based estimates of cidofovir clearance in a large cohort of kidney transplant recipients to approximate aggregate cidofovir exposure, and correlated drug exposure with virologic response. Higher systemic exposure was significantly associated with a larger reduction in the degree of BK viremia. However, only 11% of the variance in the decline in the BK viral load could be explained by variation in cumulative cidofovir exposure, indicating that other factors, likely immune-mediated, play a major role in viral clearance. Collectively, this work broadens our understanding of drug disposition in kidney transplant recipients and provides fundamental knowledge that may improve the treatment of BK virus infections.

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ABBREVIATIONS

λz	Terminal disposition rate constant
Ae	Amount of drug recovered unchanged in the urine
ANOVA	Analysis of variance
ARE	Amount remaining to be excreted
AUC	Area under the curve
AUMC	Area under the first moment curve
BKV	BK virus
BKVN	BK virus nephropathy
BSA	Body surface area
C_{12}	Plasma concentration at 12 hours
^{13}C5-FA	^{13}C5-Folic acid
CAR	Constitutive androstane receptor
CDV	Cidofovir
CKD	Chronic kidney disease
CL	Clearance
CL_R	Renal clearance
C_{max}	Maximum plasma concentration
CMV	Cytomegalovirus

COX	Cyclooxygenase
CV	Coefficient of variation
CYP450	Cytochrome P450
eGFR	Estimated glomerular filtration rate
ESI	Electrospray ionization
ESRD	End-stage renal disease
FDA	United States Food and Drug Administration
fe	Fraction eliminated unchanged in the urine
fu	Fraction unbound
GFR	Glomerular filtration rate
HPLC	High performance liquid chromatography
IL-6	Interleukin-6
IRB	Institutional review board
k_{el}	Urinary excretion rate constant
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLOQ	Lower limit of quantitation
MRM	Multiple reaction monitoring
MRP2	Multidrug resistance-associated protein 2
NAT	N-acetyltransferase
OAT	Organic anion transporter
OCT	Organic cation transporter
OATP	Organic anion-transporting polypeptide
OFV	Objective function value

P-gp	P-glycoprotein
PAH	Para-aminohippuric acid
PGE2	Prostaglandin E2
PRB	Probenecid
Q	Intercompartmental clearance
r^2	Coefficient of determination
SCR	Serum creatinine
SD	Standard deviation
SEM	Standard error of the mean
SPE	Solid phase extraction
$t_{1/2}$	Half-life
t_{max}	Time to reach maximum plasma concentration
TNF-α	Tumor necrosis factor-α
UGT	Uridine diphosphate-glucuronosyltransferase
V1	Volume of distribution of the central compartment
V2	Volume of distribution of the peripheral compartment
VP-1	Viral capsid protein-1
Vss	Volume of distribution at steady state

1.0 INTRODUCTION

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1.1 KIDNEY TRANSPLANTATION

Chronic kidney disease (CKD) is a major worldwide public health problem. An estimated 26 million adults in the United States have physiological evidence of CKD, representing 13% of the adult population [1]. Of these individuals, over 500,000 are classified as having end-stage renal disease (ESRD), defined as a glomerular filtration rate of less than 15 mL/min/1.73 m^2 [2]. The principal therapeutic options for patients with ESRD include peritoneal dialysis, hemodialysis, and kidney transplantation. Kidney transplantation is the treatment of choice because it provides the largest potential for improvement in the patient's quality of life and reduces mortality risk compared to maintenance dialysis [3]. The most common etiologies of renal disease leading to transplantation are diabetes, chronic glomerulonephritis, hypertension, and polycystic kidney disease [4].

In the United States, over 16,000 kidney transplants are performed annually [5]. Patient and graft survival rates following kidney transplantation have markedly improved over the past several decades. At present, five-year patient survival rates are 91% and 84% for living donor kidney transplantation and deceased donor kidney transplantation, respectively [5]. The high survival rates are attributed to advances in the field of transplantation, including organ preservation, surgical techniques, post-operative management, and drug therapy. Unfortunately, despite a good prognosis, kidney transplant recipients still face numerous complications, both infectious and non-infectious in nature.

1.2 INFECTIOUS COMPLICATIONS IN KIDNEY TRANSPANT RECIPIENTS

Transplant recipients are vulnerable to a variety of bacterial, fungal, and viral infections, which represent an impediment to successful clinical outcomes. Infections are a major cause of morbidity and mortality in kidney transplant patients, and it is estimated that 40-80% of recipients experience at least one infection during the first post-transplant year [6]. In fact, post-transplant infections now exceed acute rejection as a cause for hospitalization for kidney transplant patients [7]. The widespread use of potent immunosuppressive agents simultaneously reduces the risk of rejection and increases susceptibility to opportunistic infections. As a result of the growing number of immunosuppressed transplant patients with extended survival, guidelines have been established for the management of infectious diseases in this patient population [8].

At the time of presentation, infections in transplant recipients may be advanced because of compromised inflammatory and immunologic responses. Additionally, serological testing may be of little value secondary to delayed seroconversion. The overall risk of infection is considered to be a semi-quantitative relationship between (1) the epidemiologic exposure of the patient and (2) the net state of immunosuppression, characterized by the type, dose, and duration of immunosuppressive therapy, underlying disease states, and several host factors affecting the immune function [9]. Thus, maintaining an optimal degree of immunosuppression is critical in the medical management of transplant patients.

In the immediate post-operative period (< 1 month), infections derived from the donor and infectious complications of the surgical procedure prevail. Donor derived infections in the immunosuppressed host may include herpesviruses [cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV)], human immunodeficiency virus (HIV),

meningococcus, syphilis, candida, and aspergillus [10]. Antimicrobial resistant strains, such as methicillin-resistant staphylococci (MRSA) and vancomycin-resistant enterococci (VRE), may also present significant complications during this period. Common infectious postoperative complications, including typical gram (+) surgical site infections and line infections, may be amplified because of the immunocompromised status of the transplant recipient. The organisms responsible for these complications are classically the bacteria and fungi that colonize the recipient, as well as the flora in the hospital.

Following the immediate post-operative period, the nature of infectious complications changes and patients are at high-risk for contracting opportunistic infections. Opportunistic pathogens recognized to cause systemic disease in transplant recipients during the period of 1 to 5 months post-transplant include pneumocystic carinii, protozoal diseases, viral pathogens (herpesviruses, cytomegalovirus, Epstein-Barr virus), fungal infections (histoplasma capsulatum, Coccidioides species), and mycobacteria (tuberculosis) [11]. Consequently, prophylactic regimens with anti-bacterial, anti-fungal, and anti-viral agents are routinely used in the majority of patients (Table 1).

Table 1. Prophylactic anti-infective medications used in kidney transplant recipients

Infection	Medication	Dose	Notes
Cytomegalovirus	Valganciclovir	450 - 900 mg by mouth daily	- Requires dose adjustment for renal insufficiency - Risk is highest for seronegative recipients receiving an organ from a seropositive donor
Fungal infections (*Candida*, *Aspergillus*)	Voriconazole	200 mg by mouth every 12 hours	- Therapeutic drug monitoring may be used to maintain steady state trough concentrations within 1 – 6 µg/mL
PCP pneumonia (*Pneumocystis jiroveci*)	Trimethoprim/ sulfamethoxazole (TMP-SMX)	80/400 mg by mouth daily -OR- 160/800 mg by mouth three times weekly	Dapsone may be used in patients who cannot tolerate TMP-SMX

At six months post-transplant, the majority of patients receive reduced stable immunosuppression (e.g. target tacrolimus trough concentrations of 5-7 ng/mL). At this point, patients are prone to community-acquired pneumonias due to pneumococcus, respiratory viruses, and Legionella [11]. Opportunistic pathogens may also play a role, as well as delayed reactivation of latent viral infections, including HSV, CMV, VZV, HBV, and BK virus (BKV) [12]. In all cases, early identification and prompt initiation of efficacious treatment are critical to patient and graft survival.

1.2.1 BK Virus

BK virus is a member of the polyomavirus family, so named because of the viruses' ability to produce multiple (poly-) tumors (-oma). BK virus was first isolated in 1971 from the urine of a Sudanese kidney transplant recipient with the initials B.K. [13] and BK virus nephropathy (BKVN) was later diagnosed in Pittsburgh in 1993 by needle biopsy in a kidney transplant recipient suspected of having acute rejection [14]. Presently it is the most common viral disease affecting renal allografts and an important cause of graft dysfunction and graft loss following renal transplantation [15].

1.2.1.1 Biology

BK polyomavirus is a small (30 to 45 nm), nonenveloped, double-stranded, circular DNA virus that is ubiquitous in humans, occurring with a seroprevalence of approximately 70 % [16-21]. Primary infections typically occur in childhood via oral and/or respiratory exposure, though the initial infection is innocuous and the virus remains latent within the renal epithelium [22]. Healthy individuals may experience intermittent viral replication and asymptomatic shedding of the virus into the urine [23]. Viral shedding is significantly more common in immunocompromised patients, particularly immunosuppressed kidney transplant recipients and bone marrow recipients [24, 25]. During BK virus reactivation, the virus multiplies in the nucleus of renal tubular epithelial cells and eventually bursts from the host cell, causing cell lysis.

BK virus encodes six viral proteins: two early nonstructural or enzymatic proteins, an agnoprotein, and three late proteins [26]. The early proteins are the large and small tumor antigens, which are involved in cell immortalization and latency. The agnoprotein is responsible for the assembly of viral particles. The late genes encode three viral capsid proteins that make up the protein shell of the virus. These capsid proteins are termed VP-1, VP-2, and VP-3. Importantly, polymerase chain reaction (PCR) approaches to detect BK virus in biological fluids are targeted at a conserved region of VP-1 [27, 28].

1.2.1.2 Epidemiology

In the United States and Europe, 60 to 80% of adults have antibodies to BK virus, independent of gender, socioeconomic status, and rural versus urban residence [29]. In kidney transplant recipients, reactivation of BK virus carries a substantial disease burden, with an estimated incidence of viruria, viremia, and nephropathy of 35-40%, 11-13%, and 5-8%, respectively [30, 31]

1.2.1.3 Risk factors in kidney transplant recipients

The risk of developing BK virus infection in transplant recipients does not appear to be related to the use of a specific immunosuppressive agent or regimen [22, 32]. A randomized, prospective study found that the frequency of BK viremia and viruria were similar in patients receiving tacrolimus or cyclosporine, as well as patients receiving mycophenolate mofetil compared to azathioprine [30]. Further, BK virus nephropathy has been documented in patients receiving various regimens, including different combinations of cyclosporine, azathioprine, mycophenolate

mofetil, sirolimus, and calcineurin inhibitor free regimens [33-35]. Hence the overall degree of immunosuppression, encompassing both drug therapy and the humoral and cellular immunity of the patient, is believed to be the primary risk factor for viral reactivation. Retrospective analyses have revealed additional factors that increase the relative risk of developing BK viruria, viremia, or nephropathy beyond immunosuppression. These include advanced or young age, male gender, diabetes mellitus, white ethnicity, high donor antibody BK titers, and donor BK-seropositivity [36-38].

1.2.1.4 Clinical manifestations and diagnosis in kidney transplant recipients

BK virus initially presents in kidney transplant recipients as a slow progressive rise in serum creatinine [39]. Although the mean onset of disease is approximately one year after transplant, reactivation of the latent infection may begin within days or after several years [40, 41]. Patients are often asymptomatic or may have a mild non-specific fever [42].

Cytopathic changes are often observed on renal allograft biopsy; however, because of the focal nature of the infection, negative biopsy results cannot rule out BK virus nephropathy with certainty [43]. The histopathology of BKV nephropathy is characterized by intranuclear inclusion bodies in tubular epithelial cells, which are associated with necrosis, inflammatory cell infiltrates, and tubular atrophy [15]. A representative biopsy of renal medullary tissue displaying these changes is displayed in Figure 1.

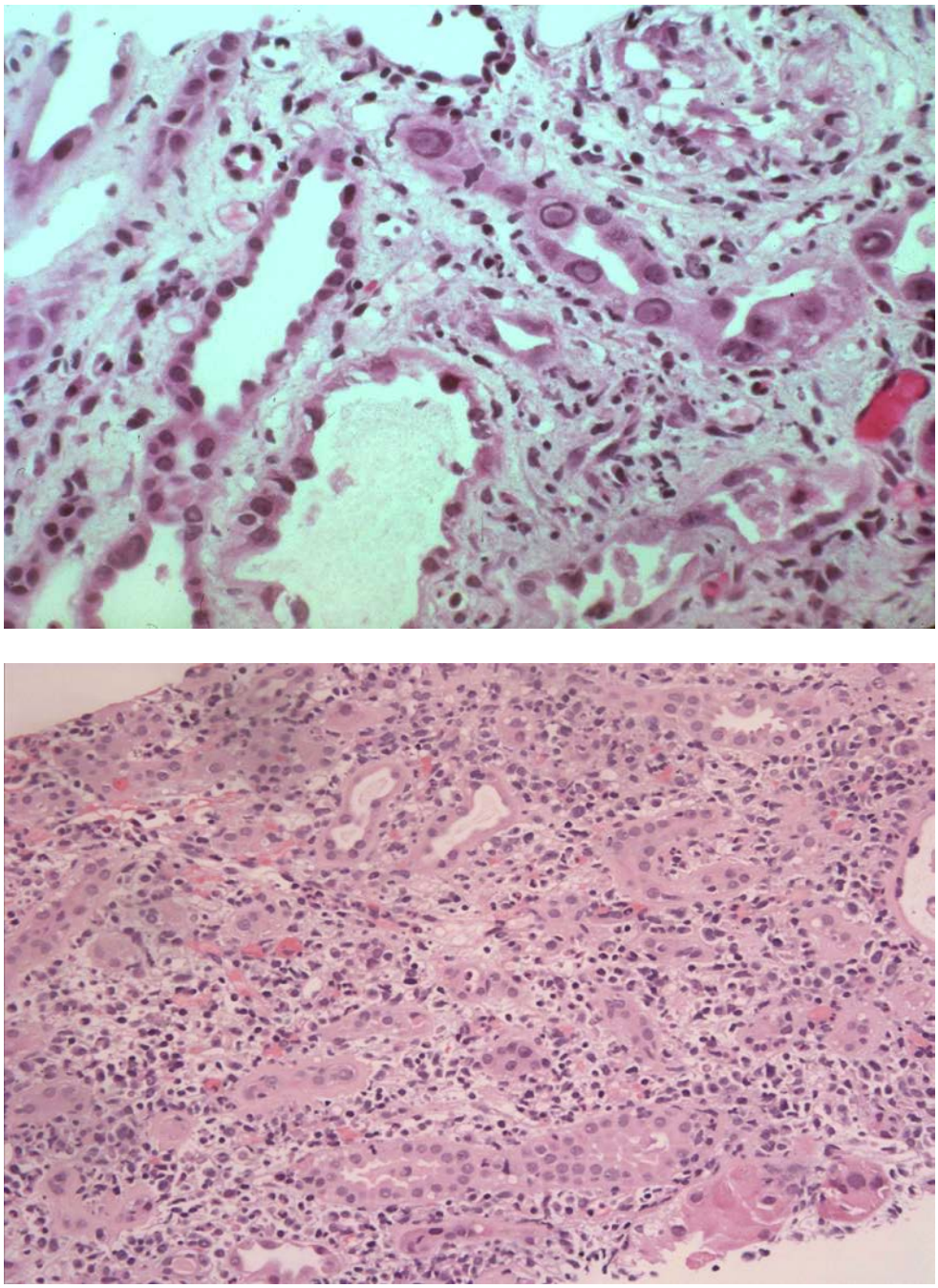

Figure 1. Biopsy of renal allograft tissue displaying cytopathic changes characteristic of BK virus nephropathy (top); renal biopsy without BK virus, for comparison (bottom)

University of Pittsburgh School of Medicine, Department of Pathology, Division of Transplantation Pathology

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As viral replication proceeds, BK virus is shed from renal tubular cells and urinalysis generally reveals pyuria, hematuria, and/or cellular casts consisting of renal tubular cells and inflammatory cells. Additionally, infected cells, referred to as “decoy cells”, may be shed into the urine from the renal tubules and detected by urine cytology. Decoy cells are not diagnostic for BK virus, however, and their presence has a low positive predictive value, because CMV infections may also lead to urinary shedding of cells with intranuclear inclusions [44]. A recent development is the detection of polyomavirus aggregates in the urine using electron microscopy. The presence of these aggregates, termed Haufen, is associated with a high sensitivity and specificity for BK nephropathy [45]. Urinary Haufen may one day serve as a noninvasive approach to diagnose BK nephropathy, but the utility of this test is presently questionable.

The identification of BK viral DNA in plasma and urine of infected patients is characteristic of the disease. Polymerase chain reaction is often employed to detect viral DNA in patients who present with a spectrum of symptoms consistent with BK virus (i.e. rising serum creatinine in the absence of evidence of rejection). Qualitatively, BK viral DNA in plasma, and to a lesser extent in urine, is associated with high sensitivity and specificity for biopsy-proven nephropathy. Additionally, quantitative PCR is a useful tool in monitoring progression of the disease and evaluating response to treatment. BK virus generally follows a course where detection is first made in the urine (secondary to lysis of renal tubular cells) and detection in plasma follows as disease severity increases.

1.2.1.5 Therapeutic interventions

Optimal therapeutic interventions for BK virus infections in kidney transplant recipients have yet to be elucidated [46]. However, several approaches are routinely undertaken. First, because BK virus is considered a disease of over-immunosuppression, the overall immunosuppression is reduced. The most common approaches include withdrawal of mycophenolate or azathioprine [47], and a decrease in target trough concentrations of tacrolimus or cyclosporine [30]. Specific immunosuppressive dosing regimens to allow immunologic clearance of BK virus and simultaneously maintain a low risk of acute rejection of the graft have not been conclusively established [15].

Antiviral regimens may be initiated if BK infection persists after reducing immunosuppression. Leflunomide, an orally administered disease modifying antirheumatic drug (DMARD) marketed for the treatment of rheumatoid arthritis, possesses immunosuppressive properties [48] and its active metabolite (A771726) has *in vitro* activity against BK virus [49]. Leflunomide is often used in the management of patients with refractory BK virus. In a case series report (n=26), 86% of patients treated with leflunomide after discontinuation of MMF had BK viral clearance and stabilization of graft function [50]. These patients received a loading dose (100 mg daily for 3-5 days) followed by 20-60 mg daily to maintain serum concentrations of the active metabolite between 50 and 100 µg/mL. The authors noted poorer outcomes in patients with concentrations of the active metabolite of less than 40 µg/mL. A recent report described the largest cohort of patients treated with leflunomide for BK (n = 61), again targeting serum concentrations of A771726 between 50 and 100 µg/mL [51]. A total of 53 patients (86.9%) had a positive response, defined as clearance of viremia (<2500 copies per mL) or

viruria (<50,000 copies per mL), and 8 (13.1%) had a negative response to therapy. The mean time to clearance of viremia was approximately 6.3 months. In light of these results, the authors conclude that leflunomide may be an effective adjunct therapy for BK virus treatment in renal allograft recipients. Although the role of leflunomide in the treatment of BKV in transplant patients is not categorically determined, the need for therapeutic drug monitoring and the long half-life of the active metabolite (> 14 days) make its use clinically challenging. A summary of the published literature on the use of leflunomide for the management of BK virus is presented in Table 2.

Table 2. Summary of published literature for the management of BK virus with leflunomide

Leflunomide dosing regimen[1]	Number of patients	Reduction in immunosupression	Results/comments	Reference
LD: 100 mg for 5 days MD: 20–60 mg daily Trough: 50–100 µg/mL	26	Discontinued MMF ↓ tacrolimus trough to 4–6 ng/mL	All patients with nephropathy prior to treatment; 7 patients received 0.25 mg/kg cidofovir every other week after failing to respond; Overall graft failure in 15%	[50]
LD: 60 mg for 3 days MD: 20–60 mg daily	21	Discontinued MMF ↓ tacrolimus trough to < 5 ng/mL	8 patients received low-dose cidofovir, overall graft loss in 19%; consistent low dose steroids used throughout in all patients	[52]
LD: 100 mg for 5 days MD: 30-70 mg daily Trough: 40 – 80 µg/mL	12	Discontinued MMF ↓ tacrolimus trough to 6–10 ng/mL	Renal function improved in 50%, remained stable in 16.6%, and deteriorated in 33.4%; Anemia in 6 cases	[53]
LD: 100 mg for 5 days MD: 20-60 mg daily Trough: 50-100 µg/mL	8	Discontinued MMF ↓ tacrolimus trough to 4–6 ng/mL	No graft loss during 12 mo follow-up; Leflunomide d/c in 1 patient due to unnamed side effects	[54]
MD: 20 mg daily	7	Discontinued MMF ↓ tacrolimus trough to 4–6 ng/mL	Graft failure in 28% at follow-up of 5-44 mo; 5 patients with stable graft function	[55]
Trough: 40–100 µg/mL	4	Discontinued MMF ↓ tacrolimus trough to 2 ng/mL	No graft loss during 14 month follow up	[56]

LD: 100 mg for 3 days MD: 40 mg daily	4	Discontinued MMF ↓ tacrolimus trough to 5–8 ng/mL	Graft loss in 50%, no monitoring of leflunomide concentrations; all patients with kidney/pancreas transplant	[57]
Trough: 40–100 µg/mL	4	Maintained only on leflunomide and corticosteroid	No graft loss; 1 patient with prior cidofovir use	[58]
LD: 100 mg for 3 days MD: 20 mg daily	2	Discontinued MMF ↓ tacrolimus trough to 4–6 ng/mL	No graft loss during 13 month follow-up	[59]
MD: 20 mg daily	1	Discontinue MMF ↓ cyclosporine	No graft loss at 12 mo follow-up	[60]

1. LD, loading dose; MD, maintenance dose

Cidofovir is a nucleotide analog antiviral drug approved by the United States Food and Drug Administration (FDA) for the treatment of cytomegalovirus retinitis in individuals with AIDS. Although the drug has *in vitro* and *in vivo* activity against a wide variety of DNA viruses, including adenoviruses, herpesviruses, papillomaviruses, poxviruses, and polyomaviruses, its clinical efficacy has only been thoroughly demonstrated against CMV [61-63]. However, cidofovir has been used as an adjunct therapy for BK virus based on several case series describing clearance of BK virus DNA from plasma and improvement or stabilization in graft function following treatment [64-68]. Cidofovir suppresses viral replication by inhibition of DNA polymerase and, unlike nucleoside analogs (i.e. acyclovir), does not require activation by viral kinases for efficacy [69]. Nevertheless, phosphorylation does occur intracellularly and the phosphorylated metabolites account for much of the antiviral activity [70]. Resistance to cidofovir has not been reported in the treatment of BK virus; however, reduced susceptibility of CMV to cidofovir has been confirmed in clinical CMV isolates from immunocompromised patients undergoing treatment, which are associated with mutations in the DNA polymerase gene [71, 72].

For CMV, cidofovir is administered as a 1-hour intravenous infusion at a dose of 5 mg/kg, which yields peak plasma concentrations of approximately 11 µg/mL [73]. In patients with normal kidney function, the majority (> 80%) of the dose is ultimately eliminated unchanged in the urine with a plasma half-life of 2.5 to 3.5 hours [74]. However, the intracellular metabolites are eliminated far more slowly [70] and allow for an extended dosing frequency of once weekly or once every two weeks. In monkeys, the terminal elimination half-life of the phosphorylated cidofovir metabolite was estimated to be 36 hr after intravenous administration of [^{14}C]-cidofovir [70], which is consistent with the long intracellular half-life of

phosphorylated cidofovir observed in both cultured human embryonic lung cells (48 hr) [75] and retinal cells *in vivo* after intravitreal injection of cidofovir to rabbits (>55 hr) [76].

In patients with normal kidney function receiving a 5 mg/kg dose of cidofovir, the total body clearance is approximately 248 mL/min and the renal clearance is 209 mL/min, or 84% of the total body clearance [73]. The nonrenal clearance (e.g. metabolic clearance) is therefore 39 mL/min. Moreover, by comparing renal clearance to baseline creatinine clearance in the same patients, the clearance of cidofovir due to active tubular secretion is approximately 84 mL/min [73]. The secretion of cidofovir is the result of an active uptake process facilitated by transport proteins localized to the basolateral membrane of renal proximal tubule cells [77]. Additionally, the drug possesses profound nephrotoxic potential that is minimized by co-administration with the uricosuric agent probenecid, which reduces renal uptake of cidofovir and prolongs the systemic exposure [78]. For this reason, the FDA-approved labeling recommends that each dose of cidofovir be given with probenecid [79]. The primary mechanism by which probenecid reduces uptake into the kidney and thereby provides nephroprotection is thought to be due to competitive inhibition of the basolateral renal drug transport of cidofovir. The effect of probenecid (90mg/kg, intravenous) on the distribution of [^{3}H]cidofovir (5 mg/kg , 20 μCi/kg) following intravenous administration was investigated in male New Zealand white rabbits. In this report, probenecid significantly reduced the distribution of total radioctivity in the kidney at 30 min, 6 h, and 24 h by 8.2%, 16.7%, and 53% respectively ($p<0.05$ for all time points) [80].

The use of cidofovir to treat BK virus in kidney transplant patients presents a challenge. On one hand, the drug is ideal because it has anti-BK activity and extraordinary affinity for the kidney via uptake into the cells where viral replication occurs. Conversely, the nephrotoxicity of cidofovir is problematic because kidney transplant recipients with BK virus usually have

underlying renal insufficiency. In light of these conflicting issues, many transplant centers use 5-10% of the FDA-approved dose (0.25-0.6 mg/kg) once per week without co-administration of probenecid [64-67, 81]. Although the low cidofovir dose was chosen to minimize nephrotoxicity, this dosing regimen has yet to be fully validated. A summary of the published literature on the use of cidofovir for the management of BK virus is presented in Table 3.

Table 3. Summary of published literature for the management of BK virus with cidofovir

Cidofovir treatment[1]	Number of patients	Outcomes/comments	Reference
Cidofovir 0.5 - 1.0 mg/kg/week for 4 – 10 weeks	8	• No graft loss occurred (median follow-up of 24.8 months) and graft function stabilized in all patients • Mean viral load at 12 months did not differ between patients treated with adjuvant cidofovir and recipients not treated with cidofovir (p=0.41). Viremia was unaltered	[66]
Cidofovir 1.5 mg/kg/week for 1.5-3 months in patients who failed reduction in immunosuppression	5	• All 4 patients had advanced BKVN (mean SCr 5.3 mg/dL) • Two patients experienced graft loss; 3 did not require dialysis after 4 months of follow-up	[82]
Cidofovir 0.25 mg/kg/dose every 2 weeks for 6 - 30 weeks	5	• BK viruria resolved within 4-12 weeks after cidofovir therapy • All patients had stable graft function at 6-26 months post therapy • 2 patients post-therapy developed recurrent viruria without viremia and with stable renal function	[83]
Cidofovir 0.33 mg/kg/dose every 2 weeks for 3 months	2	• One patient had stable renal function and undetectable viremia at 4.5 months post treatment and the second patient experienced graft loss 4 months post-therapy	[34]
Cidofovir 0.25 mg/kg/dose every 2 weeks	2	• Viremia became undetectable after 2 months of cidofovir but viruria persisted • Renal function stabilized and further biopsies did not reveal nephropathy	[84]
Cidofovir 0.3 mg/kg/dose every 2 weeks for 7 doses	2	• After 2 doses of cidofovir, renal function stabilized and viremia was undetectable at 12 months post-treatment (viruria persisted) • Renal biopsy at the end of cidofovir therapy found no BKVN	[85]

		• The second patient stopped therapy due to non-specific adverse events of headache and leg swelling after 4 doses. Renal function deteriorated and viremia increased	
Cidofovir 0.25 mg/kg/dose every 2 weeks for 8 doses	1	• After the treatment, renal function stabilized, viremia was undetectable • Viruria persisted • A biopsy 3 months post-treatment showed no BKVN	[86]
Cidofovir 0.25 mg/kg dose x 1 dose and 0.42 mg/kg/dose every 2 weeks for 7 months	1	• Four months post-treatment, viremia is undetectable, renal function is stable, viruria is persistent	[87]
Cidofovir 0.25 mg/kg/dose every 2 weeks	5	• No graft loss, clearance of viremia, persistent viruria	[88]
Cidofovir 0.25 mg/kg every 2 weeks for a total of four doses; Patients with persistent BKVN received additional doses of 0.5 mg/kg every 2 weeks for four to five doses	6	• No association between cidofovir use and viral clearance. No graft loss at 20 months follow-up	[89]
Cidofovir 0.25 - 1 mg/kg/dose every 2 - 3 weeks for 1- 4 doses	4	• BK viruria resolved within 4-12 weeks after therapy • All patients had stable graft function at 6-26 months post therapy • 2 patients post-therapy developed recurrent viruria without viremia and with stable renal function	[90]

1. In each report reduced immunosuppression was also utilized; BKVN, BK virus-associated nephropathy

The major gaps in knowledge in the use of cidofovir for the treatment of BK virus in kidney transplant recipients include the following: (1) the dose of cidofovir that will most effectively reduce the viral load without nephrotoxicity is not known; (2) the appropriateness of weekly or biweekly cidofovir dosing has not been evaluated; (3) the pharmacokinetics of cidofovir in kidney transplant recipients and the sources of intra- and inter-patient variability have not been described; (4) the relationship between cidofovir exposure and virologic response has yet to be elucidated, and the effect of probenecid on augmenting the response is not understood. As cidofovir uptake into the proximal tubules is desired to obtain high drug concentrations at the site of action, competitive inhibition of cidofovir uptake with probenecid may be deleterious; (5) it is not clear whether the expression and activity of the transporter that is responsible for uptake of cidofovir into the proximal tubule cells, OAT1, is normal or altered in kidney transplant patients. Therefore, a systematic evaluation of the pharmacokinetics and pharmacodynamics of cidofovir and an evaluation of the expression and functional activity of OAT1 in kidney transplant patients will further our knowledge and our ability to better use cidofovir in kidney transplant patients with BK virus.

The following section will review our current knowledge of drug disposition in patients with kidney disease and kidney transplantation.

1.3 EFFECTS OF KIDNEY DISEASE AND TRANSPLANTATION ON DRUG DISPOSITION

The kidney plays a major role in the disposition of cidofovir as discussed in the previous section. A discussion of various factors that can alter the pharmacokinetics of drugs in kidney disease and kidney transplantation is presented in this section. Pharmacokinetics quantitatively describes the steps involved in the processes of drug absorption and disposition (including distribution, metabolism, and excretion). Optimization of drug therapy, including immunosuppressive agents, anti-infectives, and other medications used to treat underlying medication conditions, is essential for long-term graft and patient survival in transplant patients. Therefore, it is critical to evaluate how kidney transplantation may alter the pharmacokinetic properties of medications used in this patient population.

Prior to transplantation, patients with kidney disease experience a progressive deterioration of kidney function. During this period, the clearance of drugs removed predominantly by renal filtration is reduced, which intuitively leads to a dose reduction of such drugs. As the severity of kidney disease increases, the magnitude of these effects becomes greater, as renal clearance (CL_R) is directly related to kidney function. In addition, active transporter-mediated processes involved in tubular secretion may be altered [91]. Finally, a growing body of evidence suggests that nonrenal drug clearance pathways, encompassing intestinal and hepatic metabolism and transport, may be affected in patients with kidney disease [92, 93]. The most well accepted hypothesis to explain these changes in patients with kidney disease is that accumulated toxins characteristic of uremia (uremic toxins) down regulate or directly inhibit these pathways [94]. To date, more than 110 organic compounds have been identified as uremic toxins, many of which contribute to reduce metabolic and transporter

processes [95]. Patients with CKD, particularly ESRD, exist in a chronic inflammatory state and are subject to increased oxidative stress, which is likely due to an overabundance of the accumulated uremic toxins, including pro-inflammatory cytokines [95]. In fact, the concentrations of IL-1b, IL-6, and TNF-α are increased 2.7-fold, 6.9-fold, and 8.6-fold, respectively, in uremic patients [96]. Alteration in drug disposition during inflammation is not a novel observation, as the half-life of theophylline, which undergoes hepatic oxidative metabolism, was shown over 30 years ago to be significantly longer during the acute stage of a respiratory viral illness [97]. It is now well regarded that inflammatory cytokines can down regulate drug metabolizing enzymes and transporters [98]. For instance, treatment of primary human hepatocyte cultures with cytokines has been shown to reduce both mRNA levels and enzymatic activities of CYP3A, CYP2E1, CYP1A2, and CYP2C by at least 40%, with interleukin-1b (IL-1b), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF- α) being the most potent [99]. Inflammatory cytokines suppress the expression of several metabolic enzymes and transporter proteins through the ability to serve as ligands for the nuclear transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) [100]. Reductions in the mRNA levels of PXR and CAR with a corresponding reduction in CYP enzymes have been reported in rodents with endotoxin induced (and cytokine mediated) inflammation [98]. Hence, altered metabolic enzyme and transporter function in patients with kidney disease may be a result of chronic downregulation of these proteins at the transcriptional level.

Following successful kidney transplantation, the filtration capacity is at least partially restored and the uremic state reversed. The disposition of drugs cleared principally by renal filtration may then return to normal, or at least reliably predicted based on clinical estimates of kidney function (i.e. creatinine clearance, eGFR). On the other hand, transplant recipients are

vulnerable to immune- and/or infectious-mediated inflammation that may alter the expression of various metabolic enzymes and transporters in key organs such as the intestine, liver, and kidney. In kidney transplant recipients, elevations in several pro-inflammatory cytokines, including IL-6 and TNF-α, have been documented in serum and urine during the immediate post-operative period, acute rejection episodes, periods of infection, and prior to late graft failure [101-103]. In addition, kidney transplant patients receive numerous medications, raising the possibility for drug interactions through inhibition or induction of these metabolic and transport proteins.

The subsequent sections highlight the individual steps in drug disposition and describe (1) how kidney disease (i.e. pre-transplantation) affects each process and (2) the impact of restoration of kidney function via transplantation on pharmacokinetic properties.

1.3.1 Absorption and uptake

For orally administered medications, the concerted actions of intestinal drug metabolizing enzymes and uptake and efflux transporters are important determinants of a drug's bioavailability, or the fraction of the dose that ultimately reaches the systemic circulation unchanged [104]. After oral administration, a drug encounters enterocytes lining the lumen of the gut wall where passive diffusion or active transport across the apical membrane may occur. When inside the enterocyte, the drug can be biotransformed to a more polar compound by drug metabolizing enzymes. Subsequently, the parent drug and/or metabolite may be either actively effluxed back across the apical membrane into the gut lumen to be fecally excreted or undergo repeated uptake-metabolism-efflux cycling, or translocated across the basolateral membrane into the portal circulation. Perturbation of the function of metabolizing enzymes and transporters involved in these pathways may affect bioavailability drastically, and thereby impact systemic

drug exposure. Additionally, physiological changes in gastrointestinal motility or blood flow, bile secretion, gastric acid secretion, or gastric pH may modify the bioavailability of orally administered medications.

Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing monooxygenases that are responsible for biotransformation of up to 60% to 80% of currently marketed drugs that are known to be metabolized [105], and are the primary contributors to intestinal drug metabolism [106]. CYP3A is the most abundant and clinically relevant CYP present in human small intestine [106]. The bioavailability of several CYP3A drug substrates is increased in CKD, and one frequently cited possibility for this phenomenon is reduced CYP3A-mediated intestinal metabolism [107]. However, conflicting experimental data and recent clinical studies have raised questions regarding this proposed mechanism. A 71% decrease in intestinal CYP3A2 protein expression has been observed in rats with chronic renal failure [107], yet others have reported that intestinal metabolism of the CYP3A substrate tacrolimus is not altered in rats with renal failure in which increased bioavailability was simultaneously demonstrated [108]. This may be related to the involvement of uptake or efflux transporters in the absorption of tacrolimus in the gut. In a clinical pharmacokinetic study in ESRD patients, there were no significant differences in the pharmacokinetic parameters of oral midazolam, a selective phenotypic probe of intestinal and hepatic CYP3A function, compared with healthy control subjects [109]. This finding was recently corroborated in a pharmacokinetic study of the CYP3A substrate erythromycin, which demonstrated that intestinal bioavailability of erythromycin is not altered in patients with ESRD [110]. Together, these clinical data suggest that intestinal CYP3A function is not substantially altered in patients with ESRD receiving conventional hemodialysis therapy.

Several transporters also play key roles in the clearance of drugs and have been investigated in the setting of kidney disease. The organic anion-transporting polypeptide (OATP) family of drug transporters is involved in the cellular uptake of several structurally diverse compounds [111]. OATP is expressed on the apical surface of enterocytes and serves to increase enteral drug absorption. In contrast, P-glycoprotein (P-gp) is an energy dependent transmembrane protein expressed on the apical surface of many tissues, including enterocytes, where it is responsible for efflux or extracellular transport of substrates back into the intestinal lumen [111]. P-gp is able to transport numerous structurally dissimilar neutral or cationic compounds. Multidrug resistance-associated protein 2 (MRP2) is an organic anion transporter that is expressed in human liver, intestine, and kidney [112]. Like P-gp, MRP2-mediated efflux from enterocytes limits oral bioavailability of certain xenobiotics. P-gp and CYP3A share similar substrate specificity and close proximity in enterocytes, working in concert to reduce the amount of drug reaching the systemic circulation. OATP, and to a lesser extent MRP2, also exhibits overlapping substrate specificity with CYP3A and P-gp.

Intestinal P-gp and MRP2 protein expression have been shown to be significantly reduced (40%) in rats with chronic renal failure, with corresponding reduction in the activity; however, OATP expression and activity are unchanged [113]. Decreased intestinal drug efflux activity through P-gp or MRP2 may lead to increased bioavailability and increased systemic exposure. In patients with kidney disease, the apparent oral clearance of fexofenadine, a P-gp and OATP substrate, was significantly decreased with a corresponding increase in exposure, as compared with control [109], suggesting downregulation of one or both of these transport pathways.

The available animal and human data related to the expression and activity of intestinal metabolizing enzymes and transporters following kidney transplantation is comparatively less.

The oral bioavailability of furosemide [114] and prednisone [115], which are transported by intestinal P-gp [116], is similar to that observed in normal subjects, while cyclosporine bioavailablity (a P-gp and CYP3A substrate [117]) is similar to liver and heart transplant patients [118]. While limited, these data support the complete recovery of function of intestinal transporters and metabolic enzymes after uremia is reversed. Furthermore, intestinal mRNA of the gene that encodes human P-gp (*MDR1*) is well expressed and serves as a predictive measure of tacrolimus pharmacokinetics in pediatric living-donor transplant recipients [119]. Overall, the bioavailability of both passive and carrier-mediated drug substrates in clinically stable kidney transplant recipients is expected to be similar to normal healthy individuals. However, the influences of graft dysfunction, acute rejection, and infection (including associated inflammation) have not been evaluated in kidney transplant patients.

1.3.2 Distribution

Several factors affect the distribution of drugs within the body, including blood flow to various organs, tissue partitioning, fluid status, plasma protein binding, and tissue binding. Alterations in these factors may occur pre- and post-transplantation and hence influence the pharmacokinetic properties of various drugs. Decreased perfusion of tissues and impaired tissue uptake will result in higher drug concentrations in blood and a lower volume of distribution. On the other hand, decreased plasma protein binding will result in an increased volume of distribution because more drug moves out of the vascular system and into the tissues. As the unbound concentration in blood is typically responsible for therapeutic effect, these changes may be clinically relevant. The significance of altered binding for drugs that are primarily hepatically cleared will depend upon the properties of the drug. For medications with high intrinsic

clearance, hepatic clearance is dependent on blood flow and changes in binding will have meaningful clinical consequences. Conversely, for drugs with a low intrinsic clearance, binding to blood constituents is a critical determinant of the overall hepatic clearance, though unbound concentrations responsible for therapeutic effect are not expected to be altered.

Decreased binding of several drugs has been documented in patients with kidney disease, which results in a larger apparent volume of distribution and increased distribution of drug outside of the vascular system. For example, the protein binding of acetylsalicylic acid, salicylic acid, phenylbutazone, and thiopental is decreased in patients with acute renal failure [120, 121]. This trend is also observed in patients with chronic kidney disease, where the unbound fraction of diazepam, furosemide, and phenytoin is significantly increased [122-124]. Plasma protein concentrations alone cannot account for these changes, and thus it has been suggested that accumulated organic waste products may block binding sites on plasma proteins and/or displace bound drugs, accounting for the increased unbound fraction and increased apparent volume of distribution [125].

After transplantation, drug distribution will be determined by the net effect of changes in plasma and tissue binding. The functional status of the transplanted kidney will influence the concentration of several plasma proteins as well as the clearance of uremic toxins. In the immediate post-operative period, during rejection episodes, or during infectious processes, the plasma concentrations of acute-phase proteins, including alpha-1-acid glycoprotein (AAG), are increased [126]. Because AAG serves as a primary carrier of basic drugs (i.e. propranolol, quinidine, prazosin, disopyramide), increased AAG concentrations during times of inflammation can alter the distribution volume of these medications. For example, the plasma protein binding of lidocaine is higher in renal transplant recipients compared to patients with kidney disease,

presumably related to the phenomenon of increased AAG concentration [122]. Further, the plasma protein binding of salicyclic acid, phenytoin, warfarin, diazepam, and morphine increases after kidney transplantation to a level consistent with normal healthy subjects [127-129]. This increase is seen within several days in some patients, and may take months for others. The initial increase in drug binding may be related to removal of endogenous uremic toxins in plasma. This phase is then followed by a slow increase in binding as new plasma proteins are synthesized.

1.3.3 Hepatic metabolism and transport

Hepatic drug clearance is the net result of metabolic enzyme activity and the activity of drug transport proteins responsible for hepatocellular uptake and efflux. After administration and entry of drugs and metabolites into the portal circulation, they may either diffuse or undergo active uptake across the sinusoidal membrane of the hepatocyte, followed by metabolism, then diffusion or efflux across the canalicular membrane into the bile for excretion.

CYP3A is highly expressed in human liver, constituting 30% of total CYP expression [130]. Numerous reports of decreased expression and activity of hepatic CYP3A in experimental models of kidney disease, together with clinical studies showing reduced systemic clearance of nonspecific CYP3A drug substrates in patients with kidney disease, have led some investigators to conclude that the function of hepatic CYP3A is altered in this patient population [93, 131]. However, recent studies that take into consideration enzyme-transporter interplay and the overlapping specificity of drug substrates suggest that drug transporters play a critical role. The pharmacokinetics of midazolam, a selective phenotypic probe of CYP3A, which is neither a P-gp nor an OATP substrate, and its 1-hydroxymidazolam metabolite, are not altered in patients with ESRD, suggesting that hepatic CYP3A function is unaffected [109]. Corroborating evidence

using the ^{14}C-erythromycin breath test shows lower ^{14}CO2 flux after dialysis as compared to pre-dialysis [132], which can be explained by the fact that erythromycin undergoes OATP uptake and P-gp efflux in the liver, in addition to hepatic CYP3A-mediated metabolism. Recent observations in patients with kidney disease of reduced hepatic clearance of erythromycin [110] and imatinib [133, 134], both of which are CYP3A, OATP, and P-gp substrates, further implicate drug transporter activity in reduced hepatic clearance of these drugs in patients with kidney disease. Studies have shown that OATP-mediated uptake of erythromycin (using isolated rat hepatocytes) [135] and imatinib (using *Xenopus laevis* oocytes injected with OATP1B3 cRNA) [134] is directly inhibited by the uremic toxin 3-carboxy-4-methyl-5-propyl-2-furan propanoic acid, and suggest that this may be a mechanism by which their hepatic clearance is reduced. Overall, these data indicate that altered drug disposition in CKD, previously thought to be due to a reduction in CYP3A-mediated hepatic metabolism, may in fact be a manifestation of altered drug transport pathways in the gut and liver [93].

Altered function of several other CYPs has also been reported in kidney disease. Recent clinical studies of the probe drug bupropion suggest that CYP2B6 activity is reduced in CKD. In nondialyzed patients, including individuals with advanced CKD (mean glomerular filtration rate 30 mL/min), a 63% lower apparent oral clearance of bupropion was observed which could not be accounted for by CYP2B6 genotype [136]. Reduced bupropion oral clearance was also documented in nonuremic patients with glomerular disease and mean creatinine clearance values of 102 mL/min, again suggesting altered metabolic capacity of CYP2B6 [137]. The 50% increase in the S/R-warfarin ratio in ESRD patients exhibiting the CYP2C9*1/*1 genotype compared with healthy control subjects may be reflective of decreased hepatic CYP2C9 activity [138]. These data are supported by a recent report demonstrating that patients with reduced

kidney function require lower warfarin dosages to maintain therapeutic anticoagulation independent of CYP2C9 and VKORC1 genotype [139]. Finally, experimental models indicate that expression and activity of the rat homologue of CYP2C19 (CYP2C11) is significantly affected by uremia [140].

Drug metabolism frequently involves a sequential oxidation-conjugation pathway that serves to increase the polarity of the substrate and facilitate excretion into bile or urine. Conjugation reactions are often mediated by the uridine diphosphate-glucuronosyltransferase (UGT) superfamily of enzymes or N-acetyltransferase (NAT) enzymes. UGT-mediated reactions involve the addition of glucuronides to apolar xenobiotics and the subfamilies UGT1A and UGT2B are responsible for the majority of human UGT metabolism [141]. NAT enzymes catalyze the conjugation of substrates with the acetyl moiety of acetyl-CoA. NAT1 and NAT2 are the salient isoforms in human drug metabolism [142]. Conjugative pathways of metabolism are not as extensively characterized as oxidative pathways in patients with kidney disease, but several clinical studies have reported a reduction in the activity of these pathways in this patient population. The clearance of morphine, which is primarily glucuronidated by UGT2B7 and UGT1A3, is significantly reduced in CKD patients as compared with healthy volunteers [143]. Zidovudine, an antiretroviral nucleoside reverse transcriptase inhibitor that is eliminated primarily by glucuronidation, has a significantly higher area under the plasma concentration-time curve (AUC) in patients with kidney disease as compared to patients with normal kidney function and this has been postulated to be due to reduced hepatic metabolism [144]. Similarly, the clearance of the NAT substrates isoniazid and procainamide is significantly reduced in patients with CKD as compared with healthy subjects, after stratifying both groups of patients into rapid and slow acetylators [145, 146]. Experimental models have shown that expression and

activity of NAT, but not UGT, are significantly reduced in uremic rats compared with control, suggesting that NAT-mediated metabolism may be altered in patients with kidney disease secondary to downregulation of the involved enzymes [142, 147].

Intestinal and hepatic CYP3A4 is significantly decreased at 3 months and 1 year after kidney transplantation in humans (−33% and −45%; −7% and −33%, respectively) [148]. These patients were clinically stable and had estimated creatinine clearances above 50 mL/min, potentially signifying that the mechanisms by which metabolic enzymes and transporters are down regulated may be different in patients with kidney disease when compared to transplant patients. Hypothetically, uremic toxins are implicated in kidney disease, whereas immune and inflammatory mediated changes associated with transplantation are involved in the latter. In contrast to the aforementioned data showing reduction in CYP3A4, a different report shows that antipyrine clearance in transplant recipients, a broad probe for hepatic oxidative metabolism (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4), is similar to that reported in normal subjects [149]. The expression and functional capacity of other CYP450 enzymes have not been evaluated in kidney transplant patients. Further, the influences of deterioration in the function of the transplanted organ, rejection of the allograft, and infectious processes have not been rigorously evaluated as they relate to hepatic drug metabolism in transplant patients.

In kidney transplant recipients, little is known about the functional activity of conjugation pathways in the liver. The pharmacokinetics of isoniazid, cleared primarily by hepatic NAT, were evaluated in patients with advanced CKD before kidney transplantation and again after transplantation. Isoniazid acetylation was decreased, and the corresponding half-life was significantly longer in patients with CKD, as compared with control subjects, but both parameters were normalized after successful kidney transplantation [146]. However, the

transplant recipients in this report were clinically stable, and the long-term effects of transplantation on conjugation pathways are unknown.

1.3.4 Renal excretion

The kidney is a key excretory organ for drugs and their metabolites and is capable of rapidly eliminating large quantities of xenobiotics via high capacity filtration and transport systems. Filtration through the glomerular membrane is a simple unidirectional diffusion process involved in the excretion of drugs into urine, and this pathway is impaired in patients with renal disease. Guidelines outlining dosage adjustments for patients with impaired kidney function are produced at the drug development stage and formally included in the labeling of drug products. Following transplantation, the filtration capacity rapidly improves to accommodate the physiological demand of the recipient. In a recent report of the recovery of graft function in 310 kidney transplant patients, 239 (77.1%) had immediate recovery of function after transplantation, with a mean pre-transplant serum creatinine of 7.1 mg/dL that decreased to 1.4 mg/dL at day 1 and 0.7 mg/dL at day 14 [150]. A total of 71 of these patients (22.9%) had slow recovery of graft function, with a mean pre-transplant serum creatinine of 9.4 mg/dL, which decreased to 6.4 mg/dL on day 1 and 1.4 mg/dL on day 14 [150]. Compounds that are eliminated primarily unchanged by renal filtration typically require dosage adjustments as eGFR declines below approximately 60 mL/min/1.73m^2. Thus a stable transplant recipient with a functioning kidney may not require dosage adjustments of medications that are normally filtered.

In addition to filtration, drugs may be excreted from the blood into the lumen across the proximal tubules through active transport processes. Renal drug transporters are highly specialized membrane transport systems that are capable of transporting charged organic

compounds in a specific and selective manner [151]. Many hormones, neurotransmitters, endogenous waste products, and a wide variety of drugs are classified as organic anions and cations. Renal drug transporters form a defense system by rapidly detoxifying blood of these compounds. These transporters are expressed on both the apical and basolateral (brush border) sides of the proximal tubule cells. Organic anion transporters (OATs) and organic cation transporters (OCTs) are highly expressed on the basolateral membrane, involved in uptake of compounds from the peritubular capillaries into the cell [152], as well as the apical membrane, serving to efflux drugs from inside the cell into the lumen for elimination within the urine. In addition to OATs and OCTs, other transporter families are present in the proximal tubules, such as P-gp, multidrug-resistance associated proteins (MRPs), multidrug and toxin extrusion (MATE) proteins, and oligopeptide transporters [153]. Because active secretion is often a critical step in drug elimination, alterations in this mechanism may result in increased systemic exposure or intracellular drug accumulation within the kidney. A schematic model summarizing the major drug transporters within the human kidney proximal tubule is displayed in Figure 2.

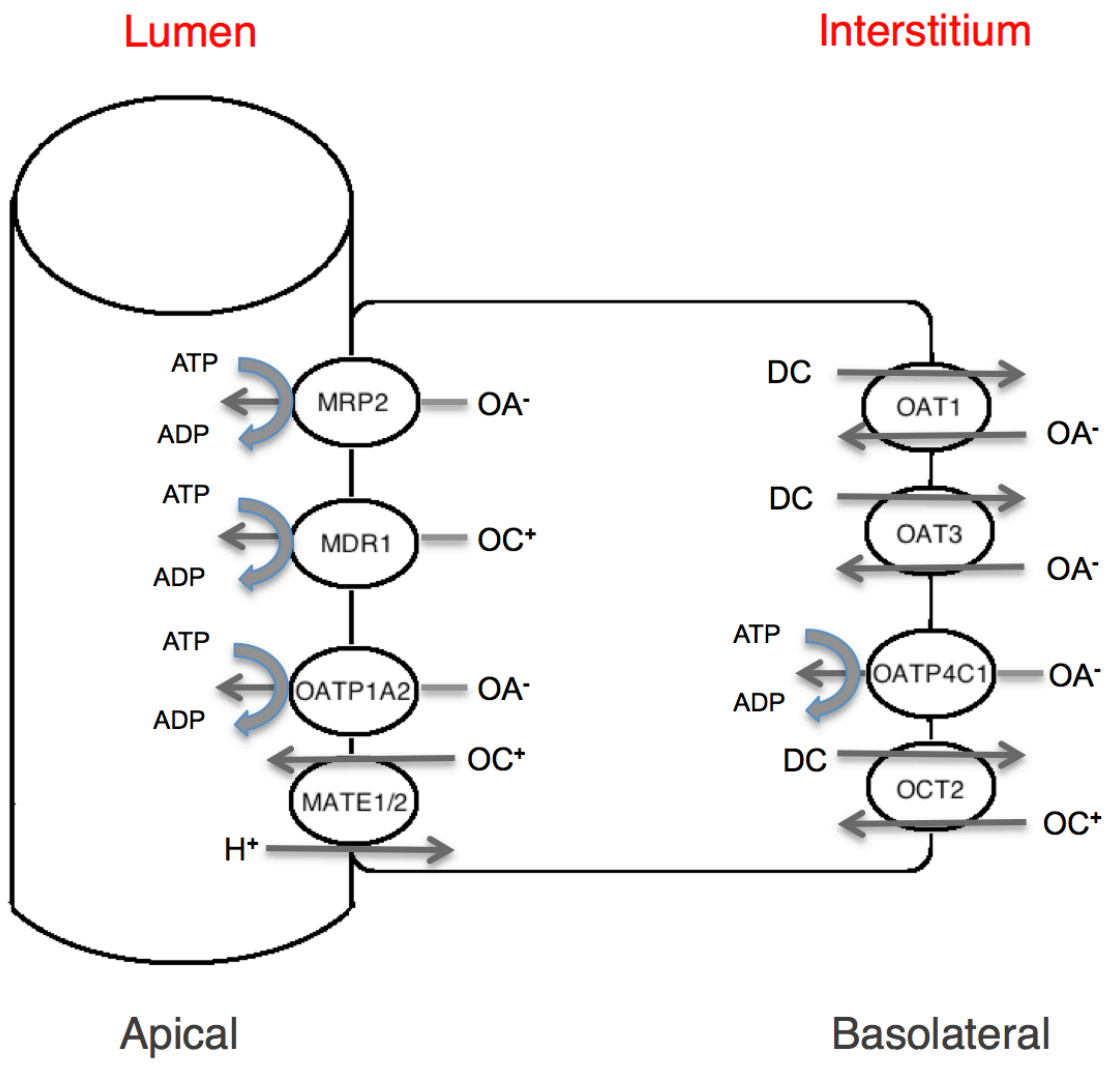


Figure 2. Schematic model of drug transporters in the human renal proximal tubule

OA^-, organic anion; OC^+, organic cation; DC, dicarboxylate; ATP, adenosine triphosphate; ADP, adenosine diphosphate

1.3.4.1 Secretion of organic anions

The organic anion transporter family of proteins mediates the uptake and efflux of small hydrophilic organic anions from plasma in the kidney [152]. This pathway includes organic anion transporters (OATs), organic anion-transporting polypeptides (OATPs), and the multidrug associated protein 2 (MRP2) [151, 154, 155].

Organic anion transporters 1 and 3 (OAT1/3) are highly expressed on the basolateral membrane of the tubules and facilitate the active uptake of drugs. Para-aminohippuric acid (PAH) is the classic prototype substrate for these transporters and has been extensively used to evaluate anion secretion in animal models, *in vitro*, and in the clinic [156, 157]. Para-aminohippuric acid transport involves energy-dependent uptake via an anion/dicarboxylate exchanger in a saturable manner, primarily by OAT1 [158]. Common drug substrates of OAT1 are presented in Table 4.

The relationship between glomerular function and tubular function was investigated by Bricker and colleagues in dogs in the 1960s [159-161]. In this model, kidney disease was induced in a single kidney while the other kidney remained intact. Separate urinary bladders were created, allowing for the clearance of probe compounds to be independently evaluated. Tubular function was assessed by the maximum reabsorptive capacity of phosphate (Tm_{PHOS}) and glucose (Tm_{GLU}), and the maximum secretory rate of PAH (Tm_{PAH}). These indices were then compared to GFR (measured by inulin clearance) to provide an index of tubular function relative to glomerular function. The ratios of tubular function indices to GFR were equivalent in the diseased and healthy kidneys, leading to the conclusion that nephron loss triggers adaptation by the remaining nephrons. This forms the basis of the so-called “intact nephron hypothesis”

which proposes that glomerular function and tubular secretion capacity are directly associated. However, numerous examples now indicate that active renal transport may be disassociated from glomerular function, perhaps through the regulation of renal transport proteins by inflammatory mediators.

In rats with cyclosporine-induced nephropathy, the withdrawal of cyclosporine after 28 days of treatment led to rapid improvement in the GFR. However, tubular injury persisted for the entire observational period (28 days) after cyclosporine withdrawal, indicating that secretion may be diminished compared to filtration [162]. In patients with renal disease who have undergone surgical nephrectomy for renal carcinoma, mRNA levels of OAT1 in biopsy samples were significantly lower than in patients without kidney disease [91]. The mRNA levels in these patients also correlated with the clearance of the anionic drug cefazolin, which is transported by organic anion transport system. These findings were confirmed in animals with renal impairment due to ischemia/reperfusion [163] or ureteral obstruction [164], where mRNA and protein levels of OAT1 were decreased. In a rat model of ischemic acute renal failure, protein expression of OAT1 and OAT3 is reduced. This effect is rescued by administration of anti-inflammatory medications, which implicates the involvement of inflammatory mediators in the down regulation of these transporters [165]. In a different study, rats with 5/6 nephrectomy-induced renal failure showed significant reductions in mRNA and protein expression of renal OAT1/3 [92]. Incubation of human proximal tubules with serum from rats with renal failure showed similar observations [166], implying a role for uremic toxins in the down regulation of drug transporters in the kidney.

On the apical membrane domain of the proximal tubule epithelia, the multidrug resistance protein 2 (MRP2) is highly expressed and serves as an ATP-dependent transporter for

organic anions across the luminal membrane [167]. MRP2 works in concert with organic anion transporters localized to the basolateral membrane to rapidly eliminate drug substrates, such as in the transport of the nucleotide analog reverse transcriptase inhibitor adefovir [168]. Regulation of MRP2 by inflammatory cytokines (TNF-α, IL-6, IL-1β) and the vasoactive hormone endothelin-1 (ET-1) has been described [169, 170]. The mRNA and protein expression of Mrp2 is induced in rats with chronic renal failure [166], perhaps signifying a nephroprotective mechanism whereby basolateral transporters undergo downregulation and apical transporters are upregulated, ultimately serving to protect the kidney by limiting further exposure to nephrotoxins.

In addition to OATs, organic anion transporting polypeptides (OATPs) are also expressed in the kidney and involved in the renal elimination of negatively charged substrates. In humans, OATP1A2 (*SLC21A3*) is localized to the brush-border membrane of the proximal tubules, in contrast to the liver, where expression is found at the basolateral membrane of the hepatocytes (94). On the other hand, OATP4C1 (*SLC21A20*) is expressed at the basolateral membrane of the tubules, in contact with the peritubular fluid [171]. The organic anion transporting polypeptide family is responsible for the disposition of a wide variety of endogenous substrates and drug substrates, including bromosulfophthalein, taurocholic acid, cholic acid, 17 β-estradiol glucuronide, leukotriene C_4, fexofenadine, and digoxin [154]. The expression of renal OATP mRNA is strongly regulated by androgens and estrogens, and in female rats, renal Oatp mRNA expression is markedly less than in males [172]. OATP-mediated transport is ATP-dependent, Na^+ independent, and bidirectional [173]. The impact of chronic renal failure on the expression of renal OATPs was recently investigated in a rat model. In this report, rats with chronic renal failure induced by 5/6 nephrectomy exhibited differential protein expression of renal OATPs,

with Oatp2 and 3 significantly increased more than 2-fold versus control, and Oatp1 and Oatp4c1 significantly reduced by at least 50% in rats with renal failure versus control [166]. To date, no information is available regarding the expression or functional activity of renal OATs, OATPs, or MRP2 following kidney transplantation.

Table 4. Drug and endogenous substrates of OAT1

	Substrate	Reference(s)
Angiotensin converting enzyme inhibitors		
	Captopril	[174, 175]
	Quanapril	[176]
Angiotensin II receptor blockers		
	Olmesartan	[177, 178]
Diuretics		
	Acetazolamide	[179]
	Bumetanide	[179, 180]
	Furosemide	[180]
Antibiotics		
	Tetracycline	[181]
	Ceftibuten	[174]
	Cephaloride	[174, 180]
	Ceftizoxime	[182]
Antivirals		
	Acyclovir	[183-185]
	Adefovir	[186]
	Cidofovir	[77]
	Ganciclovir	[184]
	Tenofovir	[187]
	Zidovudine	[185]
Antineoplastics		
	Methotrexate	[188, 189]
Histamine receptor 2 blockers		
	Cimetidine	[190, 191]
	Ranitidine	[192]
NSAIDs		
	Indomethacin	[193]
	Ibuprofen	[194]
	Ketoprofen	[195]

Table 4, continued.

Endogenous compounds	
Estrone sulfate	[196]
Folate	[197]
Prostaglandin E2	[198]
Urate	[199]
cAMP	[193]

1.3.4.2 Secretion of organic cations

The tubular secretion of cations occurs by facilitated diffusion down an electrochemical gradient via transport proteins localized to the basolateral membrane [200]. Specific transporters expressed have been isolated and cloned, including the organic cation transporters 1 and 2 (OCT1/2) [200]. The tubular secretion of cations was first described using the prototype tetraethylammonium (TEA) in a chicken [201]. As with anions, saturable transport of cations in the kidney has been reported *in vitro* and in animal models [202, 203]. Many commonly used medications undergo transport by the cationic pathway, including H2-receptor blockers (famotidine), antibiotics (trimethoprim), and antiarrhythmics (quinidine) [204]. Endogenous compounds, including creatinine, are also transported by OCT1 and/or OCT2, and may compete for common secretory mechanisms [205, 206]. As a result, the presence of competing cationic

xenobiotics can diminish the secretion of creatinine, reversibly raising the serum creatinine concentration without a corresponding decline in the glomerular filtration rate [207].

In renal failure, differential effects on anionic secretion versus cationic secretion have been reported. In dogs with bilateral ureteral-venous anastomosis, the extraction of PAH is significantly lower than that of TEA [208]. This observation indicates that anionic transport may be impacted to a greater extent, perhaps through competition with endogenous anions. On the other hand, Ji et al have shown that OCT2 protein is markedly depressed in rats with chronic renal failure, consistent with an observed reduction in cimetidine clearance in these animals [209].

Collectively, these data indicate that anionic and cationic secretory pathways in the kidney are differentially altered in various disease states, and that decline in the functional capacity of these pathways may not occur in parallel with that of glomerular filtration. Importantly, no data presently exist regarding the functional activity and expression of renal drug transporters in kidney transplant recipients. This information is particularly relevant because of the numerous medications routinely administered to this patient population that are eliminated in part by active secretion. Renally secreted drug substrates commonly used in kidney transplant patients include antibiotics (cephalosporins, penicillins), antivirals (acyclovir, cidofovir, ganciclovir), histamine receptor blockers (cimetidine, ranitidine), and several antihypertensives.

1.3.4.3 Inhibition of active secretion

Probenecid (p-dipropylsulfamoyl benzoic acid) is the prototypical inhibitor of renal drug secretion, initially developed to decrease the renal excretion of penicillin [210]. Additionally, because probenecid reduces systemic uric acid concentrations by inhibiting uric acid reabsorption in the kidney, it is widely used for the management of gout [211].

Following oral administration, probenecid is well absorbed from the gastrointestinal tract and undergoes significant hepatic metabolism, with a plasma half-life of 4-12 hours [212]. Probenecid and its metabolites are mainly renally eliminated, with 5-10% of the dose appearing in the urine as unchanged parent compound [212]. Probenecid inhibits the active transport (and reduces the renal clearance) of both anionic and cationic drug molecules in the kidney, by competitive inhibition of a wide variety of basolaterally and apically expressed drug transporters, including OAT1, OAT3, and OCT2 [213]. The contribution of these transporters to the overall elimination of drug substrates can be assessed by co-administration with probenecid.

1.4 SUMMARY AND INTRODUCTION TO DISSERTATION

Kidney transplantation is the therapy of choice for ESRD, and the aforementioned experimental and clinical evidence indicates that drug transport and metabolic pathways may be altered in kidney transplant recipients. However, to date, no information exists on the functional activity of transporters involved in the elimination of numerous endogenous and exogenous substrates in the kidney. Therefore, the body of work described in this dissertation was performed to characterize the secretion capacity in kidney transplant recipients, with the ultimate goal of improving drug therapy in these patients. The central hypothesis guiding this project is that immunogenic inflammation, local inflammation from the transplant/surgery, and nephritis resulting from BK virus infection will down regulate drug transport proteins in the kidney, which in turn will lead to decreased clearance of transporter substrates. This was investigated by employing cidofovir as a specific probe drug for renal OAT1. Cidofovir was selected because it is not metabolized by oxidative nor conjugative pathways, not bound to plasma proteins, cleared by OAT1-mediated tubular secretion, and is frequently utilized in the patient population of interest.

To examine this central hypothesis, it was first necessary to develop a novel sensitive analytical method to determine cidofovir concentrations in human plasma, as described in Chapter 2. This was essential because cidofovir concentrations were expected to be low (< 50 ng/mL) due to the low dose of cidofovir employed in kidney transplant patients with BK virus infection.

We hypothesized that secretion of cidofovir would be reduced secondary to diminished OAT1 expression in the kidney. Chapter 3 details a clinical pharmacokinetic study of cidofovir

without and with concomitant oral probenecid administration. The goal of this study was to evaluate the anionic renal secretion capacity in kidney transplant recipients with BK viremia by evaluating the effect of probenecid (an inhibitor of anionic tubular secretion) on cidofovir clearance, assessed by both noncompartmental and compartmental pharmacokinetic approaches. Next, we predicted that the expression of OAT1 in the kidney would be reduced after transplantation. An animal study was therefore used to evaluate the mRNA and protein expression of renal drug transporters and metabolic enzymes after kidney transplantation, as described in Chapter 4. The study design allowed for interpretation of the impact of the transplant independent of BK virus.

We hypothesized that in the use of cidofovir for the treatment of BK virus in kidney transplant recipients, a relationship exists between drug exposure and virologic response, and further predicted that the use of concomitant probenecid in this capacity would result in a decreased therapeutic effect due to reduced OAT1-mediated uptake of cidofovir into proximal tubule cells (the site of viral replication). Chapter 5 describes the evaluation of the acute pharmacodynamic response of cidofovir used for BK virus, both without and with concomitant probenecid administration. Chapter 6 details the utilization of the positive linear relationship between cidofovir clearance and serum creatinine (ascertained in Chapter 3) to retrospectively evaluate the use of cidofovir for BK virus at a large academic transplant center. We predicted that those patients with higher cumulative cidofovir exposure ($AUC_{0-\infty}$) would have improved outcomes compared to patients with lower exposure. Finally, the conclusions and limitations of this body of work, as well as recommended future research directions, are discussed in Chapter 7.

2.0 DETERMINATION OF CIDOFOVIR IN HUMAN PLASMA AFTER LOW DOSE DRUG ADMINISTRATION USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

[Momper JD, Zhang S, Randhawa PS, Shapiro R, Schonder KS, Venkataramanan R. Determination of cidofovir in human plasma after low dose drug administration using high-performance liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal*. 2010;53(4):1015-21.]

2.1 ABSTRACT

A sensitive and specific method for the determination of cidofovir in human plasma using high performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) was developed and validated. Plasma samples were processed by a solid phase extraction (SPE) procedure using Varian® SAX extraction cartridges prior to chromatography. The internal standard was ^{13}C5-Folic acid (^{13}C5-FA). Chromatography was performed using a Luna C8(2) analytical column, 5 μm, 150 mm × 3.0 mm, using an isocratic elution with a mobile phase consisting of 43% methanol in water containing 12 mM ammonium acetate, at a flow rate of 0.3 mL/min. The retention times of cidofovir and ^{13}C5-FA were 2.1 min and 1.9 min, respectively, with a total run time of 5 min. The analytes were detected by a Micromass Quattro Micro triple quadrupole mass spectrometer in positive electron spray ionization (ESI) mode using multiple reaction monitoring (MRM). The extracted ions monitored following MRM transitions were *m/z* 280.0→262.1 for cidofovir and *m/z* 447.0→294.8 for ^{13}C5-FA (IS). The assay was linear over the range 20–1000 ng/mL. Accuracy (101.6–105.7%), intra-assay precision (4.1–5.4%), and inter-assay precision (5.6–6.8%) were within limits proposed by the U.S. Food and Drug Administration. No significant variation in the concentration of cidofovir was observed with different sample storage conditions. This method is simple, adaptable to routine application, and allows easy and accurate measurement of cidofovir in human plasma.

2.2 INTRODUCTION

Cidofovir (VISTIDE®, CDV) is a nucleotide analog of deoxycytidine monophosphate with *in vitro* and *in vivo* activity against herpesviruses, adenoviruses, poxviruses, and polyomaviruses [49, 214-216]. Intravenous cidofovir is approved by the United States FDA for systemic treatment of cytomegalovirus retinitis in patients with AIDS. Additionally, a low dose cidofovir regimen of 0.25–0.5 mg/kg weekly is empirically used at many institutions for the treatment of BK polyomavirus virus (BKV) infections in kidney transplant recipients [65-67, 217]. Although the pharmacokinetics of cidofovir have been described in patients with normal renal function and renal insufficiency [73, 218], no data is available regarding the disposition of this drug in patients with a single transplanted kidney. In order to elucidate the pharmacokinetics and pharmacodynamics of cidofovir used for the treatment of BKV in kidney transplant recipients, it was necessary to develop a sensitive and specific assay method for the determination of cidofovir in human plasma. To date, four HPLC methods and one LC–MS/MS method have been described in the literature [219-222]. The HPLC methods require a large blood volume making intensive sampling difficult, or involve a laborious pre-column fluorescence derivatization process. Further, due to the low dose of cidofovir used in the renal transplant population, the published LC–MS/MS method, with a selective detection in the range of 78.125–10,000 ng/mL requiring 300 μL serum, cannot be used [223]. This method is also difficult to reproduce as it employs an internal standard that is not commercially available. Therefore, the objective of this study was to develop a sensitive, specific and reproducible LC–MS/MS analytical method to estimate cidofovir concentrations in human plasma following low dose intravenous administration to kidney transplant patients.

2.3 MATERIALS AND METHODS

2.3.1 Chemicals and materials

The chemical structures of cidofovir and the internal standard, ^{13}C5-Folic acid (^{13}C5-FA), are represented in Figure 3. Cidofovir reference standard was graciously supplied by Gilead Sciences, Inc. (Foster City, CA, USA). ^{13}C5-FA was purchased from Merck Aprova AG (Schaffhausen, Switzerland). Varian® Bond Elut SAX 1 mL (100 mg) extraction cartridges were purchased from Varian, Inc. (Lake Forest, CA, USA). Luna C8(2) column (150 mm x 3.0 mm, 5 μm, 100Å) and C8 SecurityGuard cartridge (4.0 mm x 2.0 mm) were purchased from Phenomenex (Torrance, CA, USA). Optima HPLC grade methanol and HPLC grade water were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Aliquots of blank human plasma used for preparation of spiked standards were obtained from the central blood bank (Pittsburgh, PA, USA).

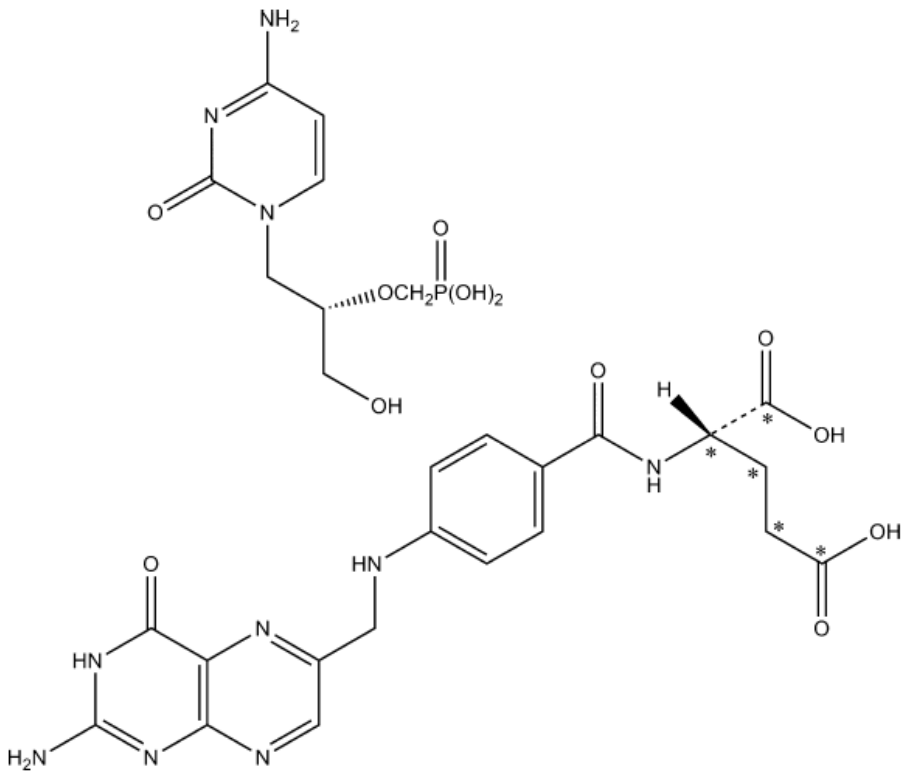


Figure 3. Chemical structures of cidofovir (top, molecular weight: 279.19) and ^{13}C5-FA (internal standard, bottom, molecular weight: 446.4).

2.3.2 Preparation of standards and quality control samples

Stock solutions of cidofovir were prepared at 1 mg/mL in water and used for a maximum of 6 months, while being stored at 4 °C in the dark. On assay days, the working solution was diluted in human plasma to produce the following cidofovir concentrations: 20, 50, 100, 200, 350, 700, and 1000 ng/mL. The stock internal standard solution (50 μg/mL) was prepared in 20 mmol/L phosphate buffer (pH 7.2) and diluted to the working standard solution (1 μg/mL) in mobile phase.

Quality control (QC) stock solution was prepared independently from a separate weighing of cidofovir and stored at 4 °C in the dark. This solution was diluted in control human plasma to produce the following QC samples: QC low (QCL) 60 ng/mL; QC mid (QCM) 400 ng/mL; and QC high (QCH) 800 ng/mL. Additionally, plasma samples were prepared from the QC stock solution at 20 ng/mL, which was the lower limit of quantitation (LLOQ).

2.3.3 Sample preparation

Routine daily calibration curves, controls, and clinical samples were thawed at room temperature. Exactly 400 μL of plasma was diluted with 500 μL of water and passed through Varian® SAX 1 mL (100 mg) extraction cartridges, previously conditioned with methanol and water. After washing with 2 mL of water, cidofovir was eluted with 2 mL of 5% acetic acid in methanol and the eluent was evaporated to dryness under air at 38 °C. The residue was reconstituted in 50 μL of 10% ammonium hydroxide in 65% methanol and 50 μL of internal standard (1 μg/mL). Following 5 min of centrifugation at 10,000 rpm at ambient temperature, 10 μL of the solution was injected into the LC-MS/MS system.

2.3.4 Chromatographic and mass spectrometer conditions

The HPLC system was a Waters 2759 model (Waters Corporation, MA). Separation was performed with a Luna C8(2) column (150 mm x 3.0 mm, 5 μm, 100Å) with a C8 SecurityGuard cartridge (4.0 mm x 2.0 mm). An isocratic mobile phase was used consisting of 43% methanol in water containing 12mM ammonium acetate. The total run time was 5 min at a flow rate of 0.3 mL/min.

Analysis was performed on a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, MA) with positive electrospray ionization mode using multiple reaction monitoring (MRM). For the analyte and internal standard, MRM settings used were as follows: capillary voltage 3.2 kV; source temperature 100 °C; desolvation temperature 500 °C; cone gas flow 50 l/hr; desolvation gas flow 550 l/hr; argon pressure 20 ± 10 psig; nitrogen pressure 100 ± 20 psig. Cone and collision energy are presented in Table 5. The extracted ions following MRM transitions were monitored at *m/z* 280.0 → 262.1 for cidofovir and *m/z* 447.0 → 294.8 for ^{13}C5-FA (IS). The LC-MS system was controlled by the Masslynx® software version 4.1, and data were collected with the same software.

Table 5. The cone and collision energy set in LC–MS for cidofovir and ^{13}C5-FA (IS)

	Parent *m/z*	Daughter *m/z*	Dwell (s)	Cone energy (V)	Collision energy (V)
CDV	280.0	262.1	0.1	28	14
^{13}C5-FA	447.0	294.8	0.1	22	11

2.3.5 Validation procedures

2.3.5.1 Calibration curve and lower limit of quantitation

Decreasing concentrations of cidofovir in human plasma, prepared as previously described, were injected into the analytical system to achieve a signal-to-noise ratio of at least 5:1. Calibration standards, blank, and zero samples were analyzed in triplicate to establish the calibration range with acceptable accuracy and precision. The response for each sample was calculated by dividing the area of the cidofovir peak by the area of the internal standard peak. Standard curves of

cidofovir were constructed by plotting the analyte-to-internal standard ratio *versus* the nominal concentration of cidofovir in each sample. Standard curves were fit by linear regression with weighting by $1/y^2$, without forcing the line through the origin, followed by the back calculation of concentrations. The deviations of these back-calculated concentrations from the nominal concentrations, expressed as percentage of the nominal concentration, reflected the assay performance over the concentration range.

2.3.5.2 Accuracy and precision

The accuracy and precision of the developed method were determined by analyzing plasma samples with cidofovir at the LLOQ, QCL, QCM, and QCH concentrations in a minimum of five replicates in 3 analytical runs together with an independently prepared, triplicate calibration curve. Accuracy was calculated at each test concentration as:

$$\frac{\text{Mean measured concentration}}{\text{Nominal concentration}} \text{ x } 100\%$$

The precision of the assay was expressed using % coefficient of variation (CV). Intra-assay and inter-assay precision were assessed by replicate analysis of specimen aliquots on a single day or successive days, respectively.

2.3.5.3 Selectivity and specificity

To investigate whether endogenous matrix constituents interfered with the assay, six individual batches of control, drug-free human plasma were processed and analyzed according to the described procedures. Responses of cidofovir at the LLOQ concentration were compared with the response in the blank samples.

2.3.5.4 Extraction recovery and matrix effect

The extraction recovery of cidofovir from human plasma was determined by comparing the absolute response of an extract of control plasma to which cidofovir had been added after extraction with the absolute response of an extract of plasma to which the same amount of cidofovir had been added before extraction. The matrix effect of plasma on cidofovir was defined as the effect on the signal when comparing the absolute response of an extract of control plasma to which cidofovir had been added after the extraction with the absolute response of reconstitution solvent to which the same amount of cidofovir had been added. Experiments were performed at the QCL, QCM, and QCH concentrations in triplicate.

2.3.5.5 Stability

The stability of cidofovir in plasma was evaluated at the QCL, QCM, and QCH concentrations in triplicate under different conditions. The control plasma samples were stored for either 24 hr at room temperature, 7 days at 4 °C, 1 month at - 20 °C, or 3 months at - 80 °C . Additionally, three freeze-thaw cycles of plasma samples prior to extraction were assessed. The reference concentration was calculated from freshly spiked plasma injected immediately post-extraction. Stability was expressed in terms of percentage of nominal concentration. The acceptance criterion for % relative recovery was set at 100 ± 10%.

2.4 RESULTS

2.4.1 Mass spectrometry and chromatography

When cidofovir and ^{13}C5-FA were injected directly into the mass spectrometer with a positive ion ESI interface, the protonated molecules $(MH)^+$ were seen in abundance. The mass to charge transition from parent ions to product ions were observed to have *m/z* 280.0 → 262.1 for CDV and *m/z* 447.0 → 294.8 for ^{13}C5-FA. The instrument parameters were selected to optimize specificity and selectivity of both parents and product ions and included capillary voltage of 3.2 kV, source temperature of 100 °C, desolvation temperature of 500 °C, cone gas flow of 50 l/hr, desolvation gas flow of 550 l/hr, argon pressure of 20 ± 10 psig, and nitrogen pressure of 100 ± 20 psig. The daughter scan mass spectra (*m/z*) for cidofovir and ^{13}C5-FA are displayed in Figure 4.

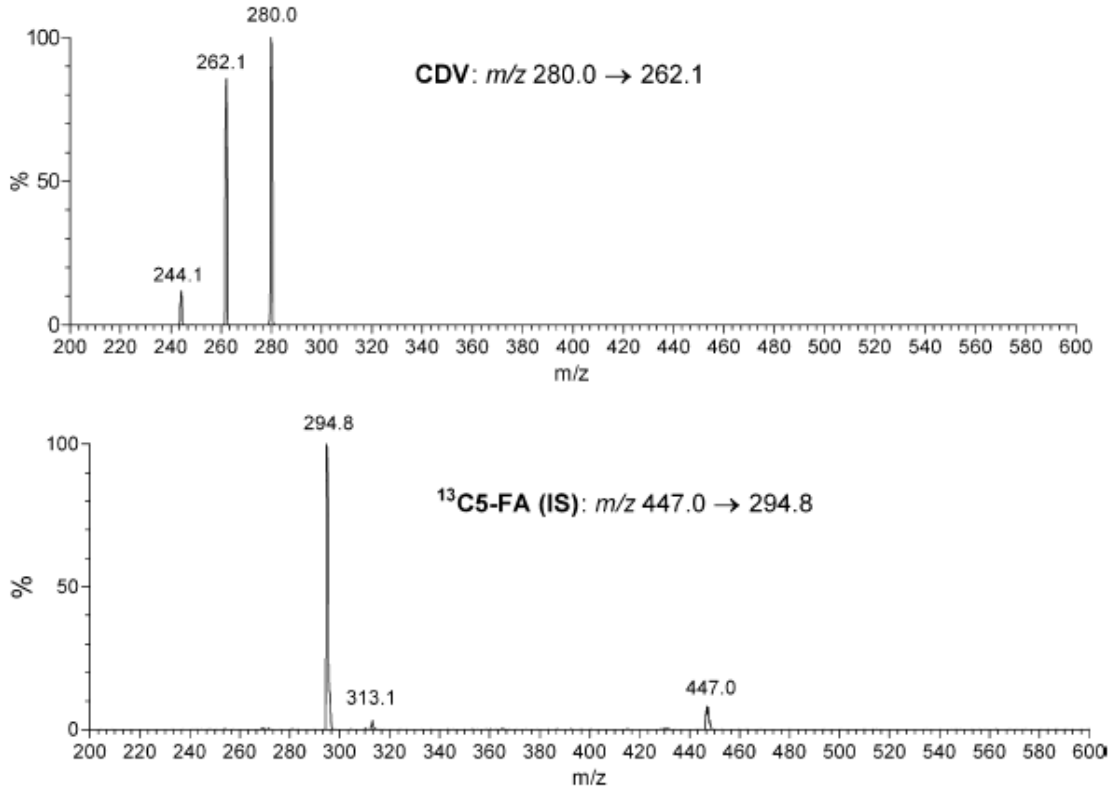


Figure 4. Representative positive ion electrospray ionization MS/MS spectrums for cidofovir (top) and ^{13}C5-FA (internal standard, bottom).

The retention times for cidofovir and ^{13}C5-FA were 2.1 min and 1.9 min, respectively, with a total run time of 5 min. Typical chromatograms of human blank plasma, plasma spiked with 20 ng/mL cidofovir, and a clinical sample collected 30 min after initiation of 0.3 mg/kg intravenous cidofovir infusion are shown in Figure 5.

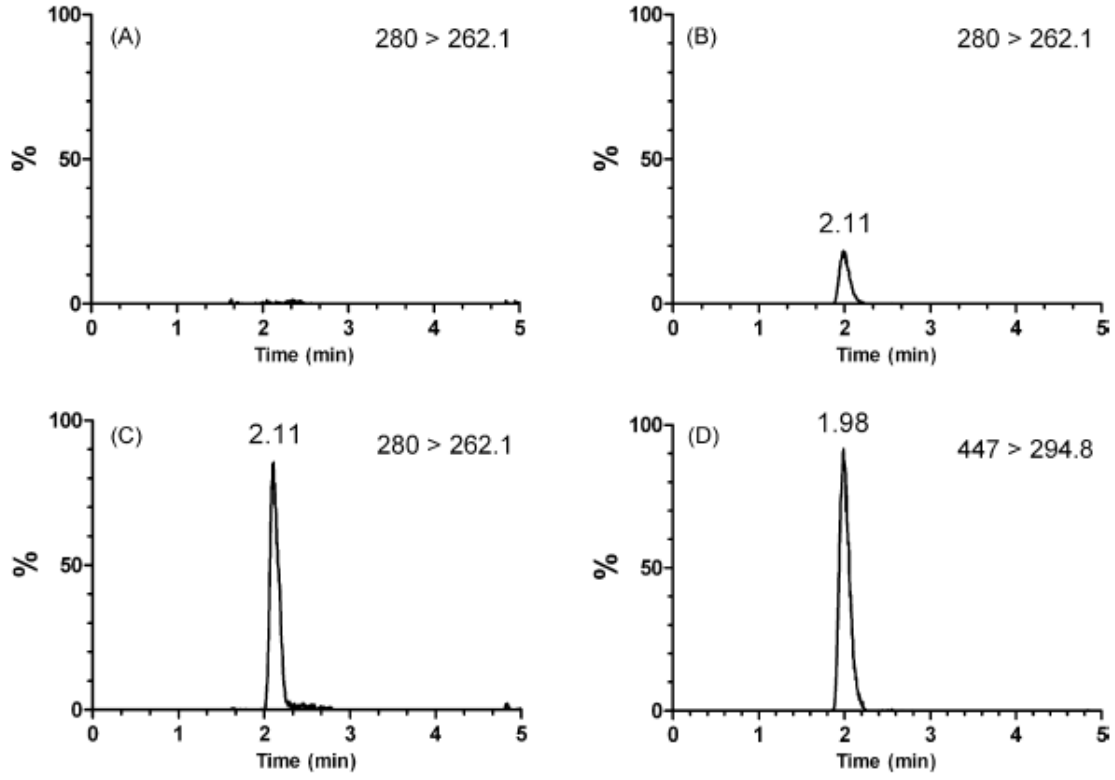


Figure 5. Representative MRM chromatograms of cidofovir and 13C5-FA in human plasma

(A) Blank human plasma without cidofovir. (B) Cidofovir spiked 20 ng/mL in blank plasma. (C) Clinical plasma sample 30 min after initiation of 0.3 mg/kg intravenous cidofovir infusion. (D) Internal standard spiked blank plasma sample.

2.4.2 Calibration curve and lower limit of quantitation

Triplicate standard curves were performed in plasma on five sequential days. The ratio of peak area of cidofovir to ^{13}C5-FA was linearly related to the concentration of cidofovir in the concentration range of 20 - 1000 ng/mL in plasma. A regression coefficient of > 0.99 was obtained in all analytical runs, with an equation of $y = 0.0016x + 0.0002$, where x = cidofovir concentration in ng/mL and y = cidofovir area/IS area. The LLOQ was 20 ng/mL using a plasma volume of 400 μL, at a signal-to-noise ratio of > 10. The individual values for the mean of the back-calculated values at each nominal concentration used in the standard curve and the accuracies calculated from those values are displayed in Table 6.

Table 6. Assay performance data of the calibration samples for cidofovir in human plasma.

Nominal concentration (ng/mL)	Mean measured concentration (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
20	21.5	107.5	3.7	7.3
50	50.8	101.6	4.8	5.8
100	105.7	105.7	2.8	4.1
200	194.2	97.1	4.5	5.3
350	352.1	100.6	3.3	4.5
700	702.8	100.4	5.5	6.1
1000	992.1	99.2	3.6	4.1

2.4.3 Accuracy and precision

The accuracies for all tested concentrations should be within ± 15%, except for the LLOQ, in which case these parameters should not exceed 20%. The accuracies and intra- and inter-assay precisions for the tested concentrations (LLOQ, QCL, QCM, QCH) were all within these pre-defined acceptance criteria (Table 7).

Table 7. Assay performance data for the quantitation of LLOQ, QCL, QCM and QCH cidofovir concentrations in human plasma.

Plasma concentration (ng/mL)	Mean measured concentration (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
20 (LLOQ)	19.6	97.9	2.7	3.8
60 (QCL)	61.9	103.3	5.4	6.2
400 (QCM)	422.8	105.7	4.9	6.8
800 (QCH)	812.8	101.6	4.1	5.6

2.4.4 Selectivity and specificity

To test for interference, 6 different sources of plasma were analyzed as blanks and after addition of cidofovir at the LLOQ (20 ng/mL). The responses in blank plasma were always less than 5% of the signal at the LLOQ.

2.4.5 Recovery and ion suppression

The extraction recovery of cidofovir was determined by comparing the absolute response of an extract of control plasma to which cidofovir had been added after extraction with the absolute response of an extract of plasma to which the same nominal concentration of cidofovir had been added before extraction [215, 224]. Recovery data and relative response when tested for matrix effects are displayed in Table 8.

Table 8. Total and ion suppression recovery of cidofovir in human plasma.

Concentration (ng/mL) *n*=4	Total Recovery		Ion suppression relative recovery	
	Mean ± S.D. (%)	CV (%)	Mean ± S.D. (%)	CV (%)
60 (QCL)	53.7 ± 2.7	4.9	107.7 ± 4.2	3.8
400 (QCM)	54.9 ± 2.6	4.7	110.1 ± 9.5	8.4
800 (QCH)	51.5 ± 2.4	5.6	94.4 ± 4.6	4.9

2.4.6 Storage stability data

There was not a significant difference in the estimated concentrations of cidofovir in plasma samples maintained and analyzed under different stability conditions as compared to freshly spiked plasma samples (Table 9).

Table 9. Stability of cidofovir under varying conditions.

Storage condition	Concentration (ng/mL)		Stability (%)	CV (%)	Replicates
Stock solution 6 months 4 °C	1,000,000		97.4	4.1	3
Plasma 24 hr Ambient temp.	QCL	60	105.5	2.3	3
	QCM	400	96.4	3.3	3
	QCH	800	101.8	5.5	3
Plasma 3 freeze-thaw cycles - 80 °C	QCL	60	93.2	6.2	3
	QCM	400	92.2	5.8	3
	QCH	800	93.5	6.7	3
Plasma 1 month - 20 °C	QCL	60	104.7	3.7	3
	QCM	400	101.9	9.6	3
	QCH	800	101.1	3.6	3
Plasma 7 days 4 °C	QCL	60	105.7	3.3	3
	QCM	400	108.1	1.2	3
	QCH	800	103	0.9	3
Plasma 3 months - 80 °C	QCL	60	93.8	3.4	3
	QCM	400	94.3	5.5	3
	QCH	800	95.2	3.8	3

2.5 DISCUSSION

Cidofovir has shown promise in the management of BK virus infections in renal transplant recipients [65-67, 217]. Low doses of cidofovir (representing 5–10% of the FDA approved dose for CMV retinitis) are typically used in this population due to the drug's nephrotoxicity. Although an LC–MS/MS assay has been previously published, its suitability for clinical pharmacokinetic studies of low-dose cidofovir is decreased by the lack of a commercially available internal standard and insufficient sensitivity [223]. The method described in the current manuscript is adequately sensitive to characterize systemic drug exposure following low dose cidofovir administration and capable of being implemented in laboratories with standard LC–MS instrumentation. Additionally, the reported assay has been validated according to the most recent FDA guidelines.

Preliminary analysis of cidofovir was performed using both positive and negative ion modes. Enhanced sensitivity was observed with positive ionization and consequently selected for the assay. We next established a lack of endogenous interference in blank human plasma using HPLC and MS techniques. Additionally, because transplant patients take a regimen of immunosuppressive and anti-infective medications, plasma from kidney transplant recipients not administered cidofovir was tested and no interference was observed.

An anion exchange solid phase extraction procedure was used to process plasma samples. Hydrophilic molecules such as cidofovir typically have low extraction recoveries from biological matrices. Accordingly, total recovery from the plasma extraction procedure averaged 53%. However, the extraction method was consistent and allowed for minimal ion suppression. Extraction recovery of all tested internal standard candidates was inconsistent and therefore the IS was added before injection to adjust for variation of LC–MS/MS analysis.

Separation of cidofovir from other components in plasma was performed using an analytical column with an isocratic profile. The selected column and mobile phase provided the most well separated and sharp peaks. Numerous compounds were evaluated as potential internal standards, including other nucleotide analogs and lipid ester analogs of cidofovir. However, ^{13}C5-FA provided the most consistent response under the conditions utilized in this method. It also eluted close to the analyte of interest and facilitated a short run time.

In pharmacokinetic studies plasma samples are stored at −20 or −80 °C until analysis and exposed to various temperatures during assay procedures. As a result, it was necessary to understand the stability of cidofovir at the conditions that samples would be subjected to prior to analysis. Stability was determined by the comparison of estimated concentrations of fresh samples to samples kept for 24 h at room temperature, 7 days at 4 °C, 1 month at −20 °C, and 3 months at −80 °C. Different sample processing conditions did not affect estimated cidofovir concentrations indicating stability under the conditions evaluated.

The majority of an intravenous dose of cidofovir is excreted unchanged in the urine. Therefore, in some instances it may be necessary to determine concentrations in urine samples in order to evaluate pharmacokinetic properties in urine. We applied the present method to both spiked blank urine (obtained from healthy volunteers) and clinical samples collected in a pharmacokinetic study. In all cases, large variability (>25%) was observed in the matrix effect in urine from different subjects. Given that cidofovir concentrations in urine are generally substantially higher than in plasma, previously published HPLC–UV methods can be used to readily quantitate cidofovir in urine.

In summary, we have developed and validated a sensitive LC–MS/MS method for quantitative assessment of cidofovir in human plasma that is useful in clinical pharmacokinetic studies in renal transplant recipients treated with low dose cidofovir.

3.0 ANIONIC TUBULAR SECRETION CAPACITY IN KIDNEY TRANSPLANT RECIPIENTS WITH PERSISTENT BK VIREMIA: CLINICAL PHARMACOKINETICS OF LOW-DOSE CIDOFOVIR WITHOUT AND WITH CONCOMITANT PROBENECID ADMINISTRATION

3.1 ABSTRACT

Transporters in the kidney mediate active tubular secretion of endogenous compounds and drugs. However, the functional activity of renal drug transporters involved in the movement of anionic drugs across the basolateral membrane of the proximal tubule has not been evaluated in kidney transplant patients. Therefore, we investigated the disposition of cidofovir, a prototypical organic anion transporter substrate used in the management of BK virus infection, in this patient population. We assessed the contribution of renal secretion, mediated by OAT1, to the overall clearance of cidofovir by evaluating the effect of probenecid, an inhibitor of anion transport, on the pharmacokinetics of cidofovir in 10 kidney transplant patients infected with BK virus. The plasma concentration of cidofovir declined with an overall disposition half-life of 5.1 ± 3.3 hr and 5.3 ± 2.9 in the absence and in the presence of probenecid, respectively ($p>0.05$). Approximately 60% of the intravenous dose was recovered unchanged in the urine in 12 h, irrespective of whether probenecid was concomitantly administered. Co-administration of oral probenecid had no significant effect on the non-compartmental pharmacokinetics or population pharmacokinetics of cidofovir in kidney transplant recipients. These data suggest that probenecid-sensitive active tubular secretion does not contribute significantly to the clearance of low-dose cidofovir in kidney transplant patients and that OAT1-mediated tubular secretion may potentially be impaired in kidney transplant recipients with BK virus infection.

3.2 INTRODUCTION

BK virus (BKV) is a human polyomavirus that is associated with premature graft failure in immunosuppressed renal transplant recipients [14, 17, 225, 226]. BKV carries a substantial disease burden, with an estimated incidence of viruria, viremia, and nephropathy of 35-40%, 11-13%, and 5-8%, respectively [30, 31]. Optimal therapeutic interventions for BKV have yet to be elucidated and a firmly established antiviral therapy is lacking.

Cidofovir (VISTIDE®) is a nucleotide analog of deoxycytidine monophosphate with *in vitro* and *in vivo* activity against herpesviruses, adenoviruses, poxviruses, and polyomaviruses [49, 214-216]. A low-dose cidofovir regimen of 0.25–1.0 mg/kg weekly is often empirically used at for the management of BKV infection in kidney transplant recipients. However, the literature on the clinical efficacy of cidofovir for BKV is conflicting, with some studies reporting apparent stabilization of renal function [64, 90], while others describe no discernible benefit [68, 89]. These contradictory data may reflect the absence of a clearly defined pharmacokinetic-pharmacodynamic relationship, which could be utilized to improve the dosing regimen of cidofovir in the treatment of BK virus infections.

The disposition of cidofovir has been investigated in healthy subjects, patients infected with human immunodeficiency virus, and patients with varying degrees of renal insufficiency [73, 74, 218, 227]. In all instances, tubular secretion plays a significant role in cidofovir clearance, as renal clearance is 60-70% higher than baseline creatinine clearance. Further *in vitro* studies have demonstrated that secretion of cidofovir involves human organic anion transporter 1 (OAT1) mediated basolateral uptake into renal proximal tubule cells in a saturable, probenecid-sensitive manner (Figure 6) [77]. However, the functional activity of OAT1 has not been evaluated in kidney transplant recipients with BK virus infection.

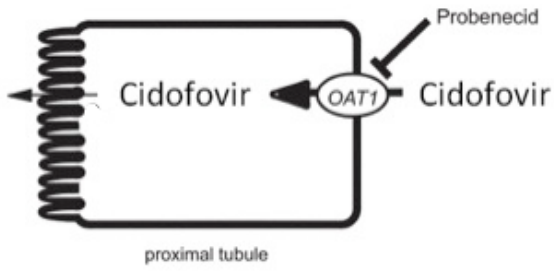


Figure 6. Model of probenecid-sensitive cidofovir transport in human proximal tubule cells

Therefore, the present study was conducted to (1) determine the pharmacokinetics of cidofovir in kidney transplant recipients with BKV infection in order to allow for future investigations into exposure-response relationships, and (2) assess the anionic renal secretion capacity in kidney transplant patients by evaluating the effect of probenecid on the systemic and renal clearance of cidofovir.

3.3 MATERIALS AND METHODS

3.3.1 Patients

This study was performed in ten adult renal transplant recipients who were diagnosed with BKV infection and received treatment with low-dose cidofovir after failing to respond to a two-week period of reduced immunosuppression. The protocol was approved by the Institutional Review Board of the University of Pittsburgh (IRB# 08060393) and written informed consent was obtained from all patients prior to participation in this study. Exclusion criteria included: (i)

hypersensitivity to cidofovir or other nucleotide analogs, (ii) hypersensitivity to probenecid or sulfonamides, (iii) currently receiving another drug known to affect renal anionic drug secretion, (vi) pregnancy or breastfeeding women.

3.3.2 Study design

Patients were studied on two separate occasions in a crossover design. In Part 1, the pharmacokinetic parameters of intravenous low-dose cidofovir were evaluated. In Part 2, following a one-week washout period, the study procedures were repeated with concomitant oral probenecid administration. Probenecid (2g) was given 1 h prior to cidofovir administration and again at 2 h and 8 h (1g each) after the completion of the cidofovir infusion. On both occasions, patients received 1 L of 0.9% sodium chloride immediately prior to cidofovir administration. Cidofovir was diluted in 100 mL of 0.9% sodium chloride and infused over 1 hr. A diagram of the study design is represented in Figure 7.

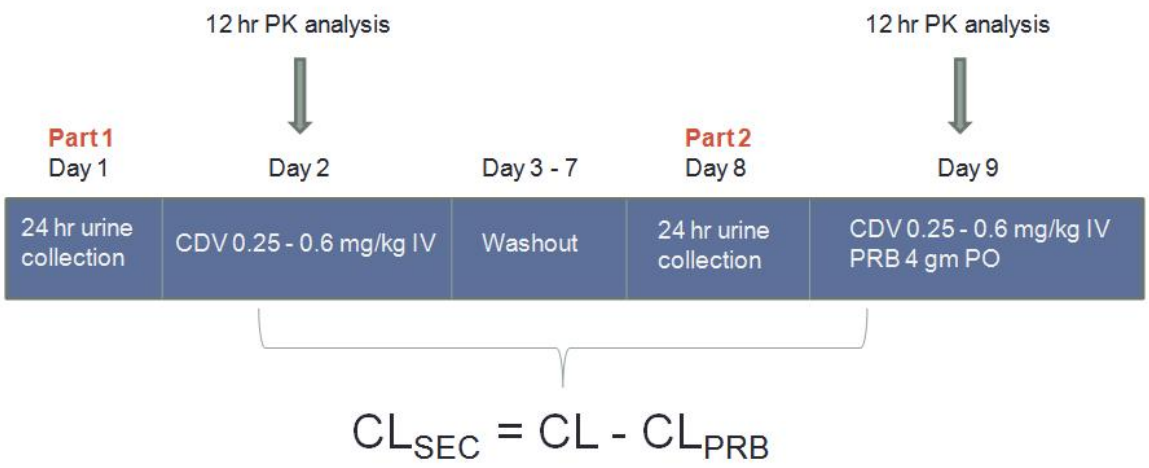


Figure 7. Schematic of the cidofovir pharmacokinetic study design

CL, clearance of cidofovir in the absence of probenecid; CL_{PRB}, clearance of cidofovir in the presence of probenecid; CL_{SEC}, clearance of cidofovir attributed to OAT1-mediated active secretion

3.3.3 Blood and urine sampling

Blood samples (7 mL) were collected in Vacutainers at 0, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after starting the cidofovir infusion. Plasma was separated and frozen at – 80 C until analysis. Urine was collected in aliquots from 0-1, 1-2, 2-4, 4-8, and 8-12 hours after the start of the cidofovir infusion and stored at – 80 C until analysis.

3.3.4 Analytical methodology

The concentrations of cidofovir in plasma were determined by the liquid chromatographic-mass spectrometric method described in Chapter 2. Briefly, plasma samples were processed by an anion exchange solid phase extraction procedure and chromatography was performed using a Luna C8(2) analytical column, 5 µm, 150mm×3.0mm, with isocratic elution. Cidofovir was detected by a triple quadrupole mass spectrometer in positive electron spray ionization mode using multiple reaction monitoring with ^{13}C5-Folic acid as the internal standard.

Cidofovir concentrations in urine samples were determined using high-performance liquid chromatography (HPLC) with UV detection at 274 nm. Chromatography was performed with a Waters 2695 separations module and Waters 2998 photodiode Array Detector set at 274 nm. The data acquisition was performed with Empower 3 Chromatography Data Software. Urine samples (100 µL) were mixed with 100 µL of mobile phase, consisting of 35% 1.5 mM of tetrabutylammonium dihydrogen phosphate and 3.5 mM of disodium hydrogenphosphate, 12% acetonitrile, and 53% water, and centrifuged at 14,000 rpm for 8 min. Fifty µL of the resulting supernatant was injected onto the HPLC. Chromatographic separation was achieved on a Symmetry 5 µm C18 column (250mm×4.6mm). The mobile phase was delivered isocraticly at 1.2 mL/min and the retention time of cidofovir was 6.5 min. Representative chromatograms of a blank urine sample spiked with cidofovir and a patient urine sample are displayed in Figure 8 and Figure 9, respectively.

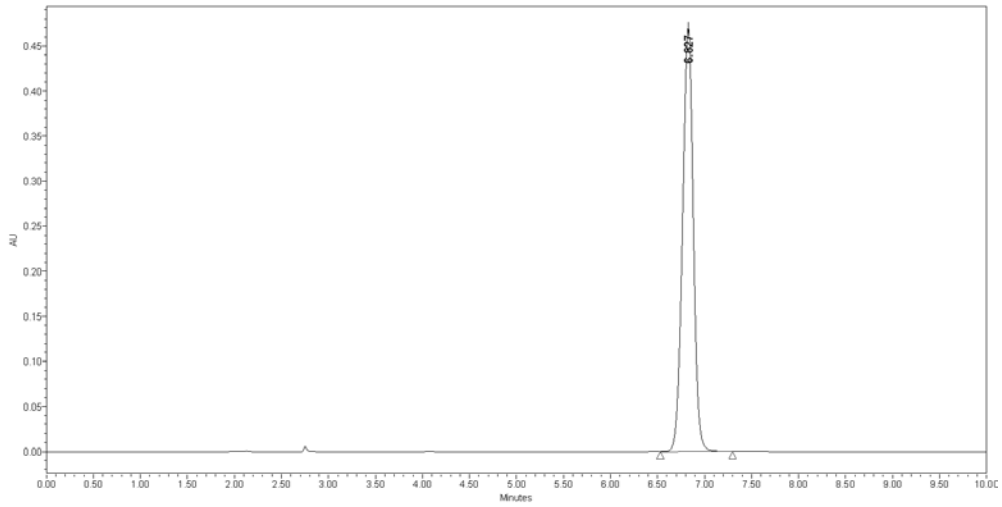


Figure 8. Representative chromatogram of blank human urine spiked with 50 µg/mL of cidofovir

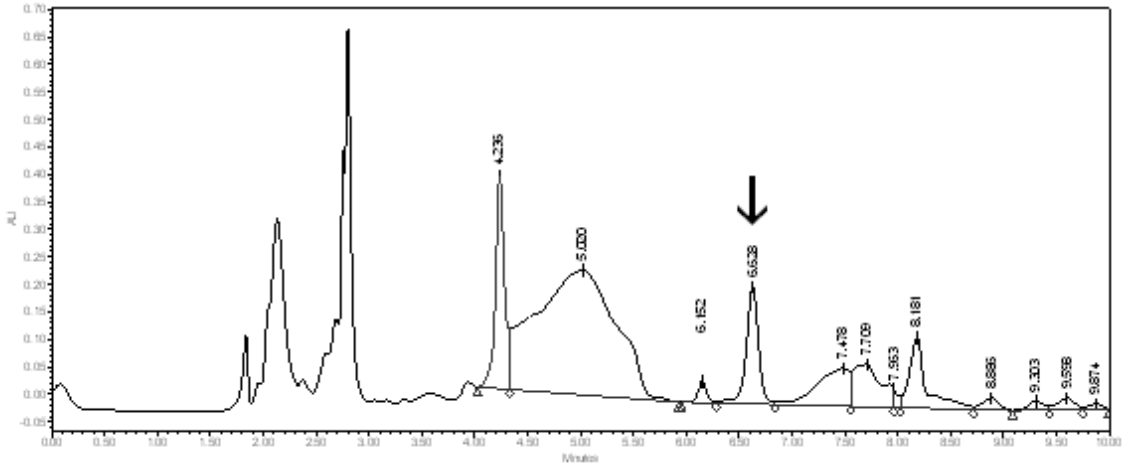


Figure 9. Representative chromatogram of a urine sample obtained from a patient following intravenous cidofovir administration.

The concentrations of probenecid in plasma were determined using high-performance liquid chromatography (HPLC) with UV detection at 242 nm. Chromatography was performed with a Waters 2695 separations module and Waters 2998 photodiode Array Detector set at 242 nm. The data acquisition was performed with Empower 3 Chromatography Data Software. Plasma samples (200 µL) were mixed with 20 µL of HCl (3N) and vortexed with 300 µL methanol for 3 min. The mixture was centrifuged for 8 min at 14,000 rpm and 100 µL of the resulting supernatant was injected onto the HPLC system, where chromatographic separation was achieved on a Symmetry 5 µm C18 column (250mm×4.6mm). The mobile phase, consisting of 0.4% ammonium acetate (solvent A) and acetonitrile (solvent B), was delivered at a gradient at 1 mL/min. The retention time of probenecid was 5.8 min. Representative standard and patient sample chromatograms are displayed in Figure 10 and Figure 11, respectively.

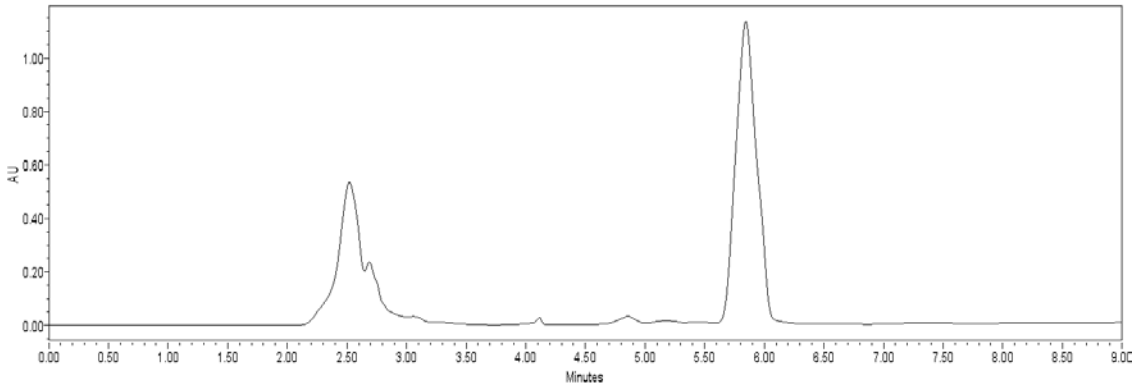


Figure 10. Representative chromatogram of blank human plasma spiked with 100 µg/mL of probenecid.

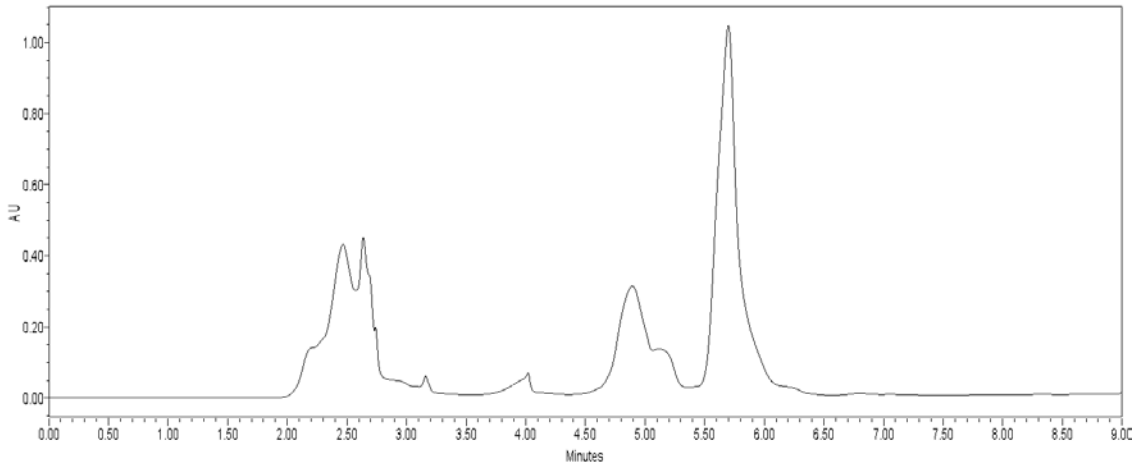


Figure 11. Representative chromatogram of a plasma sample obtained from a patient following oral administration of probenecid.

3.3.5 Noncompartmental pharmacokinetic and statistical analysis

Descriptive pharmacokinetic parameters for cidofovir were estimated by noncompartmental analysis (WinNonlin software, version 5; Pharsight Corp, Mountain View, CA). The maximum concentration in plasma (C_{max}) and the time to C_{max} (t_{max}) were estimated by visual inspection of the concentration versus time profiles. The terminal disposition rate constant (λ_z) 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 λ_z. The area under the plasma concentration-time profile from the time of dosing until infinity was calculated by the log-linear trapezoidal method with extrapolation beyond the last measured concentration, according to:

$$AUC_{0-\infty} = AUC_{0-12} + C_{12}/\lambda_z$$

Systemic clearance (CL) and the volume of distribution at steady state (Vss) were determined using the following equations:

$$CL = Dose / AUC_{0-\infty}$$

$$Vss = [(Dose)(AUMC_{0-\infty})/(AUC_{0-\infty})^2]$$

Renal clearance (CL_r) was calculated as $A_{e(0–12)}/AUC_{0–12}$, where A_e is the amount of drug recovered in the urine in 12 hours, and $AUC_{0–12}$ is the area under the plasma concentration versus time curve from 0 to 12 hours. The fraction eliminated unchanged in the urine (f_e) was calculated as the amount of drug recovered in the urine over the entire collection interval divided by the dose. Semi-logarithmic plots of the amount remaining to be excreted (A.R.E.) and the excretion rate were constructed and used to estimate the elimination rate constant (k_{el}), according to:

$$k_{el} = m = \ln(C_2/C_1)/(t_2 - t_1)$$

Results are reported as mean ± SD or SEM. Statistical comparisons between cidofovir pharmacokinetic parameters without and with probenecid were performed by a Student's paired t-test. The data were analyzed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). The threshold of statistical significance was set at 5% (α= 0.05).

3.3.6 Population pharmacokinetic analysis

The pooled dataset containing cidofovir plasma concentration-time data and the cumulative amount of cidofovir in the urine was used for modeling purposes. Plasma and urine data from all individuals were fitted simultaneously, using the first-order conditional estimation with interaction (FOCE INTERACTION) option in the nonlinear mixed-effects modeling program NONMEM, version VII (Globomax, Hanover, MD, USA). Discrimination between hierarchical models was based on the objective function value (OFV) provided by NONMEM at a significance level of 0.001, equal to a decrease of 10.8 in the OFV. Graphical analysis of residuals and predictions in model diagnostics were completed using R (version 2.1.2, Vienna, Austria). The population pharmacokinetic model for low-dose cidofovir in kidney transplant recipients was developed as follows:

Step 1: Development of the covariate-free model

Different structural pharmacokinetic models, including a one-compartment model and a two-compartment model with linear and nonlinear clearance, were tested during the model-building procedure. Pharmacokinetic parameters estimated with the model included total body clearance (CL), intercompartmental clearance (Q), volume of the central compartment (V1), volume of the peripheral compartment (V2), elimination rate constant (k), and

intercompartmental rate constants (k12, k21). The analysis allowed for both covariance between CL and V1 along with covariance between main parameters. The distribution of the parameters was assumed to be log-normal.

Interindividual variability was estimated using an exponential error model:

$$P_{ij} = TV(P_j) \times e^{\eta ij}$$

where P_{ij} is the i^{th} individual's estimate of the j^{th} pharmacokinetic parameter, $TV(P_j)$ is the typical value of the j^{th} pharmacokinetic parameter, and ηij is a random variable for the i^{th} individual and the j^{th} pharmacokinetic parameter distributed with a mean of zero and a variance of ωj^2.

The intraindividual variability was modeled using a mixed proportional and additive error model:

$$C_{obs} = C_{pred}\left(1 + \varepsilon_{ik}{}^{rel}\right) + \varepsilon_{ik}{}^{abs}$$

where C_{obs} and C_{pred} represent the observed and predicted cidofovir plasma concentration in i^{th} individual, respectively. The error terms ε^{rel} and ε^{abs} are the components of the proportional (relative error) and additive error (absolute error), respectively, and both are assumed to have a mean of zero and variance of σ^2. The adequacy of fitting was examined by plotting predicted versus observed concentrations (goodness of fit) and weighted residuals versus predicted concentrations.

Step 2: Incorporation of significant covariates

A total of fourteen covariates were considered in the analysis. Continuous variables included serum creatinine, creatinine clearance, blood urea nitrogen, weight, height, body surface area, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, and age. Patient sex and the absence of presence of comcomitant

probenecid administration were considered as categorical variables. Body surface area was calculated according to the Dubois equation as follows:

$$BSA\ (m^2) = 0.20247 \times Height(m)^{0.725} \times Weight(kg)^{0.425}$$

Covariates were tested using a forward inclusion and backward elimination approach. An initial analysis was conducted with estimates of the parameters of the two-compartment base model (i.e., without covariates). Potential covariates were added sequentially to the base model and the influence of these fixed effects was assessed with the objective function value (OFV) and the distribution of the weighted residuals. Changes in the OFV on the addition of one covariate approximate a χ^2 distribution with 1 degree of freedom (df). A patient variable was considered significant if all the following criteria were met: (i) a decrease in objective function value (OFV) of 6.63 for 1 degree of freedom ($p<0.01$), (ii) improved goodness of fit, (iii) reduced interindividual variability, and (iv) clinical plausibility for incorporation of the variable.

Precision of parameter estimation, stability of the covariate models, and normality of the distribution of the parameter estimates were evaluated using bootstrapping (resampling repeated 3,500 times) using Wings for NONMEM (http: //wfn.sourceforge.net). Nonparametric statistics (median and 95% confidence interval) of parameter estimates were obtained from bootstrapping.

3.4 RESULTS

3.4.1 Patient demographics

Patient characteristics are summarized in Table 10. All enrolled patients completed the study, with the exception of one subject who completed only Part 1 secondary to difficulty in obtaining IV access during Part 2. On average, the study participants were 55.7 ± 11.8 years of age, weighed 84.4 ± 20.3 kg, and were 13.1 ± 15.7 months post-kidney transplantation. Participants had received a median of 3 doses of cidofovir prior to enrollment in the study. All of the subjects had a reduced estimated glomerular filtration rate (eGFR), ranging from mild to severe, though none of the patients were on dialysis at the time of the study. A total of 9 subjects were Caucasian and one subject was African American. All patients had BK viremia and viruria, though none had evidence of tubulointerstitial nephropathy. The median BK viral loads in plasma and urine were 3.5 (range: 2.7 – 6.1) $\log_{10}$ copies/mL and 6.2 (range: 4.7 – 9.3) $\log_{10}$ copies/mL, respectively. The majority of patients (9/10) were on a tacrolimus-based immunosuppressive regimen, and one patient was on a cyclosporine-based regimen. Prophylactic regiments taken by most patients included valganciclovir and sulfamethoxazole-trimethoprim. Other medications commonly used to treat underlying medical conditions included antihypertensives (metoprolol, amlodipine), proton pump inhibitors (omeprazole, pantoprazole), antihyperlipidemic agents, and antidepressants. No patients were taking medications that are known to be eliminated via tubular secretion aside from cidofovir.

Table 10. Patient characteristics.

Patient	Sex	Age (years)	Graft age (months)	Body Weight (kg)	Serum Creatinine (mg/dL)	eGFR[a] (mL/min/1.73m^2)	Cidofovir dose (mg)	Cidofovir dose (mg/kg)	# of doses prior to enrollment
1	F	43	12.6	74.1	0.9	68	20	0.27	3
2	M	29	5.4	111.3	1.8	45	27	0.24	3
3	M	59	10.0	85.6	1.8	39	22	0.26	3
4	M	53	56.2	64.7	3.7	17	40	0.62	15
5	F	59	2.9	59.7	1.1	51	20	0.34	7
6	M	63	13.6	75.3	1.0	75	22	0.29	3
7	M	67	2.8	120.0	1.5	45	35	0.29	2
8	M	69	4.4	91.1	1.3	55	35	0.38	4
9	F	58	9.7	97	1.5	43	23	0.24	2
10	F	57	13.4	65.7	1.2	46	20	0.30	3
Mean (SD)		55.7 (11.8)	13.1 (15.7)	84.4 (20.3)	1.58 (0.8)	48.4 (15.8)	26.4 (7.5)	0.32 (0.11)	4.5 (3.9)

[a] Estimated glomerular filtration rate, calculated by the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation

3.4.2 Pharmacokinetics of cidofovir

3.4.2.1 Non-compartmental analysis

Linear plots of cidofovir plasma concentration versus time profiles without and with concomitant probenecid in each patient are displayed in Figure 12. The concentration-time curves were virtually superimposable, suggesting probenecid-insensitive cidofovir elimination. A positive linear relationship ($r^2 = 0.64$) was observed between cidofovir dose and the maximum plasma concentration (Figure 13).

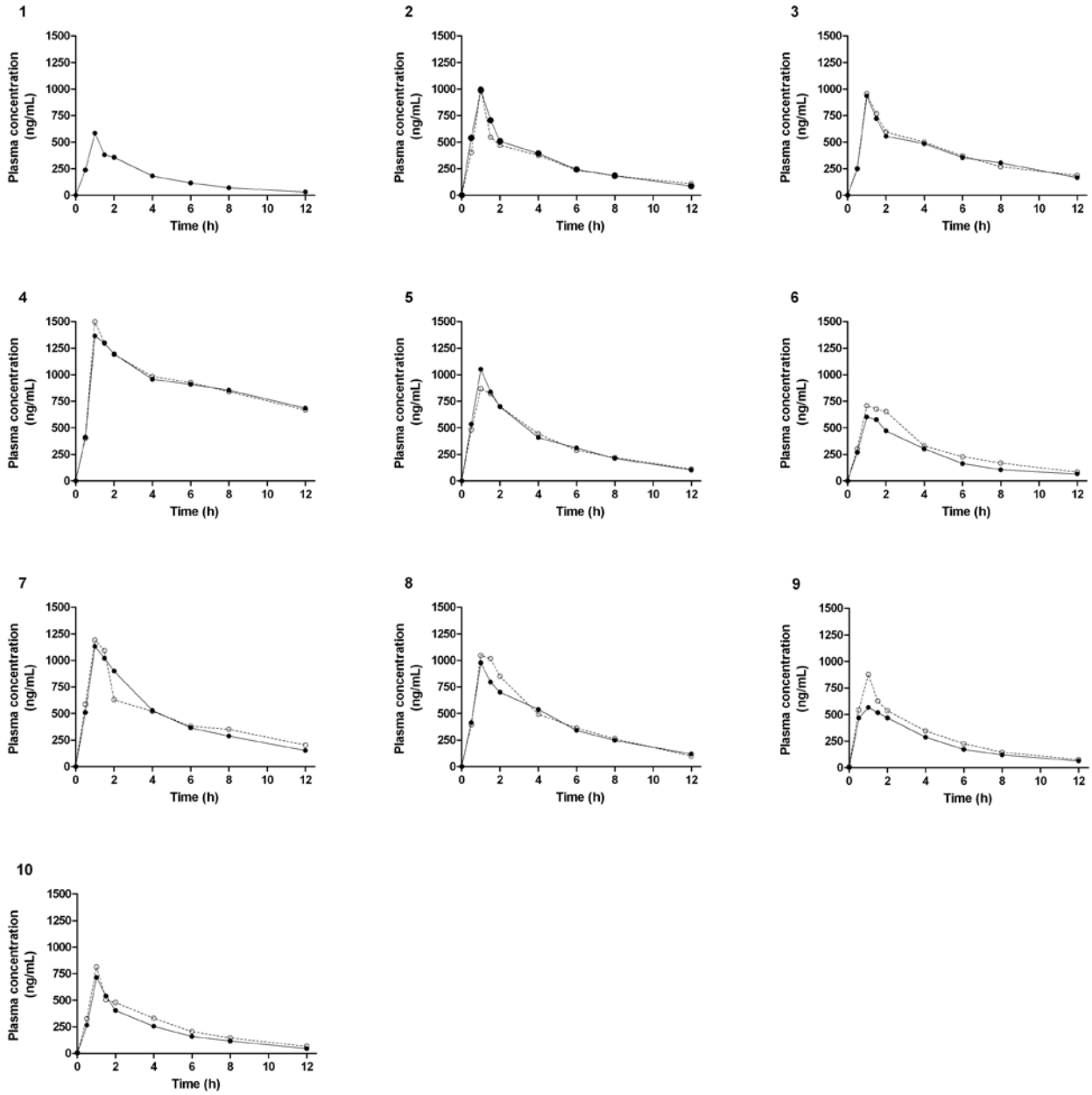


Figure 12. Observed cidofovir plasma concentrations following intravenous administration with a 1 h infusion without (●) and with (○) concomitant oral probenecid in ten individual renal transplant recipients.

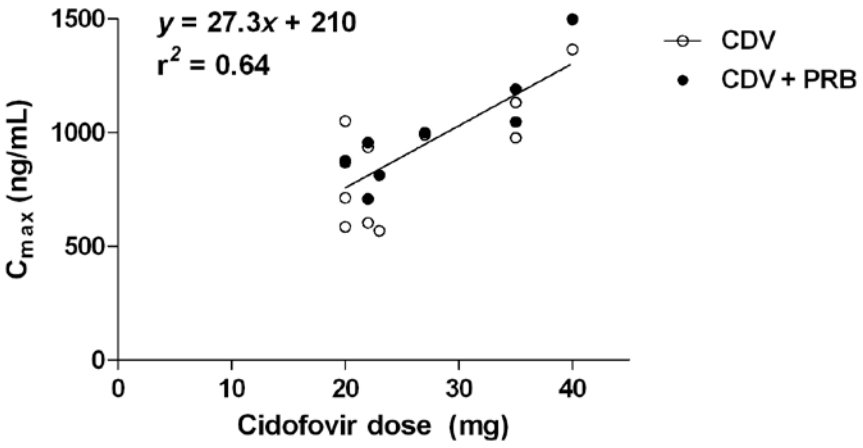


Figure 13. Relationship between cidofovir dose and the maximum plasma concentration.

CDV, cidofovir alone; CDV + PRB, cidofovir with concomitant probenecid

The percentage of the cidofovir dose eliminated unchanged in the urine from 0 to 12 hours was not significantly different when probenecid was concurrently administered (Figure 14; data presented as mean +/- SEM).

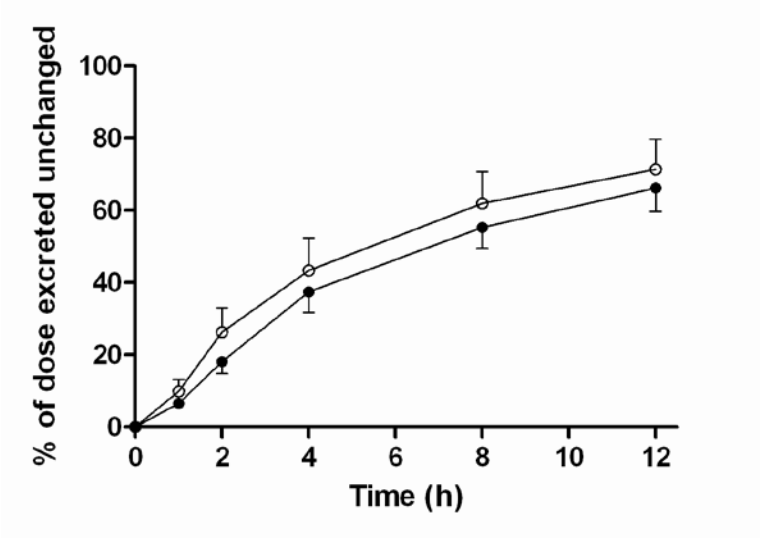


Figure 14. The percentage of the cidofovir dose excreted unchanged in the urine from 0 to 12 hours without (●) and with (○) concomitant oral probenecid.

A strong positive correlation was observed between cidofovir clearance and estimated GFR irrespective of probenecid administration (r^2 = 0.75 without probenecid; r^2 = 0.71 with probenecid). Linear regression of systemic cidofovir clearance versus estimated glomerular filtration rate is presented in Figure 15. The regression equations describing these relationships are: $y = 1.3x + 13.6$ (in the absence of probenecid); $y = 1.02x + 15.04$ (in the presence of probenecid).

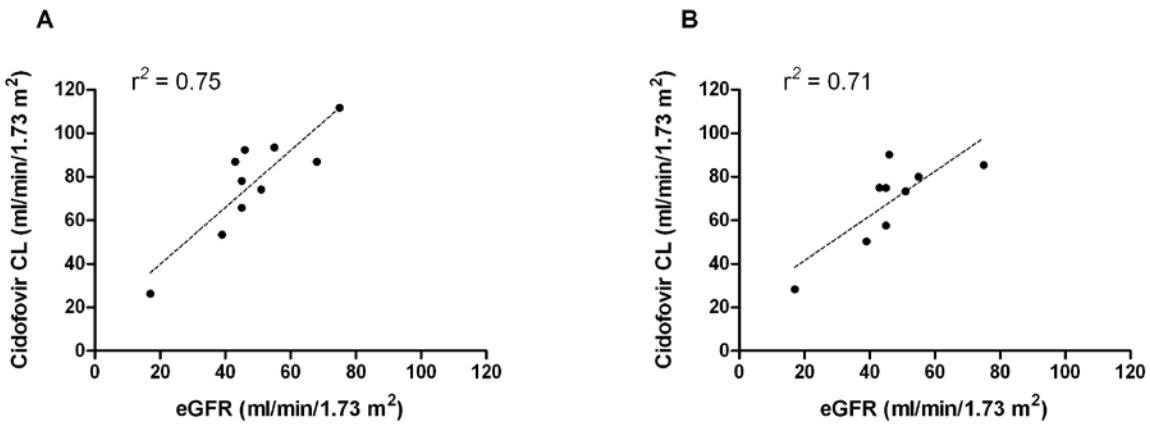


Figure 15. Relationship between systemic cidofovir clearance and estimated glomerular filtration rate without (A) and with (B) concomitant probenecid.

The urinary excretion rate plots and the amount remaining to be excreted plots used to calculate the cidofovir urinary excretion rate constant (k_{el}) are displayed below in Figure 16 and Figure 17, respectively.

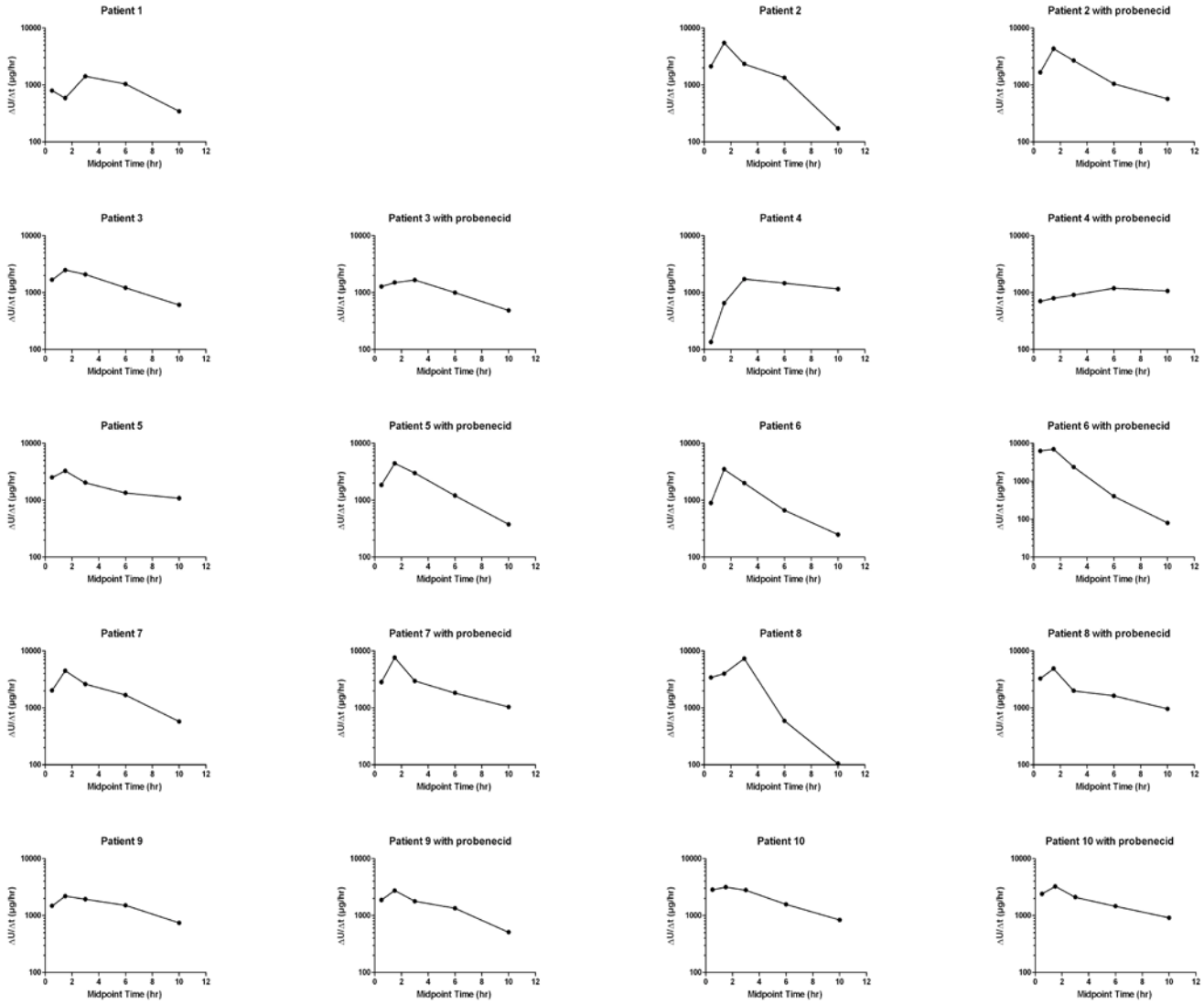


Figure 16. Semi-log plots of ΔU/Δt versus time$_{midpoint}$

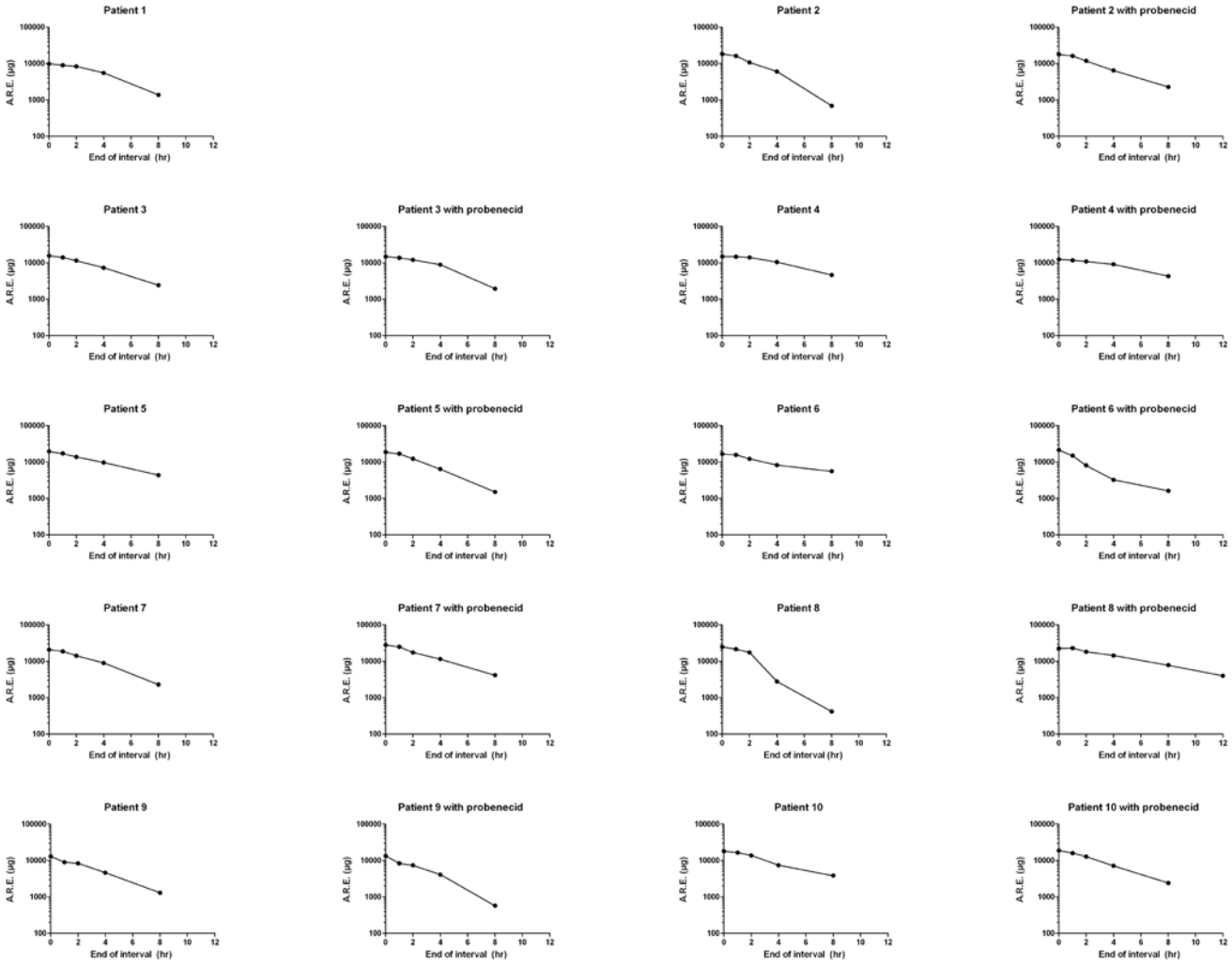


Figure 17. Semi-log plots of the amount of cidofovir remaining to be excreted (A.R.E) versus the end of the urine collection interval.

A summary of the model-independent pharmacokinetic parameters for intravenous cidofovir is presented in Table 11. Pharmacokinetic parameters in plasma (C_{max}, t_{max}, λ_z, $AUC_{0\infty}$, V_{ss}, CL) and urine (Cl_R, fe) were not significantly different when probenecid was simultaneously administered ($p > 0.05$). The distribution of calculated noncompartmental cidofovir clearance values without and with probenecid is displayed in Figure 18.

Table 11. Non-compartmental pharmacokinetic parameters of low-dose cidofovir in kidney transplant recipients without and with concomitant probenecid.

	Cidofovir (*n*=10)	Cidofovir + probenecid (*n*=9)	*p**
Plasma			
Cmax (ng/mL/mg)	33.8 ± 8.3	37.9 ± 5.2	0.17
Tmax (hr)	1.0 ± 0	1.0 ± 0	-
AUC (ng*hr/mL/mg)	223.8 ± 144.8	240.9 ± 131.4	0.31
λ_z (1/hr)	0.16 ± 0.04	0.15 ± 0.05	0.57
$t_{½}\ \beta$ (hr)	5.1 ± 3.3	5.3 ± 2.9	0.71
V_{ss} (L)	32.8 ± 5.2	30.6 ± 6.1	0.49
V_{ss}/BW (L/kg)	0.41 ± 0.09	0.36 ± 0.05	0.33
CL (mL/min)	84.7 ± 27.1	80.4 ± 24.8	0.58
CL/BW (mL/min/kg)	1.03 ± 0.37	0.96 ± 0.29	0.47
Urine			
CL_R (mL/min)	62.1 ± 9.14	57.5 ± 9.78	0.68
CL_R/BW (mL/min/kg)	0.74 ± 0.13	0.64 ± 0.11	0.58
fe	0.66 ± 0.06	0.71 ± 0.08	0.63
k_{el} (l/hr) (excretion rate)	0.12 ± 0.2	0.13 ± 0.3	0.37
k_{el} (l/hr) (A.R.E.)	0.1 ± 0.3	0.09 ± 0.2	0.41

* calculated from two-tailed student's paired t-test

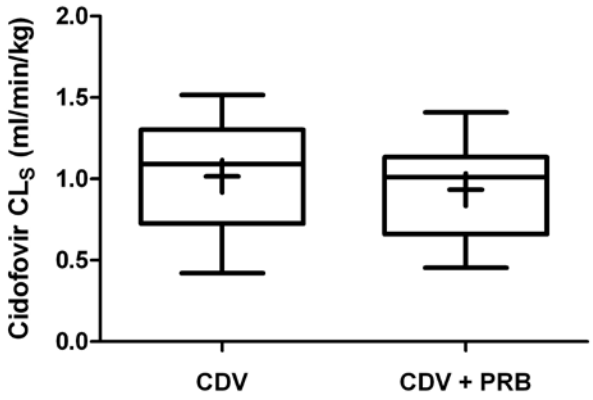
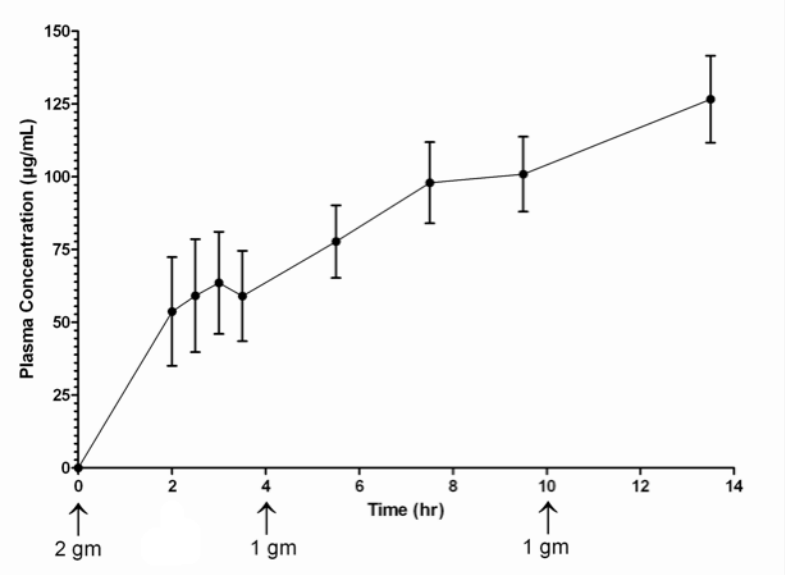


Figure 18. Box plots displaying the distribution of cidofovir clearance values, stratified according to the absence or presence of concomitant probenecid administration.

The plots display the 10th, 25th, 75th, and 90th percentiles. The median and the mean are denoted by a horizontal line and a plus sign, respectively.

Considerable systemic probenecid exposure was observed in all subjects following oral dosing with an average $AUC_{0\text{-}14}$ of 1109 ± 171.3 µg*hr/mL. One subject was incorrectly administered 4 gm of probenecid at time 0 rather than the correctly spaced regimen. The plasma probenecid concentration versus time profiles for the patients who received the correct probenecid dosing schedule (*n*=8) and the patient who received the incorrect probenecid dosing schedule are shown in Figure 19. In both instances the probenecid dosing regimen is delineated beneath the plot. Data are presented as mean ± SEM. Blood and urine sampling were not performed during the probenecid elimination phase and thus a full pharmacokinetic profile is not reported.

A.



B.

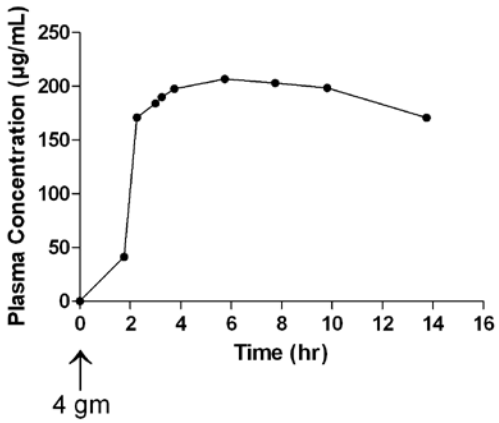


Figure 19. Plasma probenecid concentration versus time profiles following oral administration.

A) Probenecid (2g) given 1 h prior to cidofovir administration and again at 2 h and 8 h (1g each) after the completion of the cidofovir infusion (*n*=8)
B) Probenecid (4g) given 1 h prior to the cidofovir administration (*n*=1)

3.4.2.2 Population pharmacokinetic analysis

Base model

A two-compartment model with first-order elimination (ADVAN 3 subroutine in NONMEM) was chosen as the final model for the description of the plasma cidofovir concentration-time course as it best described the data as compared to a one-compartment mode (Figure 20). In the two-compartment model, population predictions and individual predictions agreed well with observations (r^2 =0.96)

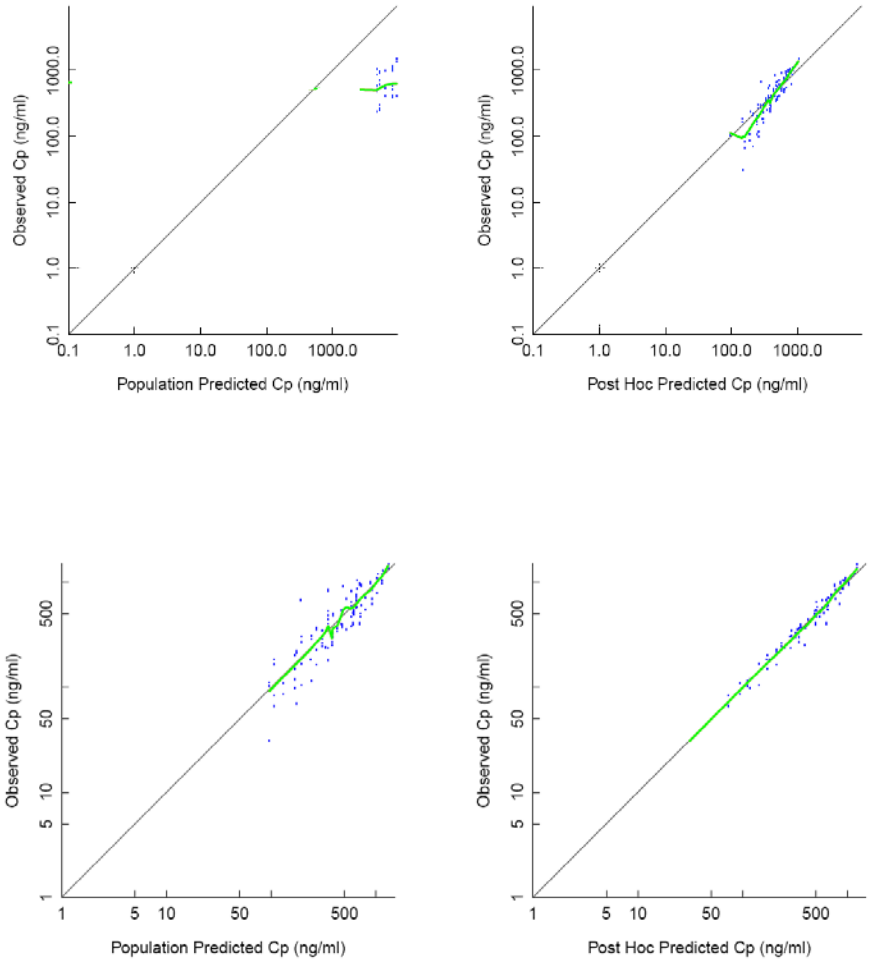


Figure 20. Goodness-of-fit of a one-compartment model (top) and a two-compartment model (bottom) for plasma cidofovir concentrations.

The structural pharmacokinetic model used the following parameters: clearance (CL), the volume of the central compartment (V1), intercompartmental clearance (Q), and the volume of the peripheral compartment (V2). The median cidofovir clearance for the study population was estimated to be 5.75 liters/h and the volume of the central compartment was 28.7 liters. Median estimates for the intercompartmental clearance and volume of distribution of the peripheral compartment were 2.06 liters/h and 5.48 liters, respectively. All pharmacokinetic parameters were precisely estimated, with relative standard errors (RSEs) of < 3%. Interindividual variabilities were estimated to be 47% for CL, 21% for the volume of the central compartment, 58% for the volume of the peripheral compartment, and 58% for intercompartmental clearance.

Covariate model

Exploratory graphical analyses revealed a direct correlation between cidofovir clearance and various markers of renal function (serum creatinine and estimated creatinine clearance) and body surface area (Figure 21). Intercompartmental clearance (in liters per hour) and the volume of the central compartment (in liters) were correlated with body weight (Figure 22) (Figure 23). There were no obvious relationships between the volume of the peripheral compartment and any of the tested covariates. Additionally, no significant association was found between estimates of cidofovir pharmacokinetics and administration of probenecid. The individual concentration versus time profiles with observed concentrations, population predictions, and individual predictions are displayed in Figure 24.

The final model for cidofovir clearance was determined to be:

$$\mathrm{CL} = 5.47 \times \left(\frac{\mathrm{CRCL}}{67.7}\right)^{1.12}$$

$$\mathrm{CL} = 5.70 - 1.94 \times (\mathrm{SCR} - 1.43)$$

$$\mathrm{CL} = 5.00 + 0.11 \times (\mathrm{BSA} - 2.02)$$

where CL = cidofovir clearance (in liters per hour), CRCL = estimated creatinine clearance, SCR = serum creatinine, and BSA = body surface area.

Both intercompartmental clearance and the volume of the central compartment were determined to be functions of body weight, as follows:

$$\mathrm{Q} = 1.21 \times \left(\frac{\mathrm{WT}}{87.6}\right)^{2.18}$$

$$\mathrm{V1} = 27.8 \times \left(\frac{\mathrm{WT}}{87.6}\right)^{0.40}$$

where WT = body weight in kilograms.

Population estimates of the pharmacokinetic parameters in the base model and final model are presented in Table 12 and a summary of the population pharmacokinetic modeling process is displayed in Table 13.

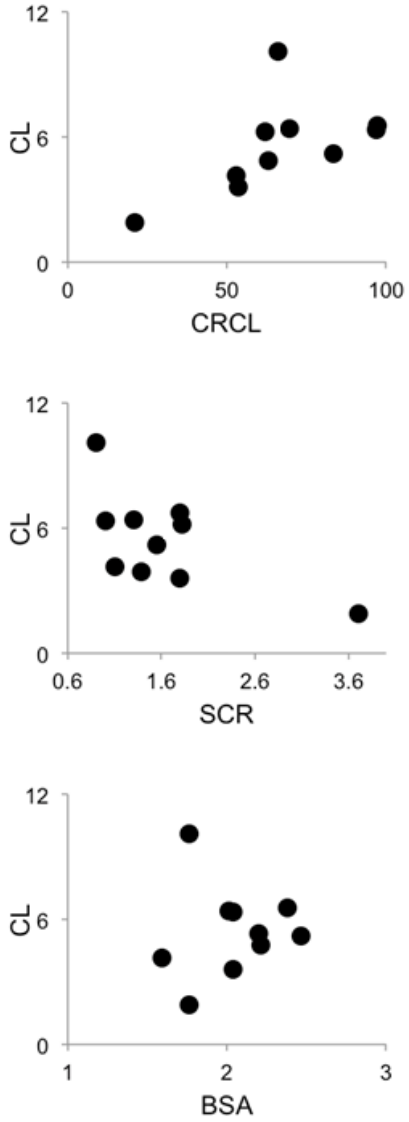


Figure 21. Correlation between apparent total body clearance of cidofovir and creatinine clearance, serum creatinine, and body surface area

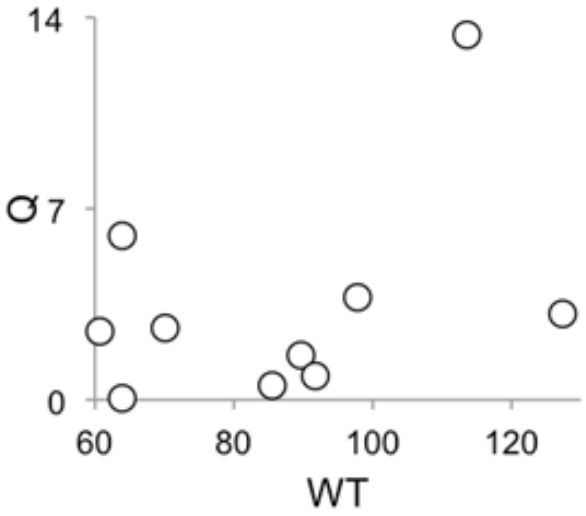


Figure 22. Correlation between intercompartmental clearance and body weight

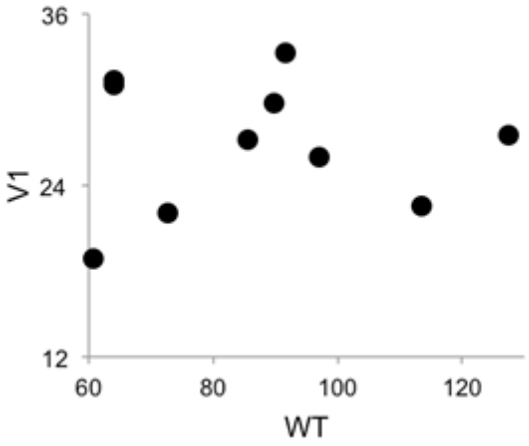


Figure 23. Correlation between the volume of the central compartment and body weight

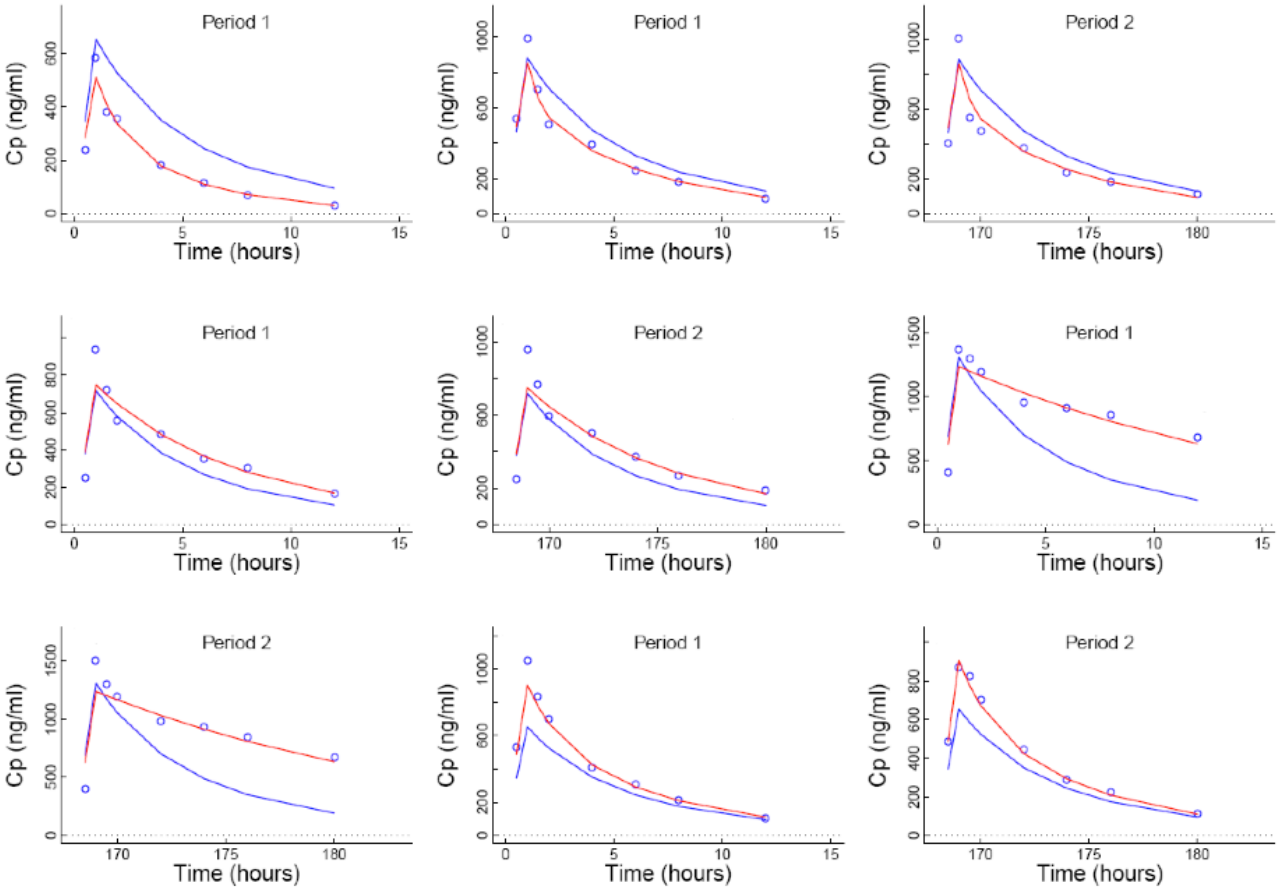


Figure 24. Individual plasma concentration versus time profiles for cidofovir following intravenous administration characterized by a two compartmental model without (Period 1) and with (Period 2) concomitant probenecid.

Note: circles represent observed concentrations; red solid line represents individual predicted concentrations; blue solid line represents population predicted concentrations

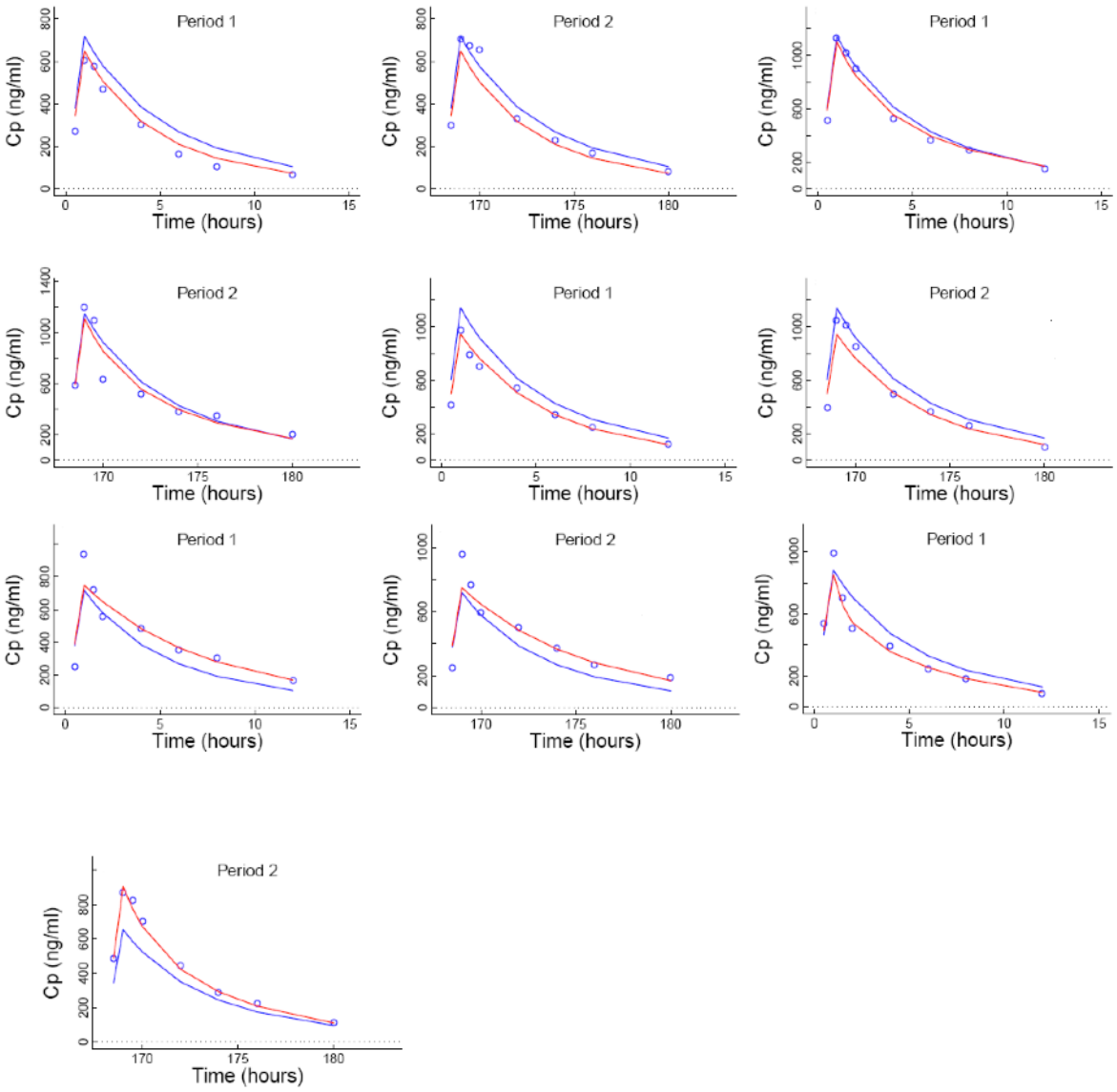


Figure 24 (cont). Individual plasma concentration versus time profiles for cidofovir following intravenous administration characterized by a two compartmental model without (Period 1) and with (Period 2) concomitant probenecid.

Note: circles represent observed concentrations; red solid line represents individual predicted concentrations; blue solid line represents population predicted concentrations

Table 12. Pharmacokinetic parameter estimates and associated inter-individual variability

					Inter-individual variability (%)				Residual error	
Population estimates	CL	Q	V1	V2	CL	Q	V1	V2	proportional	additive (µg/mL)
base model	5.75	2.06	28.7	5.48	47	58	21	58	14	0.003
final model	5.31	2.52	28.5	9.69	22	46	18	18	13	0.003

CL= apparent total body clearance of cidofovir; Q= intercompartmental clearance; V1=apparent distribution volume of the central compartment; V2=apparent distribution volume of the peripheral compartment.

Table 13. Summary of population pharmacokinetic model building process for significant covariates

Parameter	Covariate	ΔOFV	p-value	Equation
CL	CRCL	− 18.6	<0.001	Eq. 1
	SCR	− 17.9	<0.001	Eq. 2
	BSA	− 12.1	<0.001	Eq. 3
Q	WT	− 8.5	<0.01	Eq. 4
V1	WT	− 6.9	<0.01	Eq. 5

ΔOFV: change in the OFV (objective function value) compared to the base model; CL= apparent total body clearance; Q= intercompartmental clearance; V1=apparent distribution volume of the central compartment; creatinine clearance (CRCL), serum creatinine (SCR), body weight (WT), body surface area (BSA)

$$\mathrm{CL} = 5.47 \times \left(\frac{\mathrm{CRCL}}{67.7}\right)^{1.12} \quad (1)$$

$$\mathrm{CL} = 5.70 - 1.94 \times (\mathrm{SCR} - 1.43) \quad (2)$$

$$\mathrm{CL} = 5.00 + 0.11 \times (\mathrm{BSA} - 2.02) \quad (3)$$

$$\mathrm{Q} = 1.21 \times \left(\frac{\mathrm{WT}}{87.6}\right)^{2.18} \quad (4)$$

$$\mathrm{V1} = 27.8 \times \left(\frac{\mathrm{WT}}{87.6}\right)^{0.40} \quad (5)$$

3.4.3 Safety and tolerability

Overall, low-dose cidofovir and probenecid were well tolerated. No changes were observed in biochemical indices of kidney or liver function after administration. One patient experienced transient nausea and vomiting which was successfully treated with intravenous antiemetics. This incident was attributed to incorrect probenecid administration by the nursing staff (4 gm given at once).

3.5 DISCUSSION

The current study describes the pharmacokinetics of low-dose cidofovir in kidney transplant recipients with BK viremia, and assesses the active secretion capacity of this patient population by evaluating the impact of concomitant probenecid administration on cidofovir clearance.

Cidofovir is a suitable probe drug to assess anionic secretion because it is not significantly metabolized [218, 228], exhibits negligible binding to plasma proteins (< 0.5%) [227], is transported by renal OAT1 [77], and is cleared via renal filtration and secretion with no evidence of reabsorption [218]. Although the uptake of cidofovir by OAT1 is established, drug transporters responsible for cidofovir efflux across the apical membrane and into the lumen have not been identified.

The results demonstrate that renal filtration is the primary clearance mechanism, and that active secretion does not appreciably contribute to elimination of cidofovir in kidney transplant patients with BK virus infection. This conclusion is supported by: (1) cidofovir clearance is linearly related to eGFR in the absence and in the presence of probenecid; (2) probenecid, the

classical inhibitor of tubular secretion of organic anions, had no appreciable effect on the non-compartmental pharmacokinetic parameters for cidofovir, particularly systemic and renal clearance; and (3) population pharmacokinetic modeling did not identify concomitant probenecid administration as a significant categorical covariate contributing to the variability in total body clearance of cidofovir. Further, the 90% confidence interval of the relative mean of the log-transformed $AUC_{(0-\infty)}$ of cidofovir without and with probenecid is 1.02 – 1.16, which, according to U.S. Food and Drug Administration Bioequivalance Guidelines [229], represents "the absence of a significant difference in the extent to which the active ingredient becomes available at the site of drug action".

In patients with normal kidney function receiving a 5 mg/kg dose of cidofovir, the total body clearance is approximately 248 mL/min and the renal clearance is 209 mL/min [73]. The nonrenal clearance (e.g. metabolic clearance) is therefore 39 mL/min. By comparing renal clearance to baseline creatinine clearance in the same patients, the clearance of cidofovir due to active tubular secretion is approximately 84 mL/min. In this study, the average creatinine clearance was 66 mL/min, and renal clearance values of cidofovir were 62 mL/min without probenecid and 57 mL/min with probenecid. Since renal clearance of cidofovir did not exceed creatinine clearance, this suggests that active secretion of the drug did not occur.

Noncompartmental analysis revealed that renal clearance accounted for only 73.3% and 71.5% of total body clearance in the absence and presence of probenecid, respectively. This difference likely reflects some degree of intracellular phosphorylation, which produces the active cidofovir metabolites. Yet, after intravenous administration of [^{14}C]cidofovir to African green monkeys (43 mg/kg, 29.5 μCl/kg), only 5% of the dose was recovered as phosphorylated cidofovir [70]. However, the data described herein are consistent (though slightly lower) with

previously reported values in HIV-infected patients receiving 3.0 mg/kg cidofovir dosing [228], which describe renal clearance values accounting for 83% of total body clearance. Clearance pathways accounting for the difference between total body clearance and renal clearance are unknown, but could potentially be due to a small degree of hepatic metabolism or elimination in bile. In this study, co-administration with probenecid did not alter the nonrenal clearance of cidofovir. Further, discrepancy between values in this study and previous reports may indicate imprecise urine collection during sampling in some patients, as renal clearance calculations were highly dependent upon urine volume.

The nonlinear mixed-effects modeling process identified serum creatinine, creatinine clearance, and body surface area as significant covariates for cidofovir clearance ($p < 0.001$). The identification of creatinine and creatinine clearance in accounting for variability in cidofovir total body clearance is consistent with the results of the noncompartmental analysis; however, the recognition of body surface area as a significant covariate was unique. However, this finding may reflect colinearity between body surface area and both creatinine and creatinine clearance. For example, in the study population, body surface area was related to serum creatinine ($r^2 > 0.5$, $y = 0.771x - 0.2049$) and creatinine clearance ($r^2 > 0.5$, $y = 42.3x - 12.39$). As correlation between predictors should be avoided in population pharmacokinetic analyses [230] it is rational to estimate clearance based on estimates of renal filtration and avoid inclusion of body surface area. Additionally, creatinine clearance reduced the objective function value to a higher degree than both body surface area and creatinine alone, and therefore should be utilized in predictions.

One possible explanation for the lack of cidofovir secretion may be the low plasma concentrations achieved. In this study, cidofovir was dosed at 4.8% - 12.4% of the FDA approved dose for CMV retinitis in HIV-infected patients, to avoid cidofovir-associated

nephrotoxicity [227]. Accordingly, maximum plasma concentrations achieved at the end of the infusion (1 hour) were approximately 8.6% of those achieved with the 5 mg/kg dose. However, previous studies in healthy human volunteers have demonstrated concentration-independent secretion of probe drugs eliminated via this pathway [231]. Moreover, in microdosing studies of famotidine (eliminated via OCT1-mediated active renal secretion), active renal secretion is evident when only 2.5% of a typical dose is administered, with total clearance values approaching 3.2 mL/min/kg [232].

Another possible explanation may be that sufficient concentrations of probenecid were not achieved in the kidney to inhibit cidofovir secretion. Probenecid was dosed according to the FDA approved use of cidofovir for cytomegalovirus (CMV) retinitis in individuals with HIV [233]. Probenecid plasma concentrations were determined to ensure sufficient absorption from the gastrointestinal tract to a degree that would be expected to inhibit renal secretion. Significant systemic exposure of probenecid was observed, indicating the drug was adequately absorbed in renal transplant recipients. Further, previous studies have demonstrated that probenecid concentrations of 15.86 μg/mL are sufficient to inhibit the tubular secretion of diprophylline [234]. In this study, average probenecid plasma concentrations were four-fold higher than this value within two hours of oral dosing of probenecid.

Yet another potential explanation for the lack of cidofovir secretion may be related to the expression and activity or anionic renal drug transporters in kidney transplant patients. It is well established that inflammatory cytokines can down regulate drug metabolizing enzymes and transporters [98]. Transplant patients are susceptible to a high degree of inflammation, and BK virus infection in the kidney is likely to further increase the concentrations of inflammatory mediators, including circulating cytokines. This raises the possibility that renal OAT1

expression is reduced in kidney transplant patients with BK virus infections, leading to decreased secretion of organic anion substrates.

The current study has a few limitations. First, the majority of the study participants were Caucasian and genotyping of OAT1 was not performed. This raises the possibility that the results may not be dependably extrapolated to other ethnic groups. However, only six non-synonymous OAT1 variants have been identified and none are associated with loss of function. Further, these variants were identified in < 1% of a large population with diverse ethnicities [235]. Thus, polymorphisms in OAT1 do not appear to contribute to inter-individual variation in drug disposition, and the ethnic homogeneity of the study population and the lack of OAT1 genotyping do not represent major pitfalls. Another potential limitation is the relatively small sample size (n=10). Nevertheless, variability in the pharmacokinetic parameters could be reliably accounted for by clinical estimates of renal filtration.

A linear relationship was documented between cidofovir clearance and eGFR both in the absence and presence of probenecid. This relationship allows for the prediction of systemic cidofovir exposure in individual patients according to rearrangement of the equation:

$$\mathrm{Cls} = \frac{\text{Cidofovir dose}}{\mathrm{AUC}}$$

This relationship can be utilized to evaluate the pharmacokinetic-pharmacodynamic relationship between cidofovir exposure and BK virologic response. Potentially, this association can be used to improve the dosage regimen of cidofovir for kidney transplant patients with BK virus.

In the present study, the probenecid-insensitive elimination of cidofovir potentially suggests impaired renal secretion of organic anions via this mechanism. This finding may have far-reaching implications for drug therapy in renal transplant patients with BK virus. Several commonly used medications are substrates for OAT1, including angiotensin converting

enzyme (ACE) inhibitors (captopril, quinapril), angiotensin II receptor blockers (olmesartan), diuretics (bumetadine, furosemide), antibiotics (ceftibuten, ceftizoxime, cephaloride, tetracycline), antivirals (adefovir, ganciclovir, acyclovir), antineoplasics (methotrexate), histamine receptor 2 blockers (cimetidine, ranitidine), and non-steroidal anti-inflammatory agents (ibuprofen, indomethacin). Therefore, transplant recipients with BK viremia receiving the aforementioned drugs may be subject to increased plasma concentrations and potential toxicities. It is unknown if the impaired secretion observed in this study is unique to renal transplant recipients with BK viremia, or if the finding applies to kidney transplant patients in general.

4.0 EXPRESSION OF RENAL DRUG METABOLIZING ENZYMES AND TRANSPORTERS FOLLOWING KIDNEY TRANSPLANTATION IN RATS

4.1 ABSTRACT

Kidney transplantation is the treatment of choice for end-stage renal disease (ESRD) and drug therapy plays a major role in the management of transplant recipients. Active renal secretion of several commonly used drugs is mediated by drug transporters in the kidney. However, the functional activity of these transporters in transplant patients has not been systematically investigated. The objective of this work was to study the impact of kidney transplantation on the mRNA and protein expression of drug transporters (OAT1, OAT3, OCT2, MRP2, MDR1) and oxidative metabolic enzymes (CYP3A, CYP2E1) in the rat after kidney transplantation. Male Lewis rats underwent syngeneic orthotopic kidney transplantation and were sacrificed at various time points post-transplant to assess both the short-term (< 1 day) and long-term (> 60 days) effect of transplantation. The mRNA and protein expression of OAT1 and OAT3, as well as the mRNA expression of OCT2, MRP2, MDR1, CYP3A, and CYP2E1, were determined in rat kidney. A significant decrease in both OAT1 and OAT3 protein expression was observed in the transplanted kidney beginning at 6 h post-transplantation, and persisting for the entire observational period of 78 days. At 24 h post-kidney transplant, the mRNA expression of renal OCT2 and CYP2E1 were significantly reduced while MRP2, MDR1, and CYP3A were unchanged. At 78 days post-transplant, the mRNA expression of renal OCT2 was significantly reduced, while MRP2 expression was increased by 47% ($p<0.05$) and MDR1, CYP3A, and CYP2E1 were unchanged. These results demonstrate that renal transplantation differentially alters the mRNA and protein expression of various metabolic enzymes and transporters in the kidney, and may contribute to clinical complications observed in kidney transplant patients.

4.2 INTRODUCTION

Interindividual variability in drug disposition represents a significant challenge in optimizing pharmacotherapy [236-239]. The expression and activity of drug metabolizing enzymes and transporters largely dictates the degree of systemic drug exposure and consequently induction or inhibition of these proteins may lead to clinically relevant changes in drug exposure. It is well appreciated that alteration in the expression of phase I (oxidation, reduction, or hydrolysis) or phase II (conjugation) metabolic enzymes and drug transporters may occur in various inflammatory diseases, such as rheumatoid arthritis, or acute inflammatory illnesses, such as viral infections [240-243]. Inflammatory cytokines suppress the expression of several metabolic enzymes and transporters through the ability to serve as ligands for the nuclear transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) [244]. Reductions in the mRNA levels of PXR and CAR with a corresponding reduction in CYP enzymes have been reported in rodents with endotoxin induced (and cytokine-mediated) inflammation [98]. In renal transplant recipients, elevations in several pro-inflammatory cytokine, including interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α), have been documented in serum and urine during the immediate post-operative period (presumably related to ischemia/reperfusion injury), in acute rejection episodes, during periods of infection, and prior to late graft failure [101-103]. This raises the possibility that modification in the expression of renal metabolic enzymes and/or drug transporters, which mediate the influx of xenobiotics into the proximal tubule or accelerate their extrusion from the cell into the lumen, may occur in renal transplant recipients at various time points. Further, the previous chapter describes potentially impaired active renal secretion of the OAT1 probe drug cidofovir in kidney transplant recipients with BK virus infection. Therefore, the present study was conducted to mechanistically

understand this clinical observation by evaluating the impact of renal transplantation on the mRNA and protein expression of the major renal oxidative drug metabolizing enzymes and transport proteins in an animal model of kidney transplantation.

4.3 MATERIALS AND METHODS

4.3.1 Animals

Inbred male Lewis rats were used purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN) and used as donors and recipients. The animals, weighing between 200 and 250 gm, were maintained in a 12-hour light/dark cycle at the University of Pittsburgh Animal Center in laminar flow cages in a pathogen-free animal facility with a standard diet and water *ad libitum*. The University of Pittsburgh Guidelines of the Council on Animal Care and the National Research Council's Guide for the Humane Care and Use of Laboratory Animals were followed for all procedures. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

4.3.2 Orthotopic kidney transplantation

Orthotopic kidney transplantation was performed using a previously described technique [245]. Following intravenous heparinization (300 U), the left kidney was removed from the donor with the left renal artery in continuity with a short aortic segment and the left renal vein with a patch of vena cava. The excised graft was flushed with 3 ml of University of Wisconsin solution

(Viaspan, Du Pont, Wilmington, DE) and preserved for 24 h in UW solution at 4 ˚C. The kidney graft was then orthotopically transplanted into a syngeneic recipient, after 30 min of warm ischemia, by end-to-side microvascular anastomoses between graft aorta and recipient infrarenal abdominal aorta, and between graft renal vein and recipient infrarenal vena cava with 10-0 Novafil suture. Both native kidneys of the recipient were removed, and end-to-end ureteral anastomosis was performed using 10-0 Novafil suture. Recipients received prophylactic antibiotics (Cefotetan, 100 mg/kg, intramuscularly) for 3 days following the transplant. Immunosupression was not used due to the syngeneic nature of the transplant. Approximately 10% of recipients died of surgical complications such as bowel obstruction and stenosis of ureter anastomosis, and those recipients were excluded from the study

4.3.3 Experimental design

The mRNA expression of the major renal drug transporters (OAT1, OAT3, OCT2, MRP2, MDR1) and oxidative metabolic enzymes (CYP2E1, CYP3A) were evaluated at various time points after renal transplantation to assess both the short-term (< 1 day) and long-term (> 60 days) effect of the transplantation. The protein expression of OAT1 and OAT3 were also assessed at these time points. Control animals were used to establish baseline mRNA and protein expression to which all experimental groups were compared. Because an organ from a cadaveric donor typically undergoes periods of cold storage and warm ischemia, these conditions were also assessed experimentally for OAT1 and OAT3 protein abundance. A total of 3 animals were evaluated in each group, with different groups being used for mRNA and protein experiments. All kidney samples were stored immediately at – 80 ° C until analysis. To assess

the recovery of kidney function over the entire observational period, serum creatinine values were also periodically recorded.

4.3.4 Preparation of kidney tissue and protein determination for western blot analysis

Frozen kidneys (approximately 200 mg) were homogenized using a dounce hemogenizer and resuspended in 400 ul of cellular lysis buffer containing 25 mM Tris HCl adjusted to pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS. To prevent proteolytic degradation during cell lysis, 10 µL of Halt protease inhibitor cocktail (Thermo Scientific, Waltham, Massachusetts, USA) was added per 1 mL of lysis buffer. Samples were transferred to microcentrifuge tubes and centrifuged for 10 minutes at 10,000 *x g* at 4° C. The supernatant containing whole cell lysate was collected and stored in aliquots at – 80 ° C.

Protein concentrations were determined using the bicinchoninic acid (BCA) assay. Stock solutions were prepared with bovine serum albumin at protein concentrations of 0, 0.25, 0.5, 1, and 2 mg/mL. The BCA working reagent was prepared as 100:2 of Reagent A (1 gm sodium bicinchoninate, 2 gm sodium carbonate, 0.16 gm sodium tartrate, 0.4 gm NaOH, and 0.95 gm sodium bicarbonate, brought to 100 ml with distilled water, pH adjusted to 11.25 with 10 M NaOH) and Reagent B (0.4 gm cupric sulfate in 10 ml distilled water). Exactly 25 µl of each standard and sample combined with 200 µl of the working reagent was added to a 96-well microplate in replicates of three. Following 15 minutes in an incubator at 37°C, absorbance of each well was read at 570 nm using a microplate reader. Protein concentrations in samples were read from the standard curve.

4.3.5 Western blot analysis for the abundance of OAT1 and OAT3 protein

Protein expression in whole cell lysates from kidney tissue was determined using western blotting with polyclonal rabbit anti-rat OAT1 and OAT3 antibodies (Alpha Diagnostic, San Antonio, TX, USA). Samples (200 µg) were heated at 37 °C for 30 minutes in 1x loading buffer, applied to a 12% gel for separation by SDS-PAGE, and electroblotted to nitrocellulose membranes. The nitrocellulose membranes were then blocked with 5% nonfat dry milk in 1X phosphate buffered saline tween-20 and incubated overnight with rabbit anti-rat OAT1 antibody (1:500) or rabbit anti-rat OAT3 antibody (1:500) at 4 °C. After rinsing with phosphate buffered saline for 5 minutes, the membranes were incubated with a Horseradish Peroxidate conjugated goat anti-rabbit antibody (1:2000) for 1 hour at room temperature. After washing with phosphate buffered saline, membranes were incubated with a chemiluminescent reagent for 5 minutes and developed. Blots were then densitometrically analyzed using QuantityOne Analysis Software (BioRad, Hercules, CA, USA) and normalized to the optical density of beta actin in each sample.

4.3.6 RT-PCR

RNA from kidney samples (approximately 200 mg) was extracted using the TRIzol® Plus RNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, tissue samples were homogenized in 2 mL of TRIzol Reagent, mixed with 0.4 mL of chloroform, and centrifuged at 12,000 *x g* for 15 minutes at 4 °C. Approximately 600 µL of the colorless, upper phase containing RNA was transferred to an RNase-free tube and mixed with an equal volume of 70% ethanol. Samples were then purified using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's directions. RT-PCR was

performed according to the SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase system protocol (Invitrogen, Carlsbad, CA, USA). cDNA was generated at 55°C for 30 min and then the samples were denatured at 94°C for 2 min. PCR amplification was performed in 30 cycles of 94°C for 15 s, then 50°C for 30 s, and 68°C for 60 s. The final elongation step was 68°C for 10 min. Primers against OAT1, OAT3, OCT2, MRP2, P-glycoprotein (MDR1), CYP3A, and CYP2E1 were designed using the PrimerQuest Software (Integrated DNA Technologies (Coralville, IA, USA) (Table 14). Electrophoresis was performed on a 1% agarose gel and samples were imaged and optical density determined using QuantityOne Analysis Software (BioRad, Hercules, CA, USA) and normalized to the optical density of beta actin in each sample.

Table 14. Primers used for RT-PCR

Gene	Accession Number	Primers
OAT1	NM017224	Sense: 5'-AGAGTCACAGAGCCCTGCATTGAT-3' Antisense: 5'-AAGCATACAAACTTGGCAGGCAGG-3'
OAT3	NM031332	Sense: 5'-TGGATGGCTGGATCTACAACAGCA-3' Antisense: 5'-TAGATGTTGATGAGATGGCCCGCA-3'
OCT2	NM031584	Sense: 5'-ATTGGCTACCTAGCGGACAGGTTT-3' Antisense: 5'-TGTCACACATGGAGGAGCAGACAA-3'
MRP2	NM012833	Sense: 5'-TCGCTGGCACTCTTGTCATGATCT-3' Antisense: 5'-AGCTGTAGGCCAGACACAAAGGAT-3'
P-glycoprotein	NM012623	Sense: 5'-TGACAGCTTCTCAACCAAGGGACA-3' Antisense: 5'-AAGTCAACTCAGAGGCACCAGTGT-3'
CYP3A	NM001024232	Sense: 5'-TGTGGAGATTGTGGCTCAGTCCAT-3' Antisense: 5'-GCCAGCACTTTGGGTCTTTGTGAA-3'
CYP2E1	NM031543	Sense: 5'-GGTTCTTGGCATCACCATTGCCTT-3' Antisense: 5'-AGAGTTGTGCTGGTGGTCTCAGTT-3'

4.3.7 Measurement of serum creatinine in rat plasma

Serum was obtained from blood samples, and serum creatinine levels were measured using a Beckman autoanalyzer employing a modification of the Jaffe procedure (Beckman Instruments, Fullerton, CA, USA).

4.3.8 Statistical analysis

Statistical significance was determined by an unpaired Student's t-test for densitometric data obtained from western blotting and RT-PCR. Data from control animals were tested against transplanted animals and differences were considered statistically significant when $p < 0.05$. Serum creatinine values were analyzed using one-way analysis of variance with Dunnett's post-hoc test where all values from transplanted rats were compared against control. Values are presented as mean ± S.D or S.E.M. as indicated. Statistical analyses were performed using GraphPad Prism 5.0 (La Jolla, CA, USA).

4.4 RESULTS

4.4.1 Serum creatinine

The mean serum creatinine values before and periodically for a period of 72 days following kidney transplantation are displayed in Figure 25. Prior to the transplant procedure, serum creatinine values averaged 0.45 +/- 0.05 mg/dL. At 24 hr post-transplant, serum creatinine was increased 10-fold over baseline and remained elevated until day 4. At day 7, serum creatinine had stabilized with no significant difference noted between baseline. Serum creatinine continued to remain normal ($p>0.05$, 1-way ANOVA with Dunnett's post-hoc test) through day 78.

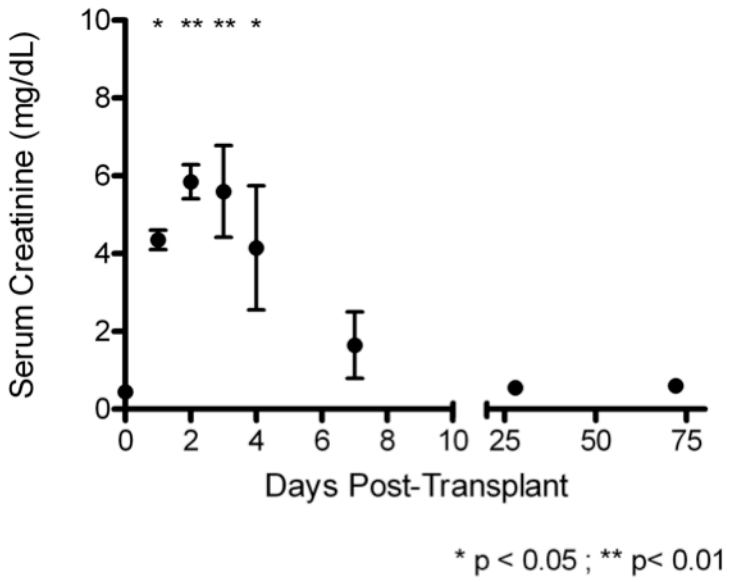


Figure 25. Stabilization of serum creatinine following kidney transplantation in rats

Results presented as mean +/- SEM

4.4.2 OAT1 and OAT3 mRNA and protein expression

Neither cold storage nor warm ischemia affected OAT1 or OAT3 protein expression. On the other hand, kidney transplantation led to a sustained reduction in the expression of renal OAT1 and OAT3 mRNA (Figure 26 and Figure 27) and protein (Figure 28 and Figure 29). Reduced expression of OAT1 and OAT3 was apparent beginning at 24h and 6h following kidney transplantation, respectively. For both anionic transporters, down regulation of both mRNA and protein was apparent at 78 days after the procedure. At 24 hours post-transplant, the mRNA expression of renal OAT1 and OAT3 were reduced by 60% and 24%, respectively (Figure 26). At 78 days post-transplant, the mRNA expression of renal OAT1 was reduced by 39% and

OAT3 was reduced by 29% (Figure 27). The relative protein expression of renal OAT1 and OAT3 expressed as a percentage of control is displayed in Figure 30. For OAT1, protein expression approached 25% of control at 24h, and recovered to a maximum of approximately 50% expression at 62 days and 78 days.

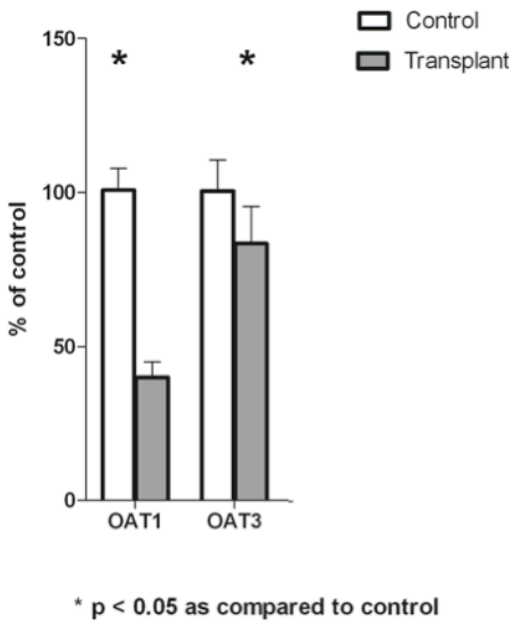


Figure 26. Renal mRNA expression of OAT1 and OAT3 at 24 h post-kidney transplantation

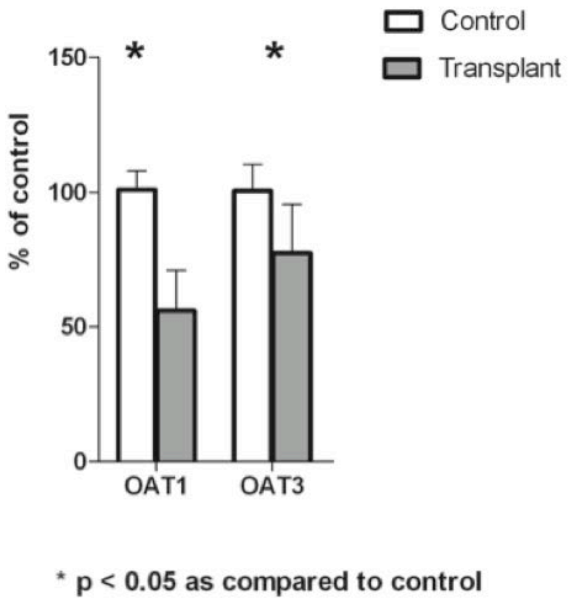


Figure 27. Renal mRNA expression of OAT1 and OAT3 at 72 days post-kidney transplantation

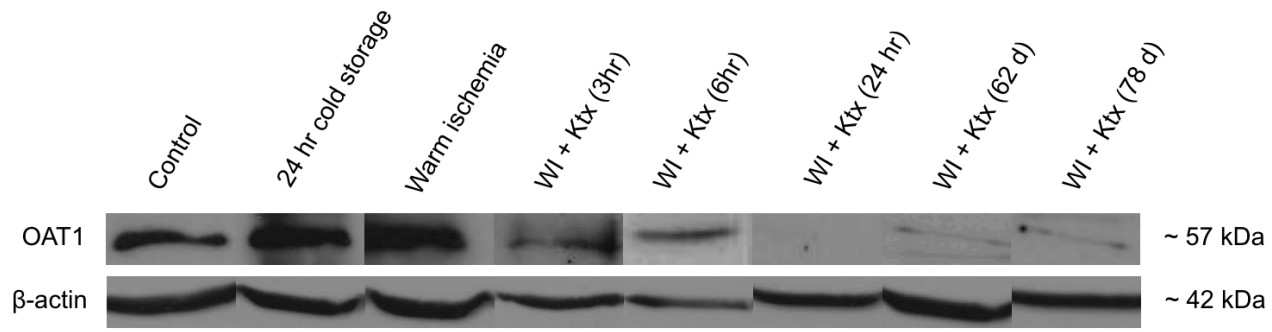


Figure 28. Representative blots displaying the effect of cold storage (24h), warm ischemia (30 min), and orthotopic syngeneic kidney transplantation on the protein levels of renal OAT1 in rats.

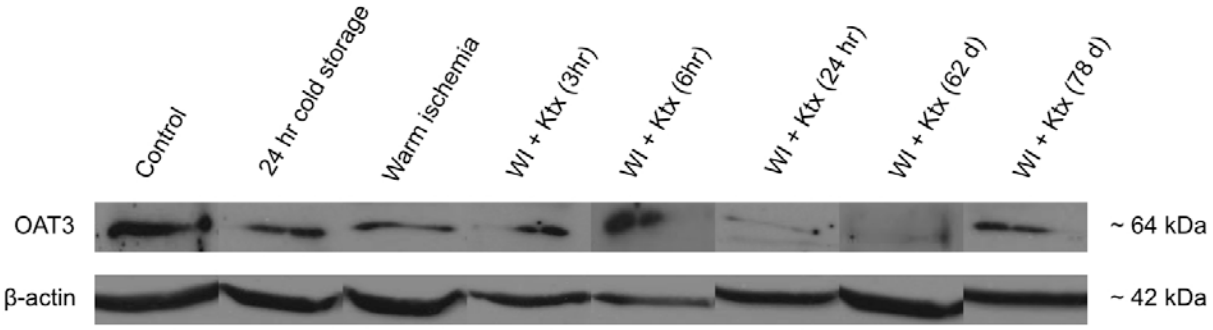


Figure 29. Representative blots displaying the effect of cold storage (24h), warm ischemia (30 min), and orthotopic syngeneic kidney transplantation on the protein levels of renal OAT3 in rats.

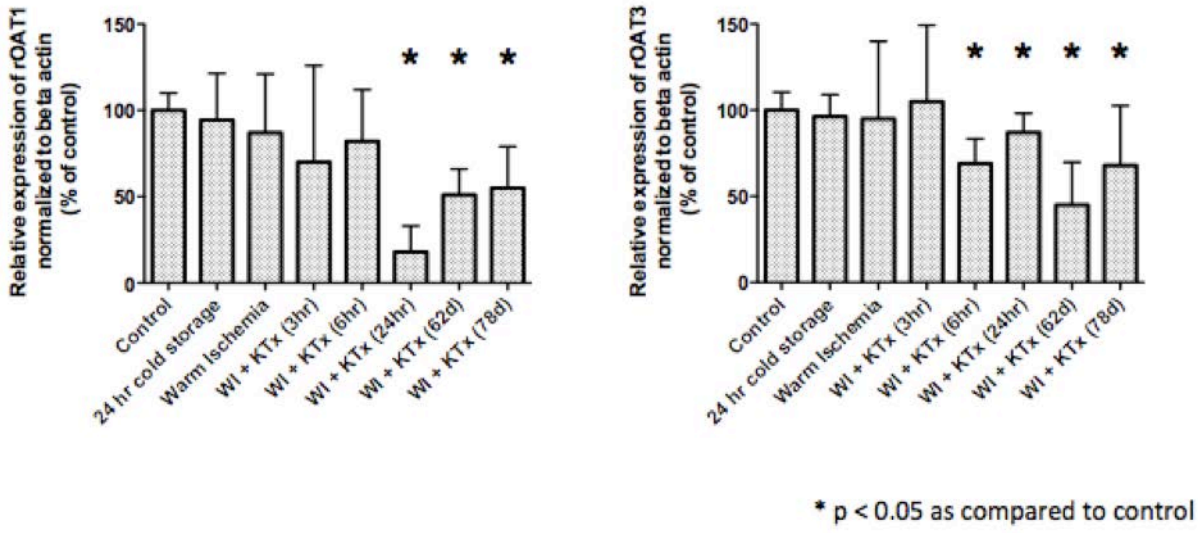


Figure 30. Protein expression of renal OAT1 and OAT3 normalized to β-actin relative to control

Results are presented as mean ± standard deviation

4.4.3 OCT2, MRP2, MDR1, CYP3A, and CYP2E1 mRNA expression

A representative image of the mRNA expression of OCT2 is displayed in Figure 31.

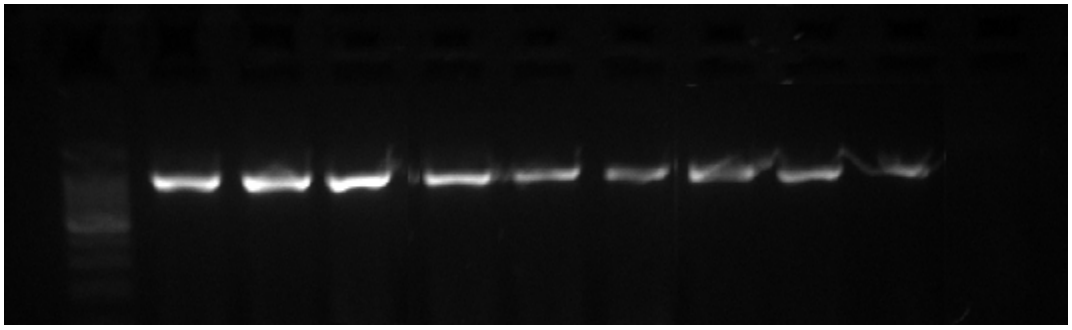


Figure 31. mRNA expression of OCT2

The first three lanes represent control animals, the middle three lanes represent animals 24 hours post-kidney transplant, and the final three lanes represent animals 78 days post kidney-transplant.

At 24 hours post-transplant, the mRNA expression of renal OCT2 and CYP2E1 were reduced by 27% and 30%, respectively (Figure 32). The mRNA expression of MRP2, MDR1, and CYP3A were unchanged.

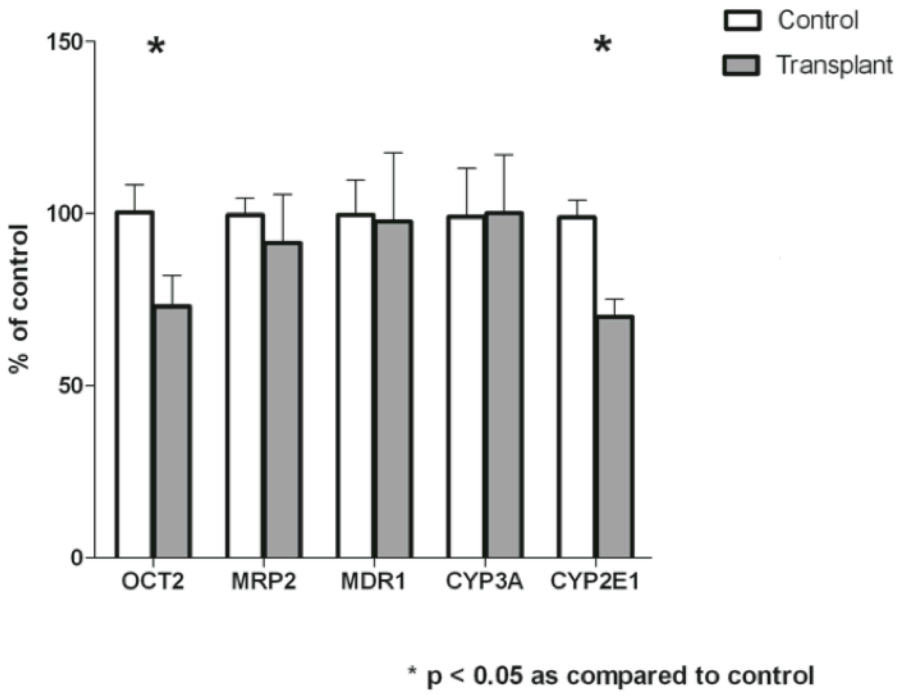


Figure 32. Renal mRNA expression of drug transporters (OCT2, MRP2, MDR1) and metabolic enzymes (CYP3A, CYP2E1) at 24 hr post-kidney transplantation

At 78 days post-transplant, the mRNA expression of renal OCT2 was reduced by 33% and the expression of MRP2 was increased by 47% (Figure 33). The mRNA expression of MDR1, CYP3A, and CYP2E1 were unchanged.

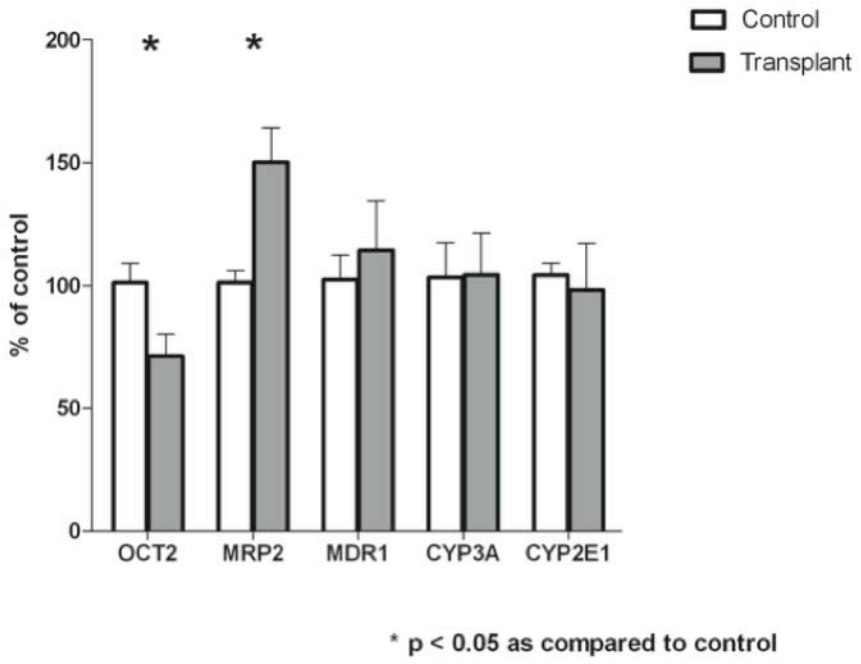


Figure 33. Renal mRNA expression of drug transporters (OCT2, MRP2, MDR1) and metabolic enzymes (CYP3A, CYP2E1) at 78 days post-kidney transplantation

4.5 DISCUSSION

The mammalian proximal tubule plays a critical role in rapidly clearing the blood of many exogenous and endogenous substrates. In this capacity, transport proteins localized to the basolateral membrane (OAT1, OAT3, OCT2) and the apical membrane (MDR1, MRP2) form the secretory pathway of the kidney and mediate the excretion of a wide variety of charged organic compounds. A number of frequently utilized drugs, including many antivirals, β-lactam antibiotics, nonsteroidal anti-inflammatory drugs, and diuretics are eliminated via these carrier-mediated pathways and altered expression of transporters may have significant consequences with respect to the pharmacokinetics of such substrates. Further, several metabolic enzymes involved in oxidative drug metabolism (CYP3A, CYP2E1) are also expressed in the kidney. The present study employed an animal model of kidney transplantation to investigate the effect of renal transplantation on the mRNA and protein expression of these renal drug transporters and enzymes.

The results described herein demonstrate decreased protein expression of OAT1 and OAT3 following orthotopic syngeneic kidney transplantation in rats. Reduced expression was noted shortly after the transplant procedure, at 24 hr for OAT1 and 6 hr for OAT3, with a sustained down regulation of both proteins over the duration of the 78-day study period. The mRNA expression of OAT1 and OAT3 was consistent with protein expression. Additionally, the mRNA expression of OCT2 was reduced at both 24 hr and 78 days post-transplant, while the

expression of CYP2E1 was diminished at 24 hr but returned to baseline by day 78. In contrast to the basolateral transporters, the mRNA expression of the apical transporter MRP2 was significantly increased at day 78. The physiological relevance of this observation is unknown, but these data may suggest a possible protective mechanism by which uptake into the proximal tubules is reduced while the efflux capacity into the lumen is increased.

Serum creatinine values were recorded at several time points after the transplant procedure to assess renal filtration. A significant increase in serum creatinine was noted in all animals after the procedure, with stabilization by day 7. Although serum creatinine returned close to baseline in the short-term, altered mRNA and protein expression of several drug transporters persisted for over two months after renal transplantation. These results potentially suggest that kidney transplant patients may have a normal or mildly reduced GFR yet still have significantly reduced renal clearance of drugs which undergo extensive renal secretion (i.e. Cl_R >> GFR) secondary to diminished transporter expression. This result is consistent with a previous report where rats with cyclosporine-induced nephropathy showed a rapid improvement in GFR upon withdrawal of the insult. However, tubular dysfunction persisted after normalization of the GFR for the entire observational period (28 days) [162], suggesting disassociation of glomerular function and tubular function.

Reduced mRNA and protein expression of OAT1 following kidney transplantation is consistent with the previously described clinical observation of the limited role of renal secretion in the clearance of the OAT1 substrate cidofovir in kidney transplant recipients. However, several limitations should be noted in translating these results to kidney transplantation in humans. First, animals in this study underwent a syngeneic transplant and immunosuppression was not utilized. The effect of increased immune response in recipients of an allogeneic kidney

transplant and the impact of varying degrees of immunosuppression was not specifically addressed in the current study. Further, participants in the clinical study were treated with cidofovir for BK virus infection. The effect of BK-associated nephritis on expression of drug transport proteins was not pursued in the current experimental approach; however, additional inflammatory mediators would likely have a cumulative rather than differential effect on decreasing transporter expression. It would have been useful to evaluate biochemical markers of inflammation in order to relate this information to protein expression (i.e. semi-quantitatively describe the relationship between the serum concentration of pro-inflammatory cytokines and the degree of down regulation). This data would be valuable in revealing underlying mechanistic insight and should be addressed in future investigations.

Few reports on the impact of kidney transplantation on the expression of drug metabolizing enzymes or transporters are available. In one human study, hepatic CYP3A4 significantly decreased at 3 months and 1 year following kidney transplantation (−33% and −45%; −7% and −33%, respectively) [148]. As certain metabolic enzymes and transporters are regulated via a similar nuclear receptor-mediated mechanism, a comparable down regulation in transporters may be expected.

Additionally, reduced renal OAT1 expression has been identified in different disease states. Sakurai, et al describe lower mRNA levels of OAT1 in biopsy samples of patients with renal disease following surgical nephrectomy for renal carcinoma [91]. Conversely, in a 5/6 nephrectomized rat model, protein expression of the cationic transporter OCT2 was down regulated, but expression of OAT1 and OAT3 was maintained [209]. These discrepant results suggest differential regulation of drug transporters in the kidney during varying disease states.

It has been reported that ischemic acute renal failure in rats leads to an increase in prostaglandin E2 (PGE2) via the cyclooxygenase (COX2) pathway, which in turn down regulates renal OAT1 and OAT3 and leads to decreased organic anion secretion [165]. COX inhibition with indomethacin or competitive inhibition of the uptake of PGE2 with probenecid rescues this effect. This report is consistent with our observation of reduced OAT1 and OAT3 protein expression in the short-term after the transplant as trauma from the surgery and transplant would be expected to cause an increase in inflammatory mediators, including PGE2. However, it remains unclear why the effect is sustained for a period of several months, at which time inflammation should be substantially reduced.

In summary, the present study demonstrates that renal transplantation alters the mRNA and protein expression of various metabolic enzymes and transporters in the kidney in rats. This finding is relevant due to the large role that drug therapy plays in the management of kidney transplant recipients and the high number of drug substrates that are eliminated via carrier-mediated tubular secretion.

5.0 PHARMACODYNAMICS OF CIDOFOVIR AND BK VIREMIA AND VIRURIA IN KIDNEY TRANSPLANT RECIPIENTS

5.1 ABSTRACT

BK virus infection in kidney transplant recipients is associated with progressive graft dysfunction and graft loss. Cidofovir, an antiviral agent that demonstrates *in vitro* activity against BK virus, has been used concurrently with reduced immunosuppression for treatment of BK virus infection in renal allograft recipients. However, the effectiveness of this approach has not been rigorously evaluated and the dosage regimen currently used is empiric. The current study, which was performed in 8 densely sampled adult renal transplant recipients, investigated both the acute pharmacodynamic response subsequent to cidofovir administration and the relationship between drug exposure and viral clearance. At 12 hr post-cidofovir infusion, viral loads in plasma and urine were significantly reduced, reflecting the short *in vivo* half-life of the virus. However, the effect was not sustained, and viremia and viruria returned to baseline at the next sampling period (8 days later for plasma; 1 day later for urine). No association was found between metrics of systemic cidofovir exposure and decline in viremia or viruria. We conclude that cidofovir transiently reduces BK viremia and viruria and may be an efficacious add-on treatment option in the pharmacological management of BK virus in renal transplant recipients. However, as viremia and viruria quickly recovered to pre-treatment levels, the dosage regimen of cidofovir requires further optimization. Prospective trials are warranted to define the optimal cidofovir dose and frequency of administration for the treatment of BK virus.

5.2 INTRODUCTION

BK virus has emerged as an important cause of allograft dysfunction in kidney transplant patients [246-249]. The use of potent immunosuppressive therapy is the primary risk factor associated with reactivation of BK virus in renal epithelium and subsequent detection in plasma and urine, which serve as quantifiable surrogate markers for the course of the disease [41, 250-255]. Consequently, the initial step in the management of this complication is a reduction in immunosuppression, including reduced calcineurin inhibitor dosing and discontinuation of antimetabolic agents [256-258]. However, this approach is only marginally successful and carries risk of graft injury due to immune-mediated rejection [259-262]. Cidofovir, an inhibitor of viral DNA synthesis, is used at low doses at many transplant centers as an add-on second-line treatment option, though the dosing regimen is empiric and its effectiveness is not fully understood [21, 39, 84-86, 90, 263-266]. Randomized controlled trials evaluating the use of cidofovir for the treatment of BK virus infection in kidney transplantation have not yet been performed; however, comparisons of cidofovir-treated patients with non-treated patients appear to indicate improved outcomes [66, 81]. Although rigorous prospective evaluations of cidofovir for BK virus in transplantation are underway [267], patients in these trials typically undergo multiple interventions, most often with reduced target tacrolimus trough concentrations of < 6 ng/mL [15]. Hence, it is difficult to independently gauge if viral clearance is the result of cidofovir, decreased immunosuppression, or both. A recent report characterizing the *in vivo* dynamics of BK virus revealed a short half-life of 1-2 hours in plasma, suggesting rapid viral turnover in the kidney [46]. Therefore, the current study was performed to assess the acute pharmacodynamic response subsequent to cidofovir administration in densely sampled renal transplant recipients. We anticipated that because of the previously demonstrated activity of

cidofovir against BKV *in vitro* [49] and the short *in vivo* half life of BKV, a reduction in the BK viral load in plasma and urine would occur directly following drug administration. The degree and duration of viral decline in relation to cidofovir exposure could then potentially be used to optimize the cidofovir dosing regimen. Additionally, as probenecid is used with cidofovir for other indications to reduce OAT1-mediated uptake into proximal tubule cells and decrease cidofovir associated nephrotoxicity, we also evaluated the effect of probenecid on the virologic response. Since BKV is localized in proximal tubule cells, decreased cidofovir uptake in the presence of probenecid should cause an attenuated response. Yet, because OAT1-mediated active secretion was not observed in the previously described clinical pharmacokinetic study in kidney transplant recipients (Chapter 3), we predicted that concomitant probenecid administration would not alter the pharmacodynamic response to cidofovir.

5.3 MATERIALS AND METHODS

5.3.1 Patients

This study was performed in eight adult renal transplant recipients undergoing treatment with low-dose once-weekly intravenous cidofovir for polymerase chain reaction-confirmed BK viremia and viruria. The protocol was approved by the Institutional Review Board of the University of Pittsburgh (IRB# 08060393) and written informed consent was obtained from all patients prior to participation. Exclusion criteria included: (i) hypersensitivity to cidofovir or other nucleotide analogs, (ii) hypersensitivity to probenecid or sulfonamides, (iii) currently receiving another drug known to affect renal anionic drug secretion, (vi) pregnancy or

breastfeeding women. On average, the study participants were 55.3 ± 13.4 years of age, weighed 85.2 ± 21.4 kg, and were 13.5 ± 17.8 months post-kidney transplant. All of the subjects had a reduced estimated glomerular filtration rate (eGFR), ranging from mild to severe, though no patients were on dialysis at the time of the study. All patients had positive BK viral loads in plasma and urine, though none had evidence of tubulointerstitial nephritis. Viral loads in individual patients at the time of enrollment are presented in Table 15.

Table 15. Patient characteristics and plasma and urine viral loads at time of enrollment.

Patient	Age (years)	Body Weight (kg)	Cidofovir dose (mg)	Cidofovir dose (mg/kg)	Plasma viral load (log_{10} copies/mL)	Urine viral load (log_{10} copies/mL)
1	43	74.1	20	0.27	3.00	9.35
2	29	111.3	27	0.24	2.73	5.54
3	59	85.6	22	0.26	3.25	5.62
4	53	64.7	40	0.62	3.05	6.80
5	59	59.7	20	0.34	4.05	5.44
6	63	75.3	22	0.29	3.80	4.74
7	67	120.0	35	0.29	4.05	8.99
8	69	91.1	35	0.38	6.12	9.31
Mean (SD)	55.3 (13.4)	85.2 (21.4)	27.6 (7.9)	0.34 (0.12)	3.76 (1.08)	6.97 (1.94)

5.3.2 Study design

The study design included two phases. During Phase 1, intravenous low-dose cidofovir was administered as a 1 hr infusion without concomitant probenecid. Phase 2 took place one week

later, when cidofovir was administered with concomitant oral probenecid. Probenecid (2g) was given 1 h prior to cidofovir administration and again at 2 h and 8 h (1g each) after the completion of the cidofovir infusion. During both phases, patients received 1 L of 0.9% sodium chloride immediately prior to cidofovir administration and cidofovir was diluted in 100 mL of 0.9% sodium chloride. Blood samples (7 mL) were collected in Vacutainers at 0, 1, and 12 hours after the start of the cidofovir infusion. Plasma was separated and frozen at – 80 °C until analysis. Midstream urine samples (~ 20 mL) were collected at 0 and 12 hours, and for three consecutive days after cidofovir administration. Urine samples were stored at – 80 °C until analysis

5.3.3 Quantitation of BK Virus DNA with TaqMan Real-Time PCR

DNA was extracted from plasma and urine samples using the QIAamp maxikit (Qiagen, Hilden, Germany) for urine or the QIAamp minikit (Qiagen, Hilden, Germany) for plasma. DNA extraction was achieved by using 5 ml of uncentrifuged urine or 200 μl of plasma. The following oligonucleotide sequences, derived from the BKV (Dunlop strain; GenBank accession no. NC001538) capsid protein-1 (VP-1) gene, were synthesized (IT BioChem, Salt Lake City, Utah): forward primer, 5’ GCA GCT CCC AAA AAG CCA AA 3’; reverse primer, 5’ CTG GGT TTAGGA AGC ATT CTA 3’.

Quantitative real-time PCR assays were performed using the Roche Light-Cycler. PCR amplifications were run in a reaction volume of 20 μl containing 2 μl of the DNA sample, Roche 10X SybrGreen FasStart mastermix, 2.5 mM magnesium chloride, and 500 nM (each) forward and reverse primers. Thermal cycling was initiated with a first denaturation step of 10 min at 95°C, followed by 40 cycles of 95°C for 10 s, 62°C for 10 s, 72°C for 5 s, and 78°C for 10 s, at the end of which fluorescence was read. Real-time PCR amplification data were analyzed with

software provided by the manufacturer. Standard curves for the quantification of BKV were constructed using serial dilutions of a plasmid containing the entire linearized genome of the BKV Dun strain inserted into the BamHI restriction site of the pBR322 plasmid (ATCC 45025). The plasmid concentrations plotted ranged from 1 to 10^9 genomic copies of BKV DNA per PCR. All patient samples were tested in duplicate and results are presented as the mean. The number of BKV copies for each plasma and urine sample was calculated from the standard curve (Figure 34). Data are expressed as copies of viral DNA per milliliter of urine or plasma. Standard precautions designed to prevent contamination during PCR were followed. No-template control lanes and negative-control samples containing DNA extracted from human peripheral blood lymphocytes were included in each run.

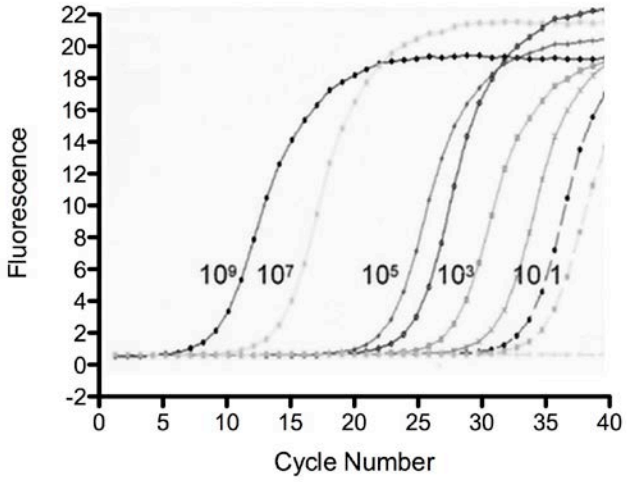


Figure 34. Quantitative PCR assay of BKV standards

5.4 RESULTS

BK viral loads were quantitated in all plasma samples and urine samples using RT-PCR. The median BK viral loads in plasma and urine prior to cidofovir dosing were 3.8 (range: 2.7 – 6.1) $\log_{10}$ copies/mL and 6.2 (range: 4.7 – 9.3) $\log_{10}$ copies/mL, respectively. In both urine and plasma, a significant reduction in the log transformed viral load was detected 12 hr after cidofovir administration for both Phase 1 and Phase 2 (Figure 35 and Figure 36). However, the effect was not sustained, and viremia and viruria returned to baseline at the next sampling period (8 days later for plasma; 1 day later for urine). In plasma, the average percent change in the viral load 12 hours after drug administration was – 21% (Figure 37). In those patients with a higher initial degree of viremia (> 6000 copies/mL) the magnitude of the effect was greater, with an average change of – 66%. No association was found between metrics of cidofovir exposure, including dose, $AUC_{0\text{-}12}$, $AUC_{0\text{-}\infty}$, and C_{max} (all identified in Chapter 3), and BK viral clearance (absolute or % change from baseline).

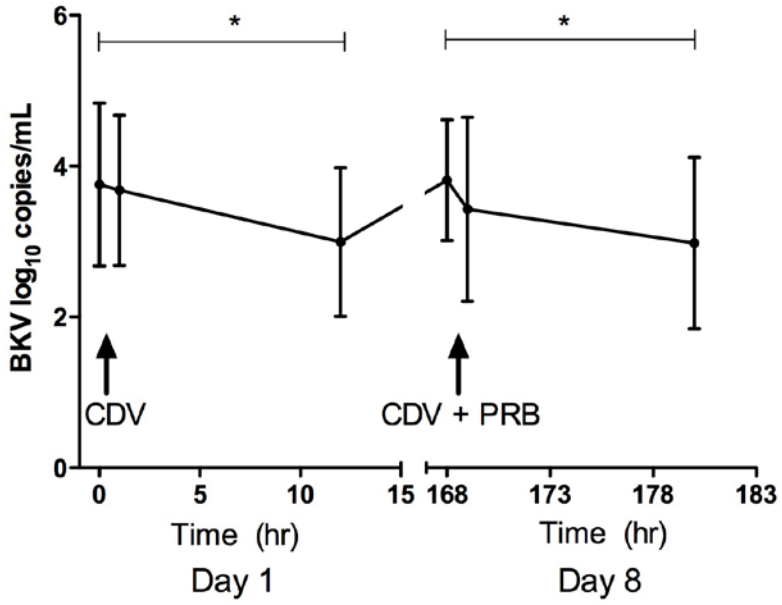


Figure 35. Acute change in BK viral load in plasma following cidofovir administration

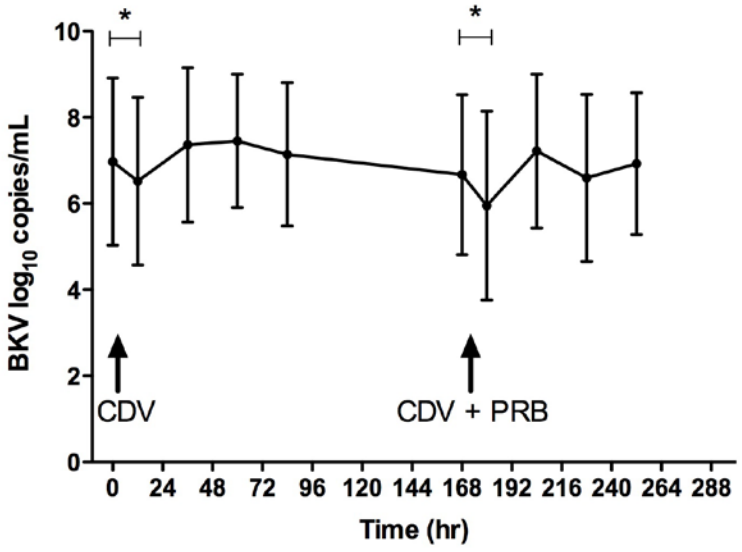


Figure 36. Acute change in BK viral load in urine following cidofovir administration

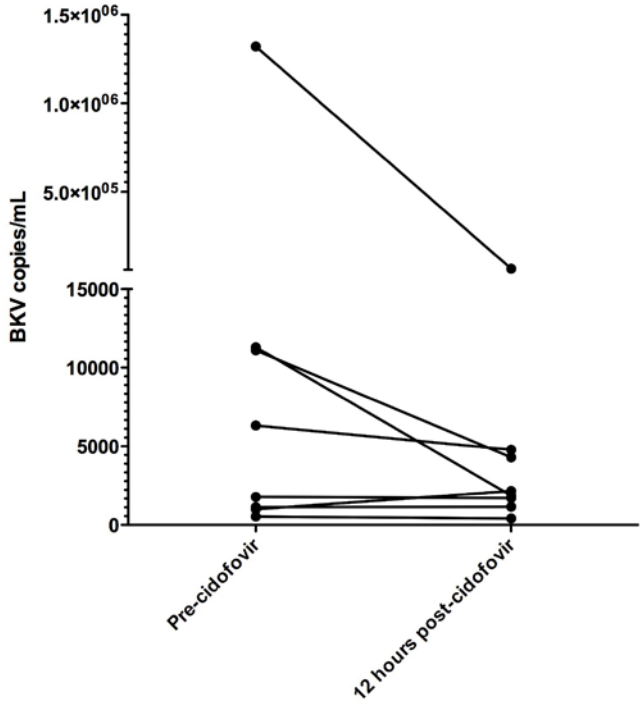


Figure 37. Change in plasma BK viral load 12 hours after cidofovir administration

Viral loads from paired plasma and urine samples were modestly correlated (r^2 = 0.41) suggesting viruria may serve as a surrogate marker for viremia when blood samples are unable to be obtained. (Figure 38)

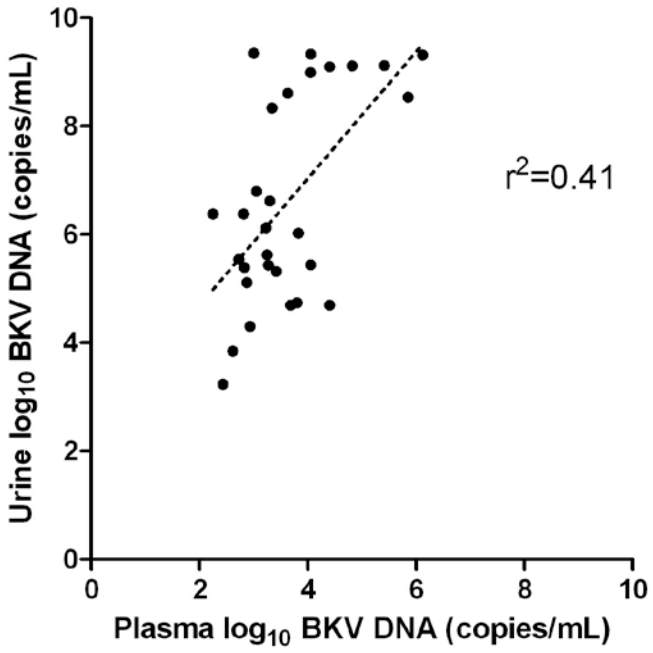


Figure 38. Linear regression analysis of log transformed BK viral loads in plasma and urine from paired samples

5.5 DISCUSSION

This study represents the first report of the acute effect of cidofovir administration on the BK viral load in plasma and urine in renal transplant recipients. Cidofovir is widely used for the management of BK virus in transplant patients, though consensus regarding the most appropriate dosing regimen is lacking. Here, we show that cidofovir transiently reduces BK viremia and viruria, but the effect is not sustained as viral loads quickly recover to baseline. Given the temporal relationship between drug administration and viral decline, this affect can be independently attributed to cidofovir therapy and not to other therapeutic interventions (i.e. reduced immunosuppression).

Our results provide *in vivo* proof of concept that cidofovir possesses some degree of efficacy for the management of BK virus infections in renal transplant recipients. However, the temporary reduction in viremia and viruria suggest the dosage regimen requires modification. Increasing the cidofovir dose and/or the frequency of administration could be an effective strategy to increase the efficacy for BK virus. The potential benefit of these approaches must be carefully weighted against the profound nephrotoxic potential of cidofovir, especially in light of the fact that probenecid is not typically used for this indication. However, in the present study cidofovir did not elicit any nephrotoxicity, as serum creatinine and BUN remained unchanged in the study population. This has been confirmed at other transplant centers over a longer duration [64, 84]. Funk et al recently proposed that a BK viral load of 1000 copies/mL corresponds to 1 lysed kidney tubular epithelial cell per day [268]. Hence, only partially impeding viral replication allows BKV to produce significant cytopathic wear over time. Modification to the

cidofovir dosing regimen to increase drug exposure may therefore be clinically warranted, and investigations into the risk-benefit ratio of this approach should be undertaken.

The 50% effective concentration (EC_{50}) *in vitro* for cidofovir against BK virus is approximately 36 μg/mL [49], though the previously described study of the clinical pharmacokinetics of low dose cidofovir in renal transplant recipients (Chapter 3) showed maximum plasma concentrations approaching only 1 μg/mL. However, in rabbits, [^{14}C] cidofovir achieved concentrations 10-fold higher in the kidney than in plasma [80]. The high affinity of cidofovir for the kidney may partially explain the initial robust virologic response observed in this study despite the low dose employed. Moreover, concomitant administration of oral probenecid did not significantly augment the pharmacodynamic effect. This finding is consistent with the observation of the limited role of active renal transport of cidofovir in transplant recipients with BK viremia, as described in Chapter 3.

This study was not designed to evaluate long-term outcomes with cidofovir treatment, such as graft survival and patient mortality, nor is it intended to define the role of cidofovir in the treatment of BK virus. Rather, we show herein that low-dose cidofovir does transiently produce a significant reduction in BK viral loads in urine and plasma and could be a suitable antiviral agent if the dose and frequency could be optimized. Unfortunately, no relationship was discovered between cidofovir exposure and response to guide this adjustment, though this may be related to the small sample size. This objective is further pursued through retrospective analysis of kidney transplant recipients treated with cidofovir for BK virus at a large academic medical center, as described in the following chapter.

Although the results of this work reveal that cidofovir reduces BK viremia and viruria, maintaining sustained inhibition of BKV replication with cidofovir could be problematic as viral

loads in urine recovered in as little as one day. Cidofovir must be administered in a hospital setting with pre-hydration with intravenous fluids for nephroprotection, and it is unrealistic for dosing to be performed more than twice per week for outpatients. The orally available lipid conjugate of cidofovir, CMX001, may represent a viable option for the treatment of BK virus since administration would note require hospitalization. This agent also inhibits BKV replication in human renal tubular epithelial cells [269], presumably by the same mechanism as cidofovir. However, the role of renal tubular drug transporters in the disposition of CMX001 needs to be examined, as active transport of antiviral drugs into the site of action is desirable to achieve maximum exposure and effect.

6.0 RETROSPECTIVE ANALYSIS OF THE CLINICAL EFFICACY OF CIDOFOVIR USED FOR THE MANAGEMENT OF BK VIRUS IN KIDNEY TRANSPLANT RECIPIENTS

6.1 ABSTRACT

BK virus is a major cause or morbidity in kidney transplant recipients and there is no therapy with proven efficacy. The use of cidofovir to treat BK virus infection remains controversial, as some case series have reported no discernable benefit. We have previously determined that baseline renal filtration is significantly correlated with cidofovir clearance in kidney transplant patients, thus affecting total drug exposure. Therefore, we hypothesized that uniform dosing with low-dose cidofovir in all patients may be inadequate, as exposure will vary widely and potentially influence treatment outcomes. The present retrospective study used serum creatinine-based estimates of cidofovir clearance to approximate aggregate cidofovir exposure in 104 individual transplant recipients, and this information was then correlated with viral response. Regression analysis demonstrated that higher estimated systemic exposure was significantly associated with a larger reduction in the degree of BK viremia, as measured by real-time quantitative RT-PCR ($p < 0.05$). However, the predicted reduction in viremia was low as compared with average pre-treatment viral loads in the study population. Only 11% of the variance in the decline in BK viral loads in plasma could be explained by variation in cumulative cidofovir exposure, suggesting that other factors, presumably immune-mediated, may play a major role in viral clearance. Finally, analyses of allograft survival suggest a marginal treatment benefit with cidofovir. These results highlight the need for continued investigations to discover and implement more effective antiviral treatment strategies for BK virus infection in transplant recipients.

6.2 INTRODUCTION

BK virus (BKV) is a polyomavirus usually acquired in childhood that remains latent in the genitourinary tract throughout life [270]. BK virus reactivation in kidney transplant recipients is associated with allograft failure, and optimal pharmacotherapeutic regimens have not been identified to effectively manage this condition [271]. Cidofovir is a potent antiviral nucleotide analog, which has broad-spectrum activity against many DNA viruses, including cytomegalovirus (CMV) and polyomaviruses [49]. Case reports and anecdotal evidence describe conflicting results on the value of low-dose cidofovir (0.25–1.0 mg/kg given intravenously once weekly or every other week) in the management of BK virus infections in renal transplant recipients, with some studies reporting clearance of the virus [64, 90] and others describing no apparent benefit [68, 89]. We have previously elucidated the pharmacokinetics of low-dose cidofovir in kidney transplant patients (Chapter 3), and demonstrated that glomerular filtration rate (GFR) is significantly correlated with cidofovir clearance, thus affecting total drug exposure. Therefore, we predicted that discrepant clinical experience with cidofovir could be related to inadequate drug exposure in some patients. Further, we anticipated that those patients with higher exposure would have improved outcomes. To test this prediction, we retrospectively evaluated all patients with BK virus at our institution, and assessed the impact of estimated cidofovir exposure on viral response.

6.3 MATERIALS AND METHODS

6.3.1 Study design and statistical analysis

This single-center, retrospective, non-randomized study was conducted at the University of Pittsburgh Medical Center in Pittsburgh, Pennsylvania. Information used for analyses was obtained through databases maintained by the University of Pittsburgh Starzl E. Transplantation Institute, under the auspices of, and with formal approval by, the Institutional Review Board of the University of Pittsburgh (IRB # 0307037). Research data were coded to prevent the identification of subjects. Adult renal transplant recipients were included if they had any of the following at any time post-transplantation: (i) detection of BK virus in plasma by real-time quantitative polymerase chain reaction (RT-PCR), (ii) detection of BK virus in urine by RT-PCR, (iii) biopsy-proven BKV infection. Descriptive statistics are used to detail the incidence of BK virus among the population by comparing to the total number of transplant recipients over the same time period. The impact of intravenous cidofovir therapy on allograft survival was evaluated by the Kaplan-Meier test using the LIFETEST procedure in SAS (Cary, NC, USA). Patients who died with a functioning graft and graft failures not due to BK (including acute and chronic rejection) were counted as non-failures.

Systemic cidofovir exposure was estimated based on previously conducted pharmacokinetic analyses of low-dose cidofovir in renal transplant recipients (as described in Chapter 3).

Cidofovir clearance was calculated as:

$$\mathrm{CL} = 5.70 - 1.94 \times (\mathrm{SCR} - 1.43)$$

where CL is cidofovir total body clearance (in liters per hour) and SCR is serum creatinine (in mg/dL). The systemic exposure from each cidofovir dose was then calculated according to:

$$\mathrm{AUC} = \frac{\mathrm{Dose}}{\mathrm{CL}}$$

where Dose is the cidofovir dose (in mg) and AUC is the estimated area under the cidofovir plasma concentration versus time curve from 0 to infinity (in mg*h/L). The cumulative cidofovir exposure in each patient was then calculated as:

$$\mathrm{AUC}_{\mathrm{total}} = \mathrm{AUC}_{\mathrm{dose\ 1}} + \mathrm{AUC}_{\mathrm{dose\ 2}} + \ldots\ \mathrm{AUC}_{\mathrm{dose}\ n}$$

where dose *n* is the final cidofovir dose received. The change in viremia was calculated as the maximum viral load prior to cidofovir treatment minus the viral load at the conclusion of the regimen (in copies/mL). Regression between cumulative systemic cidofovir exposure and the change in viremia was conducted using the REG Procedure in SAS (Cary, NC, USA).

6.4 RESULTS

6.4.1 Incidence of BK virus

Overall, 551 BKV-positive patients were identified. These patients received kidney transplants from November 1988 to March 2011. On average, subjects were 50.4 +/- 14.8 years of age, 64% were male, and the majority (88.9%) were Caucasian. A total of 66.2% of the patients had received a kidney from a cadaveric donor, while 33.8% received an organ from a living donor. The primary reason for graft failure in the study population was BK-associated chronic allograft nephropathy, which accounted for 34% of all failures. Of the patients with all-cause graft loss, 28.2% received a re-transplant.

A total of 507 patients had BK viruria, 207 had viremia, and 141 had a primary or secondary diagnosis from a needle biopsy of the kidney of BK viral infection. During the same time period, a total of 4645 patients received a kidney transplant at our institution. Thus, the overall incidence of BK viruria, viremia, and biopsy-confirmed BK nephropathy was 10.9%, 4.5%, and 3%, respectively. The demographic and clinical characteristics of the study population are reviewed in Table 16.

A total of 5787 viral loads were quantitated in urine in 507 patients. The average viral load, in $\log_{10}$ copies/mL, was 5.45 +/- 1.94 (median: 5.24). In plasma, there were 1480 samples collected in 207 patients over the observational period, with an average viral load of 3.57 +/- 0.915 $\log_{10}$ copies/mL (median: 3.39). The frequency distribution of BK viral loads in urine and plasma are displayed in Figure 39 and Figure 40, respectively.

Table 16. Characteristics of the 551 BK virus-positive kidney transplant recipients

Characteristic	Value	Range
Recipient age, y	50.4 (14.8)	18 - 82
Donor age, y	38.6	0 - 79
Male, %	64.6%	
Donor type, %		
Cadaveric	66.2%	
Living	33.8%	
Transplant type, %		
Kidney	88.9%	
Kidney/pancreas/small bowel	11.1%	
Patient race, %		
Caucasian	88.9%	
Black	8.89%	
Asian	0.36%	
Indian	1.09%	
Middle-east	0.73%	
Days post-transplant until detection of BK virus	651 (947)	0 - 5479
Prevalence of BKV in the study population		
Viruria, %	37.6%	
Viremia, %	92.2%	
Positive BK biopsy, %	25.6%	
Number of patients with graft failure	152	
Cause of graft failure, %		
Chronic allograft nephropathy	34%	
Acute rejection	11.1%	
Chronic rejection	17.7%	
Primary non-function	6.6%	
Graft functioning at death	16.4%	
Unknown/other	14.2%	
Patients with retransplantation after graft loss, %	28.2%	

Data are expressed as mean (SD) unless otherwise noted

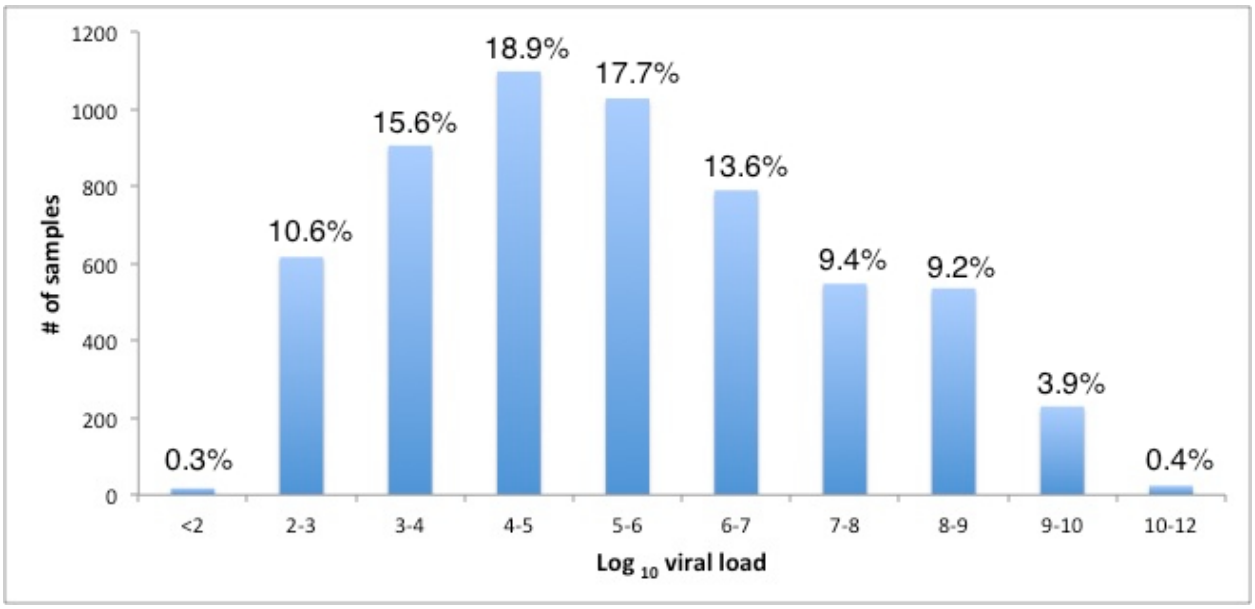


Figure 39. Frequency distribution of positive BK viral loads in urine during routine monitoring

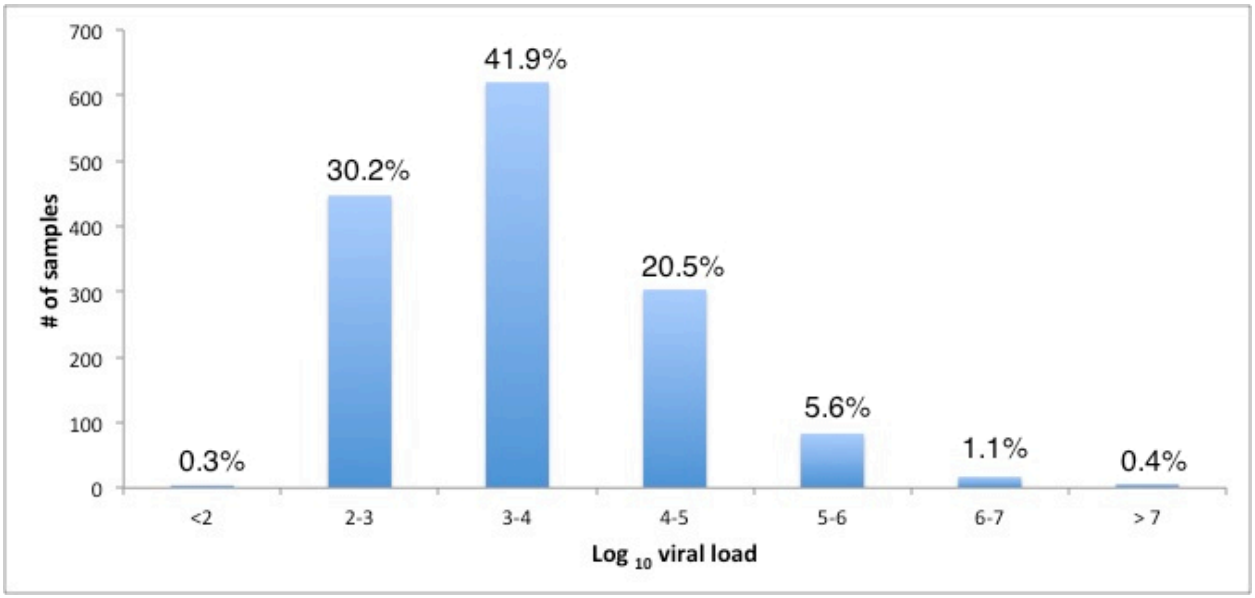
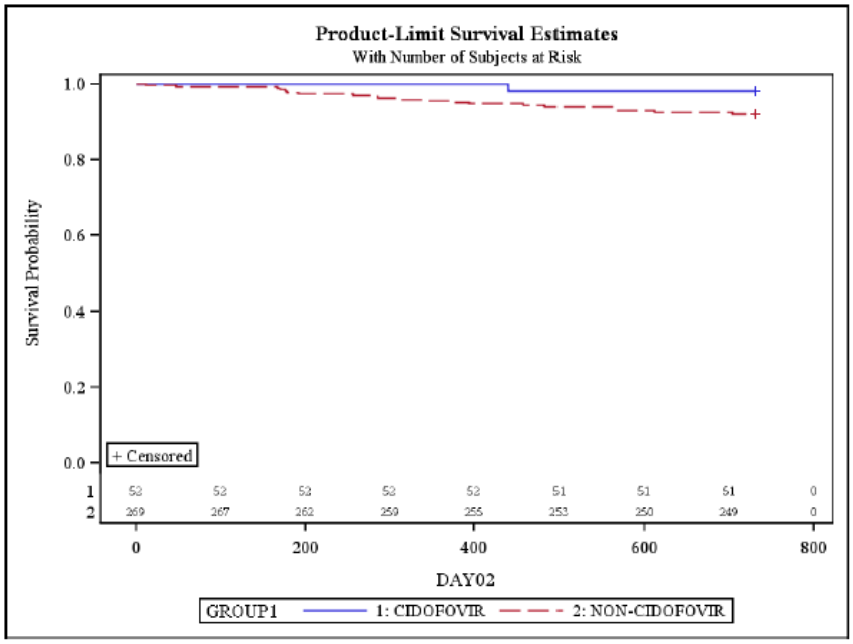


Figure 40. Frequency distribution of positive BK viral loads in plasma during routine monitoring

6.4.2 Impact of treatment with cidofovir on allograft survival

A total of 104 BK-virus positive patients (18.9%) received treatment with low-dose cidofovir. The use of cidofovir was at the discretion of the treating physician and no standardized algorithm was followed. The average cidofovir dose was 28.18 ± 31.17 mg, or 0.37 ± 0.37 mg/kg. The median number of cidofovir doses received was 20, which ranged from 1 dose to 94 doses. The Kaplan-Meier estimator was used to evaluate two-year allograft survival stratified by the use or non-use of cidofovir in renal transplant recipients with persistent BK viremia. Patients who died with a functioning graft and graft failures not due to BK (including acute rejection, chronic rejection, primary non-function, or unknown/other) were counted as non-failures. In cidofovir treated patients, the percent survival at two-years from the detection of viremia was 98.08% versus 92.19% in patients without cidofovir treatment, though this difference did not reach statistical significance ($p > 0.05$) (Figure 41). Due to the inherent bias of stratifying patients based on the use or non-use of cidofovir therapy (i.e. those patients with higher severity of illness are more likely to receive the drug), we also tested the survival function in an identical number of kidney transplant recipients (161) with viremia detected before and after January 1, 2007. This date was chosen because the majority of low-dose cidofovir use (85%) occurred during or after 2007. In patients with viremia detected pre-2007, the percent survival at two years was 89.4% versus 96.8% in patients with viremia detected post-2007 ($p < 0.05$). As the primary difference between the two groups was the more frequent use of cidofovir after January 1, 2007, this indicates a possible additional benefit of cidofovir use for graft survival in patients with BK virus infection.



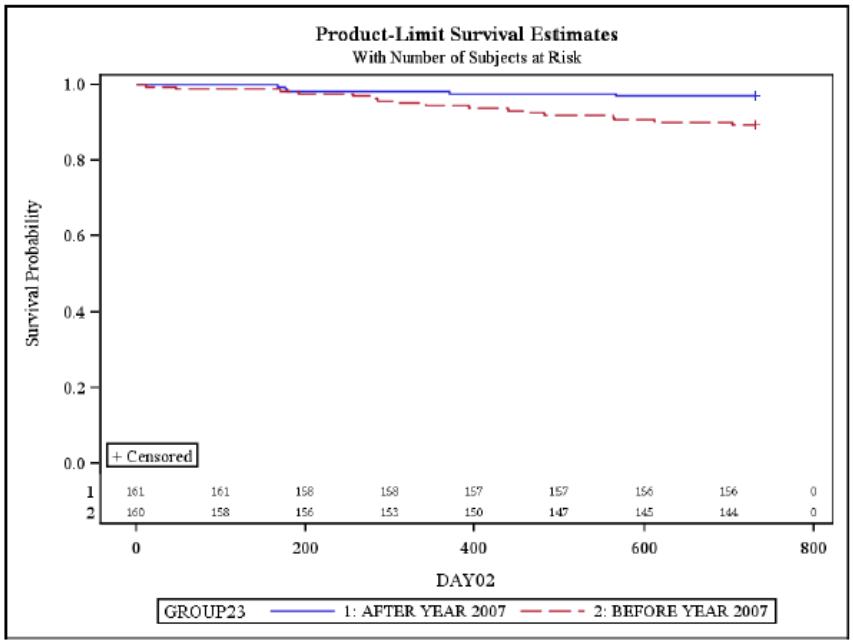


Figure 41. Kaplan-Meier estimates of two year graft survival in renal transplant recipients from the time of detection of viremia

Top: patients stratified according to use or non-use of cidofovir
Bottom: patients stratified according to date of detection of viremia (pre- and post- January 1, 2007)

6.4.3 Relationship between cidofovir exposure and viral decline

Overall, 2288 administrations of low-dose cidofovir were given over the 10-year time period between July 2001 and May 2011. A high degree of variability was present in the total degree of aggregate exposure, ranging from a minimum of 2 mg*h/L to a maximum of 758 mg*h/L. Regression analysis between cumulative cidofovir exposure and change in viremia from prior to initiation of the cidofovir regimen to the completion of the regimen revealed that higher systemic exposure was associated with a larger reduction in the degree of BK viremia (p=0.0238) (Figure 42). The equation describing this relationship is:

$$\Delta \text{ BK viremia} = -[(\text{AUC}total * 2407) + 32059]$$

where AUC_{total} is the cumulative cidofovir exposure in mg*h/L and the change in viremia is expressed in copies/mL. However, the goodness of fit of the linear model was poor, with a coefficient of determination (r^2) of only 0.11. Further, predicted reduction in viremia was low as compared with average pre-treatment viral loads.

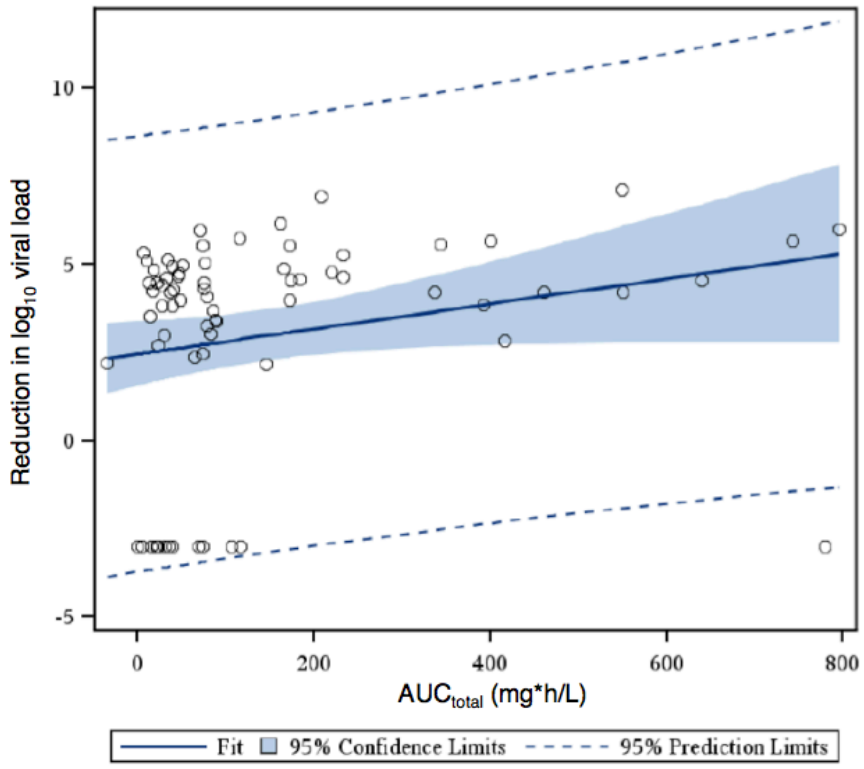


Figure 42. Linear model of the relationship between cumulative cidofovir exposure and change in viremia

6.5 DISCUSSION

In the past two decades, the human polyomavirus BK has surfaced as a significant pathogen in kidney transplant recipients. At the present time, optimal treatment regimens are lacking, though cidofovir is often used on an empiric basis with mixed results. Although several case reports and anecdotal evidence support the use of cidofovir for this indication, the utility of cidofovir in the treatment of BK virus has not been meticulously explored in randomized controlled trials. We have previously demonstrated that the disposition of low-dose cidofovir in kidney transplant recipients is highly dependent on glomerular filtration rate. Patients with varying degrees of kidney function receiving the same cidofovir dose, as is often done clinically, will consequently have vastly different systemic exposure to cidofovir, which could contribute to variability in treatment response. The objectives of this study were to evaluate the incidence of BK viruria, viremia, and biopsy-proven nephropathy at a large academic transplant center over an approximately 15 year period, and to assess the use of the antiviral agent cidofovir in the management of BK virus infection. Our results regarding the incidence of BKV are consistent with those of other centers, which describe BK affecting a substantial number of renal allografts. We found viremia, viruria, and nephropathy of 10.9%, 4.5%, and 3%, respectively. These results are slightly lower than other centers have reported; however, we evaluated a long-term period, at the beginning of which routine surveillance for BK was not common. This likely contributed to an underestimation of the total prevalence. The lack of routine surveillance in the early period may also explain why the average number of days post-transplant until identification of BK virus in this study is longer than previous reports (1.8 years versus 1 year) [40, 41].

Cidofovir-treated patients did not have significantly higher allograft survival than non-treated patients, though it is probable that patients receiving cidofovir were more ill and therefore

with higher risk of graft loss. We did discern, nevertheless, that in the 5-year period when cidofovir use was more prevalent, allograft survival was higher as compared to the period prior, suggesting a potential benefit with the use of the drug. On the other hand, this finding could signify more vigilant monitoring for BKV in plasma and urine, as well as prompt initiation of reduced immunosuppression upon detection, including lowered target trough concentrations for calcineurin inhibitors and discontinuation of antimetabolic agents. Additionally, practice patterns have changed over time, and since 2007 treatment is routinely initiated upon the detection of viremia and/or significant viruria, whereas in the period prior to 2007 treatment was generally withheld pending evidence of nephropathy on kidney biopsy.

This study retrospectively employed serum creatinine-based estimates of cidofovir clearance to approximate cidofovir exposure in individual transplant recipients. Predictions were then correlated to the microbiological response. We found that those patients with higher exposure did have an improved response, though the reduction in viremia was low when compared to the baseline values prior to treatment. For instance, the average maximum plasma viral load in the 207 patients with viremia was 3.89E+06 copies/mL. Assuming a cidofovir dose of 0.37 mg/kg in a 80 kg patient with a serum creatinine of 2 mg/dL, the single dose AUC would be estimated as 6.43 mg*h/L. Therefore, based on the regression analysis, a 6-month treatment course of once-weekly low-dose cidofovir encompassing 24 doses would be expected to reduce the degree of viremia by only 10.4%. Further, only 11% of the variance of the decline in BK viral loads in plasma could be explained by variation in cumulative cidofovir exposure. This indicates that other factors, presumably reduced immunosuppression allowing for an increased immune response, play a major role in viral clearance. Moreover, this data suggests that the low

doses of cidofovir used in renal transplant recipients may be inadequate to produce optimal therapeutic concentrations in the kidney at the site of action.

The current study has several limitations. First, we used predicted rather than measured cidofovir AUC estimates. However, we have demonstrated that accurate estimation of cidofovir clearance is possible using the renal filtration rate. Next, when analyzing the relationship between drug exposure and virologic response, we used cumulative exposure, which incorporated each of the doses received. Those patients with a higher cumulative AUC would have received more doses over a longer period of time, allowing for a longer time frame for immune-mediated viral clearance. Third, no institutional protocol was used to guide the use of cidofovir for BK virus infection. In the future, it is recommended that an algorithm be followed, which would remove variance from the patterns of practice and provide more meaningful data to advance patient care. Finally, we did not obtain the characteristics of the entire kidney transplant population at our institution over the study period, which would have been useful in identifying independent risk factors for the development of BK virus and its complications. However, this has been done previously and several reports are available in the literature [36-38].

In summary, BK virus continues to represent a major cause of morbidity and mortality to kidney transplant recipients, and cidofovir use as practiced currently only marginally improves outcomes and viral clearance. Although patients with higher cumulative cidofovir exposure had a larger decline in plasma BK viral loads, the reduction may not be sufficient to consistently bring viremia below the limit of detection in the long-term in the majority of cidofovir-treated patients. These results highlight the need for continued investigations to discover and implement more effective treatments.

7.0 SUMMARY AND FUTURE DIRECTIONS

7.1 DISCUSSION AND SUMMARY

The objective of the work carried out in this dissertation was to characterize anionic tubular secretion capacity in kidney transplant recipients and to evaluate the role of actively secreted antiviral agent cidofovir in the treatment of BK virus infection in this patient population. This was investigated by (i) development of a novel analytical technique to determine low concentrations of the OAT1 probe drug cidofovir in human plasma, (ii) conducting a clinical pharmacokinetic study of cidofovir in renal transplant recipients without and with inhibition of active secretion, (iii) performing a translational animal study that explored the impact of kidney transplantation on expression of drug transporters and metabolic enzymes in the kidney, (iv) analyzing the acute pharmacodynamic response after administration of cidofovir in kidney transplant recipients with BK viremia, and (v) retrospectively assessing the use of cidofovir for management of BK virus in kidney transplant recipients at a large academic transplant center.

In the first part of the study, we used non-compartmental and population pharmacokinetic approaches to elucidate the disposition of low-dose cidofovir in densely sampled kidney transplant recipients with BK viremia. The OAT1-dependent active secretion capacity was evaluated by assessing the impact of concomitant probenecid administration on cidofovir total body clearance and renal clearance. Cidofovir was selected as a probe substrate for anionic secretion because it is not significantly metabolized [228], exhibits negligible binding to plasma proteins (< 0.5%) [227], undergoes transport by renal OAT1 [77], is cleared via renal filtration and secretion with no evidence of reabsorption [218], and is frequently administered to the patient population of interest. Potential correlations between pharmacokinetic parameters and patient variables were also considered to explain sources of variability. We demonstrate that renal filtration is the predominant clearance mechanism and that active secretion likely does not

appreciably contribute to cidofovir elimination in kidney transplant patients, despite the fact that previous studies in healthy volunteers and HIV-infected patients with normal kidney function show that renal clearance is 60-70% higher than baseline creatinine clearance. Noncompartmental estimates of cidofovir clearance were linearly related to eGFR in both the absence and presence of probenecid. In the population analysis, a two-compartment model with first order elimination adequately described the data and estimates of CL and Vd were 5.31 L/hr and 28.5 L, respectively. Probenecid administration was not identified as a significant categorical covariate for cidofovir clearance. Overall, our data suggest that both systemic clearance (CL_S) and renal clearance (CL_R) were lower in kidney transplant recipients than in patients with normal renal function, and probenecid did not alter the renal clearance of cidofovir. The metabolic clearance (CL_M; e.g. $CL_M = CL_S - CL_R$) was also not significantly altered, accounting for approximately 22% of systemic clearance. These data indicate that OAT1-mediated drug secretion may be impaired in renal transplant recipients with BK viremia, on the basis that cidofovir (a prototypical OAT1 substrate) was not transported by a probenecid-sensitive mechanism. Possible explanations for this finding include altered intracellular phosphorylation of cidofovir in kidney transplant patients, the presence of probenecid-insensitive drug elimination pathways, lack of intracellular α-ketoglutarate stores in transplant patients necessary for counter transport of cidofovir, or reduced expression and/or activity of drug transporters in the renal epithelium

In the second part of the study, we conducted a translational animal study to understand the mechanistic basis of the above-mentioned clinical observation. Specifically, we evaluated if kidney transplantation itself alters the expression of anionic transporters in the kidney. An orthotopic, syngeneic rat transplant model was employed and the mRNA and protein expression

of the primary drug transporters and oxidative metabolic enzymes were evaluated at various time points after transplantation. We demonstrate a significant decrease in both OAT1 and OAT3 protein expression in tissue homogenates beginning at 6 h post-transplantation and persisting for the entire observational period of 78 days. At 24 h post-renal transplant, the mRNA expression of renal OCT2 and CYP2E1 were significantly reduced while MRP2, MDR1, and CYP3A were unchanged. At 78 days post-transplant, the mRNA expression of renal OCT2 was significantly reduced while MRP2 expression was increased by 47% ($p<0.05$) and MDR1, CYP3A, and CYP2E1 were unchanged. All animals had stabilization of serum creatinine to pre-transplant levels with 7 days, indicating that alteration in filtration and secretion may not occur in parallel. These results show that renal transplantation alters the mRNA and protein expression of various metabolic enzymes and transporters in the kidney, and the data regarding OAT1 in particular are in agreement with the clinical pharmacokinetic study of cidofovir.

In the third part of the study, we appraised the pharmacodynamics of cidofovir used for the treatment of BK virus in renal transplant recipients. This study also evaluated the inhibition of OAT1-mediated uptake of cidofovir (with probenecid) on the virologic response in plasma and urine. As BK virus is localized in proximal tubule cells, decreased cidofovir uptake in the presence of probenecid should cause an attenuated response. Yet, because OAT1-mediated active secretion was not observed in the pharmacokinetic study in kidney transplant recipients and reduced expression of OAT1 was noted in the rat transplant model, we hypothesized that concomitant probenecid administration would not alter the response. The results demonstrate a significant reduction in viruria and viremia at 12 hr post-cidofovir infusion, reflecting the short *in vivo* half-life of the virus. However, the effect was not sustained, and viremia and viruria returned to baseline at the next sampling period (8 days later for plasma; 1 day later for urine).

We observed no association between metrics of systemic cidofovir exposure and decline in viremia or viruria. We conclude that cidofovir transiently reduces BK viremia and viruria and may be an efficacious add-on treatment option in the pharmacological management of BK virus in renal transplant recipients; however, as viremia and viruria quickly recovered to pre-treatment levels, the dosage regimen may require modification. Additionally, probenecid did not change the degree or duration of decline in viruria and viremia, providing further evidence to support a lack of cidofovir uptake into the renal epithelium in kidney transplant recipients.

In the last study, serum creatinine-based estimates of cidofovir clearance were used to retrospectively approximate aggregate cidofovir exposure in 104 individual transplant recipients, and this information was then correlated with microbiological response. Regression analysis demonstrated that higher estimated systemic exposure was significantly associated with a larger reduction in the degree of BK viremia, though the predicted reduction in viremia was low as compared with average pre-treatment viral loads in the study population. Further, only a small percentage of the variance in the decline in BK viral loads in plasma could be explained by variation in aggregate cidofovir exposure, suggesting that other factors, presumably immune-mediated, play a major role in viral clearance.

The major site of BK viral replication *in vivo* is within the kidney tubules, and our results suggest that OAT1-facilitated uptake of cidofovir into these cells may be reduced in kidney transplant recipients. This potentially implies that cidofovir should not be expected to achieve adequate concentrations at the site of pharmacological action. Yet our results demonstrate that cidofovir does elicit a therapeutic response when used for the treatment of BK virus infection in this patient population. Potential explanations for these two seemingly contradictory observations include: (1) cidofovir crosses the basolateral membrane of the proximal tubules in a

passive, probenecid-insensitive manner, (2) cidofovir is actively transported into the tubules by a probenecid-insensitive pathway, (3) cidofovir is efficiently transported across the basolateral membrane into the tubules by a probenecid-insensitive mechanism and efflux across the apical membrane into the collecting duct is impaired such that active secretion of the drug was not detected upon pharmacokinetic analysis, or (4) cidofovir produces an anti-BKV effect locally within the blood and urine, and not within the kidney.

In summary, the work detailed in this dissertation collectively broadens our understanding of drug disposition in kidney transplants recipients and provides fundamental knowledge that may improve the treatment of BK virus infections in kidney transplant recipients.

7.2 CLINICAL IMPLICATIONS

1) The LC-MS/MS method that was developed and validated for quantitative assessment of cidofovir in plasma is suitable for implementation in laboratories with standard instrumentation and would be useful in future studies of low-dose cidofovir, especially where high sensitivity is required.

2) Renal anionic drug secretion facilitated by OAT1 may be compromised in kidney transplant recipients with BK viremia, possibly secondary to diminished OAT1 expression in the kidney. Renal clearance of several OAT1 substrates may be reduced in this patient population and dosage adjustments may be warranted to avoid toxicities associated with these drugs.

3) Endogenous prostaglandins, including the proinflammatory prostaglandin E2 (PGE_2), are OAT1 substrates and reduced elimination in the setting of diminished renal OAT1 expression in kidney transplant recipients may lead to increased inflammation, which in turn may further down regulate OAT1 in a cyclical nature.

4) The clearance of cidofovir after low-dose administration could be accurately predicted using clinical estimates of renal filtration (SCR, eGFR, CrCl). The linear relationship between these variables can be utilized to guide drug dosing for BK virus, provided an optimal degree of cidofovir systemic exposure is identified through future investigations.

5) The administration of low-dose cidofovir on a once weekly basis to transplant recipients with BK virus infection only transiently reduced the degree of viruria and viremia. Once weekly dosing may be insufficient to produced a sustained response in order to clear the virus in the majority of treated patients. Increasing the dosing frequency to at least twice weekly may be justified in light of this finding. Additionally, we demonstrate a lack of nephrotoxicity from once weekly low-dose cidofovir. However, the benefit of this approach should be thoroughly categorized in prospective, randomized controlled trials.

7.3 LIMITATIONS AND RECOMMENDED FUTURE RESEARCH DIRECTIONS

1) Drug transport facilitated by renal OAT1 is potentially reduced in kidney transplant recipients based on the absence of cidofovir secretion as compared to estimates of renal filtration. The anionic tubular function in this population should be confirmed by characterizing

the OAT1 functional secretory capacity with the gold standard substrate para-aminohippuric acid (PAH). Ideally, the glomerular filtration rate should be precisely measured with markers such as inulin or iothalamate. While this approach would be more invasive than the probe drug approach employed in this dissertation, the results would conclusively establish the activity of this pathway. Further, such a study could be expanded to include patients with various stages of CKD, as well as transplant recipients without and with BK virus infection.

2) The pharmacokinetic-pharmacodynamic studies were carried out in a relatively ethnically homogenous patient sample, potentially limiting the applicability of the conclusions to other populations.

3) Altered expression of various drug transporters and metabolic enzymes localized to the proximal tubule of the kidney was observed following renal transplantation in a rat model, though it is unknown how this finding translates to humans. To understand the clinical relevance, the mRNA and protein expression of anionic and cationic drug transporters and oxidative enzymes should be evaluated in kidney biopsy samples from renal transplant recipients when such samples are available through the normal scope of care.

4) The results of the work herein suggest that renal transplantation and BK virus reduce OAT1 expression and activity, though the impact of each of these factors was not thoroughly studied independently. To address this limitation, the OAT1-dependent basolateral-to-apical transport of cidofovir should be evaluated in an *in vitro* model system using Madin-Darby canine kidney (MDCK) type II distal tubular polarized cells stably expressing the human OAT1

isoform. Modification of the model system by inclusion of varying concentrations of inflammatory cytokines or viral infection with BKV would allow for discrimination between the effects of each factor on OAT1 expression and functional activity.

5) A prospective, randomized trial to establish the safety, tolerability, and effectiveness of cidofovir for the management of BK virus in renal transplant recipients is warranted. Such a trial should include cohorts receiving escalating cidofovir dosages. Additionally, the results of the work in this dissertation demonstrate that more frequent dosing would potentially be advantageous, and this approach should be incorporated into future investigations.

6) The use of low-dose cidofovir for the treatment of BK virus produced only modest benefit in retrospective analyses and was not definitively associated with improved allograft survival. Continued investigations to discover and implement more effective and less-toxic treatments for the management of BK virus in renal transplant recipients are necessary.

7) The orally available lipid conjugate of cidofovir CMX001 is under investigation for the treatment of BK virus. Although the anti-BKV activity of this agent has been demonstrated in human renal tubular epithelial cells [269], the role of renal tubular drug transporters in the disposition of CMX001 should be explored, as active transport into the site of action is desirable to achieve maximum therapeutic effect.

8) We hypothesized that the altered expression of drug transporters and metabolic enzymes observed following kidney transplantation in rats is secondary to increased inflammatory

mediators. However, circulating cytokine concentrations were not measured. Future investigations should determine cytokine concentrations and relate to the degree and duration of renal transporter mRNA and protein down regulation.

APPENDIX A

[IRB PROTOCOL]

Abstract

Polyoma BK virus causes destructive nephropathy in approximately 8% of renal allografts and is an important cause of renal graft dysfunction. Currently no firmly established treatment regimen exists for BK virus infection in renal transplant patients. Cidofovir is an acyclic nucleotide analog antiviral agent with activity against BK virus. A low dose of 0.25 to 0.5 mg/kg of cidofovir (representing 5-10% of the standard dose for other indications) is empirically used in kidney transplant patients due to concern of the drug's nephrotoxicity at normal doses. However, it is unclear whether this dose is adequate to treat BK virus in this patient population. Recently there have been methods developed to measure BK viral load in plasma and urine. Since adequate levels of cidofovir in the kidney are important to treat BK virus, it is important to understand the urinary excretion and pharmacokinetics of cidofovir and changes in BK viral load in plasma and urine in renal transplant patients receiving cidofovir in order to optimize its use in this patient population.

In this study we will evaluate the BK viral load, and characterize the pharmacokinetics of cidofovir in renal transplant patients on two separate occasions - while it is administered alone and again when it is administered along with probenecid, a drug that blocks the active excretion of cidofovir in the kidney. During each study section, eligible consenting patients will be asked to provide blood sampling over the course of 12 hours just before, during, and after initiation of the infusion of cidofovir. Urine will also be collected throughout this time period. If patients reside locally, we will obtain a 24, 48, and 72-hour blood and urine sample. If patients are not local we will attempt to obtain the samples if possible. Plasma will be analyzed for cidofovir and probenecid concentrations and BK virus DNA. Urine will be analyzed for cidofovir and probenecid concentrations and BK virus DNA. When available,

kidney biopsy samples will be subjected to mRNA and protein analysis of the cidofovir transport protein, OAT1. BK viral load from plasma and urine is presently assessed routinely in the clinics. Viral load measurements will be incorporated into this study to aid in the development of a pharmacokinetic-pharmacodynamic model. Subjects will also be asked to provide a 24-hour urine collection before initiation of cidofovir dose and after the last dose or after the tenth dose, whichever occurs first. Urine will be analyzed for creatinine concentration to assess the effect of cidofovir on creatinine clearance and corresponding creatinine clearance (CrCl).

Hypotheses and Specific Aims

We hypothesize that there will be a relationship between BK viremia and BK viruria and cidofovir plasma and urine concentration.

Specific Aim #1. To quantify changes in plasma and urine BK virus DNA using real-time quantitative polymerase chain reaction, and to correlate with cidofovir concentrations in plasma and urine. This will be pilot study to evaluate relationship between drug concentration and clinical outcome.

We hypothesize that cidofovir pharmacokinetics will be altered and the renal secretion of cidofovir will be impaired in renal transplant patients due to impaired renal secretion process.

Specific Aim #2: To evaluate the pharmacokinetics of cidofovir in renal transplant patients

with BK viremia. To assess the contribution of renal secretion to the overall clearance of cidofovir by evaluating the effect of probenecid (an inhibitor of the renal secretion pathway) on cidofovir pharmacokinetics.

We hypothesize that protein principally involved in cidofovir transport (organic anion transporter 1-OAT1) will be down-regulated secondary to increased pro-inflammatory cytokines associated with organ transplantation. We hypothesize that the low expression of OAT1 will lead to the low urinary secretion of cidofovir.

Specific Aim #3. To evaluate the mRNA / protein expression of organic anion transporter 1 (OAT1) in kidney biopsy samples (whenever available) from renal transplant recipients.

We hypothesize that the use of low dose of cidofovir will lead to minimal alteration in the glomerular filtration rate.

Specific Aim #4. To evaluate the effect of low-dose cidofovir on glomerular filtration rate (GFR) of the transplanted kidney.

Background and Significance

The ultimate goal is to develop an improved dosing regimen for cidofovir using pharmacokinetic/pharmacodynamic (viral load) data to optimize therapy for BK virus in renal transplant recipients.

BK polyomavirus has emerged as an important complication following kidney transplantation. Currently no firmly established therapy exists for the treatment of BK virus infection. Standard practice includes lowering immunosuppression and monitoring BK virus DNA in plasma and urine. If infection persists, cidofovir therapy may be initiated. Cidofovir, an acyclic nucleotide analog antiviral agent, has been shown to be effective against BK virus *in vitro*. A low dose of 0.25 to 0.5 mg/kg given weekly or biweekly is commonly used due to concern of the drug's nephrotoxicity. However, it is unclear if this dose/frequency is adequate. The cidofovir dose used is empiric and represents only 5-10% of the recommended dose used for patients with normal renal function undergoing treatment for CMV. Additionally, the relationship between changes in BK viral load and cidofovir concentration has not yet been elucidated.

There is limited data on the course of BK viral load in kidney transplant patients. Nothing is known about the pharmacokinetics and pharmacodynamics of cidofovir in renal transplant patients. This pilot research study is important to validate cidofovir as an effective treatment for BK virus infection and to establish appropriate dosage regimen in kidney transplant patients.

Patients who receive cidofovir for clinical treatment will be enrolled in this study. Cidofovir will not be administered for research purposes. Cidofovir is standard of care for BK virus infection in patients not responding to a reduction in immunosuppression.

Research design and methods

Study 1:

Part 1: 24 hr urine collection:

This may be performed from 5 days to 1 day prior to IV cidofovir administration

Study 1:

Part 2: BK viral load and pharmacokinetics of Cidofovir when administered alone:

This will take place within 5 days after study 1 part 1. On the day of cidofovir administration, participants will come to Montefiore Hospital Clinical and Translational Research Center by 7 am. One liter of 0.9% sodium chloride solution will be given intravenously over a 1 hr period immediately prior to cidofovir infusion. Cidofovir 0.25-6 mg/kg (based on the discretion of the treating physician) will be administered intravenously over 1 hr. Blood samples (approximately 8 ml) will be collected in vacutainers just before and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after the start of the cidofovir infusion. Urine will be collected in aliquots from 0 to 1, 1 to 2, 2 to 4, 4 to 8, and 8 to 12 hours after the start of the cidofovir infusion. All procedures will be performed by CTRC nursing staff. Subjects will receive meals during the stay at the CTRC. If patients reside locally we will obtain additional blood (approximately 8 ml) and urine samples at 24, 48, and 72 hours at the CTRC, however, these samples may be drawn during routine clinical visits. If patients are not local we will attempt to get blood and urine samples at 24, 48, and 72 hours if possible. These additional samples will be helpful to fully understand the effect of cidofovir on viral replication. Only blood and urine collection is done for research purposes. Concentrations of cidofovir and probenecid in each plasma and urine sample will be measured by high performance liquid chromatography mass spectrometry (HPLC-MS).

Each plasma and urine sample will be analyzed for BK virus DNA measured using RT-PCR and correlated with cidofovir concentrations in plasma and urine. The instrumentation and methodology for the RT-PCR is available within Transplantation Pathology. This PCR test is routinely done clinically but will be carried out more often for

research purposes on blood and urine samples already collected for pharmacokinetic study. This will be pilot study to evaluate relationship between drug concentration and clinical outcome. If available through the normal scope of clinical care, kidney biopsy samples may be analyzed for expression of the cidofovir transport protein OAT1 using western blot techniques. No biopsy samples will be taken strictly for research purposes.

Study 2: BK viral load and Pharmacokinetics of Cidofovir when administered with probenecid:

Part 1:

This study will take place either one week or two weeks after Study 1 Part 2. However, if due to clinical judgment or a patient who is unable to attend the study session, this time period may be extended to 1 month. On the day of cidofovir administration, participants will come to Montefiore Hospital Clinical and Translational Research Center by 7 am. Participants will receive two grams of probenecid orally approximately 1-2 hours prior to the infusion of cidofovir. One liter of 0.9% sodium chloride solution will be given intravenously over a 1 hr period immediately prior to cidofovir infusion. Cidofovir will be administered intravenously over 1 hr. One gram of probenecid will be given orally at 2 hours and again at 8 hours following completion of the cidofovir infusion. Blood samples (approximately 8 ml) will be collected before and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after the start of the cidofovir infusion. Urine will be collected in aliquots from 0 to 1, 1 to 2, 2 to 4, 4 to 8, and 8 to 12 hours after the start of the cidofovir infusion. All procedures will be performed by CTRC nursing staff. Subjects will receive meals during they stay at the CTRC. If patients reside locally we will obtain additional blood (approximately 8 ml) and urine samples at 24, 48, and

72 hours at the CTRC, however, these samples may be drawn during routine clinical visits. If patients are not local, we will attempt to get blood and urine samples at 24, 48, and 72 hours if possible. The dispensing and administration of probenecid and blood/urine collection is done for research purposes. However, probenecid is normally used with cidofovir in other patient populations. Concentrations of cidofovir and probenecid in each plasma and urine sample will be measured by high performance HPLC-MS. Each plasma and urine sample will be analyzed for BK virus DNA using RT-PCR by clinical lab and correlated with cidofovir concentrations in plasma and urine. This test is clinically done routinely but will be carried out more often for research purposes on blood and urine samples already collected for pharmacokinetic study. This will be pilot study to evaluate relationship between drug concentration and clinical outcome. If available through the normal scope of clinical care, kidney biopsy samples may be analyzed for expression of the cidofovir transport protein OAT1 using western blot techniques. No biopsy samples will be taken strictly for research purposes.

Study 3:

Part 1: 24 hr urine collection:

This will be performed after ten doses of IV cidofovir administration have been given or after the patient's last cidofovir dose, whichever occurs first. This information will be used to access changes in GFR in the patients pre and post cidofovir use. This can be done at patients home or in the hospital. Participants will be given containers and asked to collect urine for a 24-hour period.

Creatinine clearance will be calculated as [(UCr * UVol * 1/1440min) / (Plasma Cr)]

Human Subjects

Inclusion criteria

- Age greater than or equal to 18 years and less than or equal to 70 years

- Recipient of kidney transplant

- BK virus infection diagnosed by a positive plasma or urine PCR assay for BK virus DNA or renal biopsy demonstrating BK virus within 30 days prior to receipt of first dose of cidofovir

- Ordered treatment with cidofovir as part of standard medical care

Exclusion criteria

- Unable to provide informed consent.

- Hypersensitivity to cidofovir or other nucleotide analogues

- Hypersensitivity to probenecid or sulfonamides

- Hemoglobin less than 9 gm/dL and/or hematocrit less than 26%

- Currently receiving another drug known to affect active renal secretion of anionic drugs

- Pregnant or breast feeding women.

APPENDIX B

[CONSENT FORMS AND STUDY SHEETS]

CONSENT TO ACT AS PARTICIPANT IN A RESEARCH STUDY

TITLE: Pharmacokinetics and Pharmacodynamics of Cidofovir Used for the Treatment of BK Virus in Renal Transplant Recipients.

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Source of Support: Partial support from NIH grant; Clinical Pharmacokinetics Laboratory.

Why is this research being done?

Cidofovir is an antiviral drug that is approved by the Food and Drug Administration (FDA) for the treatment of cytomegalovirus (CMV) infections. It is commonly used with another drug called probenecid in order to prolong (extend) its effect to treat this type of infection and to protect the kidney.

In some kidney transplant patients, a virus called BK virus, can cause dysfunction (make the kidney not to work properly) or possible failure of the kidney. Currently, Cidofovir is not FDA approved for the treatment of BK virus but it has been used in small doses for the treatment of the BK virus (off-label) in kidney transplant patients. We are looking to gather more information to check whether the current dose and dosing schedule is appropriate for kidney transplant recipients. This study will be the first study to look at blood and urine levels of these medications during treatment with Cidofovir, both with and with out the addition of the second medication probenecid, in kidney transplant patients.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you are a male or female between the ages of 18-70 years, have had a kidney transplant and have been diagnosed with the BK virus. Your transplant physician is recommending treatment with the medication cidofovir, since a reduction of your immunosuppressive medication(s) has not been adequate for the treatment of the virus. We will be enrolling approximately 14 individuals at this medical center for this study.

How will the study be done?

If you decide to take part in this research study, you will undergo screening procedures that are not part of your standard medical care.

Screening Procedures:

Procedures to determine if you are eligible to take part in a research study are called "screening procedures." For this research study, the screening procedures include:

For women who could possibly be pregnant, a urine sample will be collected for a pregnancy test. Pregnant women or women who are currently breast feeding will not be allowed to take part in this study.

Treatment Procedures:

Your treatment plan will be very similar to the routine treatment plan for patients with BK virus (the treatment you would receive regardless if you participate in this study), but who may not participate in this study. However, for the purpose of this study we will be evaluating the concentration of the drug in your blood and urine during two of your treatments with cidofovir.

One treatment will be with cidofovir only and one treatment will be with cidofovir plus 3 doses of a medication, called probenecid (that is normally used along with Cidofovir in other patient populations to protect the kidney). You will be asked to provide blood and urine samples before, during and after these treatments.

You may receive Cidofovir once a week or every other week, as per the decision of your physicians. Once you are enrolled, you will be scheduled for two visits that will last

approximately 12 to 14 hours, at our Clinical and Translational Research Center (CTRC) located on the 6th floor of Montefiore Hospital. You will also be scheduled to return to the CTRC at 24, 48, and 72 hours after you are given cidofovir for additional blood draws and urine samples. You will complete your participation within a maximum of six months. On your first visit you will receive only cidofovir intravenously (through a vein in your arm). On your second visit, the medication probenecid will be given orally (by mouth) once before and two times after the Cidofovir administration. During your stay, there will be blood sampling and urine collection at specific times throughout the day.

1. Prior to scheduled treatment with cidofovir: A 24 hour urine collection will be carried out within 5 days of the first dose of scheduled Cidofovir treatment. You will be given a plastic container to collect all of your urine for a 24 hour period. When you are finished with the collection, you will bring the container to your next scheduled transplant clinic visit.
2. Treatment with cidofovir only (Study 1): On the day that you are scheduled for your Cidofovir treatment you will arrive at the CTRC by 7 AM. The nurses will place an IV catheter (a thin plastic tube) into your arm and begin to give you an IV of normal saline (a fluid) to keep you hydrated. Prior to starting the treatment, you will have a blood sample taken (about 1 teaspoon). They will then give your medication, cidofovir through the IV, which will take approximately 1 hour. Blood samples (approximately 2 teaspoons each time) will also be collected at 30 minutes, 1, 1 ½, 2, 4, 6, 8, and 12 hours after the start of the medication. You will be asked to collect your urine in plastic containers during the entire stay. After leaving the CTRC you will be asked to return to provide blood (about 2

teaspoons) and urine samples at 24, 48, and 72 hours after you have been given cidofovir.

3. Treatment with cidofovir and probenecid (Study 2): On the day that you are scheduled for your treatment you will arrive at the CTRC by 7 AM. On your arrival, you will be given the medication probenecid to take by mouth 1 to 2 hours prior to the start of cidofovir. The nurses will place an IV catheter (a thin plastic tube) into your arm and begin to give you an IV of normal saline (a fluid) to keep you hydrated. During this time, a blood sample will be taken (about 2 teaspoons). One to two hours after taking the probenecid, they will start giving you the cidofovir through your IV. This will take approximately 1 hour. After finishing the cidofovir, probenecid will be given 2 hours later and again 8 hours later. Blood samples (1 teaspoon each time) will also be collected at 30 minutes, 1, 1 ½, 2, 4, 6, 8, and 12 hours after the start of the cidofovir. You will be asked to collect your urine in plastic containers during the entire stay. After leaving the CTRC you will be asked to return to provide blood (about 1 teaspoon) and urine samples at 24, 48, and 72 hours after you were given cidofovir.
4. 24 hour urine collection (Study 3): Additionally, a 24 hour urine collection will be done after you have received several treatments with cidofovir (up to 10 treatments) or after your last cidofovir dose, whichever occurs first. You will be given a plastic container to collect all of your urine within 24 hours. When you are finished with the collection, you will bring the container to your next scheduled transplant clinic visit. If at any time during your participation in this study a kidney biopsy is done for your routine clinical care, additional testing may be performed on the biopsy sample. No biopsies will be taken for research purposes only.
5. Follow-up procedures: You will be followed as per your routine visits to the Starzl

Transplantation Clinic.

What are the possible risks, side effects, and discomforts of this research study?

There may be certain risks associated with participation in this study. These may include:

Risks of Venipuncture:

Common risks (occurs in 10-25% or 10 to 25 out of 100 people): Discomfort, bruising, bleeding or slight swelling at the site of where the blood was drawn.

Infrequent (rare) risks (occur in 1-10% or 1 to 10 out of 100 people): Infection may occur at the site of where the blood was drawn or fainting may occur after you have had your blood drawn.

Risks of intravenous catheter insertion:

Common risks (occurs in 10-25% or 10 to 25 out of 100 people): Discomfort, bruising, bleeding or slight swelling at the site of the IV catheter.

Infrequent (rare) risks (occur in 1-10% or 1 to 10 out of 100 people): Clot formation or infection may occur at the site of the IV catheter.

Risks of Probenecid:

Common risks (occurs in 10-25% or 10 to 25 out of 100 people): Headache, nausea/vomiting, anorexia (loss of appetite).

Infrequent (rare) risks (occur in 1-10% or 1 to 10 out of 100 people): Dizziness, flushing, alopecia (loss of hair), polyuria (frequent urination), nephrotic syndrome (swelling of the kidney), interstitial nephritis (inflammation of the kidney), leukopenia (low white blood cell count), anemia (low red blood cell count), allergic reaction (itching, swelling, hives), anaphylactic shock (severe/life threatening allergy).

As with the use of any drug there may be adverse events or side effects that are currently unknown and it is possible that certain of these unknown risks could be permanent, serious and life threatening.

Being a part of this study while pregnant or breastfeeding may expose the unborn child or nursing infant to risks that are known and unknown. Therefore, pregnant and nursing women will not be included in this study. If you are a woman of childbearing potential, a urine pregnancy test will be done during a screening visit. It must be negative before you can enter this study. While receiving study drug, and for a period of 30 days after that you must agree to use appropriate methods of birth control. Medically acceptable birth control methods include: (1) surgical sterilization, (2) approved hormonal contraceptives (such as birth control pills or Lupron Depot®), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) an intrauterine device (IUD).

What are possible benefits from taking part in this study?

There is no guarantee that you will receive any benefit from participating in this study. However, your participation may help others in the future by what the doctors learn from your involvement in this study.

What treatment or procedures are available if I decide not to take part in this research study?

If you decide not to take part in this research study, you will undergo normal procedures associated with the treatment of BK virus in kidney transplant recipients. No routine treatment will be withheld.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate in this study.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

All costs and tests done to treat you before and after your kidney transplant should be covered by your medical insurance. The tests included in this study are those that would normally be performed in kidney transplant recipients with the BK virus.

Some of the services you will receive during this are “research only services” that are being done only because you are in the study. These services will be paid for by the study and will not be billed to your health insurance company or you. The administration of the medication probenecid, pregnancy tests, the blood and urine testing done on study 1 and 2 and the 24 hour urine collection test done twice will be paid for by the research study.

Some of the services you will receive during this study are considered to be "routine clinical services" that you would have even if you were not in the study. An example would be receiving the cidofovir medication as your treatment. These services will be billed to your health insurance company or you, if you do not have health insurance.

You will be responsible for paying any deductibles, co-payments or co-insurance that are a normal part of your health insurance. You may want to get more detailed information about what "routine clinical services" your health insurance is likely to pay for. You may want to talk to a member of the study staff and/or a UPMC financial counselor to get more information.

Will I be paid if I take part in this research study?

You will receive payment for parking during your participation in this study. In addition, you will receive meals during your 12-hour stay at the CTRC.

Who will pay if I am injured as a result of taking part in this study?

University of Pittsburgh researchers and their associates who provide services at University of Pittsburgh Medical Center (UPMC) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

Who will know about my participation in this research study?

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use or disclosure of my identifiable medical information?

This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The

information that will be recorded will be limited to information concerning demographics (age, gender, and race) and concurrent conditions and medications you are receiving.

This research study will result in identifiable information that will be placed into your medical records held at the Starzl Transplantation Institute. The nature of the identifiable information resulting from your participation in this research study that will be recorded in your medical record includes your age, gender, date of transplant, and lab values. Note that this information may already be in your medical records regardless of whether or not you agree to participate in this study.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives from the Food and Drug Administration may review and or obtain your identifiable (which may include your identifiable medical information) related to your participation in this research study for the purposes of monitoring the accuracy and completeness of the research data. While the U.S. Food and Drug Administration

understands the importance of maintaining the confidentiality of your identifiable research and medical information, the UPMC and University of Pittsburgh cannot guarantee the confidentiality of this information after it has been obtained by the U. S. Food and Drug Administration.

Authorized representatives of UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information)

related to your participation in this research study for a minimum of 7 years and for as long (indefinite) as it may take to complete this research study.

May I have access to my medical information that results from my participation in this research study?

In accordance with UPMC Notices of Privacy Practices document that you have been given, you are permitted access to information (including information resulting from your participation in this research study) contained within your medical records filed with your health care provider.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed to participate in the research study.) Whether or not you provide your consent for participation in this research study will have on effect on your current and future care at a University or Pittsburgh or UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

Your doctor may be an investigator in this research study, and as an investigator, is interested both in your medical care and in the conduct of this research. Before entering this study or at any time during the research, you may discuss your care with another

doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

If you decide to withdraw from study participation after you have received the study drug, you should participate in described monitoring follow-up procedures directed at evaluating the safety of cidofovir and probenecid.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if, for example, your pregnancy test proves to be positive. You may be removed from the study if you experience unexpected or life-threatening side effects and in the opinion of the investigators that it is in your best interest. If you are withdrawn from participation in this research study, there will be no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

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VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

☐ By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise."

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

STUDY FLOW SHEETS

CHART COPY - THIS MUST REMAIN IN CHART

UPMC HEALTH SYSTEM

PHYSICIAN'S ORDER SHEET

AUTHORIZATION IS GIVEN TO THE PHARMACY TO DISPENSE AND TO THE NURSE TO ADMINISTER THE GENERIC OR CHEMICAL EQUIVALENT WHEN THE DRUG IS FILLED BY THE PHARMACY OF PRESBYTERIAN UNIVERSITY HOSPITAL OR MONTEFIORE UNIVERSITY HOSPITAL - UNLESS THE PRODUCT NAME IS CIRCLED.

Revised 7-28-09

Date	Time	ORDERS
		IRB# PRO08060393 Pharmacokinetics and Pharmacodynamics of Cidofovir Used for the Treatment of BK Virus in Renal Transplant Recipients. **Short Title: PK Cidofovir** PI: Dr. Ron Shapiro: Phone# 412-647-5800 Pager #7300 Co-PI: Jeremiah Momper, Pharm.D.: Phone# 412-648-2377 Cell# 724-309-7629 Coordinator: Laurie Hope, RN: Phone# 412-682-2208 Pager# 10983 Subject ID# _____ <u>Cidofovir Only Visit</u> 1) Admit to MUH CTRC – outpatient. Verify copy of signed consent. 2) Obtain Vital signs at the following time points: on admission, prior to start of Cidofovir infusion, 30 minutes after start of Cidofovir infusion, at end of Cidofovir infusion, 6 hours after start of Cidofovir infusion and prior to discharge. 3) Activity: OOB ad lib 4) Diet – general (unless otherwise noted) 5) May administer the following meds from home: 6) Obtain a STAT urine pregnancy test on women of child bearing potential __N/A. Document results and notify PI if positive. 7) Send 24 hour urine for creatinine clearance (if not already done – subject will bring in with them) ___ N/A. 8) Place two IV heplocks (one for the infusion and one for PK blood draws). 9) Infuse NaCl 0.9% 1000 cc IV over 1 hour prior to starting Cidofovir. 10) Collect two, 4 ml purple top (EDTA) tubes for PK prior to giving Cidofovir. 11) Obtain Pre-dose blank urine sample. Place into four, 4 ml provided aliquot tubes and freeze at -70º C.

1 Physician's Signature _____

CHART COPY - THIS MUST REMAIN IN CHART

UPMC HEALTH SYSTEM

PHYSICIAN'S ORDER SHEET

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Revised 7-28-09

Date	Time	ORDERS
		IRB# PRO08060393 **Cidofovir Only Visit** **Page 2/2**

12) Administer Cidofovir (dispensed by MUH Pharmacy) _____ mg in 100 cc IV over one hour. 0 time (for the PK draws) is the start of the Cidofovir infusion. **Place IV start and infusion charge to insurance.**

13) Collect two, 4 ml purple top (EDTA) tubes for PK at 30 minutes and 1, 1.5, 2, 4, 6, 8, and 12 hours after the start of Cidofovir infusion.

14) Instructions for processing PK blood samples:
   a. Can be kept at room temperature until centrifuged.
   b. Centrifuge tubes at 3000 rpm for 10 minutes
   c. Divide plasma evenly between the three labeled vials that will be provided (each should contain at least 1 ml of plasma per tube)

15) Collect urine for the following time intervals: 0-1; 1-2; 2-4; 4-8 and 8-12 hours after the start of Cidofovir infusion. For each collection, document total volume, place into four, 4 ml aliquot tubes, and freeze at -70º C.

16) Discharge to home after +12 hr samples are obtained if vitals are stable.

2 Physician's Signature _____

CHART COPY - THIS MUST REMAIN IN CHART

UPMC HEALTH SYSTEM

PHYSICIAN'S ORDER SHEET

AUTHORIZATION IS GIVEN TO THE PHARMACY TO DISPENSE AND TO THE NURSE TO ADMINISTER THE GENERIC OR CHEMICAL EQUIVALENT WHEN THE DRUG IS FILLED BY THE PHARMACY OF PRESBYTERIAN UNIVERSITY HOSPITAL OR MONTEFIORE UNIVERSITY HOSPITAL - UNLESS THE PRODUCT NAME IS CIRCLED.

Revised 7-28-09

Date	Time	ORDERS
		IRB# PRO08060393 Pharmacokinetics and Pharmacodynamics of Cidofovir Used for the Treatment of BK Virus in Renal Transplant Recipients. **Short Title: PK Cidofovir** PI: Dr. Ron Shapiro: Phone# 412-647-5800 Pager #7300 Co-PI: Jeremiah Momper, Pharm.D.: Phone# 412-648-2377 Cell# 724-309-7629 Coordinator: Laurie Hope, RN: Phone# 412-682-2208 Pager# 10983 Subject ID# _____ <u>Cidofovir and Probenecid Visit</u> 1) Admit to MUH CTRC – outpatient. Verify copy of signed consent. 2) Obtain Vital signs at the following time points: on admission, prior to start of Cidofovir infusion, 30 minutes after start of Cidofovir infusion, at end of Cidofovir infusion, 6 hours after start of Cidofovir infusion and prior to discharge. 3) Activity: OOB ad lib 4) Diet – general (unless otherwise noted) 5) May administer the following meds from home: 6) Obtain a STAT urine pregnancy test (if woman of childbearing age) ___N/A. Document results and notify PI if positive. 7) Send 24 hour urine for creatinine clearance (if not already done – subject will bring in with them) ___ N/A. 8) Place two IV heplocks (one for the infusion and one for PK blood draws). 9) Administer Probenecid (dispensed by IDS pharmacy), two grams PO, 1-2 hours prior to starting Cidofovir 10) Infuse NaCl 0.9% 1000 cc IV over 1 hour prior to starting Cidofovir 11) Collect two, 4 ml purple top (EDTA) tubes for PK prior to giving Cidofovir 12) Obtain Pre-dose blank urine sample. Place into four, 4 ml provided aliquot tubes and freeze at -70° C.

1 Physician's Signature _____

CHART COPY - THIS MUST REMAIN IN CHART

UPMC HEALTH SYSTEM

PHYSICIAN'S ORDER SHEET

AUTHORIZATION IS GIVEN TO THE PHARMACY TO DISPENSE AND TO THE NURSE TO ADMINISTER THE GENERIC OR CHEMICAL EQUIVALENT WHEN THE DRUG IS FILLED BY THE PHARMACY OF PRESBYTERIAN UNIVERSITY HOSPITAL OR MONTEFIORE UNIVERSITY HOSPITAL - UNLESS THE PRODUCT NAME IS CIRCLED.

Revised 7-28-09

Date	Time	ORDERS
		IRB# PRO08060393 Cidofovir and Probenacid Visit Page 2/2

13) Administer Cidofovir (dispensed by MUH Pharmacy) _____ mg in 100 cc IV over one hour. 0 time (for the PK draws) is the start of the Cidofovir infusion. **Place IV start and infusion charge to insurance.**

14) Administer Probenecid (dispensed by IDS), one gram PO at 2 hours and again at 8 hours **after the completion of the Cidofovir infusion.**

15) Collect two, 4 ml purple top (EDTA) tubes for PK at 30 minutes and 1, 1.5, 2, 4, 6, 8, and 12 hours **after the start of Cidofovir infusion.**

16) Instructions for processing PK blood samples:
   a. Can be kept at room temperature until centrifuged.
   b. Centrifuge tubes at 3000 rpm for 10 minutes
   c. Divide plasma evenly between the three labeled vials that will be provided (each should contain at least 1 ml of plasma per tube)
   d. Freeze at -70º C

17) Collect urine for the following time intervals: 0-1; 1-2; 2-4; 4-8 and 8-12 hours after the start of Cidofovir infusion. For each collection, document total volume, place into four, 4 ml aliquot tubes, and freeze at -70º C.

18) Discharge to home after +12 hr samples are obtained if vitals are stable.

2 Physician's Signature _____

UPMC HEALTH SYSTEM
CLINICAL TRANSLATIONAL RESEARCH CENTER

ADDRESSOGRAPH

IRB: PRO08060393 Short Title: Pk Cidofovir
Title:Pharmacokinetics and Pharmacodynamics of Cidofovir Used for the Treatment of BK Virus Transplant Recipients

PI: Ron Shapiro, MD Phone:412-647-5800 Pager:7300
Co-PI: Jeremiah Momper,Pharm D. Phone:412-648-2377
Cell:724-309-7629
Coordinator: Laurie Hope, RN: 412-682-2208, Pager:10983

DATE:_____ SUBJECT ID#_____

HGT:_____ WGT:_____

NEG POS N/A
Urine Pregnancy results _____ _____ _____
Notify the physician if positive

Cidofovir Only Visit

Send 24 hour urine for creatinine clearance (If not already done - pt will bring)*

TIME	ACTUAL TIME	(2) 4 ml PURPLE	VITALS	URINE	COMMENTS
PRE				Blank 0-1 start	Predose blank urine. Place in four - (4)ml aliquot tubes Then START 0 - 1hr Urine just prior to Cidofovir TIME:_____
1 HR prior to Cidofovir					Infuse NaCl 0.9% 1000cc IV over 1 hr. prior to starting Cidofovir TIME:_____
"0" TIME start of inf			just prior to Cidofovir		Admin. Cidofovir _____mg in 100 ml IV over 1 hr. (From MUH Pharm. Place IV start & infusion charge to insurance)
(+) 30 min after start of inf					
(+) 1 HR EOI				1-2 start	END of Cidofovir infusion TIME:_____ END 0 -1 hr Urine TIME:_____ TOT VOL_____ START 1 - 2 hr Urine
(+) 1.5HR				2-4 start	END 1 -2 hr Urine TIME:_____ TOT VOL_____ START 2 - 4 hr Urine
(+) 2 HR					
(+) 4 HR				4-8 start	END 2 -4 hr Urine TIME:_____ TOTAL VOL._____ START 4 - 8 hr Urine
(+) 6 HR					
(+) 8 HR				8-12 start	END 4-8 hr Urine TIME:_____ TOT VOL _____START 8 -12 hr Urine
(+) 12 HR				8-12 END	END 8 - 12 Urine TIME:_____ TOTAL VOL._____ Discharge to home after 12hr blood pk samples are obtained if vitals stable

SIGNATURE:_____ SIGNATURE:_____

SIGNATURE:_____ SIGNATURE:_____

UPMC HEALTH SYSTEM
CLINICAL TRANSLATIONAL RESEARCH CENTER

ADDRESSOGRAPH

IRB: PRO08060393 Short Title: Pk Cidofovir
Title:Pharmacokinetics and Pharmacodynamics of Cidofovir Used for the Treatment of BK Virus Transplant Recipients

PI: Ron Shapiro, MD Phone:412-647-5800 Pager:7300
Co-PI: Jeremiah Momper,Pharm D. Phone:412-648-2377
Cell:724-309-7629
Coordinator: Laurie Hope, RN: 412-682-2208, Pager:10983

DATE:_____ SUBJECT ID#_____

HGT:_____ WGT:_____

Urine Pregnancy results NEG _____ POS _____ N/A _____
Notify the physician if positive

Cidofovir and Probenecid Visit

****Send 24 hour urine for creatinine clearance (If not already done - pt will bring)*****

TIME	ACTUAL TIME	(2) 4 ml PURPLE	VITALS	URINE	COMMENTS
PRE				Blank 0-1 start	Predose blank urine. Place in four - (4)ml aliquot tubes Then START 0 - 1hr Urine just prior to Cidofovir TIME:_____
1 - 2 hr prior to Cidofovir					Administer Probenecid 2 grams (From IDS Pharm.) PO 1-2 hrs prior to starting Cidofovir TIME:_____
1 HR prior to Cidofovir					Infuse NaCl 0.9% 1000cc IV over 1 hr. prior to starting Cidofovir TIME:_____
"0" TIME start of inf			just prior to Cidofovir		Admin. Cidofovir _____mg in 100 ml IV over 1 hr. (From MUH Pharm. Place IV start & infusion charge to insurance)
(+) 30 min after start of inf					
(+) 1 HR EOI				1-2 start	END of Cidofovir infusion TIME:_____ END 0 -1 hr Urine TIME:_____ TOT VOL_____ START 1 - 2 hr Urine
(+) 1.5HR				2-4 start	END 1 -2 hr Urine TIME:_____ TOT VOL_____ START 2 - 4 hr Urine
(+) 2 HR					
(+) 3 HR 2 hr post Cido					Administer Probenecid 1gram PO (From IDS) 2 hrs after the COMPLETION of Cidofovir infusion. TIME:_____
(+) 4 HR				4-8 start	END 2 -4 hr Urine TIME:_____ TOTAL VOL._____ START 4 - 8 hr Urine
(+) 6 HR					
(+) 8 HR				8-12 start	Administer Probenecid 1gram PO (From IDS) TIME:_____ END 4-8 hr Urine TIME:_____ TOT VOL _____START 8 -12 hr Urine
(+) 9 HR 8 hr post Cido					Administer Probenecid 1gram PO (From IDS) after the COMPLETION of Cidofovir infusion. TIME:_____
(+) 12 HR				8-12 END	END 8 - 12 Urine TIME:_____ TOTAL VOL._____ Discharge to home after 12hr blood pk samples are obtained if vitals stable

SIGNATURE:_____ SIGNATURE:_____

SIGNATURE:_____ SIGNATURE:_____

UNIVERSITY OF PITTSBURGH MEDICAL CENTER

PHYSICIAN'S ORDER SHEET

PATIENT NAME:_____

PATIENT NUMBER:_____

DATE/TIME STUDY MEDICATION IS DUE:

_____

PATIENT LOCATON:

_____**CTRC or**

IN-PATIENT UNIT/ROOM #: _____

AUTHORIZATION IS GIVEN TO PHARMACY TO DISPENSE AND TO THE NURSE TO ADMINISTER THE GENERIC OR CHEMICAL EQUIVALENT WHEN THE DRUG IS FILLED BY THE PHARMACY OF PRESBYTERIAN UNIV HOSPITAL OR MONTEFIORE UNIV HOSPITAL - UNLESS NAME IS CIRCLED

TITLE: Pharmacokinetics and Pharmacodynamics of Cidofovir Used in the Treatment of BK Virus in Renal Transplant Recients

IRB# PRO08060393

PRINCIPAL INVESTIGATOR: Ron Shapiro, MD

COORDINATOR: Laurie Hope, RN **Phone Number:** (412) 692-2208

FAX ORDER TO IDS PHARMACY AT (412) 647-9651

PATIENT CONSENT SIGNED: YES or NO (circle one)

DISPENSE: Probenecid 500 mg tablets
 # 8 tablets

DIRECTIONS: On Study Day 2 patients will receive 2 grams (4 tablets) of Probenecid by mouth approximately 1-2 hours prior to the infusion of Cidofovir. 1 gram (2 tablets) of Probenecid will be given at 2 hours and again at 8 hours following completion of the of the Cidofovir infusion

PHYSICIAN SIGNATURE:_____**DATE:**_____

INVESTIGATIONAL DRUG SERVICE
PATIENT ENROLLMENT SHEET

Study Title: Pharmacokinetics and Pharmacodynamics of Cidofovir Used in the Treatment of BK Virus in Renal Transplant Recients

IRB# PRO08060393

PI: Ron Shapiro, MD

Patient Name:_____ ____ _____
first mi last

Patient Address:_____
_____
_____

Patient Phone number: (_____) _____

Social Security Number: _____

Date of birth:_____

Allergies:_____

Patient signed informed consent on file:YES / NO(circle one)

Scheduled first day of therapy:_____

Enrollment completed by:_____

Phone number for questions:_____

Fax completed form to the IDS Office at 7-9651
Please call to IDS Office with any questions at
7-5350 or 7-3178 (pharmacists) or 7-9065 (technician)

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