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The incidence of polyomavirus infections, particularly that of BK virus (BKV), in kidney transplant recipients has been increasing steadily. The diagnosis of this disease has been made more difficult because of the pathological similarities between BK viral nephropathy and acute cellular rejection. Despite reduced immunosuppression, the authors have seen persistence of BKV, which can result in gradual loss of kidney function. Cidofovir, an antiviral agent with known nephrotoxic effects, has been used, in very low doses, to treat patients with BKV while monitoring blood and urine with DNA-PCR assays. The authors report the outcome of 16 renal transplant patients treated with cidofovir for BKV-associated nephropathy.

ABBREVIATIONS:

BKV	BK virus
BKVAN	BK virus–associated nephropathy
PCR	Polymerase chain reaction
SV40	Simian virus 40

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Introduction

BK virus–associated nephropathy (BKVAN) emerged as a major cause of renal allograft dysfunction worldwide in the 1990s and seems to have coincided with the widespread use of potent immunosuppressive drugs such as tacrolimus, cyclosporine for microemulsion, mycophenolate mofetil, and sirolimus. Although BKVAN can be a diagnostic challenge in the initial stages of the disease, the treatment is even more complex and demanding. Since the recognition of BKV as a major cause of both acute and chronic allograft dysfunction, different treatment modalities have been proposed in an attempt to prolong graft survival in patients who develop BKVAN.

In 1993, the clinical diagnosis of BKV was first made in one of our renal transplant recipients who was treated aggressively for presumed cellular rejection. This patient subsequently lost his graft. Our initial experience, in other similar patients who were treated with steroids and increased maintenance immunosuppression, instituted as treatment of the variable tubulitis seen in these patients, also resulted in graft loss in nearly 50% of the cases.[1] Since then, the incidence of BKV infection has been steadily climbing in our transplant patient population. In view of the dismal experience associated with the ongoing treatment of tubulitis, we have attempted to treat BKVAN with reduced immunosuppression alone for the past 5 to 6 years. Others[2-4] have also reported prolongation of graft function following an approach that involves a careful reduction of the immunosuppressive therapy with the aim of eliminating the viral load before significant scarring develops in the kidney. This approach, though partially successful, can still be associated with graft loss, either to rejection or to ongoing viral nephropathy, confirmed by repeated kidney biopsies or by plasma and urine polymerase chain reaction (PCR) assays. This has led to the search for alternative therapies in the treatment of BKVAN.

The development of quantitative viral load–monitoring tools with real-time PCR technologies has provided a way to monitor the course of this viral infection, as well as the response to the various treatment modalities. Several therapies have been tried or shown to be effective in vitro for BKV infections, and these include vidarabine, cidofovir, retinoic acid derivatives, intravenous immunoglobulin (IVIG), DNA gyrase inhibitors, and so on. We

first used one such therapy, anecdotally in 1999, when low-dose cidofovir was tried on a child with BKVAN, with an encouraging outcome. Since then, low-dose cidofovir has been used in a number of our patients to treat BKVAN. Plasma and urine PCR assays were used as monitoring tools once they became available in the clinical laboratory. Cidofovir, an acyclic nucleoside phosphonate antiviral agent known to be nephrotoxic, is generally contraindicated in patients with renal dysfunction. However, we found that when used at very low doses, effective eradication of BKV from the plasma along with marked reduction in urinary viral loads, could be achieved. The results of our experience at the University of Pittsburgh using cidofovir treatment are summarized here.

Patients and Methods

Between June 1999 and January 2002, 16 renal transplant recipients (15 adult, 1 pediatric) diagnosed with BKVAN underwent treatment with low-dose cidofovir at the University of Pittsburgh Medical Center. Two of these patients have been reported previously.[5] One patient diagnosed at the time of initiation of dialysis was excluded from this analysis. All patients underwent transplantation at the University of Pittsburgh Medical Center. Diagnosis was made either by immunostaining for polyoma antigen (SV40) and/or in situ hybridization for BK virus on renal biopsy tissue. Percutaneous biopsies were performed under ultrasound guidance for elevated serum creatinine levels or as part of a protocol biopsy schedule.

Quantitative PCR testing for the BK virus in plasma and urine was performed by the Molecular Diagnostics Division at the University of Pittsburgh Medical Center–Presbyterian Hospital. Treatment with low-dose cidofovir was administered to patients between August 1999 and March 2002. The outcome in this patient population was assessed to determine the effects of cidofovir treatment on graft function and BKV infection.

Results

Sixteen renal transplant recipients (15 adult, 1 pediatric) with functioning grafts received low-dose cidofovir for the treatment of BKV. The initial diagnosis was made either by pathological staining for the presence of BKV in tissue from renal biopsy specimens (10 patients) or by plasma DNA-PCR assay (6 patients). Diagnosis was made by both methods simultaneously in 2 patients. The time to diagnosis of BKVAN was 3 to 73 months post-transplantation.

The median age of the recipients was 48.5 years (range = 6–70). The male-to-female ratio was 13:3. All patients were recipients of kidney grafts; however, 2 also received simultaneous pancreas grafts, and 1 patient had a previous liver transplant. The first step in the treatment of BKVAN was to decrease immunosuppression. The dose of tacrolimus was decreased, and steroids were decreased or discontinued. If patients were on mycophenolate mofetil or sirolimus, these agents were nearly always discontinued after the diagnosis of BKV was established. Low-dose cidofovir was given, after reduction in immunosuppression, in multiple doses ranging from 0.20 mg/kg/dose to 1.0 mg/kg/dose. Treatment was administered every 1 to 4 weeks based on results of renal biopsy initially and then guided by the results of the DNA-PCR assay in plasma and urine.

BKVAN was confirmed in 8 patients by renal biopsy alone prior to our ability to perform blood and urine DNA-PCR assays. These patients were all treated with decreased immunosuppression initially. Cidofovir treatment was administered at the time of initial diagnosis by biopsy in 1 patient or in response to worsening renal function (4 patients) or positive BKV stains on repeat biopsy (3 patients). Five patients received treatment with cidofovir (1-5 doses) at varying dosages prior to the development of our plasma PCR technology. Subsequent assays performed on the 5 patients varied from negative to low intermittent positive, with peak values of 2.5×10^3 to 8×10^3 copies/ml of plasma after treatment with cidofovir therapy. These low positive values were either preceded and/or followed by negative PCR values (the limit of detection for this PCR assay is 1000 copies/ml of plasma and 200 copies/ml of urine). The time course for follow-up varied from 6 to 29 months (mean = 15 months) after diagnosis of BKVAN. For the 2 patients with positive plasma PCR after treatment (patients 1 and 4), each received a single dose of cidofovir, with subsequent improvement in renal function, which has

Table 1 | **Diagnosis and Cidofovir Treatment Data**

Patient	Initial Method of BKV Diagnosis (PCR = Plasma Titers)	Plasma PCR Assay Post-Rx	Urine PCR Assay Pre-Rx	Urine PCR Assay Post-Rx	Cidofovir Treatment, # Doses (Total mg/kg)		Cr Levels Pre-Rx	Cr Levels Post-Rx
1[a]	Biopsy	Negative	9.6×10^{9}	7.8×10^{4}[b]	1	(1.0)	2.2	1.7
2	Biopsy	Negative	7.3×10^{8}	4.2×10^{7}	3	(0.93)	1.4	1.6
3[c]	Biopsy	Negative	1.3×10^{5}	2.1×10^{6}[b]	5	(2.14)	2.8	Dialysis
4	Biopsy	3.4×10^{4}	9.6×10^{5}	8.6×10^{5}	1	(0.27)	3.1	1.8
5	Biopsy	Negative	4.9×10^{5}	8.3×10^{4}	7	(3.78)	1.7	5.2
6	Biopsy	Negative	1.1×10^{4}	4.9×10^{4}	2	(0.53)	2	2.3
7	Biopsy	Negative	2.1×10^{7}	2.4×10^{3}	2	(0.40)	3	3.8
8	Biopsy	Negative	2.9×10^{9}	1.9×10^{3}	4	(1.55)	2.2	1.8
9[c]	PCR: 8.2×10^{4}	Negative	6.1×10^{8}	930	3	(> 3.0)	4	Dialysis
10[c]	PCR: 7.4×10^{5}	Negative	3.5×10^{10}	663	3	(0.91)	1.8	Dialysis
11	PCR: 5.0×10^{5}[d]	Negative	1.3×10^{10}	2.3×10^{4}	3	(0.85)	2.4	2.4
12	PCR: 4.3×10^{3}[d]	Negative	5.9×10^{9}	5.6×10^{5}	7	(1.74)	2.7	2.3
13[c]	Bx/PCR: 7.4×10^{5}	1.3×10^{6}	3.5×10^{9}	1.4×10^{8}	3	(0.96)	1.4	Dialysis
14	PCR: 2.5×10^{7}	Negative	10×10^{9}	663	4	(1.17)	10.5	7
15	Bx/PCR: 1.3×10^{6}	Negative	1.7×10^{10}	2.1×10^{6}	3	(0.85)	1.6	4.7
16	PCR: 3.1×10^{6}[d]	Negative	1.5×10^{7}	7.0×10^{5}	2	(0.50)	2.4	2.4

The limit of detection for this PCR assay is 1000 (1×10^{3}) copies/ml for plasma and 200 copies/ml for urine.
a. Pediatric patient with transiently positive plasma PCR post-cidofivir.
b. Patients with transient clearance of viruria.
c. Patients with subsequent graft loss.
d. Previous biopsy that was negative for BKV.

remained stable (Table 1). Documented spontaneous clearance of viremia occurred in patient 1. Despite persistent positive plasma levels for BKV in patient 4, renal function has improved; no further therapy was given due to other coexisting complications. Only 1 other patient (patient 13) failed to clear the viremia despite multiple doses of cidofovir therapy. Persistent BKV nephritis and acute rejection subsequently led to graft failure within 5 months of BKV diagnosis (details discussed below).

Of the remaining 8 patients, the initial diagnosis of BKV was made by renal biopsy in 2 patients but confirmed by plasma and urine PCR assays at 2 weeks after diagnosis. Four patients were diagnosed by positive PCR blood and urine assays; 3 had previous biopsies showing acute rejection and with no evidence of BKV 2 to 4 weeks prior to BKV onset. The diagnosis by PCR and biopsy was confirmed in 2 patients, 1 (patient 13) of whom lost his graft because of ongoing rejection (detailed below). Cidofovir treatments were initiated, and the course of BKVAN followed, with further treatments based on the PCR results. The time course for the clearance of viremia (as detected by plasma PCR) was 1 to 5 months (mean = 3.34 months). Although 2 patients received 7 doses of cidofovir, most patients averaged between 2 and 5 doses (mean = 2.7 doses/patient) before viremia resolved.

Graft Outcome

Four patients (25%) lost their allografts. One patient (patient 3), diagnosed with BKVAN for 19 months, sustained graft loss to chronic allograft nephropathy 28 months after transplantation. Despite initial positive PCR levels, aggressive treatment with escalating doses of cidofovir resulted in clearance of her viremia and viruria along with transient improvement in renal function. She was reported previously.[5] Subsequent plasma PCR levels remained negative throughout her later course, despite persistent positive stains on renal tissue biopsy. All 4 biopsies performed in this patient showed evidence of ongoing moderate acute cellular rejection with intimal arteritis, along with the presence of BKV nephritis. With borderline renal function even at the time of diagnosis of BKV, we believe graft loss occurred due to ongoing chronic allograft nephropathy. The nephrectomy

specimen confirmed ongoing rejection and severe chronicity.

Three other patients, all diagnosed with BKVAN after July 2001, sustained graft loss 2 to 8 months after diagnosis. Two of these patients had documented acute cellular rejection (mild to moderate) following treatment for BKV nephritis. One patient (patient 13) suffered rapid deterioration of renal function without any response to antirejection or antiviral therapy. Both plasma and urine PCR levels remained elevated. Resulting allograft nephrectomy confirmed ongoing severe rejection and BKV infection. One patient with graft failure received a high dose of cidofovir (3 mg/kg) inadvertently, which led to the need for temporary dialysis. His renal function improved transiently but subsequently deteriorated, and he resumed permanent dialysis within 2 months of high-dose cidofovir treatment.

Twelve (75%) patients have retained graft function. Eleven of these 12 patients have documented evidence of absent viremia. Plasma PCR levels were not repeated in 1 patient. Eight patients have remained with stable renal function. Serum creatinine remained less than 2 mg/dl in 4 patients, 3 of whom improved from pretreatment levels. Five of 12 patients have remained with serum creatinine levels between 2.3 and 3.8 mg/dl, all in a range that can be considered unchanged or improved when compared to the serum creatinine levels prior to the diagnosis and treatment of BKVAN (Table 1). One patient, who was lost to follow-up temporarily, returned with severe renal dysfunction. Further antiviral therapy resulted in improvement of his renal function, which remains stable at the most recent follow-up. A second patient (patient 14) with poor initial graft function due to donor disease has maintained the same degree of renal dysfunction, not yet requiring dialysis. Only 1 patient (patient 15) with negative plasma PCR titers has experienced a significant decline in renal function. Repeat renal biopsy has demonstrated mild acute rejection and moderate allograft nephropathy; BKV has been negative by in situ hybridization.

Discussion

Since the first report of BKV in the urine of a renal transplant recipient nearly 30 years ago, BKVAN has been emerging as an important cause of renal allograft dysfunction. The diagnosis of BKV nephropathy is usually made by renal biopsy, which shows viral inclusions and is often associated with interstitial nephritis changes that may resemble acute rejection.[6,7] Given the challenges posed by the diagnosis and management of BKVAN, the development of noninvasive quantitative techniques to monitor viral load and newer therapeutic options can have a significant impact on the clinical management of these cases. Although various therapeutic strategies have been suggested and tried, the results have often been variable and dismal. Most often, the therapy for BKVAN is based on renal allograft biopsy findings. The difficulty in clinical management is compounded by the fact that even when biopsies show tubulitis, suggesting the possibility of underlying rejection, there is little or only transient response to corticosteroids in many cases. Another possibility for clinical management is reduction of immunosuppression. Although reducing immunosuppression decreases the viral load, it increases the risk of rejection. An area of critical need is development of drugs effective against polyoma virus. Several drugs, including retinoic acid derivatives, DNA gyrase inhibitors, cytosine arabinoside, and cidofovir, have been shown to inhibit polyoma viral DNA replication in vitro.[8] Our experience shows that specific antiviral therapy with low-dose cidofovir may be effective in BKVAN.

Cidofovir (HPMPC, Vistide™, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine) is an antiviral agent that is a synthetic purine nucleotide (phosphorylated nucleoside) analogue of cytosine. It is converted via cellular enzymes to the pharmacologically active diphosphate metabolite (cidofovir diphosphate). This metabolite has in vitro and in vivo inhibitory activity against a large number of herpes viruses, as well as activity against adenovirus, human papilloma virus, and human polyoma virus. Cidofovir diphosphate exerts its antiviral effect by interfering with DNA synthesis and inhibiting viral replication. Because this drug has an extended half-life, there is a prolonged antiviral effect as well as an extended protection against subsequent viral infections in the uninfected cells. Initial indications for the use of cidofovir have been for the treatment of human cytomegalovirus (CMV) infection,[9] partic-

ularly CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). It has also been shown to be effective in the therapy of bone marrow transplant patients with hemorrhagic cystitis due to BKV and CMV infections.[10,11] Although probenecid (a uricosuric agent) is recommended for concomitant use with cidofovir to reduce renal excretion and possibly nephrotoxicity, withholding probenecid possibly allows for higher concentration of cidofovir to be achieved within the proximal tubules, with an increased amount of drug excreted from the kidney.

To prevent the nephrotoxicity of cidofovir, the dosages used were reduced to levels of 5% to 20% of the recommended dose that is given to patients without renal disease. These patients were also adequately hydrated. By withholding probenecid while using low-dose cidofovir in the range of 0.20 to 0.50 mg/kg/dose, we expect that adequate doses can effectively be delivered to the renal tubules. Because of concern about the nephrotoxicity of this drug, the dosages given were deliberately lowered to prevent significant side effects. As a result, many patients remained positive for months before clearance of the virus could be established by antiviral treatment.

Because the drug is secreted via the renal tubules, one would expect clearance or significant reduction in the viral load of BKV from the urine. Cidofovir therapy was associated with significant reduction in urine viral load (4–6 log orders) in more than half of the patients. Cidofovir therapy was associated with a reduction in viral load to a level that we would consider insignificant (< 10,000 copies/ml) in 9 of the 16 patients. Two of 3 patients who cleared their viruria went on to develop graft failure. However, most patients remained with positive urine PCR levels despite multiple courses of low-dose cidofovir (Table 1) and clearance of virus from the plasma. Our data show little correlation between disease and shedding of BKV in the urine, although no patient with BKVAN had a negative urinary PCR. Similarly, no correlation between plasma and urinary PCR titers for BKV could be observed in about 50% of the patients. However, during acute BKV infection, the urinary viral load is several log orders (> 4–6 log orders) higher than the plasma load. Of the 4 patients who sustained graft loss, urinary excretion of BKV varied from low positive to more than 100 million copies/ml. Although it is true that graft loss occurred in 1 patient with this very high load, many patients have maintained good graft function despite persistent high titers of virus in the urine.

Cidofovir was effective in rendering the plasma PCR negative in a significant majority of the patients treated for BKVAN. However, a few patients continued to demonstrate BKV interstitial nephritis despite negative plasma PCR levels. This might be explained by the ongoing presence of the virus in the kidney and urine but without spillover into the bloodstream. Whether this represents a low-grade and ongoing viremia, not detectable by PCR, is unclear. However, the continued presence of the virus in the kidney can lead to progressive destruction of the graft. This can possibly be monitored by urinary titers or tissue biopsy for BKV. One patient (patient 13) who did not respond at all to cidofovir therapy may represent infection with a drug-resistant virus. Future studies on the correlation of viral genotypes with clinical outcomes and drug sensitivity may shed more light on this phenomenon.

Conclusion

In summary, low-dose cidofovir therapy administered every 1 to 3 weeks at a dose of 0.25 to 1 mg/kg without probenecid may be useful in the management of BKVAN patients. Our experience demonstrates the ability to use cidofovir safely and efficaciously. A gradual increase in the dosage of cidofovir with each treatment for BKVAN would perhaps allow for an earlier clearance of the virus and a shorter course of infection. This therapy should, however, be carefully monitored by serial assessment of renal function and viral load. The quantitation of the viral load in blood and urine can allow a physician to monitor a patient's response to specific antiviral therapy. Systematic assessment of the kidney by biopsy is important in determining the progression and/or deterioration of renal function. The role of cidofovir in BKVAN, despite the ability to clear BKV from the plasma, needs to be further investigated. This might be possible in a larger cohort of patients, perhaps in a multicenter study. Careful monitoring of patients diagnosed with BKV will allow for the analysis of

those unresponsive to current methods of treatment and perhaps early determination of the emergence of resistant virus.

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References

1. Scantlebury V, Shapiro R, Justin, G, Re L. Graft function after diagnosis of BK virus in adult kidney transplant recipients under tacrolimus–based immunosuppression. **Am J Transplant 2001;1(suppl. 1):404**.
2. Hurant de Ligny B, Etienne I, Francois A, Toupance O, Buchler M, Touchard G, et al. Polyoma virus induced acute tubulo-interstitial nephritis in renal allograft recipients**. Transplant Proc 2000;32:2760–1**.
3. Mylonakis E, Goes N, Rubin RH, Cosimi AB, Colvin RB, Fishman JA. BK virus in solid organ transplant recipients: an emerging syndrome. **Transplantation 2001;72(10):1587–92**.
4. Barri YM, Ahmad I, Ketel BL, Barone GW, Walker PD, et al. Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. **Clin Transplantation 2001;15(4):240–6**.
5. Vats A, Shapiro R, Randhawa PS, Scantlebury V, et al. Quantitative viral load and cidofovir therapy for the management of BK virus-associated nephropathy in children and adults. **Transplantation; in press**.
6. Randhawa PS, Finkelstein S, Scantlebury V, Shapiro R, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. **Clin Transplantation 1999;67(1):103–9**.
7. Mathur VS, Olson JL, Darragh TM, Benedict Yen TS. Polyomavirus-induced interstitial nephritis in two renal transplant recipients: case reports and review of the literature. **Am J Kidney Dis 1997;29:754–8**.
8. Andrei G, Snoeck R, Vandeputte R, et al. Activities of various compounds against murine and primate polyomaviruses. **Antimicrobial Agents and Chemotherapy 1997;41:587**.
9. Lea AP, Bryson HM. Cidofovir. **Drugs 1996;52(2):225–30**.
10. Held TK, Biel SS, Nitsche A, Kurth A, Chen S, et al. Treatment of BK virus-associated hemorrhagic cystitis and simultaneous CMV reactivation with cidofovir. **Bone Marrow Transplant 2000;26:347–50**.
11. Gonzalez-Fraile MI, Canizo C, Caballero D, Hernandez R, et al. Cidofovir treatment of human polyomavirus-associated acute haemorrhagic cystitis. **Transplant Infect Dis 2001;3:44–6**.