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CASE REPORT

Successful outcome of ganciclovir-resistant cytomegalovirus infection in organ transplant recipients after conversion to mTOR inhibitors

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Summary

Ganciclovir-resistant (GanR) cytomegalovirus (CMV) infection after organ transplantation is emerging as a significant therapeutic challenge. We report two cases of GanR CMV infection successfully managed by switching immunosuppression from calcineurin inhibitors to an mTOR inhibitor-based regimen. This salvage therapy should be considered when other options are not available.

Cytomegalovirus (CMV) is one of the most significant pathogens in solid-organ transplant (SOT) recipients. Ganciclovir and valganciclovir have become the antiviral agents of choice for prevention and treatment of CMV infections in these patients [1,2]. However, with the widespread use of these drugs, an increase in the incidence of ganciclovir-resistant (GanR) CMV strains has been reported [3].

Among SOT recipients, GanR CMV infections may be associated with aggressive clinical courses, organ dysfunction, and mortality [4]. Moreover, GanR CMV poses particular management difficulties because foscarnet and cidofovir can be extremely toxic [4]. Therefore, alternative therapeutic options for GanR CMV are urgently needed.

Interestingly, several studies have reported a reduction in the incidence of CMV infections in SOT recipients treated with mammalian target of rapamycin (mTOR) inhibitors [5–7]. Although it has been hypothesized that mTOR inhibitors could be useful in treating GanR CMV-infected patients, clinical data supporting this strategy is lacking.

We report two cases of SOT recipients with GanR CMV infection who had a successful outcome after switching immunosuppression from a calcineurin inhibitor to

an mTOR inhibitor-based regimen. Table 1 summarizes characteristics of patients. Additional information is provided in the case reports.

Case 1

Patient 1, a 57-year-old male underwent heart transplantation for ischemic cardiomyopathy. The donor was positive and the recipient presented CMV negative serostatus. Two weeks after transplantation, the patient suffered an acute graft rejection, managed with pulses of methylprednisolone. A week later, a positive CMV antigenemia was detected [22 cells/10^5 peripheral blood mononuclear cells (PBMC)]. The patient received oral valganciclovir 900 mg every 12 h for 2 weeks. CMV antigenemia fell to undetectable levels. Secondary prophylaxis with valganciclovir 900 mg daily was continued for 2 months. One month after finishing prophylaxis, the patient was admitted for a 2-week history of weakness, abdominal pain, and weight loss. At admission, medications included cyclosporine 300 mg/day, prednisone 7.5 mg/day, and mycophenolate mofetil 3000 mg/day. Vital signs were: temperature 36 °C, blood pressure 104/76 mmHg, and heart rate 95/min. Physical examination was normal. Laboratory evaluation revealed 2300×10^9/l leukocytes with 782×10^9/l granulocytes, 690×10^9/l lymphocytes and 736×10^9/l monocytes, hemoglobin 10.0 g/dl, and platelets 203×10^9/l. Renal and liver function were normal. CMV antigenemia was $145/10^5$ PBMC. Intravenous ganciclovir (5 mg/Kg/12 h) was then initiated and mycophenolate mofetil was reduced to 2000 mg daily. An esophagogastroduodenoscopy including a gastric biopsy was performed, with normal results.

Despite ganciclovir treatment, symptoms persisted and pp65 antigenemia remained above $150/10^5$ PBMC (Fig. 1). CMV genetic analysis revealed the presence of

Table 1. Characteristics of two organ transplant recipients with Ganciclovir-resistant cytomegalovirus (CMV) infection successfully managed by switching immunosuppression from calcineurin inhibitors to mTOR inhibitor-based regimen.

Variable	Patient 1	Patient 2
Sex/age, years	Male/57	Male/49
Type of transplant	Heart	Kidney
Donor/recipient CMV sero-status	Positive/Negative	Positive/Positive
Previous valganciclovir	Yes	Yes
CMV infection manifestations	Viral syndrome	Viremia
Immunosuppressive regimen	Prednisone; cyclosporine; mycophenolate mofetil	Prednisone; tacrolimus; mycophenolate mofetil
CMV mutation	A594V in UL97 gene	A595S in UL97 gene
Antiviral treatment, before switching to mTOR inhibitor	Ganciclovir; foscarnet	Valganciclovir; ganciclovir
Drug-related adverse effects	Neutropenia; genital ulcers; encephalopathy; renal failure	Neutropenia
Switching from calcineurin inhibitors to mTOR inhibitor	Cyclosporine; everolimus	Tacrolimus; sirolimus
Outcome	Negativization of CMV antigenemia; cure	Negativization of CMV antigenemia; cure

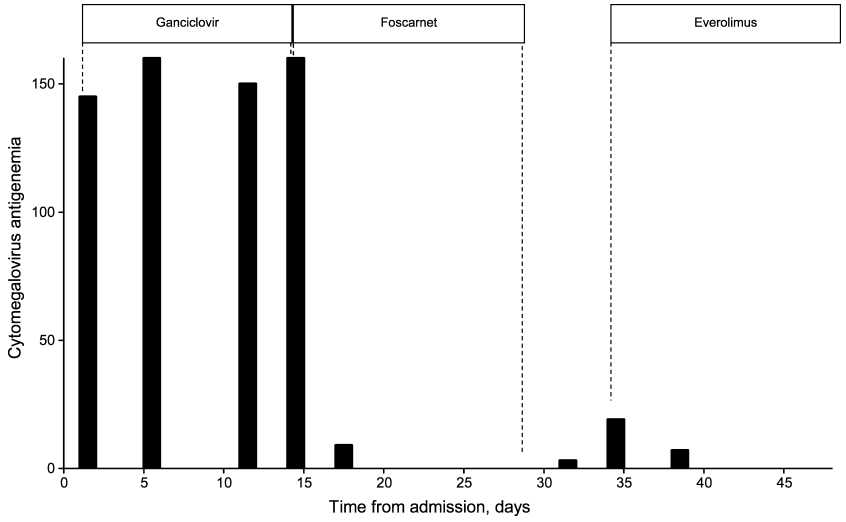

Figure 1 Treatment and pp65 CMV antigenemia levels in a heart transplant recipient with GanR CMV infection.

mutation A594V in UL97 phosphotransferase gene which conferred ganciclovir resistance. Ganciclovir was stopped after 2 weeks and intravenous foscarnet (60 mg/Kg/8 h) was initiated. Foscarnet treatment reduced CMV antigenemia to undetectable levels within 5 days, but the patient developed extensive genital ulcers, encephalopathy, and renal failure. Because of the neurologic symptoms, a magnetic resonance imaging of the brain and a lumbar puncture were performed; the spinal fluid findings were normal, including a negative PCR for CMV. Foscarnet was then discontinued after 2 weeks of therapy because of the severe adverse effects.

After foscarnet treatment was stopped, CMV antigenemia rose to $19/10^5$ PBMC within 4 days. At this point, everolimus 1.5 mg daily was initiated and cyclosporin was discontinued without other changes in immunosuppressive regimen. Patient symptoms improved, renal function returned to normal values, and CMV antigenemia decreased and became negative within 5 days. The patient was discharged from hospital. No positive pp65 antigenemia has been reported since, and the patient remains well after 4 years of follow-up.

Case 2

Patient 2, a 49-year-old male underwent renal transplantation for polycystic kidney disease. The donor and the recipient had CMV positive sero-status. Two months after transplantation, bacteremic pyelonephritis was diagnosed. During hospitalization, CMV antigenemia above $250/10^5$ PBMC was detected. Oral valganciclovir 900 mg every 12 h was given and surveillance antigenemias were negative.

Two weeks after hospital discharge, the patient was admitted again for bacteremic pyelonephritis. At admission, medications included tacrolimus 16 mg/day, prednisone 7.5 mg/day, mycophenolate mofetil 1000 mg/day, and valganciclovir 900 mg every 12 h. Vital signs were: temperature 37.3 °C, blood pressure 134/70 mmHg, and heart rate 85/min. Physical examination was normal. Laboratory evaluation showed 8700×10^9/l leukocytes with 8270×10^9/l granulocytes, 110×10^9/l lymphocytes and 240×10^9/l monocytes, hemoglobin 7.8 g/dl, and platelets 276×10^9/l. Renal and liver function were normal. At admission, oral valganciclovir was continued with negative surveillance antigenemias.

The patient's in-hospital course was complicated by recurrent bacteremic pyelonephritis caused by polycystic kidney disease. For this reason, a bilateral nephrectomy was performed a month after admission, with resolution of urinary infections.

A week after nephrectomy was performed, CMV antigenemia rose to $52/10^5$ PBMC. Oral valganciclovir was stopped. Intravenous ganciclovir (5 mg/Kg/12 h) was then initiated and mycophenolate mofetil was reduced to 500 mg daily. In spite of the intravenous ganciclovir therapy, pp65 antigenemia continued between 40 and $50/10^5$ PBMC (Fig. 2). CMV genetic analysis demonstrated the presence of mutation A595S in UL97 phosphotransferase gene conferring ganciclovir resistance. Ganciclovir was stopped 6 days after initiation. At this point, sirolimus 6 mg daily was given and tacrolimus was discontinued without any other changes in immunosuppressive regimen. CMV antigenemia become negative within a week. The patient was discharged from hospital. No positive pp65 antigenemia has been reported since, and the patient remains well after 2 years of follow-up.

Discussion

The most widely documented gene mutations responsible for CMV resistance are in the CMV UL97 gene, which

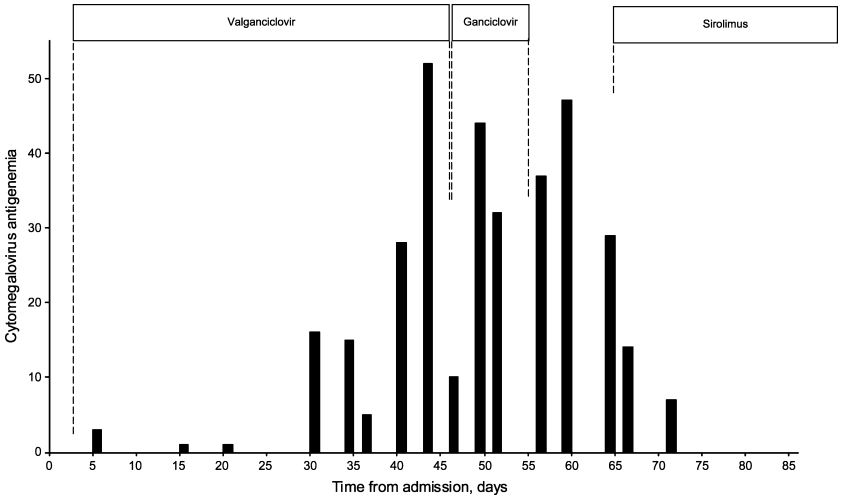

Figure 2 Treatment and pp65 CMV antigenemia levels in a kidney transplant recipient with GanR CMV infection.

codes for the viral protein kinase responsible for initial phosphorylation of ganciclovir. Different mutations in the UL97 gene confer various degrees of ganciclovir resistance. Less commonly, resistance may be because of mutations in the CMV UL54 gene, which codes for CMV DNA polymerase. Ganciclovir, foscarnet, and cidofovir target the viral DNA polymerase, and cross-resistance may result from some UL54 mutations [8].

Current guidelines on the management of CMV in SOT recipients recommend that treatment of GanR CMV infection must be based on genotypic analysis, patient immune state, and disease severity [1,2]. Reducing immunosuppressive therapy may be effective in some patients. However, depending on the severity of CMV disease, empirical treatment may be necessary until the genotypic analysis is available. These alternative treatments include increasing the dose of ganciclovir in patients with mild disease, combining ganciclovir and foscarnet, or administering foscarnet separately to patients with severe CMV disease [1,2]. Cidofovir is not recommended as alternative therapy for GanR CMV unless genotypic analysis is available. However, these treatments are associated with significant adverse effects: high doses of ganciclovir often produce neutropenia caused by marrow toxicity, and foscarnet and cidofovir are associated with kidney damage and may produce synergistic renal injury with calcineurin inhibitors [4].

Little evidence is currently available regarding the role of alternative treatments for CMV disease. The investigational drug maribavir inhibits the viral UL97 kinase and has proved to be active against GanR CMV. Nevertheless, maribavir resistance has already been reported in *in vitro* studies [9]. Moreover, compared with placebo, maribavir prophylaxis did not prevent CMV disease in a recent trial involving allogeneic stem-cell transplant recipients [10]. Leflunomide and the antimalarial drug artesunate have been shown to have anti-CMV effects; however, data regarding the use of these drugs are scarce and the results contradictory [11,12]. Passive immunotherapy with immunoglobulins containing CMV antibodies and adoptive infusions of CMV-specific T cells may improve antiviral hosts' defenses, but have not been adequately evaluated in the treatment of GanR CMV infections [1,2,13].

mTOR inhibitors are potentially a less nephrotoxic form of immunosuppression than calcineurin inhibitors [14]. Interestingly, recent studies of immunosuppressive regimens in SOT recipients have encountered a lower incidence of CMV disease among those receiving mTOR inhibitors, prompting the proposal that these drugs could be used as adjunctive therapy in patients with GanR CMV infection [5–7]. CMV is an intracellular virus that must utilize the intrinsic metabolic pathways of the host cell to synthesize the proteins essential for its replication. The mTOR is a kinase that is a key regulator for protein synthesis. Compelling data suggest that mTOR inhibitors may affect viral amplification by blocking cellular proliferation and impairing pathways critical for CMV infection, signaling, and replication [15]. It has also been speculated that the association of fewer CMV events with use of mTOR inhibitors might be explained by less potent immunosuppression. However, in several trials the acute rejection rates between SOT recipients receiving mTOR inhibitors and those receiving other immunosuppressive regimens were similar, suggesting that the ''net state of immunosuppression'' for any group did not vary greatly [6].

At present, little information is available regarding the clinical use of mTOR inhibitors for the management of GanR CMV. A Brazilian study reported nine transplant patients with GanR CMV infection who responded to a change to a sirolimus-based immunosuppression plus ganciclovir therapy [16]. However, the introduction of mTOR was associated with the withdrawal of mycophenolate mofetil in four patients, and intravenous ganciclovir was continued in all cases. In contrast, our two SOT recipients with GanR CMV infection reported here were successfully managed merely by switching immunosuppression to mTOR inhibitors. Everolimus replaced cyclosporine in the first case and sirolimus replaced tacrolimus in the second case suggesting that if there is an anti-CMV effect of mTORs, it is a class effect and not specific to either of the two available agents. Moreover, in these cases no other antiviral drugs were coadministered and mycophenolate mofetil was continued in both. Nor were any deleterious effects found on graft outcomes.

In conclusion, physicians should be aware that switching to mTOR inhibitor-based regimen may be a useful salvage therapy to manage GanR CMV in SOT recipients, when other options are not available.

Authorship

NS: responsible for concept/designed study, collected data, analyzed data, and wrote the paper. JG-C, IR, JR, JMC, and NM: collected data and carried out critical revision of the manuscript. JN: performed microbiological studies and carried out critical revision of the manuscript. MB: analyzed data and wrote the paper. JC: responsible for concept/designed study, analyzed data and wrote the paper.

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