

Teaching Point (Section Editor: W. Herrington)

Ganciclovir-resistant cytomegalovirus infection in renal transplantation

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Introduction

Cytomegalovirus (CMV) infection is an important cause of morbidity in renal transplant recipients, due to both direct and indirect effects of the virus on the graft and patient [1].

Ganciclovir (GCV) remains the most common first-line therapy, but its low oral bioavailability was identified as a risk factor for the emergence of resistant strains [1], and intravenous (IV) administration is inconvenient for use in prophylactic or pre-emptive therapy. Valganciclovir (VGCV) is a prodrug of GCV with a much higher oral bioavailability, which makes it very useful for prophylaxis and pre-emptive therapy, as well as for treatment in selected patients [2–4]. Although initially associated with a negligible risk of drug resistance [5], subsequent papers identified patients with GCV-resistant CMV infection after VGCV prophylaxis and treatment [6, 7].

Patients with drug-resistant CMV strains often have more tissue-invasive disease and unfavourable clinical outcomes [8, 9], but there is some heterogeneity, and reports of asymptomatic infection, particularly in non-lung transplant recipients, suggest that some mutations are less pathogenic than others [10]. The therapeutic approach is a challenge and must balance the severity of the infection against the risks of drug toxicity and reduction of immunosuppression.

We report two cases of GCV-resistant CMV from our unit: the first is a case of invasive CMV disease in a low-risk renal transplant recipient while on therapy with VGCV, and the second is a high-risk patient who developed GCV-resistant CMV infection while on VGCV prophylaxis.

Case 1

A 47-year-old male patient, with obstructive end-stage renal disease, underwent renal transplantation from a deceased donor in September 2009. There were three HLA mismatches between donor and recipient (2AB and 1DR),

and the peak panel reactive antibody (PRA) was 11%. Both donor and recipient were seropositive for CMV. The immunosuppressive induction therapy consisted of basiliximab, and the maintenance immunosuppression of tacrolimus, mycophenolate mofetil (MMF) and prednisolone (PDN).

One month after transplant, the patient presented with a 1-week history of epigastric pain and vomiting. He also noticed weight loss and fever. Physical examination was unremarkable, and blood tests only showed a small increase in liver transaminases up to 1.5 times the normal limit. Upper gastrointestinal endoscopy revealed gastric erosions with positive immunohistochemical staining for CMV, establishing the diagnosis of CMV gastritis. Despite the negative CMV antigenaemia on admission, it rose to 26 cells on the seventh day of hospitalization. After 14 days of IV GCV 5 mg/kg twice daily, the patient showed clinical and virological improvement and was discharged with oral VGCV at a therapeutic dose (900 mg twice daily, adjusted for renal function).

Two months later, and still on a therapeutic dose of VGCV, the patient was admitted with diarrhea; CMV colitis was confirmed by positive immunohistochemical staining for CMV in a colonic biopsy. In the face of a recurrence of invasive CMV gastrointestinal disease, MMF was suspended and IV GCV 5 mg/kg twice daily was resumed; however, 2 weeks later, he retained persistently high CMV antigenaemia (136 cells). On suspicion of GCV-resistant CMV disease, the IV GCV dose was increased to 7 mg/kg twice daily and anti-CMV immunoglobulin was added (initial dose 150 mg/kg, followed by 100 mg/kg every other day, for a total of seven doses). Consequently, the CMV antigenemia became negative, and there was a progressive decrease in serum CMV DNA, as measured by polymerase chain reaction (PCR), from 2.1×10^5 to 1.8×10^3 copies/mL. The patient was discharged, again maintaining the therapeutic dose of oral VGCV (900 mg twice daily, adjusted for renal function), without MMF and with a serum creatinine of 1.2 mg/dL.

One month later, the patient was admitted because of severe allograft dysfunction and fever. CMV antigenaemia and PCR for CMV DNA were negative. A kidney biopsy was performed and showed grade IIA acute cellular rejection and grade II acute antibody-mediated rejection with C4d-positive staining (according to the Banff 07 classification). Donor-specific antibodies were negative. He was treated with thymoglobulin (cumulative dose of 6 mg/kg), plasmapheresis and human immunoglobulin (total dose 2 g/kg). Mycophenolate mofetil was restarted. He progressed well with prompt apyrexia and a decline in serum creatinine to baseline.

A decision was made to prolong therapy with VGCV (900 mg twice daily, dose adjusted for renal function) for up to 8 weeks following the return to negative CMV antigenaemia. The patient then started secondary prophylaxis (900 mg once daily, dose adjusted for renal function). He remains asymptomatic, without relapse of CMV infection or rejection episodes, after 44 months of follow-up.

Case 2

A 61-year-old female patient, with a prior history of end-stage renal disease of unknown aetiology, was transplanted in August 2008 from a deceased donor. Both donor and recipient CMV serostatus were positive. There were six HLA mismatches, and the peak PRA was 62%. She received thymoglobulin (total dose 6 mg/kg) for induction immunosuppression and cyclosporine (CsA), MMF and PDN for maintenance, in addition to CMV prophylaxis with VGCV (900 mg once daily, adjusted for renal function).

CMV infection was diagnosed by positive antigenaemia (40 cells), without organ involvement, three months after transplant and while the patient was still under CMV prophylaxis with oral VGCV. The initial treatment was oral VGCV adjusted to a therapeutic dose (900 mg twice daily, adjusted for renal function), but MMF was suspended due to persistently high antigenaemia (91 cells). After 14 days of treatment, the patient's viraemia levels remained high, and she was therefore admitted in order to start IV GCV (5 mg/kg twice daily). There appeared to be a mismatch between antigenaemia and viraemia: antigenaemia continued to increase up to 420 cells, despite a decrease in CMV DNA as detected by PCR (from 8.5×10^4 to 2.2×10^3 copies/mL). On suspicion of GCV resistance, the dose of IV GCV was adjusted to 7 mg/kg twice daily, in combination with anti-CMV immunoglobulin (initial dose 150 mg/kg, followed by 100 mg/kg every other day, for a total of seven doses). Although she remained asymptomatic, with stable graft function and no cytopaenia, CMV antigenaemia and DNA levels detected by PCR continued to increase. Rescue therapy was based on switching from CsA to everolimus and a combination of low-dose foscarnet (FOS) (50 mg/kg once daily) with a conventional dose of IV GCV (5 mg/kg twice daily). Negative antigenaemia was detected on the tenth day of this approach. The patient completed 3 weeks of therapy with no side-effects and was discharged with a serum creatinine of 1.1 mg/dL, maintaining the use of everolimus (1.5 mg twice daily) and PDN (10 mg once daily) as immunosuppressive agents. The patient's graft function remains stable with no relapse of viral replication after 55 months of follow-up.

Discussion

The cases reported here are two examples of the issues raised by CMV resistance in renal transplant recipients,

namely the relation to VGCV exposure and the difficulties of treatment and eradication.

Our first patient was a low-risk recipient for CMV infection, not only due to the donor-recipient CMV serostatus but also because he was a non-lung transplant recipient and did not receive lymphocyte-depleting antibody as an immunosuppressant [1]. For this reason, we used a pre-emptive approach, as has been suggested by many authors, with weekly monitoring of viraemia for 12 weeks after transplant and initiation of treatment when viraemia reaches a specified threshold [1]. However, our patient presented with clinical disease before the detection of a viral load, as frequently happens in invasive gastrointestinal CMV disease. Our second case was a high-risk patient for CMV infection due to exposure to lymphocyte-depleting antibodies. A decision was therefore made to use antiviral prophylaxis for a 3- to 6-month period beginning soon after transplant [1]. Nevertheless, our patient developed CMV infection during prophylaxis and was presumed to have a resistant strain. In fact, CMV drug resistance has been observed with both strategies (prophylaxis and pre-emptive treatment), and there are contradictory data regarding which is associated with a higher risk [11, 12].

Screening for CMV IgG in donors and recipients should be performed before transplant to allow for risk stratification and to guide the posterior approach. If the result is negative and a significant amount of time elapses after testing, serology should be repeated at the time of transplantation. Serological testing only reveals prior exposure, and it is not useful either for diagnosis of active disease or to provide guidance on the therapeutic response.

CMV infection can be detected by testing for antigenaemia or DNAemia (by PCR analysis). These are good methods for diagnosis and treatment monitoring, but each has its limitations. The antigenaemia assay is a rapid semi-quantitative immunofluorescence test that detects phosphoprotein 65 (pp65), produced in CMV-infected polymorphonuclear leukocytes in peripheral blood [13, 14]. It has good sensitivity and high specificity, but cannot be performed when the neutrophil count is $<200/\mu\text{L}$ [14–16]. The PCR assay detects CMV DNA. It has higher sensitivity than pp65, resulting in a higher negative predictive value and a lower positive predictive value [14, 16]. It therefore allows us to detect more cases of CMV infection, but these may represent latent viral DNA status instead of active disease. To be sure of the meaning of a positive DNA result, we have to assess either the total viral load (a higher viral load is typically associated with active viral replication) or changes in value over time (at least a 3-fold change in value is necessary to confirm significant changes in viral replication) [17]. International guidelines recommend the use of either method, depending on the availability and the technical capacity of the laboratory, and neither test has been shown to be clinically superior [1].

CMV drug resistance is clinically suspected when high or rising viral loads and progressive disease are observed despite the administration of adequate antiviral therapy for >2 weeks. Genotypic tests for resistance should be performed, if available, when there is a suspicion of resistance [13, 15]. Unfortunately, these are not available in our centre, and so the cases reported here are examples of presumed CMV drug-resistant disease, based on the lack of clinical and virological response to therapy.

Resistance to GCV can be explained by mutations in two CMV genes: UL97, encoding a kinase responsible for the initial phosphorylation and activation of GCV; and UL54,

encoding the viral DNA polymerase that is the target for GCV. Mutations in UL97 are far more common, and different mutations in this gene confer various degrees of GCV resistance and response to antiviral dose increments [1]. Mutations in UL54 appear in the presence of continued GCV exposure and after UL97 mutations. These confer cross-resistance to cidofovir (CDV) and, less commonly, FOS [9]. Therefore, viral sequencing for CMV resistance, apart from its value in the diagnosis of resistant strains, is a useful tool to guide treatment and predict which cases will respond to increases in the dose of GCV or when to expect cross-resistance with other drugs [1, 18].

Therapeutic options for GCV-resistant CMV are limited and, in some cases, extremely toxic, but there are no controlled trial data to support the best alternative therapy. Although limited by haematological toxicity, the use of higher doses of IV GCV (up to 10 mg/kg twice daily) [15] is a good option and makes sense in patients with UL97 mutations with low-grade resistance [18] or in mild-to-moderate disease. Foscarnet is an alternative agent for GCV resistance and cases of GCV intolerance due to severe myelosuppression. Foscarnet also targets viral DNA polymerase but, unlike GCV, FOS does not need to be activated by UL97 kinase. It can replace or be added to GCV when high-grade UL97 mutations are present, with or without UL54 mutations; however, nephrotoxicity can be a limiting factor [19]. Cidofovir is another alternative, but highly nephrotoxic and additionally restricted by GCV-CDV cross-resistance in UL54 mutations.

In addition to the choice of antiviral agents, reduction of immunosuppression must be considered in GCV-resistant CMV disease. Switching to mTOR inhibitors may be an option, based on the lower CMV incidence that was reported when these regimens were used [20]. The mechanism is unclear, but mTOR inhibitors seem to interfere with viral amplification by blocking cellular proliferation [21]. On the other hand, they confer less potent immunosuppression, and hence lower susceptibility to opportunistic infections. Nonetheless, evidence of the therapeutic efficacy of switching to mTOR inhibitors in GCV-resistant CMV infection is limited to case reports [21, 22].

The role of intravenous immunoglobulin (IVIG), particularly in GCV-resistant infection, is not well established due to a lack of data. Some authors suggest that IVIG may be used as adjunct to antiviral drugs, especially in severe forms of CMV disease [18].

Returning to the cases presented here, Patient 1 responded well to a GCV dose increment, suggesting a low-grade UL97 mutation, whereas in Patient 2, combination therapy with FOS was necessary, in addition to switching to mTOR inhibitors, suggesting a high-grade UL97 mutation with or without a UL54 mutation.

Conclusion

We describe two cases of GCV-resistant CMV infection/disease, during prophylaxis and treatment with oral VGCV, which illustrate the complexity of management necessary for resistant CMV strains in renal transplant recipients: the risk of morbidity from CMV must be balanced against the risk of nephrotoxicity and graft loss consequent to treatment. Effective monitoring, minimization of the risk of resistance and early and careful intervention, guided if possible by genotypic resistance testing, are crucial for prognosis.

Teaching points

- (i) Testing for antigenaemia (pp65) and testing for DNAemia (by PCR analysis) are equally good methods for diagnosis of CMV infection and monitoring the response to treatment, but antigenaemia has limited utility in leukopenic patients, and CMV DNA detected by PCR may represent latent viral DNA and not active disease. The results of both methods must be carefully interpreted.
- (ii) GCV resistance should be suspected when increasing or high-level CMV viraemia or progressive clinical disease is observed after 2 weeks of adequate antiviral therapy.
- (iii) Genotypic resistance testing should be performed, when available, to guide treatment of CMV drug-resistant infection and reduce the risk of treatment failure and drug toxicity.
- (iv) Oral VGCV at a prophylactic or therapeutic dose is a risk factor for the development of GCV resistance; however, the risk is lower than that observed with oral GCV.
- (v) Increased doses of GCV and a combination of antiviral agents may be used in an attempt to resolve GCV-resistant CMV infection in solid organ transplants.

Conflict of interest statement. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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