DR PO YEE MIA LEUNG (Orcid ID : 0000-0002-3736-0777)

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Ganciclovir-resistant post-transplant cytomegalovirus infection due to combined deletion mutation at codons 595-596 of the UL97 gene

Po Yee Mia Leung[1], Thomas Tran[2], Adam Testro[3], Kathy Paizis[1], Jason Kwong[4] and John B Whitlam[1, 5]

[1]*Department of Nephrology, Austin Health, Melbourne, Australia*

[2]*Virus Identification Laboratory, Victorian Infectious Diseases Reference Laboratory, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia*

[3]*Liver Transplant Unit, Austin Health, Melbourne, Australia*

[4]*Department of Infectious Diseases, Austin Health, Melbourne, Australia*

[5]*Department of Medicine, University of Melbourne, Australia*

Corresponding author: Po Yee Mia Leung, Department of Nephrology, Austin Health, Melbourne, Australia. Email: mialeung@gmail.com

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Abstract

The development of antiviral resistant cytomegalovirus (CMV) infection complicates the management of transplant recipients. We describe the case of a 65-year-old male who developed CMV disease on valganciclovir prophylaxis (donor CMV IgG positive, recipient CMV IgG indeterminate) 30 days after combined liver-kidney transplantation for alcoholic cirrhosis and hepato-renal syndrome. After an initial complete response to treatment dose oral valganciclovir, he developed recurrent CMV viraemia. Resistance testing revealed a UL97 mutation with in-frame deletions of codons 595-596. He was treated successfully with foscarnet and reduction in immunosuppression. This mutation has not been described previously and was suspected to confer ganciclovir resistance. Ganciclovir resistance occurs most commonly due to mutations in the UL97 or UL54 genes, which encode a protein kinase and a DNA polymerase, respectively. The UL97-encoded protein kinase phosphorylates ganciclovir to ganciclovir-triphosphate, which competitively inhibits viral replication. Mutations in the UL97 gene are typically point mutations or deletions. We describe a new mutation, del595-596 in the CMV UL97 gene, occurring in the context of clinical treatment failure with standard and double-dose ganciclovir, and successful virological control achieved with foscarnet. This mutation is likely to result in ganciclovir resistance, although recombinant phenotyping is required for confirmation.

Keywords

Cytomegalovirus infection, ganciclovir resistance, mutation, UL97 gene, kidney transplant, liver transplant

Introduction

Cytomegalovirus (CMV) infection is an important complication in the intermediate to late post-transplantation period. It may present in a spectrum from asymptomatic viraemia to invasive organ disease. CMV infection is also associated with indirect

effects, such as chronic allograft nephropathy, hepatic artery thrombosis after liver transplantation and elevated risk of other infections[1].

Development of antiviral resistant CMV disease poses clinical challenges. The mainstay of treatment is with ganciclovir. Alternative treatments, such as foscarnet and cidofovir, carry significant toxicities. Newer antivirals, such as maribavir and letermovir, are yet to be proven in clinical trials. Early identification of resistance genotypes and the corresponding resistance profile provides opportunity for earlier institution of effective antiviral therapy. Thus, reducing the risks of progression to life-threatening disease and minimising drug toxicities from agents of limited therapeutic benefit.

Case report

A 65-year-old man underwent a combined liver and kidney transplant for alcoholic cirrhosis and end-stage renal failure secondary to hepato-renal syndrome. The immunological matching was 6/6 HLA mismatch without donor-specific antibodies. The donor was CMV seropositive and the recipient had indeterminate serology (≤0.5-1.0AU/mL). Induction immunosuppression included methylprednisolone and intravenous basiliximab on days 0 and 4. He was commenced on maintenance immunosuppression of prednisolone, mycophenolate mofetil and tacrolimus. Our institution employs universal valganciclovir CMV prophylaxis, and so he was commenced on prophylactic oral valganciclovir 450mg twice weekly based on Cockcroft-Gault creatinine clearance[2]. His immediate post-transplant course was complicated by delayed graft function, aspiration pneumonitis and delirium. Renal biopsies at days 9, 20 and 37 showed resolving acute tubular necrosis without evidence of rejection.

30-days post-transplant, he reported symptoms of odynophagia. A gastroscopy showed viral oesophagitis and inclusion bodies consistent with CMV oesophagitis. The whole blood CMV viral load was 3.867 $\log_{10}$ IU/mL. He was commenced on a therapeutic dose of oral valganciclovir 200mg three times a week after dialysis[2]. However, the viral load continued rising over the next three weeks, reaching a peak viral load of 4.609 $\log_{10}$ IU/mL at day 44. During this time, intermittent haemodialysis was progressively weaned. Immunosuppression was reduced in the

setting of neutropenia. His symptoms of odynophagia improved and CMV viraemia became undetectable at day 72 indicating successful treatment. Due to his high-risk serostatus, valganciclovir prophylaxis was continued at 450mg daily in the setting of a creatinine clearance estimate of 40mL/min. Adjustment of valganciclovir dose was based upon twice-weekly Cockcroft-Gault estimates of glomerular filtration rate.

At day 107, routine monitoring revealed recurrence of CMV viraemia again without symptoms suggestive of CMV disease. Oral valganciclovir dose was deliberately doubled from 450mg daily to twice daily with a creatinine clearance of 36mL/min. Mycophenolate was ceased and he was admitted to hospital for intravenous ganciclovir (5mg/kg every 12 hours) at day 135 due to worsening viraemia, raising suspicion of resistant CMV. Prednisolone 7.5mg daily and tacrolimus continued. His viral load continued to increase over the next ten days to 4.826 $\log_{10}$ IU/mL. Genotype testing detected a complete mutant population with in-frame deletions at codons 595-596 in the UL97 gene. There were no ganciclovir resistance mutations detected in the UL54 gene. Intravenous foscarnet was commenced and continued for 18 days, with regular monitoring of fluid status and electrolyte balance. The CMV viral load rapidly became undetectable (Figure 1).

He was discharged home on day 171 without antiviral prophylaxis. He had stable renal function (224 μmol/L, eGFR 25 mL/min/1.73m^2). Ongoing weekly monitoring of CMV viral load revealed a recurrence of CMV viraemia to 4.307 $\log_{10}$ IU/mL without symptoms suggestive of CMV disease. Subsequently, his immunosuppression was changed to prednisolone, tacrolimus and everolimus (TRANSFORM regimen), which has resulted in undetectable CMV viral load without specific antiviral treatment.

Discussion

Transplant recipients are a risk of developing primary CMV infection or reactivation based on the interplay of host and viral factors. Significant emphasis is given to an individual's antibody status and level of immunosuppression, with the highest risk in donor seropositive and recipient seronegative combinations[3,4].

Mechanism of ganciclovir resistance

CMV is a double-stranded DNA virus with a genome consisting of 235 kilobases[3]. Ganciclovir resistance is most commonly due to mutations in the UL97 or UL54 genes, which encodes a viral UL97 kinase or DNA polymerase respectively. Intracellular ganciclovir undergoes an initial phosphorylation by the viral UL97 kinase. Ganciclovir monophosphate then undergoes subsequent phosphorylation by host cellular kinases to result in ganciclovir triphosphate. This competitively inhibits the CMV DNA polymerase, terminating DNA elongation[3]. Therefore, as ganciclovir and its metabolites are substrates of UL97 kinase and DNA polymerase, mutations in UL97 or UL54 genes can confer resistance. The most common mutations are typically point mutations or small deletions which alter the ability of UL97 kinase to phosphorylate ganciclovir[4].

CMV has high degree of genetic heterogeneity. There is a lack of genetic linkage among divergent genes, suggesting that recombination in superinfected individuals may occur[3]. Different body sites within a patient can harbour CMV strains with different resistance profiles[3]. Moreover, valganciclovir dosing is dependent on renal function, which may be inaccurately estimated using convenient approaches based on serum creatinine alone[5]. Thus, inadequate dosing of valganciclovir, especially during active infection or fluctuating renal function, can select ganciclovir-resistant virions from the wild type virus[5]. In this patient, improving creatinine clearance after initial delayed graft function may have resulted in under-dosing despite stringent monitoring. CMV resistance should be suspected in the setting of poor clinical or virological response to treatment.

Diagnosis of ganciclovir resistance

Viral load testing is required for diagnosis and monitoring of CMV infection and disease. Detection by quantitative nucleic acid amplification can be performed with whole blood or plasma. At this institution, whole blood testing is used, which detects DNA at a lower viral load compared to plasma samples[2]. There are significant variations in reference ranges and assay designs between laboratories[4,6].

Genotypic testing employs Sanger sequencing to detect mutations in the UL97 (codons 400-670) and UL54 (codons 300-1000) genes. When a novel mutation is

discovered, it cannot be presumed to confer resistance. Recombinant phenotyping (also known as marker transfer) should be performed to distinguish polymorphisms which are clinically insignificant from mutations that confer antiviral resistance[7]. This technique involves introducing a DNA fragment containing the specific mutation of interest into a control laboratory wild type virus strain and then testing its susceptibility to an antiviral to confirm resistance.

Implications of a combined deletion at codons 595-596

There is an increasing international database of CMV mutations which have been shown to confer resistance to ganciclovir[8]. The most common mutations of the UL97 gene are clustered at codons 460, 520 or 590-607, and there are less frequent independent in-frame deletions at codons 595, 596 and 600[2].

Chou et al (2017) performed recombinant phenotyping using CMV strains with various mutations or deletions, and analysed the corresponding ganciclovir resistance with EC_{50} ratio. Deletions of three or more consecutive codons conferred at least 8-fold ganciclovir resistance. Single or two codon deletions had variable ganciclovir resistance. Deletions of codons 595 and 596 each individually have been shown to confer ganciclovir resistance. The most resistant single deletion was at codon 595, with a ganciclovir EC_{50} ratio of 8-8.5, whereas deletion of codon 596 conferred an EC_{50} ratio of 4-4.7[9].

The combined deletion at codons 595-596 has not been previously described[7,9,10]. It is not known whether this mutation makes the virus more virulent. Based upon the phenotypes of the individual deletion mutations at codons 595 and 596, it is probable that the combined deletion of codons 595-596 confers at least a five-fold ganciclovir EC_{50} increase.

Treatment

When ganciclovir-resistance is confirmed, one treatment option is to use a high dose ganciclovir (10mg/kg every 12 hours) rather than standard dose therapy (5mg/kg every 12 hours)[2]. UL97 mutations confer varying degrees of ganciclovir resistance and a low level of ganciclovir resistance may be overcome with dose escalation[2]. In this patient, valganciclovir therapy was empirically doubled but this was complicated by bone marrow suppression. The predicted ganciclovir EC_{50} increase for the codon

595-596 deletion mutation suggests it is not a candidate for ganciclovir dose escalation.

Another treatment option is to change to an antiviral with a different mechanism of action. Foscarnet does not require activation by the UL97 kinase and binds directly to the CMV DNA polymerase's pyrophosphate binding site to impede viral replication[4]. Foscarnet is administered intravenously and common side effects include nephrotoxicity, hypocalcaemia, hypomagnesaemia and hypokalaemia[2]. Similarly, cidofovir does not require UL97 kinase for activation. It undergoes phosphorylation by viral cellular kinases and then binds to DNA polymerase to inhibit replication[4]. There is limited evidence for the use of cidofovir in solid organ transplant recipients but it can be considered in the setting of both ganciclovir and foscarnet resistance[11]. Nephrotoxicity is a significant concern.

Other antiviral drugs have been under investigation. Letermovir binds to the CMV terminase complex to inhibit viral replication[4]. It is the newest antiviral approved by the US Food and Drug Administration for CMV prophylaxis in haematopoietic-cell transplant recipients, following a successful phase III randomized control trial in 2017[12]. A phase III trial of letemovir for prophylaxis in kidney transplant recipients is underway (ClinicalTrials.gov identifier: NCT03443869). Maribavir is an oral UL97 kinase inhibitor which failed to prevent CMV infections in haematopoietic-cell and liver transplant recipients in phase III trials[13,14]. A phase II study of maribavir as salvage treatment for resistant CMV disease in haematopoietic-cell and solid organ transplant patients showed it was efficacious[15]. Currently, phase III trials are being conducted (ClinicalTrials.gov identifier: NCT02927067 and NCT02931539). Brincidofovir, an oral version of cidofovir, failed in phase III trials for CMV prophylaxis in CMV-seropositive recipients after allogeneic stem cell transplant recipients and resulted in significant gastrointestinal side effects[16].

Reducing immunosuppression or switching to a mammalian target of rapamycin drug should be considered. The TRANSFORM study demonstrated one-third of the incidence of CMV disease in the everolimus and low-dose calcineurin inhibitor group compared to the standard immunosuppression arm[17].

Drugs approved for other indications have been used although there are no randomized controlled trials supporting this. Leflunomide, indicated for treatment of

rheumatoid arthritis, have been described in case reports as an adjunctive treatment in resistant CMV disease[18]. Artesunate, an anti-malarial, may have anti-CMV effects in vitro[19].

Adjunctive immunotherapies include CMV immunoglobulin, which have been used for treatment of CMV infection and disease in heart or lung transplant recipients[20]. Its use in kidney or liver transplantation is not well studied but it may have a role in the setting of concomitant hypogammaglobulinaemia[21]. A novel therapy under investigation harnesses chimeric antigen receptors on T cells (CAR-T cell therapy) to recognise CMV-encoded proteins on host cells as a therapeutic target[22].

Conclusion

A novel mutation del595-596 of the CMV UL97 gene was detected in this patient. This is in the context of clinical treatment failure with standard and double-dose ganciclovir. Successful virological control was subsequently achieved with foscarnet. This mutation is likely to result in ganciclovir resistance but recombinant phenotyping is required for confirmation.

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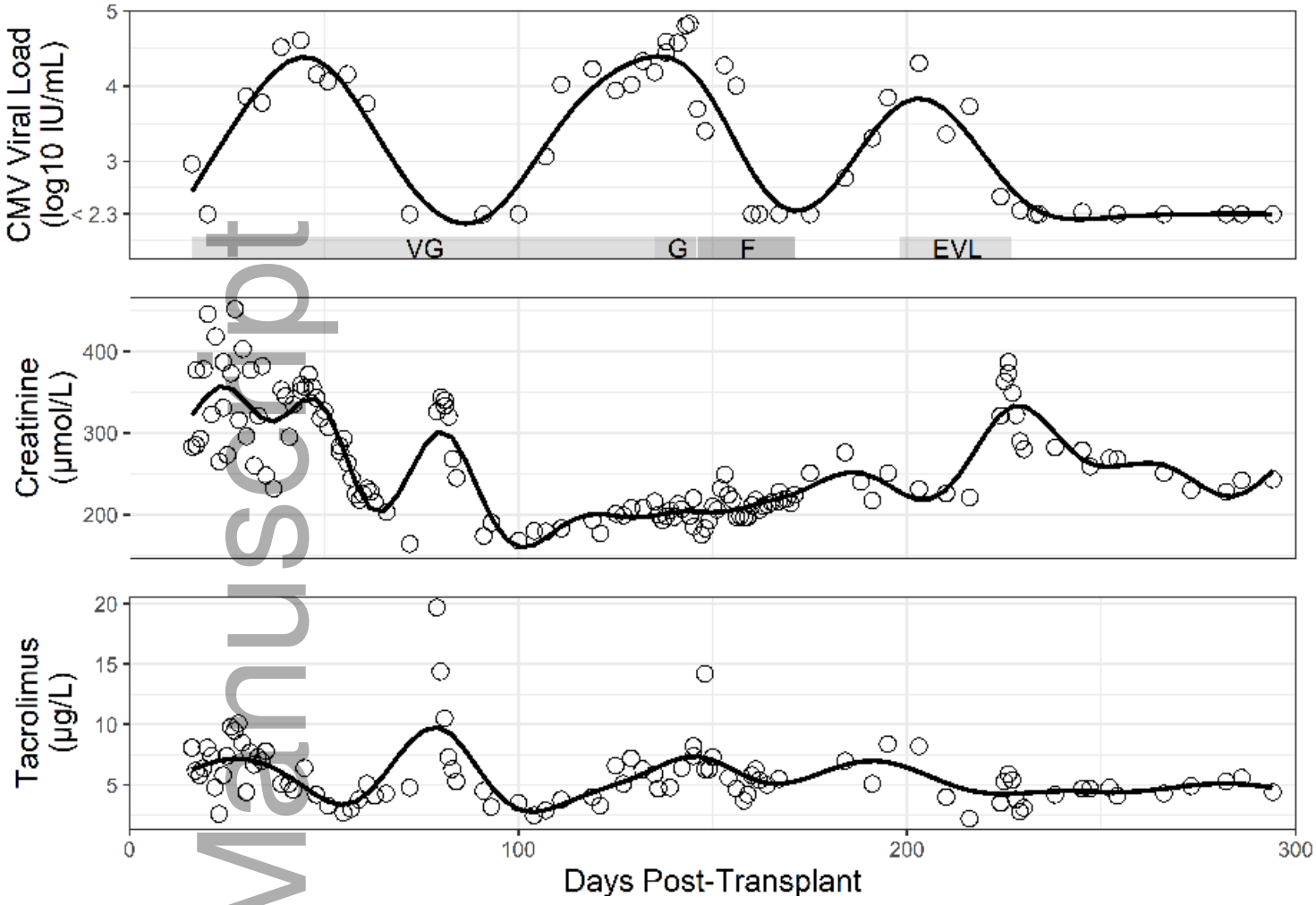
Authors' contributions

P.Y.M.L. wrote the manuscript. T.T., A.T., K.P., J.K., and J.B.W. provided specialist advice, edited and reviewed the manuscript. J.B.W. created the figure and oversaw writing of the manuscript.



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