Case report

Recurrent and persistent cytomegalovirus infection in a kidney recipient caused by the L595S mutation in UL97 phosphotransferase gene

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Ganciclovir (GCV) is the first therapeutic choice for prevention and treatment of active cytomegalovirus (CMV) infection in solid organ transplant recipients in Bahia state, Brazil. Prolonged and repeated GCV therapy may result in drug-resistant virus, associated with progressive and disseminated disease. We present a case report of a young male kidney recipient, who was CMV-seronegative with a CMV-seropositive donor (D+/R-), and who developed clinical GCV resistance, confirmed by mutation in viral UL97 phosphotransferase responsible for GCV activation. Under prophylactic therapy with intravenous GCV for 6 weeks post-transplantation, he developed severe anaemia and hepatic enzyme increases, probably due to drug side effects. At this moment, the drug was discontinued and he started to be monitored by pp65 antigen test. At week 10 post-transplantation, he presented fever, myalgia, thrombocytopenia and neutropaenia, with a positive CMV antigen test. During treatment with intravenous GCV, antigenaemia assay demonstrated a higher number of positive cells, requiring GCV at higher doses. Pre-emptive therapy lasted for 31 days and he started the maintenance therapy with oral GCV. However, antigenaemia assay demonstrated an extremely high number of positive cells, and he was rehospitalized and prescribed intravenous GCV. Severe leukopaenia led to GCV interruption, but immunosuppressive dose reduction helped to control the active CMV infection. GCV-resistant CMV infection resulted in increased morbidity, rehospitalization episodes and increased costs; therefore, implementation of resistance diagnostic tests in the transplantation routine is of great importance. We documented the first case of GCV-resistant CMV infection due to the L595S mutation in UL97 phosphotransferase gene in a kidney recipient from Bahia state, Brazil.

Introduction

More than two-thirds of solid organ transplant (SOT) recipients have evidence of cytomegalovirus (CMV) infection during the post-transplant period. However, only a few of those who are actively infected develop CMV symptomatic disease [1]. Ganciclovir (GCV), a deoxyguanosine analogue, is the first therapeutic choice for the prevention and treatment of CMV infection in SOT. In infected cells, a viral phosphotransferase encoded by the UL97 gene phosphorylates GCV to GCV monophosphate, with cellular kinases carrying out further phosphorylations to GCV di- and triphosphate. The active drug represents a false substrate for viral DNA polymerase, inhibiting its enzymatic function and blocking CMV replication [2].

Prolonged and repeated GCV therapy may result in the emergence of drug-resistant virus, which is associated with therapeutic failure and persistent and disseminated infections. Resistance mainly occurs due to a decrease in GCV phosphorylation, and mutations in the UL97 gene have been identified in >90% of CMV resistant strains clinically isolated [3].

This report is the first GCV resistance case observed in a kidney transplant recipient from Bahia state, in the northeast area of Brazil, and describes the risk...
factors and outcomes associated with CMV-resistant infection.

Case report

A young man with chronic glomerulonephritis associated to systemic hypertension underwent an HLA-haploidentical (donor A3A68–B35B63 and recipient A3A23–B35B44) living related donor kidney transplantation at the age of 28, in March 2008. Immediately after transplantation, he initiated immunosuppression with mycophenolate mofetil (1.5 mg/day), prednisone (30 mg/day) and tacrolimus (8 mg/day). This initial immunosuppressive regimen was individualized and monitored by laboratory exams for dose verification. He was CMV-seronegative, but the donor was CMV-seropositive (DR/ R), which indicated the need for prophylaxis with GCV, 5 mg/kg twice daily, intravenously for 4 weeks, followed by 5 mg/kg on alternative days for 10 weeks [4]. However, the drug was discontinued 6 weeks after the surgery because he developed severe anaemia (haemoglobin 6.0 g/dl and haematocrit 16.7%) and hepatic enzymes increases (aspartate aminotrasf erase 122 Ul, alanine aminotransferase 370 Ul, g-glutamyltransferase 79 Ul and alkaline phosphatase 78 Ul). He received blood transfusion and erythropoietin for treatment of anaemia. As an alternative to GCV prophylaxis, he was monitored weekly with a pp65 antigen test to detect CMV active infection for a preemptive approach.

During week-10 post-transplantation, the patient presented fever (38°C), myalgia, rhinorrhea, thrombocytopaenia (33,800 platelets/Ul) and neutropaenia (1,451 neutrophils/Ul). CMV antigenaemia presented nine positive cells in 2×10⁵ peripheral leukocytes. Clinical presentation along with the laboratory confirmation of active CMV infection indicated intravenous GCV therapy, 5 mg/kg daily. After 3 days under treatment, the dose was adjusted to 5 mg/kg twice daily, as no side effects occurred. During week 12, the antigenaemia presented 238 positive cells in 2×10⁵ leukocytes, even after 9 days under treatment, which was maintained for 31 days. In the next 2 weeks, he achieved good clinical response to the antiviral therapy, without any symptoms, and clearance of CMV antigenaemia. The patient was discharged during week 16 post-transplantation and prescribed maintenance treatment with oral GCV, 1,000 mg twice daily.

At week 21 post-transplantation, during treatment with oral GCV, the patient complained of fever (38°C), diarrhoea and anorexia, and presented a positive antigenaemia with an extremely high level of 405 positive cells in 2×10⁵ leukocytes. He was rehospitalized to receive treatment with intravenous GCV. After 1 week of treatment (week 22), antigenaemia decreased to one positive cell in 2×10⁵ leukocytes. Laboratory data showed severe leukopaenia (white blood cells count 1,800 cells/Ul) and reduction in platelet counts (113,000 cells/Ul) during this week that could be associated either to the CMV myelosuppression or to the antiviral myelotoxicity. These alterations led to interruption of GCV. Nevertheless, after 4 days in the absence of the drug, the antigenaemia increased to 34 positive cells in 2×10⁵ leukocytes without recovering normal bone marrow function. During this period, the use of erythropoietin prevented the occurrence of anaemia. Intravenous GCV therapy was restarted, leading to infection control, with antigenaemia presenting 10 positive cells in 2×10⁵ leukocytes during week 24 and negative in week 25. He also recovered platelet and leukocyte counts near normal levels (123,000 platelets/Ul and white blood cell count 5,000 cells/Ul), suggesting that myelosuppression was caused by viral immunomodulation.

At week 27 post-transplantation, the patient finished the preemptive therapy with GCV, presenting negative antigenaemia, and was discharged from the hospital. During the following weeks, antigenaemia presented 5, 7 and 1 positive cells in 2×10⁵ leukocytes, and he developed thrombocytopaenia (75,000 platelets/Ul) and gastritis symptoms (abdominal pain, indigestion and nausea). To avoid GCV side effects, active infection was controlled with a reduction of immunosuppression. Antigenaemia clearance was achieved at week 35, with leukocytes and platelets reaching normal levels (white blood cell count 11,900 cells/Ul and 146,000 platelets/Ul). Follow-up with CMV antigenaemia was carried out until week 42 post-transplantation, without any symptoms of CMV disease.

Clinical resistance to GCV was corroborated by multiple high positive cell counts in antigenaemia tests during drug administration. Resistance to GCV was confirmed by retrospective molecular analysis of UL97 gene sequences that were compared with CMV Towne strain sequences (GenBank accession number U07355) by using BioEdit version 5.0 (Hall, Raleigh, NC, USA). Sequencing was performed by fluorescent dyes chain-terminator on PCR products (fragment comprising codons 439–696, nt1312–2017) obtained with primers described elsewhere [5] using an automatic genetic analyser (ABI 3100, Applied Biosystems, Foster City, CA, USA).

The sequence obtained from week 9 post-transplantation, before exposure to a long term of GCV therapy, did not have any amino acid replacement. However, the sequence obtained from week 21 post-transplantation, when there was a higher number of positive cells in the antigenaemia assay (405 positive cells in 2×10⁵ peripheral leukocytes), presented a nucleotide change 1784T>C (Thymine to Cytosine) at codon 595 (Leu-595-Ser), which represents the L595S mutation in UL97 phosphotransferase gene, commonly associated with GCV resistance [6].

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The local ethical committee of Portuguese Hospital (Salvador, Brazil) and of Oswaldo Cruz Foundation (Brazil) approved this study and the patient signed a consent form obtained in accordance with the Declaration of Helsinki.

Discussion

CMV infection is the most important cause of morbidity and mortality among transplant recipients, associated with disseminated disease, opportunistic infections and graft rejection [7]. Clinical management of CMV infection in SOT recipients can be divided into prophylaxis, preemptive therapy or therapeutic therapy, with dose variations of the available antiviral drugs GCV or valganciclovir. In some parts of the world, prophylaxis with either intravenous or oral GCV or valganciclovir is recommended for CMV D+/R- SOT patients, who are at major risk of CMV disease, mainly during the first 3 months after transplantation [8].

In this case, the patient presented with a high risk for CMV disease (D+/R) and started prophylaxis with oral GCV just after the transplantation. However, the drug was interrupted early because he developed severe anaemia and hepatic enzyme abnormalities. Drug adverse effects occur due to accumulation of GCV triphosphate that result in myelosuppression, with severe neutropenia in approximately 30% of patients, besides other relevant side effects, such as hepatocellular dysfunction, thrombocytopenia, gastrointestinal, neurological and renal disturbances [9]. Major limitations to antiviral prophylaxis are the exposure of a significant proportion of patients who will never develop CMV disease to prolonged courses of antiviral therapy and related side effects, enhancing the chance of developing drug resistance or the late onset of CMV disease after prophylaxis discontinuation [10,11]. By contrast, a recent study has shown that treatment failure of CMV infections occurred less frequently in D+/R renal transplant patients on a sequential prophylaxis-pre-emptive regimen than in patients on a purely pre-emptive regimen, which reaffirms the importance of prophylaxis [12].

The use of prolonged or repeated courses of GCV predisposes to the emergence of resistant CMV strains [13]. The occurrence of CMV resistant disease may vary according to the type of transplantation, the immunosuppressive drugs and the donor/receptor serological status. Limaye et al. [14] detected 2.1% of GCV resistance in a heterogeneous group of transplanted patients, and the percentage increased to 7% when only D+/R- SOT recipients were evaluated. The patient presented in this case (D+/R) developed GCV-resistant CMV disease, associated with alternated periods of symptoms and increased rate of viral replication. In addition to his serological risk group, his immunosuppressive scheme contained mycophenolate mofetil, which raises the risk of CMV activation and has been associated with severe and prolonged disease [15]. The high rates of viral replication and exposure to suboptimal systemic GCV doses during oral GCV therapy might be the major factors that contributed to the emergence of GCV-resistant CMV [16].

Drug resistance to GCV results from mutations in either the UL97 phosphotransferase gene or in the UL54 gene coding for the DNA polymerase, or in both, and impaired drug phosphorylation is the most important mechanism of GCV resistance in CMV. The UL97 gene has a highly conserved region, and mutations occur more frequently at codons 460, 594 and 595 [17]. Detection of drug-resistant strains from clinical specimens could be done by phenotypic or genotypic assays; among them, DNA sequencing is the method of choice for assessment of the presence of new and known mutations [18]. The case detected L595S mutation, which has been widely associated with GCV resistance, identified in ≥55% of resistant clinical isolates [19]. It is located at a gene region responsible for enzymatic substrate recognition, associated with a residual GCV phosphorylation level of approximately 10–20% of those observed in wild strains [20].

Resistant CMV infections have been associated with disseminated disease, graft loss and increased morbidity. They also increase the cost of GCV treatment because higher doses and extended lengths of therapy are needed to control viral infection, which means greater risk for developing drug side effects and longer periods of hospitalization [16]. In the present study, the resistant CMV infection promoted a mild disease, with fever and gastrointestinal symptoms, but the patient showed a persistent active infection, that could be evaluated by monitoring antigaemnaemia during the post-transplant period, with positive antigaemnaemia even after many weeks of GCV therapy. In addition, he developed severe leukopaenia and thrombocytopenia that may be associated either to viral myelosuppression or GCV myelotoxicity. These could also intensify the immunocompromised status of the patient, increasing risks of others opportunistic infections. Therefore, he needed to be rehospitalized several times and was subjected to long treatment periods, increasing the transplant financial costs. The CMV infection could be controlled by increasing GCV doses combined with immunosuppressive dose reduction. No alternative antiviral drug was prescribed as GCV is the only one available in the transplantation programme of Bahia state.

Laboratory evaluation of CMV resistance through genotypic assays is of great value because it allows results to be available in a clinically relevant time.
frame, thus improving the management of patients with alternative choices of treatment when therapeutic failure and persistent disease cases occur during the post-transplantation period for SOT recipients.

We documented the first case of GCV-resistant CMV infection in a kidney recipient from Bahia state, in Brazil, which resulted in increased morbidity with many hospitalizations and increased costs. In conclusion, post-transplantation monitoring with antigenemia assay was essential for the correct and early diagnosis of CMV disease, and it also demonstrates the clinical resistance to GCV. CMV resistant strains occur in SOT recipients in Bahia, which points to the importance of implementing resistance diagnostic tests in the transplantation routine to optimize CMV treatment and the choice of alternative immunosuppression.

Acknowledgements

The authors would like to thank Jurema Garrido and Silvana Paixão for technical support. Our thanks are also due to Paula Durant Andrade and Dulcineia Albuquerque for stimulating discussion about CMV detection. This work was supported by the Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Pesquisa (CNPq), PRODOC grant number 5090.

Disclosure statement

The authors declare no competing interests.

References


Accepted 25 July 2011; published online 27 October 2011