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Resistant cytomegalovirus infection in renal transplant recipients

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Abstract

Resistant cytomegalovirus infection is a significant problem in the transplant population including renal transplant recipients. A combination of factors including receipt of potent immunosuppression, high viral loads and suboptimal levels of anti-cytomegalovirus antivirals leads to emergence of resistant strains. Reports of associated poor graft survival and mortality demonstrate the potential pathogenic nature of such strains. Genotypic and phenotypic resistance testing are available for laboratory diagnosis of resistant cytomegalovirus infection and may help guide therapy. Various agents, including novel and newly minted antivirals and treatment approaches have been employed, with variable success. Thus, in spite of major advances in both diagnostics and therapeutics, management of resistant cytomegalovirus infection in renal transplant recipients remains a challenging prospect.

Keywords

cytomegalovirus, antiviral resistance, renal transplant

Introduction

Cytomegalovirus (CMV) resistance is an increasing problem in the transplant recipient. Ganciclovir (GCV), introduced in the mid-1980s, was the first antiviral compound with activity against CMV. GCV-resistant CMV strains, however, quickly ensued. This was well recognized in acquired immunodeficiency disease syndrome (AIDS) patients with CMV retinitis requiring prolonged courses of ganciclovir.[1] At the same time, only sporadic cases of CMV resistance were reported among solid organ transplant (SOT) patients. The introduction of more immunosuppressive transplant protocols and the practice of CMV prophylaxis or pre-emptive therapy post-transplant have changed this landscape. This article aims to provide an overview of CMV resistance in the setting of renal transplantation.

Pathogenesis of resistance

The prerequisite for the development of resistance is the occurrence of sustained CMV replication in the presence of the antiviral drug. During antiviral treatment, these CMV mutant strains have a survival advantage over wild-type CMV, emerging as the dominant population and potentially resulting in failure of treatment and disease progression. The environment that promotes the emergence of resistance is likely to be a culmination of factors. In the renal transplant patient, this includes CMV donor-positive recipient-negative serostatus (D+/R–), receipt of potent immunosuppression, prolonged exposure to anti-CMV agents, especially at suboptimal levels, and the presence of high CMV virus load.[2]

Mechanisms of resistance

The mechanisms of resistance are best understood by considering the mechanisms of action of the three main CMV antivirals in use (Figure 1) – GCV, foscarnet (PFA) and cidofovir (CDV).

GCV is a guanosine analogue that exerts anti-CMV activity by inhibiting viral DNA polymerase. Conversion to its active form requires triphosphorylation. The first phosphorylation is performed by the virus-encoded enzyme phosphotransferase, which is the product of the viral UL-97 gene. The remaining two steps of phosphorylation are performed by cellular enzymes. The active triphosphorylated GCV then inhibits CMV DNA polymerase, which is encoded by the viral UL-54 gene.

CDV is a nucleotide analogue that requires a two-step phosphorylation performed exclusively by cellular enzymes

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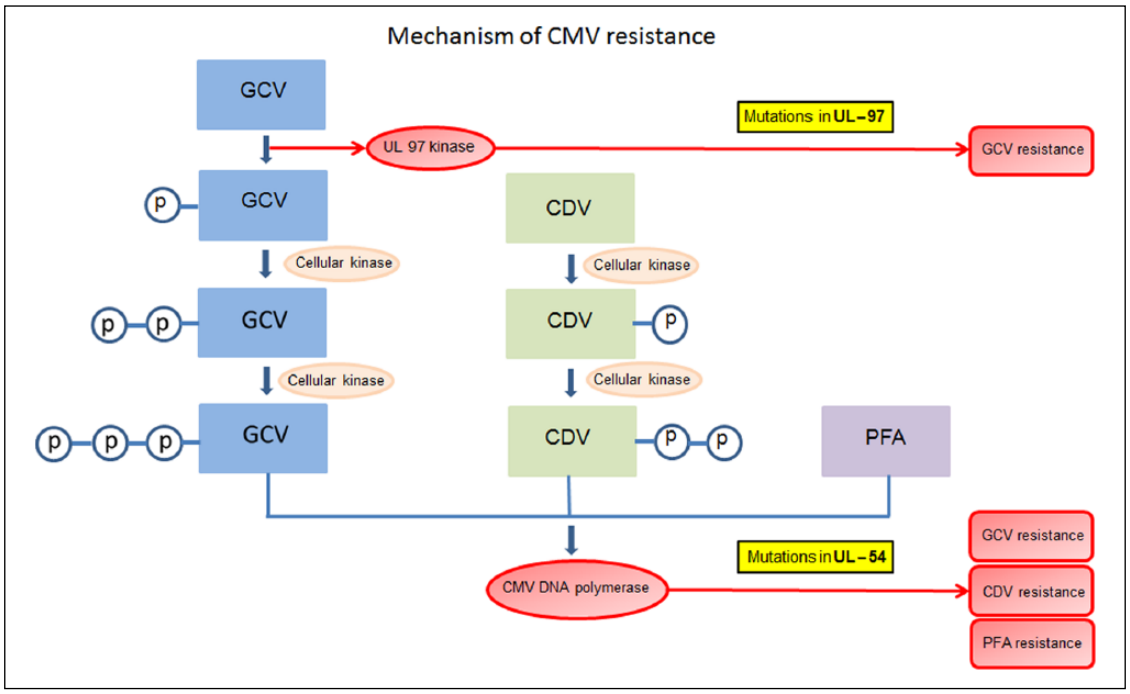

Figure 1. Sites of action of three main cytomegalovirus (CMV) antivirals.
The first phosphorylation (p) of ganciclovir (GCV) is mediated by viral UL-97 kinase; mutations in certain codons of the UL-97 gene will confer GCV resistance. Cellular kinases perform the final two phosphorylations that convert GCV to the active ganciclovir triphosphate. Cidofovir (CDV) as a monophosphate analogue only requires phosphorylation by cellular kinases for conversion to the active cidofovir diphosphate while foscarnet (PFA) is a pyrophosphate analogue that does not require activation. All three substrates have CMV DNA polymerase as the final site of action. Mutations of viral UL-54 gene encoding this polymerase may confer resistance to any of the three agents; cross resistance between agents is also seen.

converting it to its active form, which exerts its inhibitory effect on viral DNA polymerase. Thus, CDV is not dependent on the UL-97 gene encoded viral phosphotransferase for its activation. PFA is a pyrophosphate analogue that directly inhibits CMV DNA polymerase and does not require activation via phosphorylation.

Mutations in UL-97 and UL-54 mediate CMV resistance to these antivirals. UL-97 mutations prevent effective triphosphorylation of GCV, resulting in low levels of the active drug. The majority of GCV-resistant CMV result from UL-97 mutations. Mutations in UL-54 produce mutant CMV DNA polymerases that are less inhibited by these antiviral compounds. CMV DNA polymerase being the common pathway of action of these antivirals, it is easy to understand how UL-54 mutations can potentially confer cross resistance among GCV, PFA and CDV while mutations in UL-97 confer resistance to GCV alone.

What of the newer antiviral agents, such as maribavir? Maribavir is a benzimidazole L-riboside that directly inhibits the UL-97 kinase, causing impaired viral replication via mechanisms not yet well understood. Mutations in UL-97 gene separate from those associated with GCV resistance as well as mutations in UL-27, a betaherpesvirus-specific early gene, have been shown to mediate maribavir resistance.[3,4]

Diagnosis

Resistant CMV may be clinically suspected when there is progressive disease and/or rising viral loads despite adequate antiviral CMV therapy for >2 weeks. It is important to note that CMV antigenaemia may rise in the first 2–3 weeks of therapy without connoting resistance. The reason for this is uncertain but likely related to corticosteroid use.[5] Phenotypic and genotypic assays can be done to obtain a laboratory diagnosis.

The gold standard for phenotypic testing uses plaque reduction assay and is expressed as the concentration that inhibits growth of 50% of viral plaques (IC_{50}). Phenotypic assays have to contend with issues of interlaboratory and interassay variability, lack of agreement for cut-off IC_{50} values that define resistance, reduced sensitivity in detecting low-level resistance and the possibility of selection bias when there is mixed viral population growth. More important, these assays require time-consuming viral isolation, thus the long turn-around time negates its ability to guide clinical decision-making with regards to CMV treatment and the choice of antiviral agent.[6]

Genotypic assays detect the presence of mutations that are known to be associated with resistance. This is done via DNA sequencing of UL-97 and UL-54 viral genes or restriction fragment length polymorphisms of polymerase chain reaction-amplified DNA fragments. In clinical GCV resistance associated with UL-97 mutations 80%–85% is found in codons 460, 520 and 591–607. Using recombinant phenotyping, the different mutations and the associated level of GCV resistance conferred can be characterized.[7] Resistance mutations in the UL-54 gene have also been characterized, with the majority being found in codons 395–540. As with UL-97, different mutations in UL-54 confer different levels of resistance as well as different degrees of cross resistance. For example, UL-54 mutations in codons 981–987 are able to result in cross resistance to all three antivirals.[8]

As the genotypic assay may be directly performed on clinical specimens, its shorter turn-around time allows its use as a tool to guide treatment of resistant CMV infections. While genotypic assays are faster and more sensitive, their interpretation is limited to currently known resistance associated mutations. New uncharacterized mutations may confer clinically significant resistance and can only be confirmed by phenotypic testing. Zhang et al.[9] described a new mutation, C518Y, in two renal transplant patients that resulted in high grade GCV resistance. Another practical point to note is the minimum viral load requirement for genotypic assays that precludes its use in cases of clinically suspected resistance but with quantitatively low viral loads.

Clinical characteristics

The incidence of resistant CMV among SOT has been estimated as between 0% and 13% from various studies which reported mainly GCV resistance.[2] The incidence varies according to type of SOT. Lung and small bowel transplants have the highest incidence, likely related to more intense immunosuppression and higher amount of lymphoid tissue being transplanted. Limaye reviewed GCV resistant CMV among SOT, summarizing data from five studies: overall GCV resistance was estimated at 0.54%–1% among renal transplants.[2] Myhre et al. conducted a retrospective single centre study of 1244 kidney and kidney–pancreas transplant recipients and documented GCV-resistant CMV incidence of 2.2%.[10]

Risk factors

CMV serostatus. D+/R– CMV serostatus is the most consistent risk factor found across studies. In the cohort of Myhre et al., comprising 1244 kidney transplant recipients, GCV-resistant CMV was found in 12.5% of D+/R– patients whereas this incidence was only 0.15% among D+/R+ and none were found in D–/R+ and D–/R– patients. The high risk conferred by D+/R– reflects the role of high viral loads that occur in primary CMV infection and the lack of pre-existing CMV specific immunity in mediating emergence of resistant CMV.

On the other hand, the occurrence of resistant CMV among R+ recipients implies the interplay of other factors that bring about resistance. These other factors are discussed below.

Immunosuppression. The intensity of immunosuppression is likely to be one of these factors. Higher rates of resistant CMV among lung transplant recipients are partly attributed to the intense immunosuppression received by these patients as compared with other SOTs. The use of lymphocyte depleting agents such as antithymocyte globulin (ATG), OKT3 (anti-CD3 antibody) and alemtuzumab (anti-CD 52 antibody) has been associated with an increased risk of developing CMV disease. The degree of risk varies widely according to the agent, the dose, CMV serostatus and practice of CMV prophylaxis. For instance, studies involving renal transplant recipients receiving ATG at induction reported rates varying from 1% to 51% dependent on these factors, especially the receipt of CMV prophylaxis.[11] There is, however, no established direct link between the specific immunosuppressive agents and the development of resistance. A French cohort study of transplant recipients that included 287 SOT recipients, of which 224 were kidney transplants, failed to find an association between the use of anti-lymphocyte agents at induction and resistant CMV disease.[12]

Duration of treatment and drug levels. Prolonged duration of exposure to systemic anti-CMV agents, especially with suboptimal drug levels, is an important factor in the emergence of resistance. This was readily demonstrated in early studies on human immunodeficiency virus (HIV) infected patients receiving prolonged courses of GCV for treatment of CMV disease. A prospective study of 72 HIV patients on GCV for CMV disease reported the prevalence of GCV resistant CMV to be 38% in the subgroup of patients receiving treatment for more than three months.[13] Another prospective study of 108 HIV patients with CMV retinitis on GCV reported a baseline GCV resistance rate of 2.7% at diagnosis which increased to 11.4% and 27.5% at six months and nine months of treatment respectively.[14] The suboptimal levels of antivirals achieved in sanctuary sites such as the eye and central nervous system increase the risk of resistance developing within these sites. Valganciclovir (VGCV), with its higher bioavailability compared with oral GCV, has replaced oral GCV for CMV prophylaxis of SOT patients. Boivin et al. in his prospective multicentre study of high risk SOT subjects found an incidence of 1.9% for UL-97 resistance mutations in patients on oral GCV prophylaxis compared with none in patients on VGCV.[15] Nevertheless, in the series of Myhre et al., the use of VGCV at 900mg/day for pre-emptive therapy was associated with the development of resistance.[10] This dose is possibly inadequate, as, in the pre-emptive strategy, viral replication is already occurring. Accordingly, then, the 2013 international consensus guideline on CMV prevention in transplant recipients emphasizes the need to use full-dose VGCV in pre-emptive therapy.[16]

CMV prophylaxis and pre-emptive strategies. There are conflicting findings comparing prophylaxis and pre-emptive strategies with regard to the risk of resistance. Centres employing a prophylactic approach have described GCV resistance.[17,18] A pre-emptive approach which reduces the cumulative exposure to anti-CMV agents intuitively should reduce the rates of resistant CMV compared with prophylaxis. Couzi et al., however, found the opposite. In a study comparing D+/R– renal transplant recipients receiving VGCV using these two strategies, they reported higher rates of treatment failure and CMV drug resistance mutations in the pre-emptive group.[19] On current evidence, increased or reduced risk of promoting resistant CMV cannot be attributed to either strategy

Knowing that the emergence of resistance depends on a complex interplay of multiple factors, it is too simplistic to apply the prophylaxis or pre-emptive approaches as a 'one-size-fits-all' solution to the prevention of CMV among SOT. Rather, some degree of risk stratification should be done in deciding the prevention strategy.[20] In high risk D+/R– recipients and R+ recipients receiving anti-lymphocyte agents at induction, the prophylaxis approach should be favoured. A pre-emptive strategy in these patients may potentially result in suboptimal drug

levels in the presence of high viral loads thus promoting resistance. Conversely, in the low risk D–/R– recipients, a pre-emptive approach is a reasonable consideration.

Outcomes

CMV has both direct and indirect effects in SOT. The direct effects of CMV are a spectrum from asymptomatic viraemia and CMV syndrome to tissue invasive disease such as hepatitis, pneumonitis, gastrointestinal disease and encephalitis.[21] CMV has also been indirectly implicated in allograft rejection, post-transplant infections, accelerated atherosclerosis, development of post-transplant lymphoproliferative disease and reduced patient survival.

Clinical outcomes of patients with resistant CMV are similarly varied. Some case series have demonstrated significant mortality and morbidity associated with resistant CMV disease.[16,17,22,23] In contrast, the study of Myhre et al. involving renal transplant recipients reported good clinical outcomes among the 27 patients with GCV-resistant CMV.[10] Do patients with resistant CMV disease, then, do better or worse compared with patients with non-resistant CMV? On the balance of current evidence, no definite conclusions can be drawn on this matter.

Treatment

There are no controlled trials that support a particular approach or agent in the treatment of resistant CMV disease. Due to the diversity of both host and viral factors, it is not possible to have a standardized treatment protocol for treatment of resistant CMV disease.

The various treatment approaches may involve a combination of a reduction or modification of immunosuppression, modifying the use of currently available antiviral agents, immunotherapy and the use of new and novel agents.

Modifying immunosuppression

Reducing the net state of immunosuppression helps the treatment of CMV infection and this approach should similarly be applied in resistant CMV infections. In all such cases, a review of the patient's immunosuppression regimen should be done with the aim of reducing it as much as is safely possible, balanced against the risk of allograft rejection. Mammalian target of rapamycin (mTOR) inhibitors such as sirolimus and everolimus have less immunosuppressive effect and may indirectly affect CMV replication.[24] Switching to mTOR inhibitors has been reported in small case series to be successful as salvage treatment of GCV-resistant CMV infections in SOT recipients.[24,25]

CMV antivirals

GCV, PFA and CDV are the mainstays of CMV antiviral treatment. In patients with UL-97 resistance mutations alone, switching from GCV to either PFA or CDV should be the main treatment approach. Among renal transplant recipients, however, this is tempered by the significant nephrotoxic effects of CDV and PFA and close monitoring of renal function is required. Increasing the dose of GCV from the standard 5mg/kg q12h to 7.5–10mg/kg q12h is another approach that has been used.[26] This can be considered especially in cases with UL-97 mutations known to confer only low-level GCV resistance. GCV and PFA combination therapy utilizes the different pathways of action of these two agents and in vitro synergy has been demonstrated.[27,28] Mylonakis et al. successfully used a combination of daily PFA with GCV (at 50% of therapeutic dose) in six SOT patients who had persistently rising CMV viraemia after three weeks of full dose intravenous GCV.[29]

Immunotherapy

CMV hyperimmunoglobulin (CMVIG) is a pooled serum containing high titres of anti-CMV antibodies. While proven to be effective as CMV prophylaxis in high risk renal transplant recipients,[30] its role in treatment of CMV disease remains unclear. It has been widely used in severe and difficult to treat CMV disease. However, no clear evidence of benefit attributable to CMVIG has been forthcoming, including that of resistant CMV disease.[31] Similarly, the more widely available intravenous immunoglobulin (IVIG) has been used effectively as CMV prophylaxis but there is minimal evidence of its role in treatment of CMV disease, much less resistant CMV disease. Combining intravenous GCV with IVIG has shown better outcomes in the treatment of CMV pneumonia in a small cohort of 10 bone marrow transplant patients.[32] While not specifically limited to patients with drug-resistant CMV, a European review of haematopoietic stem cell transplant patients with CMV pneumonitis found no difference in outcome between those who received IVIG and those who received CMVIG.[33] Thus, IVIG may have a role to play in treatment of difficult CMV disease but its exact treatment effect and whether this can be replicated in different cohorts including renal transplant recipients remain to be seen. CMV specific T-cell adoptive therapy is another form of immunotherapy that has gained recent interest especially within the haematopoietic stem cell transplant cohort. Its use in SOTs, specifically in renal transplant recipients, has been limited to single case reports in the literature.[34]

New and novel agents

Novel agents including the antimalarial agent artesunate and leflunomide, a disease-modifying anti-rheumatic drug (DMARD), have been utilized as part of salvage treatment in CMV resistant cases with varying degrees of success. The evidence for these agents are currently limited to case reports and small case series.[35–37] Maribavir as a direct inhibitor of UL-97 kinase is an attractive option in drug resistant CMV but clinical experience is currently limited and reports of maribavir-resistant CMV have quickly followed its use.[38,39] Another new agent, CMX001 (brincidofovir), is an oral prodrug of CDV without the significant nephrotoxic effects of CDV. Where the use of CDV in the renal transplant cohort has been severely limited by its nephrotoxicity, CMX001 can potentially bypass this issue. As with the rest of the novel agents, we await more clinical data on CMX001.

Conclusion

Resistant CMV in renal transplantation is increasingly recognized with the widespread use of GCV or VGCV as prophylaxis post-transplant. CMV D+/R– serostatus is the most important risk factor for the development of resistance while potent immunosuppression, high viral loads and prolonged duration of treatment with suboptimal drug levels play contributory roles. Resistant CMV has pathogenic potential for invasive disease and may cause significant mortality and morbidity. We have a limited treatment armamentarium that is further limited by its respective adverse effects. Thankfully, there have been encouraging developments in the field of novel anti-CMV agents and we await further clinical evidence. In the meantime, minimizing the risk factors that promote resistance, prompt clinical diagnosis of resistant CMV, collaboration with an Infectious Diseases team even before resistance develops, and the appropriate use of a limited arsenal remain our best bet to overcome this condition.

Conflict of interest

None declared.

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