

Ganciclovir Resistant Cytomegalovirus in Solid Organ Transplant Recipients: An Update

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Abstract

Ganciclovir-resistant cytomegalovirus (CMV) infection is an emerging problem in solid organ transplant (SOT) recipients. Treatments such as foscarnet and cidofovir are fraught with serious side effects that may limit their use in this condition. The aim of this paper is to elucidate the mechanisms on how resistance occurs, when to suspect it clinically and what special tests are necessary to diagnose it. Based on recent literature, the paper also brings to light two medications, maribavir and leflunomide, which have been described to have anti-CMV activity.

Keywords: Ganciclovir resistance; Cytomegalovirus; Leflunomide; Kidney transplantation; Solid organ transplant

Abbreviations: CMV: Cytomegalovirus; GCV: Ganciclovir; SOT: Solid Organ Transplant; MBV: Maribavir

Introduction

Antiviral resistance in CMV poses an important therapeutic challenge in SOT recipients. Ganciclovir (GCV) is the current mainstay of treatment for CMV infections. We summarize the current understanding of epidemiology, pathogenesis, clinical manifestations, diagnosis and management strategies for GCV resistant CMV infections in SOT recipients, particularly kidney transplants.

While the precise incidence of GCV resistance is undefined, it may affect up to 7% of SOT recipients, with highest risk among kidney-pancreas and lung transplant patients [1-15].

Mechanisms of Resistance

In order to understand the mechanisms of resistance to antivirals in CMV, it is important to review the mode of action of GCV. It is a guanosine analogue that must be converted into its triphosphorylated form to be active. Once triphosphorylated, it inhibits viral DNA synthesis by inhibition of CMV DNA polymerase (encoded by viral gene, UL54). After cell entry, the phosphorylation of GCV occurs in three sequential steps, first of which requires a virally encoded phosphotransferase, a product of UL97. The following phosphorylation steps are performed by host cellular enzymes (Figure 1). Mutations in UL97 leading to reduced levels of active form of GCV, and those in UL54 leading to CMV polymerase resistance to GCV, are the major mechanisms of resistance.

Many of the common mutations in UL97 and UL54 correlate with phenotypic resistance to GCV by in vitro studies [16]. The correlation between genotypic mutations, phenotypic resistance and clinical refractoriness to GCV has formed the basis for the development of genotypic screens for resistance [17]. Furthermore, it appears that UL97 region mutations are more common than those of UL54. However, UL54 mutations are typically associated with higher levels of resistance to GCV and cross resistance to cidofovir and foscarnet [18,19].

Risk Factors

New understanding into the emergence of resistant CMV strains has developed after studies were done by Emery et al. [20,21]. They have shown that in the presence of GCV, mutant CMV strains have a survival advantage compared to wild type CMV. During prolonged exposure to GCV, especially when the systemic drug levels are low (oral GCV or valganciclovir), these mutant strains may become the

dominant population leading to treatment failure. This information helps understand several clinical observations. For instance, antiviral resistance is associated with high viral titers since there is a greater opportunity for selection of resistant mutant strains. Furthermore, resistance is usually encountered after prolonged periods of exposure to GCV, presumably because there is a growth advantage for mutant strains in the presence of the drug. Lastly, GCV resistance develops more commonly after oral rather than intravenous administration. This is because the blood levels achieved with oral GCV are much lower than those obtained after intravenous therapy, leading to incomplete suppression of viral replication.

Risk factors include a donor seropositive and recipient seronegative (D+/R-) status for CMV, prolonged GCV exposure, potent immunosuppression and high viral titers [22]. The observation that D+/R- status almost seems necessary (except in lung transplant recipients) in the development of CMV resistance narrows the population at risk. This predisposition is likely due to lack of previous immunity to CMV.

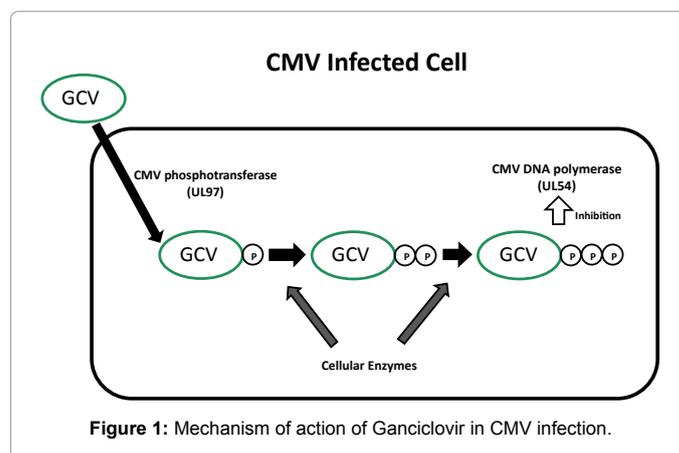


Figure 1: Mechanism of action of Ganciclovir in CMV infection.

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Received March 29, 2013; Accepted May 24, 2012; Published May 27, 2012

Citation: Takkar C, Gamilla-Crudo AK, Kadambi PV (2013) Ganciclovir Resistant Cytomegalovirus in Solid Organ Transplant Recipients: An Update. J Transplant Technol Res S6: 001. doi:[10.4172/2161-0991.S6-001](https://doi.org/10.4172/2161-0991.S6-001)

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Lung transplantation seems to be an exception to this, since antiviral resistant CMV has also been noted in R+ setting [22]. It is possible that these patients have other issues that are conducive to development of CMV resistance.

Clinical Features

The spectrum of clinical manifestations of GCV resistant CMV can range from asymptomatic viremia to the CMV syndrome as well as tissue invasive disease. Since these strains have been demonstrated in diseased tissue [23], it could be concluded that despite reduced potential to replicate [20,21], they maintain their pathogenicity. Based upon published cases of GCV resistant CMV in SOT patients, about 87% have symptomatic infection, with 55% of those having tissue invasive disease [1-15]. The mortality rate in affected patients approaches 20%, although the mortality directly attributable to resistant CMV is difficult to ascertain given the anecdotal nature of most data [1-15].

Due to routine prophylaxis against CMV, the timing of CMV infection and disease has shifted later in the post transplant period compared to the era when CMV prophylaxis was not widely used [22].

Compared with other SOT patients, lung transplant patients seem to have an earlier onset of resistant CMV infection (median time post transplant 146 days vs. 279 days) [22].

Diagnosis

The diagnosis of GCV resistant CMV involves either documentation of reduced susceptibility of the CMV isolate to GCV *in vitro* by one of the phenotypic methods or direct identification of established genetic mutations known to correlate with resistance.

In contrast to genotypic analyses, which utilize the knowledge of genetic mutations that confer resistance to antiviral agents, phenotypic methods depend upon measuring inhibition of viral growth in the presence of varying drug concentrations compared to a control strain. Some of the phenotypic methods include measurement of viral plaques, DNA synthesis or antigen production [19]. It is proposed that on plaque reduction assay, a 50% inhibitory concentration of GCV exceeding 6 $\mu\text{mol/L}$ be considered the threshold for diagnosis of resistant CMV [22].

Genotypic methods involve restriction enzyme analysis of polymerase chain reaction products from clinical CMV isolates and looking for known mutations associated with antiviral resistance.

One of the shortcomings of most available methods is the need for viral culture, which takes several weeks thus delaying the diagnosis. Despite recent technical advances in phenotypic and genotypic methods allowing for a quicker diagnosis [24], they are not widely available and are not standardized, thus limiting their utility in clinical decision making.

Therefore, in a high risk patient, a high index of suspicion must be maintained. Based on expert opinion, antiviral resistance should be suspected in a high risk patient when viral load fails to decline or rises after appropriate intravenous GCV administration for 14 days. Similarly, persistence of positive viral cultures or failure of clinical improvement after 14 days of intravenous GCV should suggest resistance [22]. Under such circumstances, empiric changes in antiviral regimen must be made before laboratory confirmation is available.

Treatment

Unfortunately, there are no controlled studies to guide the treatment of GCV resistant CMV specifically in the setting of SOT.

Data derived from studies in HIV population and clinical experience in SOT patients provides some guidance regarding this issue. It is important to note that not all clinical failure to therapy in CMV infection is due to resistance to antivirals. Conversely, GCV resistant infections may respond to GCV, especially when used in combination with other treatments. The degree of GCV resistance, host immune response and disease severity should all be taken into account when making therapeutic decisions. Accordingly, a multifaceted approach targeting all these factors is likely to be helpful in the management of resistant CMV infections.

Based upon genotypic studies of resistant CMV isolates, mutations in UL97 alone usually do not show cross resistance to foscarnet or cidofovir [16,17]. Due to the fear of nephrotoxicity with cidofovir, foscarnet is considered the first line alternative for management of resistant CMV infections. CMV hyperimmune globulin is usually added in patients with tissue invasive and severe disease. In patients who are not severely ill, an increase in GCV dose from 5 mg/kg/dose to 7.5 mg/kg/dose can be utilized as well [25]. However, neutropenia and renal impairment often limit this dose increase. Mylonakis et al. showed that GCV at a dose of 5 mg/kg when combined with escalating doses of foscarnet was another useful strategy [26]. More recently, two medications have come to light in the treatment of resistant CMV disease.

Maribavir (MBV) is an investigational benzimidazole antiviral agent (1H-beta-L-ribofuranoside-2-isopropylamino-5,6-dichlorobenzimidazole) that has *in vitro* activity against CMV strains that are resistant to other therapeutic agents [27]. Its effects include prevention of viral encapsidation and exit of viral particles by binding to UL97 viral protein kinase [27,28]. Avery et al. reported outcomes on 6 patients (5 SOT and 1 hematopoietic stem cell transplant) with treatment failure to conventional agents and/or known GCV resistance. Initially, patients received oral MBV at 400 mg twice daily for a median duration of 207 days. Four of the six patients had no detectable viremia within six weeks of starting MBV. The authors recommended an MBV concentration of at least 6 $\mu\text{g/mL}$ for CMV treatment [29]. MBV was generally well tolerated with no significant renal, hepatic or hematologic toxicity.

Leflunomide, (N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide) is a drug that is approved for use in rheumatoid arthritis. It not only has immunosuppressive properties but also has anti-CMV activity. Waldman and colleagues first described the use of leflunomide against CMV. Their study found that leflunomide acts through inhibition of tegument acquisition by viral nucleocapsids [30]. Evers and colleagues investigated FK 778, a drug structurally similar to the active metabolite of leflunomide (A77 1726), and reported that the drug inhibited protein tyrosine phosphorylation and *de novo* pyrimidine biosynthesis [31]. Studies have been reported demonstrating the efficacy of leflunomide against CMV [32,33]. John and colleagues administered leflunomide to four renal transplant recipients with symptomatic CMV disease. They utilized a loading dose of 100 mg daily for 3 days followed by 20 mg daily for three months [33]. After median treatment duration of a month, all patients had an undetectable viral load with improvement in their symptoms. Leflunomide in combination with foscarnet was reported by Avery and colleagues to have successfully treated a multidrug resistant case of CMV disease in a bone marrow transplant recipient. The target serum level for A77 1726 was set at 60-80 $\mu\text{g/mL}$ [34]. Based on these successful reports, larger studies are warranted to elucidate the efficacy, dosing and long-term safety of leflunomide.

Conclusion

GCV resistant CMV is associated with significant morbidity and poses diagnostic and therapeutic challenges in SOT recipients. Definitive diagnosis can be established by sophisticated lab tests; however, clinical suspicion in the right host is of paramount importance for rapid therapeutic decisions. Given the side effects of conventionally employed second line agents, Leflunomide, due to its immunosuppressive and antiviral activity appears to be an attractive option.

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This article was originally published in a special issue, **Solid Organ Transplant: The Pediatric Perspective** handled by Editor(s). Dr. Christian Benden, University Hospital Zurich, Switzerland

Citation: Takkar C, Gamilla-Crudo AK, Kadambi PV (2013) Ganciclovir Resistant Cytomegalovirus in Solid Organ Transplant Recipients: An Update. J Transplant Technol Res S6: 001. doi:10.4172/2161-0991.S6-001