Combination Antiviral Therapy for Ganciclovir-Resistant Cytomegalovirus Infection in Solid-Organ Transplant Recipients

Eleftherios Mylonakis, Wendy M. Kallas, and Jay A. Fishman

Infectious Disease Division and Department of Microbiology, Massachusetts General Hospital, Harvard University, Boston

The resistance of cytomegalovirus (CMV) to ganciclovir is a factor in therapeutic failure and disease progression. The clinical significance of such resistance in solid-organ transplantation has not been completely established. Six patients who developed persistent infection due to ganciclovir-resistant CMV were treated with a combination of ganciclovir (50% of the therapeutic dose) and a daily dose of intravenous foscarnet that gradually increased to a maximum of 125 mg/kg. All patients responded clinically within 72–96 hours. Magnesium depletion occurred in all patients. No clinical or laboratory relapses have been observed in 6–30 months of follow-up. Gradually increasing doses of foscarnet combined with half-dose regimens of ganciclovir are safe and can be beneficial in organ transplant recipients with ganciclovir-resistant CMV infection. Larger studies are needed to identify the patients who are most likely to benefit from this regimen.

Cytomegalovirus (CMV) infection in humans is a major cause of morbidity and mortality in immunocompromised hosts. Among bone marrow and solid-organ transplant recipients, the incidence of active infections is 15%–60% and is related to (1) the presence of latent CMV infection (as determined by positive results of serologic tests) in the donor or recipient, (2) the organ transplanted, and (3) the intensity of immunosuppressive therapy [1, 2]. Ganciclovir (9-[1,3-dihydroxy-2-propoxy)methyl]guanine, or “DHPG”) is a common first-line therapy for the management of CMV infection. Recently, however, clinical CMV isolates that are resistant to ganciclovir in vitro have been reported and the molecular basis of drug resistance has been investigated [3]. The factors associated with the emergence of ganciclovir resistance include high virus load, immunosuppression, and intermittent exposure to antiviral agents, often at subtherapeutic levels [4–6]. Clinically, the transplant recipient with ganciclovir-resistant CMV infection who receives full-dose ganciclovir therapy (5 mg/kg iv b.i.d.) experiences persistent or increasing virus loads in the circulation or tissue.

Foscarnet (trisodium phosphonoformate hexahydrate, or “PFA”) therapy, with or without CMV hyperimmunoglobulin, has been used to treat ganciclovir-resistant CMV infection in some transplant recipients. This use of foscarnet has been associated with significant side effects—nephrotoxicity and neurotoxicity (including seizures), in particular—in individuals receiving calcineurin inhibitors to prevent allograft rejection [5]. Cidofovir has been tolerated poorly in renal transplant recipients. Ganciclovir and foscarnet have demonstrated synergy against CMV in vitro, even for ganciclovir-resistant isolates of CMV [7]. In patients with AIDS for whom ganciclovir monotherapy for CMV retinitis is failing, the addition of foscarnet to the treatment regimen has been shown to be superior to either continuing ganciclovir monotherapy or switching to foscarnet monotherapy. Similarly, for patients who are...
unsuccessfully treated with foscarnet monotherapy, the addition of ganciclovir is preferable to switching to ganciclovir monotherapy [8]. Similar results have been obtained in hematopoietic stem cell transplant recipients [9, 10]. On the basis of these in vitro and in vivo data, we have investigated combination therapy with reduced doses of ganciclovir and foscarnet for the management of ganciclovir-resistant CMV infection in solid-organ transplant recipients.

METHODS

At the Massachusetts General Hospital (Boston), CMV-seronegative recipients of transplanted organs from CMV-seropositive donors receive intravenous ganciclovir prophylaxis (5 mg/kg q.d., corrected for renal function) while they are hospitalized and oral formulations (1 g b.i.d. for 3 months) after they have been discharged. The same prophylaxis is administered to CMV-seropositive recipients who are receiving antilymphocyte antisera for induction or graft rejection. In addition, these patients are monitored monthly by use of a CMV antigenemia assay. Individuals who develop persistently positive antigenemia assay results receive ganciclovir (5 mg/kg b.i.d., corrected for renal function) and monthly doses of CMV hyperimmunoglobulin (150 mg/kg iv once followed by 100 mg/kg iv 3 times) [1, 11].

The CMV antigenemia assay was performed as follows: leukocytes were harvested from anticoagulated peripheral blood samples by means of dextran sedimentation. RBCs were lysed with ammonium chloride, followed by a wash with phosphate-buffered saline. A cell suspension was made of 1.8–2.0 × 10⁶ cells/mL. Three slides, each of which had 180,000–200,000 leukocytes, were prepared by means of cytocentrifugation. Before fixation, cells were observed by light microscopy for morphologic characteristics and confluence. Slides were fixed and air dried. Two slides were selected and stained by use of the CMV pp65 Antigenemia Immunofluorescence Kit (Chemicon International), and the third slide was frozen for future reference. Slides were read, and either the results were classified as negative or the number of CMV-positive cells per 2 slides was noted.

Virus isolates were obtained from each patient’s peripheral blooduffy coat cultures while the patients were receiving intravenous ganciclovir therapy. The in vitro susceptibility of each clinical isolate to ganciclovir and foscarnet was evaluated at The Children’s Hospital of Philadelphia, by use of an ¹²⁵I-labelled human CMV probe (Diagnostic Hybrids). All isolates had MICs of ganciclovir of ≥3 μg/mL (12 μM), which is defined by the laboratory as “high-level ganciclovir resistance.” Plaque reduction assays are considered the “gold standard,” despite the technical complexity of the method and, until recently, the lack of consensus regarding the definition of resistance [4, 12]. Multiple investigators have found that the MIC method used in this study has similar efficacy to the plaque reduction assay [4, 13]. High-level viral resistance is now defined as either an MIC of ganciclovir of ≥3 μg/mL, as determined by the ¹²⁵I-labelled human CMV probe, or an inhibitory concentration of 50% (IC₅₀) of ganciclovir of ≥12 μM/L, as determined by the standardized plaque reduction assay used in studies of CMV-ganciclovir resistance in patients with AIDS [12, 14, 15]. The protocol was approved by the human studies review committee of the Massachusetts General Hospital.

RESULTS

In the past 3 years, we have treated 6 patients (3 men and 3 women) who developed persistent CMV antigenemia and viremia while receiving intravenous ganciclovir therapy (table 1). The patients were 42–57 years old (mean, 54.5 years) and had received liver (2 patients), lung (2 patients), renal (1 patient), or heart (1 patient) transplants.

The diagnosis of infection with ganciclovir-resistant CMV was made 3 months to 2 years after transplantation (median, 5 months). During the development of ganciclovir resistance, all patients were receiving either oral ganciclovir for prophylaxis (5 CMV-seronegative recipients of transplants from CMV-seropositive donors) or intravenous ganciclovir treatment (1 CMV-seropositive recipient). Despite the administration of full-dose intravenous ganciclovir, patients progressed to develop fever (4 patients), weight loss (2 patients), diarrhea (1 patient), nausea (1 patient), generalized malaise (1 patient), neutropenia (2 patients), thrombocytopenia (1 patient), and abnormal liver function test results (1 patient) attributed to CMV infection. The CMV antigenemia assay titer increased, and virus was isolated from the blood samples obtained from each patient, despite the administration of ≥3 weeks of full-dose intravenous treatment.

Table 1. The results of susceptibility testing of cytomegalovirus (CMV) isolates recovered from solid-organ transplant recipients for whom ganciclovir therapy was unsuccessful.

<table>
<thead>
<tr>
<th>Organ transplanted</th>
<th>CMV antigenemia level,a no. of cells per 2 slides</th>
<th>MIC, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ganciclovirb</td>
<td>Foscarnetc</td>
</tr>
<tr>
<td>Liver</td>
<td>500</td>
<td>5.2</td>
</tr>
<tr>
<td>Liver</td>
<td>&gt;1000</td>
<td>4.9</td>
</tr>
<tr>
<td>Lung</td>
<td>&gt;1000</td>
<td>3.1</td>
</tr>
<tr>
<td>Lung</td>
<td>450</td>
<td>10.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>700</td>
<td>3.7</td>
</tr>
<tr>
<td>Heart</td>
<td>600</td>
<td>49.0</td>
</tr>
</tbody>
</table>

NOTE. ND, not determined.

a The CMV antigenemia level was determined during intravenous ganciclovir therapy; it decreased to zero cells per 2 slides while the patients were receiving combination therapy. CMV was isolated from bloody coat cultures during ganciclovir monotherapy.

b Resistance was denoted by an MIC of ≥3 μg/mL (12 μM).

c Resistance was denoted by an MIC of ≥90 μg/mL.
ganciclovir monotherapy. This was the first episode of CMV infection in 5 of the 6 patients, and it was the second episode in 1 patient. None of the patients underwent invasive testing for the diagnosis of CMV, and genotyping of the isolates was not performed. The results of the CMV antigenemia assay had been negative in monthly evaluations for ≥2 months before this episode (table 1). The results of the CMV antigenemia assays at the beginning of treatment with combination therapy are summarized in table 1.

Patients were treated with the combination of ganciclovir (5 mg/kg q.d., adjusted for renal function to 50% of the therapeutic dose). In addition, each patient received gradually increasing dosages of intravenous foscarnet, starting with 4 g on day 1 of therapy. The dosage was increased by 2 g every other day, to a maximum of 125 mg/kg per day. Dosages that were ≥8 g per day were divided into 2 doses administered at 12-h intervals. Patients with serum creatinine values of ≥2 mg/dL received a maximum of 8 g per day. Patients received each daily dose in a minimum of 500 mL of solution with ≥1 L of normal saline hydration. Serum magnesium, potassium, blood urea nitrogen, and creatinine levels and complete blood counts were assessed daily. Magnesium supplementation (4 g of magnesium gluconate per day taken orally) was initiated on day 1, and the dosage was increased, as needed, to maintain normal magnesium levels. All patients also received CMV hyperimmunoglobulin.

All patients had a clinical response in 72–96 h. Antigenemia levels decreased by >50% in all patients within 2 weeks of therapy and to zero by 4–8 weeks of therapy. Patients continued to receive therapy for ≥10 days after the first negative result of the antigenemia assay was obtained. All 6 patients required significant magnesium supplementation during treatment (10–24 g per day given orally or intravenously). Patients continued to receive oral ganciclovir and monthly doses of intravenous CMV hyperimmunoglobulin (100 mg/kg per dose) for 3 months after the completion of therapy. No clinical or laboratory relapses have been observed after 12–36 months of follow-up.

**DISCUSSION**

Resistance of CMV to antiviral agents is an increasingly important factor in therapeutic failure and disease progression in patients who undergo transplantation. The clinical significance of such resistance has not been established, but it has been associated with invasive disease of the tissue and graft rejection [1, 11, 16]. In practice, most patients with CMV infection respond both clinically and virologically to intravenously administered therapy with ganciclovir. Previously, most CMV isolates that are resistant to antiviral agents have been seen in association with AIDS. The incidence of infections caused by drug-resistant CMV in solid-organ transplant recipients is not known [4]. In 1999, Kruger et al. [17] reported that 18 (5.2%) of 348 lung transplant recipients had isolates recovered from specimens of blood or bronchoalveolar lavage fluid that exhibited some degree of resistance to ganciclovir.

This study evaluated the efficacy of a combination of ganciclovir with foscarnet for the management of ganciclovir-resistant CMV infection among organ transplant recipients. Ganciclovir resistance was documented by the isolation of CMV from blood samples obtained while patients were receiving ganciclovir therapy and a progressive increase in the CMV antigenemia titer. Although this study was uncontrolled, each patient had been unsuccessfully treated with intravenous ganciclovir for ≥3 weeks before the initiation of combination therapy. CMV infection responded to treatment with the combination of foscarnet and ganciclovir. All patients tolerated therapy, and the most common side effect was magnesium wasting.

Ganciclovir and foscarnet inhibit CMV DNA synthesis by inhibiting the viral DNA polymerase. Resistance to ganciclovir is typically, but not invariably, associated with mutations in the genes encoding UL97 (phosphotransferase) and/or UL54 (DNA polymerase mutations) [18–20]. The functional consequence of the UL97 mutations is impaired phosphorylation of ganciclovir in virus-infected cells, with the consequent lack of synthesis of ganciclovir triphosphate, the active form of the drug [4]. Isolates with low-level ganciclovir resistance are usually associated with UL97 alterations and short durations of ganciclovir treatment, whereas isolates with high-level ganciclovir resistance are frequently associated with both UL97 and polymerase alterations and are most often isolated after patients have received prolonged ganciclovir therapy [21]. Ganciclovir resistance emerges over time as resistant strains supplant wild-type, susceptible strains under antiviral pressure. Thus, most patients are infected with a mixed population of virus strains [16]. Resistance to foscarnet and cidofovir is associated with mutations in conserved regions of the viral DNA polymerase. Some of these isolates with DNA polymerase mutations are cross-resistant to ganciclovir, foscarnet, and cidofovir [4].

The emergence of ganciclovir-resistant CMV strains is a particular threat after exposure to antiviral drugs, particularly in patients who have received intermittent therapy and/or treatment with oral agents that have relatively poor bioavailability [6, 22]. The effect of low levels of ganciclovir may be exacerbated by high levels of virus and viral replication in the CMV-seronegative (naive) recipient of a CMV-seropositive organ. Thus, 5 of our patients were CMV-seronegative recipients of organs from CMV-seropositive donors, which is predictive of higher CMV virus loads once CMV infection is activated from latency, compared with CMV-seropositive recipients who have some preformed immunity. Greater virus load, coupled with exposure to potentially inadequate levels of the drug, may increase the selection of drug-resistant mutants [5, 6]. It is also
possible that ganciclovir-resistant CMV isolates emerge under selective pressure during exposure to acyclovir [3]. The recent addition of valganciclovir, a new L-valyl ester prodrug of ganciclovir with 10-fold higher bioavailability than oral ganciclovir, might be expected to reduce the incidence of antiviral resistance. A recent study of valganciclovir for the treatment of CMV retinitis in patients with AIDS suggests that ganciclovir resistance is less frequent and develops later with valganciclovir than it has historically with oral ganciclovir [23]. Further studies are needed.

The combination of ganciclovir with foscarnet has demonstrated additive or synergistic effects against both ganciclovir- and foscarnet-resistant strains in vitro [7]. Clinical studies involving both bone marrow transplant recipients and HIV-infected patients suggest that (1) the combination of ganciclovir and foscarnet may produce clearing of CMV infection, and (2) compared with monotherapy, it may reduce morbidity and mortality rates. Bacigalupo et al. [9, 10] evaluated 32 recipients of allogeneic hematopoietic stem cell transplants who had positive results of CMV antigenemia assay (i.e., ≥5 positive cells) and who were given combination therapy with foscarnet (180 mg/kg per day) and ganciclovir (10 mg/kg per day) for 15 days. Maintenance therapy with foscarnet and ganciclovir was given on alternate days for an additional 2 weeks. All patients cleared CMV antigenemia by day 15 of treatment. Twenty-six patients survived for 119–1051 days after transplantation. Eighteen of these patients were compared with 15 matched control subjects who had been treated with a single drug (either foscarnet or ganciclovir) for CMV antigenemia (i.e., ≥5 positive cells). The actuarial 1-year transplant-related mortality rate was 13% for patients who received combination therapy, compared with 47% for control subjects who received monotherapy (P = .02) [9, 10]. Dual therapy for clinical ganciclovir resistance also improved the outcome for patients with CMV retinitis and AIDS, compared with monotherapy with either ganciclovir or foscarnet [8].

Before the introduction of this combination regimen, our patients with persistent CMV infection after transplantation had multiple relapses of invasive infection, including CMV retinitis, CMV colitis with bleeding, and CMV hepatitis in recipients of lung, kidney, and liver transplants, respectively [11]. A lung transplant recipient died of CMV pneumonitis and graft rejection after experiencing seizures while receiving full-dose regimens of foscarnet. None of the patients in the present study cleared infection while receiving ganciclovir therapy alone. It is not known whether these patients would have had CMV infection clear while receiving low-dose foscarnet monotherapy. Full-dose foscarnet therapy (usually administered intravenously at a minimum dosage of 60 mg/kg q8h) has been successfully used to treat CMV disease caused by ganciclovir-resistant CMV strains, but this efficacy is compromised by side effects that include nephrotoxicity, anemia, electrolyte imbalance, nausea, vomiting, and seizures [24]. The 2 patients in the present study who were infected with CMV isolates resistant to both foscarnet and ganciclovir might well have experienced treatment failure with foscarnet monotherapy (table 1). This suggests that, like the treatment of CMV retinitis in patients with AIDS [8], the combination of ganciclovir and foscarnet was more likely to be effective in our patients than is monotherapy with either agent. The use of combination therapy for infection with ganciclovir- and foscarnet-resistant strains of CMV merits further study.

We recently used the combination of ganciclovir with foscarnet for the treatment of a an allogeneic bone marrow transplant recipient who had active graft-versus-host disease (GVHD). Although the CMV antigenemia titer continued to increase despite the administration of full-dose intravenous ganciclovir, combination therapy resulted in regression of CMV infection and appeared to contribute to amelioration of GVHD. In addition to the 6 patients described in this report, we have successfully treated 5 additional solid-organ transplant recipients with ganciclovir and foscarnet combination therapy. In these patients, CMV infection progressed despite the administration of ganciclovir monotherapy; however, data regarding virus susceptibility were not obtained. The review of our experience suggests that this approach may be useful in the treatment of solid-organ transplant recipients. Use of reduced-dose foscarnet was not associated with the development of drug resistance or clinical relapse in our patients; larger studies are needed to confirm this observation. It should be noted that the dose of foscarnet must be corrected for renal function, the patients must be well hydrated, and renal function and magnesium levels must be closely monitored. The possible impact of calcineurin inhibitors on renal function must be considered, even in patients with normal serum creatinine values.

In conclusion, therapy with gradually increasing doses of foscarnet combined with half-dose ganciclovir therapy can be beneficial in organ transplant recipients infected with ganciclovir-resistant CMV. The toxicity of this regimen was minimal in our small series. Larger studies are needed to identify the patients who are most likely to benefit from this approach. The larger studies should include genotyping of isolates and parallel studies of both humoral and cellular immunity to CMV. Presently available viral culture systems and susceptibility testing assays are costly, labor intensive, and too slow to be useful in making therapeutic decisions. Rapid molecular assays are needed to optimize antiviral therapy.

Acknowledgments

We would like to acknowledge Dr. Martin S. Hirsch, for his critical review of the manuscript, and the Clinical Microbiology...
Department of the Massachusetts General Hospital, for their assistance.

References

In an article published in the 1 January 2006 issue of the journal (Anzueto A, Niederman MS, Pearle J, Restrepo MI, Heyder A, Choudhri SH. Community-acquired pneumonia recovery in the elderly (CAPRIE): efficacy and safety of moxifloxacin therapy versus that of levofloxacin therapy. Community-Acquired Pneumonia Recovery in the Elderly Study Group. Clin Infect Dis 2006;42:73–81), an error appeared in footnote b in table 2. The footnote should read “Six strains were fluoroquinolone susceptible, and 1 strain from a patient in the levofloxacin arm was intermediate (levofloxacin MIC, 4 mg/L)” (not “Six strains were fluoroquinolone susceptible, and 1 strain from a patient in the levofloxacin arm was intermediate (moxifloxacin MIC, 4 mg/L).”) The authors regret this error.

In an article published in the 15 May 2002 issue of the journal (Mylonakis E, Kallas WM, Fishman JA. Combination antiviral therapy for ganciclovir-resistant cytomegalovirus infection in solid-organ transplant recipients. Clin Infect Dis 2002;34:1337–41), an error appeared in the third sentence of the third paragraph of the Discussion section. The corrected sentence should read as follows:

“The functional consequence of the UL97 mutations is impaired phosphorylation of ganciclovir in virus-infected cells, with the consequent lack of synthesis of ganciclovir triphosphate, the active form of the drug” [4, p. 286].

not

The functional consequence of the UL97 mutations is impaired phosphorylation of ganciclovir in virus-infected cells, with the consequent lack of synthesis of ganciclovir triphosphate, the active form of the drug [4].

The authors regret this error.