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Emergence of Antiviral Resistance During Oral Valganciclovir Treatment of an Infant with Congenital Cytomegalovirus (CMV) Infection

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Abstract

Congenital infection with human cytomegalovirus (CMV) is a major cause of morbidity, including sensorineural hearing loss (SNHL), in newborns. Antiviral therapy with ganciclovir (GCV) and its oral prodrug, valganciclovir (VAL-GCV) are increasingly being administered to infected infants, toward the goal of improving neurodevelopmental and auditory outcomes. In this case report, we describe a symptomatic congenitally infected infant treated with VAL-GCV in whom GCV resistance was suspected, based on a 50-fold increase in viral load after 6 weeks of oral therapy. Analyses of CMV sequences from both blood and urine demonstrated populations of viruses with M460V and L595F mutations in the *UL97* phosphotransferase gene. In contrast, analysis of viral DNA retrieved from the newborn dried blood spot demonstrated wild-type *UL97* sequences. DNAemia resolved after the discontinuation of VAL-GCV. Long-term VAL-GCV therapy in congenitally infected infants can select for resistant viral variants, and anticipatory virological monitoring may be warranted.

Keywords

congenital cytomegalovirus infection; antiviral therapy; ganciclovir resistance; *UL97* mutation

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Competing interests

None declared.

Ethical approval

Genotypic analyses were performed on DNA from clinical samples collected in the context of standard care for the infant described in this report. All studies of viral DNA and viral strains were performed with approval of the University of Minnesota Institutional Review Board (study number: 0709M17161) and parental consent.

1. Why this case is important

Human cytomegalovirus (CMV) is an important cause of disease following solid organ and hematopoietic stem cell transplantation (HSCT), in the setting of HIV infection, and in newborn infants with congenital infection^{1–4}. Most experience with treatment of CMV has been accrued with the nucleoside analog, ganciclovir (GCV), and its oral prodrug, valganciclovir (VAL-GCV)^{5–11}. Although useful, these drugs cause toxicities, particularly neutropenia, and long-term therapy can lead to the emergence of antiviral resistance^{12–14}. Most reports of GCV resistance have been in adult patients, and less information is available for pediatric patients, particularly infants. However, with the increasing use of GCV for treatment of congenital CMV infection^{15–19}, GCV resistance may be identified more commonly in infants in the future. An ongoing study by the Collaborative Antiviral Study Group (CASG) is examining potential benefits of prolonged (six months) VAL-GCV therapy on the neurodevelopmental and audiologic status of infants with congenital CMV infection²⁰. If long-term VAL-GCV therapy becomes increasingly utilized for the treatment of congenitally infected infants, anticipating and identifying the emergence of resistant CMV strains may increasingly become an important clinical management issue.

2. Case description

A 30-year-old *gravida 2 para 1* woman was noted by ultrasound at 25 weeks gestation to be carrying a fetus with intrauterine growth retardation, hepatomegaly, and brain calcifications. Amniocentesis was positive for CMV by PCR. Caesarian section was performed at 35 weeks gestational age due decreased fetal movement. At birth, the male infant weighed 1270 g, with a length of 40.5 cm and a head circumference of 27.5 cm (all values <3rd percentile). The placenta was positive for CMV antigen by immunohistochemistry and demonstrated histological findings of CMV placentitis.

Neonatal urine and blood specimens were both positive by PCR for CMV DNA, confirming congenital infection. Renal insufficiency was noted, with reduced urine output and elevated serum creatinine. Because of documented CMV end-organ disease, including central nervous system involvement, GCV therapy was initially commenced at a 3 mg/kg dose (6 mg/kg/day), adjusted for prematurity and renal insufficiency. After the infant's weight reached 1500 grams, the dose was increased to 7.5 mg/kg/day. After 4 weeks of IV therapy, GCV was discontinued because of neutropenia [Fig. 1], which resolved. At approximately 3 months of age, following hospital discharge and on an outpatient basis, VAL-GCV therapy was commenced at a dose of 20 mg/kg twice daily. A duration of six additional months of treatment was planned, based on the regimen employed in the ongoing CASG study of long-term oral VAL-GCV therapy in congenitally infected infants ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00466817) identifier: NCT00466817)²⁰.

The dynamics of CMV DNAemia in this patient are summarized in Fig. 1. Initially, the magnitude of DNAemia decreased from 14,500 to 3000 copies/ml on GCV therapy. Within 4 weeks after GCV was discontinued, viral load had increased to 13,000 copies/ml, essentially identical to the pre-treatment level. Following the initiation of VAL-GCV, viral load remained essentially stable, at 9500 copies/ml, for the next two weeks. The parents reported adherence to the VAL-GCV regimen, and the patient had no symptoms of CMV disease. At 5 months of age (8 weeks into oral therapy), viral load dramatically rose to 476,000 copies/ml [Fig. 1], prompting an evaluation for possible GCV resistance.

All studies were done with parental consent and with approval of the University of Minnesota Institutional Review Board. To test for genotypic resistance, DNA extracted from blood at day of life 150 (at the time of peak DNAemia, Fig. 1) was PCR-amplified and sequenced to test for *UL54* (DNA polymerase) and *UL97* (ganciclovir kinase) mutations²¹.

For *UL97*, two primer sets were used to amplify regions covering codons 377–653. For *UL54*, 4 primer sets were used to amplify codon regions 276–1004. PCR products were cloned into the TA cloning vector (Invitrogen) and, following transformation in *E. coli*, 15–20 clones from each PCR amplification were sequenced. These analyses demonstrated two *UL97* mutations in the majority of the PCR-amplification products. In total, 60% of sequenced clones (12/20) demonstrated a M460V mutation, and 75% of clones (12/16) demonstrated a L595F mutation [Fig. 2]. No *UL54* mutations were observed.

To confirm that these resistance mutations had emerged while on GCV therapy, the newborn viral culture isolate was obtained, and CMV DNA was amplified by PCR. PCR was also performed, following parental consent, on the archived newborn dried blood spot. All sequences were wild-type in the *UL97* and *UL54* genes, confirming that a GCV-susceptible genotype was present at birth (Fig. 2B).

In light of documented GCV resistance, VAL-GCV therapy was discontinued. Viral load declined to 300 copies/ml at 13 months of age, and DNAemia ceased by 20 months of age. Cerebral palsy and bilateral severe-to-profound sensorineural hearing loss, requiring cochlear implantation, were observed on long-term follow-up.

3. Other similar and contrasting cases in the literature

GCV resistance has been reported as early as 1–3 weeks after the initiation of preemptive therapy in young children with immunodeficiency disorders undergoing T-cell-depleted haploidentical stem-cell transplantation²². Resistance to GCV, cidofovir and foscarnet was described in an infant with severe combined immune deficiency with severe CMV disease, probably acquired via breast milk²¹. Multiple GCV resistance mutations, detected by PCR and pyrosequencing, have been described in an infant with symptomatic congenital CMV infection treated sequentially with GCV, VAL-GCV, and foscarnet²³. Detection of GCV-resistant variants of CMV using pyrosequencing was also described in fatal case of a preterm infant with congenital CMV infection²⁴.

4. Discussion

GCV and, more recently VAL-GCV, have been increasingly employed in the treatment of congenital CMV infection, toward the goal of improving neurodevelopmental and auditory outcomes^{15–20}. In this case, we describe GCV resistance in an infant being treated sequentially with intravenous GCV and long-term oral VAL-GCV for congenital CMV infection. The emergence of GCV-resistance most likely occurred during oral VAL-GCV therapy, since the initial viral load decreased on intravenous therapy. However, it is possible that emergence of resistant strains occurred during initial GCV therapy, which was given at a reduced dose in light of prematurity and renal insufficiency. There is little information available about drug dosage in premature infants with renal insufficiency, and, unfortunately, GCV drug levels were not available for this patient. The VAL-GCV dose prescribed for this patient, 20 mg/kg twice daily, achieves therapeutic levels in both premature and term infants^{25, 26}, making it unlikely that GCV resistance emerged due to inadequate dosing during oral therapy.

In this case, clinical and genotypic evidence of resistance emerged and was detectable after 8 weeks of oral VAL-GCV therapy, [Fig. 1]. There is precedent for resistance emerging after comparable long-term treatment courses in adults. Following long-term GCV therapy for AIDS-associated CMV retinitis, the prevalence of GCV resistance was reported to be 38% in patients still shedding CMV after 3 months of therapy, and estimated to be 7.6% overall, although these studies assessed for phenotypic, not genotypic, resistance²⁷. In a prospective study comparing intravenous GCV and oral VAL-GCV in transplant patients,

the risk of developing a GCV resistance mutation was 2.3% for GCV, and 3.6% for VAL-GCV, after 7 weeks of therapy²⁸. In pediatric solid organ transplant patients receiving VAL-GCV prophylaxis for 100 days, 2 of 46 patients (4%) demonstrated *UL97* mutations in blood samples²⁹. Our case is similar to a case described by Campanini and colleagues²³, who also described GCV resistance in an infant with congenital CMV infection treated sequentially with GCV and VAL-GCV. Our study relied on sequencing of subcloned PCR amplification products, and demonstrated that 67% of clones demonstrated drug-resistance mutations, using DNA purified from blood obtained on VAL-GCV therapy. This is similar to the 47% of positive clones noted during GCV therapy when pyrosequencing was used to identify *UL97* mutations in virus subpopulations²³. *UL97* mutations continued to be detected in our patient months after discontinuation of VAL-GCV therapy; although the M460V mutation had reverted to wild-type sequence by 14 months of age, the L595F mutation persisted. This observation was surprising in light of mathematical models showing a reduced *in vivo* fitness of GCV-resistant *UL97* mutants in the absence of the drug^{30, 31}. A recent review suggested a range of 3–8 months is required for repopulation of GCV-resistant CMV by wild-type virus following discontinuation of GCV in the setting of documented resistance³². Long-term molecular virological follow-up will be required to evaluate whether our patient again sheds a GCV-susceptible strain.

Our case differs from that described by Campanini in that our patient was commenced on VAL-GCV on an outpatient basis, at a time that he was asymptomatic, solely toward the goal of maximizing the likelihood of a favorable neurodevelopmental outcome^{16, 20}. Indeed, the resurgence of high-grade DNAemia on VAL-GCV therapy in this infant was not associated with symptoms. DNAemia rapidly cleared in this infant following discontinuation of antiviral therapy, presumably because of the development of an effective immune response. Low-level viral replication (and low grade DNAemia) may promote development of cytotoxic T-lymphocyte immunity, and complete ablation of viral replication with GCV may retard the development of effective long-term immunity³³. Whether prolonged VAL-GCV therapy delays the development of an effective cellular immune response in congenital CMV infection requires further study.

In summary, although most available data on the emergence of GCV resistance in the setting of long-term therapy suggests that the risk is low^{34–36}, our case demonstrates that GCV resistance can nonetheless emerge in asymptomatic infants being treated on an outpatient basis for congenital CMV infection with oral VAL-GCV suppression. The potential risks of treatment, coupled with the emergence of resistance as demonstrated in this report, provide support for a recommendation that prolonged VAL-GCV should not be administered to infants with congenital CMV infection outside of controlled clinical trials^{20, 37}. Strategies to enable rapid, “real-time” detection of genotypic resistance will be needed to more precisely define the risk and aid in the clinical management of infants on long-term therapy^{24, 38}.

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CMV DNAemia Analysis

Fig. 1. CMV DNAemia Analysis

Time course of DNAemia and evolution of GCV resistance. The initial course of parenteral GCV was limited to 4 weeks of therapy due to neutropenia. After resolution of neutropenia, oral VAL-GCV was commenced, with emergence approximately 8 weeks later of a resistant isolate associated with high systemic viral load (476,000 copies/ml).

A.)	460 no mutation	ACA CCC ATG AAC GTG
		T P M N V
		458 459 460 461 462
	460 mutation	ACA CCC G TG AAC GTG
		I P V N V
		458 459 460 461 462
	595 no mutation	CGC GCG TTG GAG AAC
		R A L E N
		593 594 595 596 597
	595 mutation	CGC GCG TTT GAG AAC
		R A F E N
		593 594 595 596 597

B.)

Fig. 2. Antiviral Resistance Mutational Analyses

Demonstration of GCV resistance mutations. Blood PCR at 5 months of age (while on oral VAL-GCV therapy) demonstrated resurgence of high viral load. PCR of *UL97* coding regions associated with GCV resistance followed by DNA sequence analysis demonstrated simultaneous appearance of both an M460V (left panel, post-GCV) and a L595F mutation (right panel, post-GCV). These mutations were not noted when PCR and subsequent sequence analyses were performed using DNA purified from the original viral isolate obtained at birth, indicating that resistance mutations emerged after the initiation of antiviral therapy.