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Case Report

Onset of valganciclovir resistance in two infants with congenital cytomegalovirus infection

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ABSTRACT

Ganciclovir and its prodrug valganciclovir are elective treatments for cCMV. Neonates with important symptoms undergo 6 months of therapy to ameliorate/prevent symptoms and late sequelae, but evidence of resistance is emerging. Over the last 5 years, we took care of 59 cCMV infants and experienced two cases of resistance among nine cCMV infants receiving long-term valganciclovir therapy. In the first case, valganciclovir therapy was prolonged beyond 6 months due to severity of symptoms, control of viral load, and absence of adverse events. Resistance was detected in the 8th month of therapy. In the second case, after a significant reduction following valganciclovir administration and no adverse events, CMV viral load suddenly increased in the 6th month of therapy due to resistance. Both events were associated with UL97 gene mutation. The cCMV infants, affected by severe symptoms, remained in a steady state during treatment, and their later neurological development was coherent with initial seriousness of diagnosis. Prolonged therapeutic exposure may therefore be a risk for resistance, suggesting that constant dosage/weight adjustments, monthly surveillance of viral load, and therapeutic drug monitoring could be proposed to monitor resistance onset and optimize the therapy regime. The risk–benefit ratio for long-term therapy, including the possibility of resistance onset, alongside SNHL and neurodevelopmental improvement, should also be evaluated.

Introduction

Congenital cytomegalovirus (cCMV) infection is the leading cause of non-genetic sensorineural hearing loss (SNHL) and a contributor to psychomotor impairment.

Only 10–15% of cCMV infants are symptomatic at birth, 40–58% of whom, and about 12% of the asymptomatic children, develop late-onset sequelae (Dollard et al., 2007). In 2017, two consensus papers recommended that antiviral treatment should only be considered for cCMV neonates with moderate-to-severe symptoms (Rawlinson et al., 2017), or with evidence of CNS disease (Luck et al., 2017). The two groups suggested the use of antiviral therapy for at least 6 months (long-term therapy).

Ganciclovir (GCV) IV and the oral prodrug valganciclovir V-GCV are the chosen treatments for cCMV in the neonatal period (Rawlinson et al., 2017; Luck et al., 2017). After absorption, V-GCV is converted to GCV by hepatic esterases, then phosphorylated to monophosphate by the CMV-encoded UL97 protein kinase, and finally transformed by cellular kinases to the active triphosphate form, which competitively inhibits the binding to viral DNA polymerase, and thus synthesis of the viral DNA (Biron, 2006). The primary mode of cCMV resistance is a mutation in the UL97 gene, preventing phosphorylation of GCV. In a minority of cases resistance is caused by mutations in the viral DNA polymerase encoded by UL54, which prohibits the binding of GCV and other antiviral agents not usually applied in neonatal cCMV patients (Biron, 2006; Bauters et al., 2016; Sun et al., 2012).

Materials & methods

From January 2015 to December 2019, 59 cCMV infants were referred our centre. Thirteen were symptomatic, needing treatment: four received 6-week therapy; nine were allocated 6-month therapy, with resistance detected in two of the nine cases.

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Standards of care relating to CMV PCR viral load and adjustment of antiviral drugs with weight gain consist of monthly and fortnightly measurements, respectively.

Case 1

A 31-year-old primiparous, seroimmune woman gave birth to a term male infant. Ultrasound fetal assessment had revealed a reduced cranial circumference (CC) and altered Doppler findings. IUGR diagnosis led to a cesarean section at 37 weeks + 5 days gestation. The SGA infant was born with microcephaly (<3°centile) hypertonia, chorioretinitis, left intraventricular hemorrhage, and severe left SNHL. MRI revealed polymicrogyria, ventriculomegaly, bilateral calcifications in the ependymal and periventricular area, and dilated temporal horns. Pathological EEG tracings were recorded, leading to a confirmation of epilepsy at 4 months of life. cCMV infection was established by PCR in blood and urine at birth, with CMV counts of 1.8×10^5 copies/mL and 62×10^6 copies/mL, respectively, and thus V-GCV therapy (15 mg/kg twice daily) was started. The CMV DNA level was under the detectable limit until the 5th month, when it increased to 30 copies/mL) No adverse events (AEs) were reported. After mindful counseling with the parents, the physician staff decided to prosecute the therapy, owing the severity of the clinical picture. The 8-month testing underlined that CMV DNAemia had increased significantly to 5.3×10^4 copies/mL in blood and 4.5×10^4 copies/mL in urine. The sequencing UL97 single-nucleotide mutation M460I was identified in one of the three UL97 hot spots for GCV/V-GCV resistance, and therapy was discontinued.

Case 2

A 41-year-old woman after 39 + 5 weeks of physiological gestation delivered a severe symmetrical SGA infant. At birth, she suffered from severe petechie and thrombocytopenia (PTL = 26.000/mL; range: 150.000–450.000/mL) and received multiple platelet transfusions. Cerebral ultrasound revealed germinolytic cysts, vasculopathy of the striatal thalamus, and ventriculomegaly, confirmed by MRI. Bilateral SNHL was diagnosed with the need for cochlear implant. cCMV was diagnosed at birth by PCR, with the CMV DNA level in blood at 6.2×10^4 copies/mL and in urine at $>1.0 \times 10^6$ copies/mL. GCV (6 mg/kg, twice daily) was administered for 15 days, followed by V-GCV (15 mg/kg, twice daily). Viral load decreased to under positive limits, with no AEs. At about 6 months of age, CMV DNA turned positive (6236 copies/mL in blood). Consultation with our Foundation's cCMV specialist was required, who measured CMV DNA at 23.850 copies/mL in blood and 59.850 copies/mL in urine. Subsequent investigation of GCV resistance revealed UL97 multiple nucleotide mutations (M460V and A594AV) in one of the three UL97 hot spots for GCV/V-GCV. Therapy was stopped.

Discussion

Studies on cCMV symptomatic cases requiring long-term GCV/V-GCV therapy with onset of UL97 gene resistance are briefly summarized in Table 1

We described two additional cases requiring long-term therapy in which, with V-GCV treatment, the viral load remained under the detectable limit for a long period until the UL97 gene mutation. In the first case, the clinical staff decided to prolong therapy beyond 6 months due to the severity of the symptoms and the decreased viral load in the absence of AEs, until the detection of resistance at 8 months. In the second case, after a significant reduction, the CMV viral load suddenly increased at the end of the 6-month V-GCV therapy, also in the absence of AEs. To avoid sub-therapeutic quantities, the full dosage was maintained throughout the whole therapy period in both the cases.

Data from transplanted patients highlighted that high initial CMV viral replication, prophylactic sub-therapeutic dosage, and highly prolonged (>2.5 months) therapeutic dosage of GCV are determinants for an increased risk of resistance and rejection (Majeed et al., 2018). Nevertheless, Amir et al. (2010) treated cCMV patients for 1 year, reducing V-GCV dose after 12 weeks of full-dosage GCV/V-GCV, and reported no cases of resistance as well as better auditory outcome.

One of the aims of long-term antiviral treatment is to ameliorate or prevent the worsening of symptoms and late sequelae. Kimberlin et al. (Kimberlin et al., 2015; James and Kimberlin, 2016) performed a controlled trial that demonstrated a better outcome in hearing and a modest improvement in developmental outcomes at the age of 2 years with 6-month therapy compared with a the 6-week regime. Regarding our study, available data at 2 years of age indicated that both patients had retained the same degree of SNHL. In the first child (case 1) development resulted in severe neurological delay, epilepsy, and cerebral palsy. The second child (case 2) reported a modest developmental delay. We agree with other authors (Campanini et al., 2012; Benzi et al., 2012; Choi et al., 2013; Morillo-Gutierrez et al., 2017) that a prolonged therapeutic exposure may be a risk factor for resistance in cCMV patients, and thus viral load surveillance is the key in monitoring early onset of resistance to V-GCV/GCV. Time-scheduled viral load assessments allow prompt identification of resistance, avoiding ineffective therapy and related ADs, even if the current literature mainly indicates that long-term therapy, after the initial period, does not account for ADs

Table 1
Summary of previous resistance cases.

	GA at birth	Antiviral treatment	Resistance onset	Gene conferring resistance	Notes
Kampmann et al. (2011)	28 weeks + 6 days	Ganciclovir/valganciclovir therapy from 5th day of life to 113th (exitus)	Resistance detected post-mortem	CMV UL97	–
Campanini et al. (2012)	33 weeks	Ganciclovir/valganciclovir from 2nd day of life	113th day of life	CMV UL97	Gap between ganciclovir and valganciclovir due to neutropenia
Benzi et al. (2012)	Not reported	Ganciclovir/valganciclovir – 4 months of therapy	120th day of life	CMV UL97	–
Choi et al. (2013)	35 weeks	4 weeks ganciclovir + 8 weeks valganciclovir	5 months of age	CMV UL97	Gap between ganciclovir and valganciclovir due to neutropenia
Morillo-Gutierrez et al. (2017)	35 weeks	valganciclovir from 5th day of life	After 4 months of valganciclovir	CMV UL97	valganciclovir dose was up to 140% of the standard treatment dose

while ongoing (Amir et al., 2010; James and Kimberlin, 2016). In our opinion, constant dosage adjustment with weight increase and a monthly surveillance of viral load could be proposed to monitor onset of resistance and direct adherence to optimum therapy. We suggest that it may be useful to perform therapeutic drug monitoring (TDM) when viral load starts to increase, even with ongoing antiviral therapy, while also considering that bioavailability could be different between neonates and older infants. However, while managing AEs (e.g. neutropenia) during therapy is feasible, predicting risks in childhood through to adulthood, such as gonadal dysgenesis or carcinogenicity, until now known only in animal models, is not possible. While the described cases highlight the issue of resistance to CMV during long-term therapy, the scientific community should also evaluate the risk–benefit ratio of long-term therapy, considering possible onset of resistance alongside SNHL and neurodevelopmental improvement.

Author contributions

FG and GL were responsible for the study concept, interpretation of the data, and manuscript writing. MA performed data acquisition and is responsible for their integrity. MZ, FB, and GC were responsible of the virology data, while MB and FB supervised every case report stage and critically revised the manuscript in terms of important intellectual content. All authors approved the final manuscript as submitted, and are accountable for all aspects of the work.

Availability of data and materials

All materials and data described in this manuscript are available upon reasonable request to the corresponding author, and if complying with patients' privacy.

Consent for publication

Written informed consent was obtained from both the patients' parents for scientific publication of these case reports.

Ethical approval and consent to participate

Written informed consent was obtained from both the patients' parents for scientific and anonymous use of the clinical data.

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Conflicts of interest

None.

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