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ORIGINAL PAPER

Treatment of symptomatic congenital cytomegalovirus infection with intravenous ganciclovir followed by long-term oral valganciclovir

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Abstract Congenital cytomegalovirus infection is the most common cause of nonhereditary sensorineural hearing loss and an important cause of psychomotor retardation. Earlier studies showed that 6-weeks' treatment with ganciclovir, starting in the neonatal period, prevented hearing deterioration at 6 months, but in one-fifth of the infants, the effect was not sustained at age 12 months. The aim of this preliminary retrospective study was to investigate the effectiveness and safety of long-term treatment with ganciclovir/valganciclovir for congenital cytomegalovirus infection. Twenty-three infants with culture-proven symptomatic congenital cytomegalovirus infection were treated with ganciclovir for 6 weeks followed by oral valganciclovir to age 12 months. Audiometry was performed at least three times in the first year, in addition to physical examination including neurological and developmental assessment. At age ≥1 year, hearing was normal in 76% of affected ears compared to baseline (54%). In 25 normal ears at birth no deterioration was found at ≥1 year. These results were significantly better than reported in a historical control group of similar infants treated for 6 weeks only (P=0.001). Viral load monitoring demonstrated sustained virological response. Four of the children (18%) had mental retardation. The main side effect of treatment was transient neutropenia. In conclusion, prolonged therapy of symptomatic congenital CMV infection with intravenous ganciclovir followed by oral valganciclovir is safe, and it appears to lead to a better auditory outcome than short-term treatment.

Keywords Ganciclovir · Valganciclovir · Congenital CMV

Abbreviations

CMV	Congenital cytomegalovirus
BSER	Brainstem-evoked response
PICC	Peripherally inserted central venous catheter
PCR	Real-time polymerase chain reaction

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Introduction

Congenital cytomegalovirus (CMV) infection is the most common cause of nonhereditary sensorineural hearing loss [6, 7, 13] and an important cause of psychomotor retardation in developed countries [2–4, 9]. The reported incidence in the United States is ~1%; approximately 1 in 750 of all live-born American children suffered of permanent disabilities [3].

Ganciclovir has been used for the treatment of congenital CMV disease for almost two decades [5, 14, 15]. However, there is only one large randomized controlled study of its effect on hearing loss [11]. The results showed that the administration of intravenous ganciclovir for 6 weeks

starting in the neonatal period prevents hearing deterioration at 6 months. In some infants, there was even an improvement in audiometric parameters compared to untreated infants, of whom 41% showed hearing deterioration. However, at age 12 months, hearing had deteriorated in 21% of the treated infants [11]. This finding suggests that 6 weeks of treatment may not be sufficient to prevent hearing loss. Accordingly, in a retrospective study, Michaels et al. [12] treated nine children with CMV infection with intravenous ganciclovir for 6–18 months followed by oral ganciclovir for a median of 6 months [12]. None of the children showed hearing deterioration, and three children with neurological abnormalities improved on follow-up.

Valganciclovir was recently approved in Israel for use as CMV prophylaxis in pediatric transplant recipients. This treatment was also used in an infant with congenital CMV [16] and is currently offered to parents of infants with congenital CMV infection. Since 2005, treatment for symptomatic infants with congenital CMV has been ganciclovir and valganciclovir. The aim of the present preliminary study was to determine the effectiveness and safety of a protocol of intravenous ganciclovir followed by a long-term oral valganciclovir for the treatment of infants with symptomatic congenital CMV disease.

Methods

Subjects

The files of all children with congenital CMV infection who were treated with ganciclovir/valganciclovir at Schneider Children's Medical Center of Israel between March 2005 and February 2009 were reviewed. The diagnosis was based on a positive urine culture for CMV (shell vial method) at age up to 2 weeks. The following clinical data were collected from the files: birth weight, head circumference, physical findings, and laboratory results. Only infants with any sign of central nervous system involvement were treated. The criteria of CNS involvement included: 1. microcephaly; 2. hearing impairment detected by brainstem-evoked response (BSER) test; 3. chorioretinitis; and 4. abnormal findings on brain ultrasound (US) compatible

Table 1 Baseline clinical data of 23 infants with symptomatic congenital CMV

Patient #	Sex	Birth weight	Head circumference	Brain US findings	Fundoscopic examination	BERA[a]	
						Rt ear	Lt ear
1.	M	3,000	30.5	Positive	Normal	≤20	≤20
2.	M	2,900	33.5	Positive	Normal	≤20	≤20
3.	F	2,050	31.1	Positive	Normal	≤20	≤20
4.	F	2,300	31.0	Positive	Normal	80	≤20
5.	F	3,500	36.1	Positive	Normal	≤20	≤20
6.	F	2,690	31.0	Positive	Normal	≤20	≤20
7.	M	2,380	33.0	Positive	Normal	≤20	≤20
8.	M	2,360	31.2	Positive	Normal	≤20	≤20
9.	M	2,520	31.0	Positive	Normal	65	80
10.	M	3,590	34.5	Positive	Retinitis	≤20	≤20
11.	M	2,470	31.9	Positive	Normal	80	40
12.	M	2,680	34.0	Positive	Normal	≥90	80
13.	M	2,990	34.0	Negative	Normal	≥90	≥90
14.	M	3,630	35.2	Positive	Normal	40	35
15.	F	3,600	35.5	Positive	Normal	50	40
16.	F	2,085	31.8	Positive	Retinitis	60	50
17.	M	3,000	31.9	Positive	Normal	≤20	30
18.	M	1,580	27.9	Positive	Normal	≤20	≥90
19.	M	2,520	33.0	Negative	Normal	85	30
20.	F	3,020	35.0	Negative	Retinitis	≤20	40
21.	M	3,450	34.0	Positive	Normal	≤20	≤20
22.	F	2,950	31.8	Positive	Normal	≤20	≤20
23.	F	2,770	32.0	Positive	Normal	≥90	≤20

[a] BSER—threshold in dB

Table 2 Ultrasound findings of children with congenital CMV infection

Patient #	
1	[a]PHE, [b]LSV, pseudocyst
2	Calcification, pseudocyst
3	PHE, LSV
4	Calcification, PHE, LSV
5	Calcification
6	Calcification, ventriculomegaly, LSV
7	Calcification, LSV
8	PHE, LSV
9	Calcification, ventriculomegaly
10	LSV
11	Pseudocyst, ventriculomegaly, LSV
12	LSV
14	Calcification, PHE, LSV
15	LSV
16	LSV
17	Calcification, PHE
18	PHE
21	Calcification, ventriculomegaly, PHE
22	PHE, LSV
23	Ventriculomegaly, PHE

[a] Periventricular hyperechosity—PHE

[b] lenticulostriated vasculopathy—LSV

with congenital CMV infection. Positive ultrasonographic signs included calcification, periventricular hyperechosity, ventricular dilatation, pseudocyst, and lenticularstriated vasculopathy (LSV) [17].

The study was approved by the Institutional Helsinki Committee.

Treatment plan

Initially, intravenous ganciclovir, 5 mg/kg, was administered every 12 h for 6 weeks. Most of the infants had a central venous catheter, mainly a peripherally inserted central venous catheter (PICC), and part of the treatment was given at home by the parents. Thereafter, all infants received an oral suspension of valganciclovir prepared by our pharmacy from 450 mg tablets according to the manufacturer's recommendation. The dose was calculated using the equation provided by the drug's manufacturer (Hoffmann-La Roche, Zurich): Dose (mg)=body surface area × creatinine clearance (Schwartz equation) × 7. Two daily doses every 12 h were given for the first 6 weeks of oral treatment and then one daily dose up to age 1 year. The dose was adjusted to the individual child's growth after every kilogram of weight gain.

Follow-up

The infants were followed at our clinic once a month up to age 3 months and every 3–4 months thereafter. A full physical examination including neurological and developmental assessments was performed at each visit.

Blood count was performed every other week to age 3 months and then only during the clinical visits.

Besides the initial BSER test in the first month, audiometry was performed at least twice in the first year and later every 6 months up to age of 2 years. In infants with suspected conductive hearing loss, tympanometry was performed after the BSER. Infants found to have moderate or severe hearing loss (threshold >46 dB) were referred to the audiology clinic for further treatment.

Viral load

In four infants, viral load was measured before initiation of treatment and during the 6 weeks of intravenous therapy. A proportion of this group, and some of the other infants, underwent convenience sampling during oral treatment. Whole blood was collected into EDTA tubes and kept at −80°C until assayed.

For CMV DNA quantitation, DNA was extracted from 200 µl of whole blood with the QIAamp DNA blood Mini kit (QIAGEN, Hilden, Germany). Five microliters of the 50 µl of purified DNA solution was subjected to real-time polymerase chain reaction (PCR) using primers and probe derived from the CMV glycoprotein B (gB) gene, as previously described [1]. Viral DNA loads were recorded

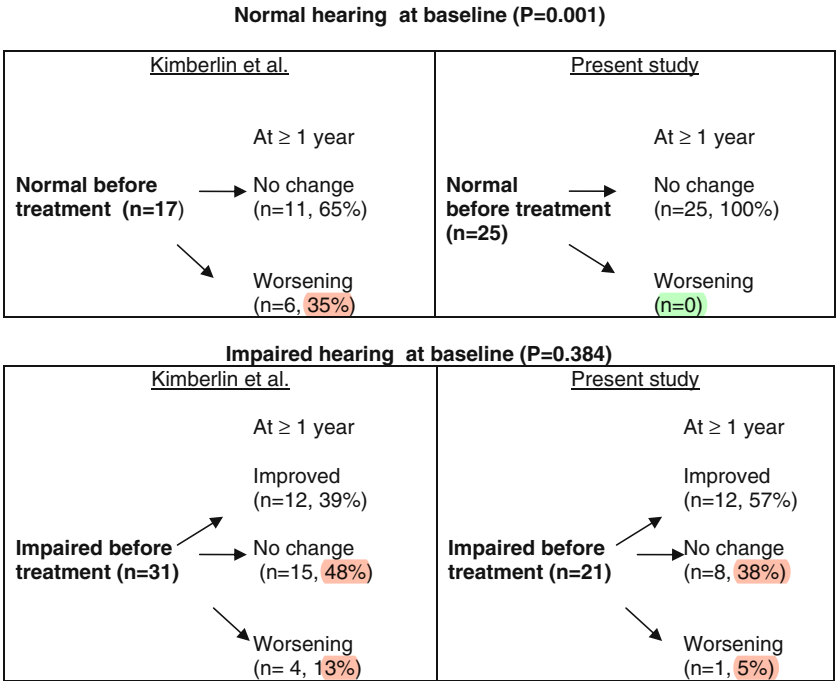
Table 3 Hearing assessment by BSER[a] at baseline and age ≥1 year in 23 children (46 ears) treated for congenital symptomatic CMV infection

	Baseline	At ≥1year[c]
Best ears (n=23)		
Normal[b]	15 (65%)	20 (87%)
Mild	4 (17%)	1 (4%)
Moderate	2 (9%)	1 (4%)
Severe	2 (9%)	1 (4%)
Total ears (n=46)		
Normal	25 (54%)	35 (76%)
Mild	7 (15%)	3 (7%)
Moderate	4 (9%)	3 (7%)
Severe	10 (22%)	5 (11%)

[a] BSER, brainstem-evoked response

[b] BSER thresholds were defined as follows: normal, ≤20 dB; mild hearing loss, 21–45 dB; moderate hearing loss, 46–70 dB; and severe hearing loss, >71 dB

[c] 22 patients had hearing assessments after 1 year



Si lésions auditives au départ => le traitement n'y change rien dans 50% des cas

Fig. 1 Study outcomes according to BSER status before treatment, total ears analysis

as genome copies/milliliter. The assay demonstrated a linear quantitation over a six-log range with a sensitivity of 50 viral DNA copies/ml.

Historical controls

Given that both our criteria for antiviral treatment and the hearing assessment protocol were similar to those used in the short-term ganciclovir treatment group of Kimberlin et al. [11], we compared the auditory outcome in our patients with that in the earlier study.

Statistical analysis

Chi-square test or Fisher's exact test was used to compare rates between groups. A p value of <0.05 was considered statistically significant. We compared our findings with Kimberlin et al. [11] using chi-square tests. Similar to Kimberlin et al. [11], data were analyzed both by the best-ear approach and by the total-ear approach. Since, by definition, normal hearing ears cannot improve, analyses were stratified according to hearing level pre-treatment (normal or impaired hearing).

Results

Thirty-two infants started long-term treatment for CMV infection during the study period, this group includes all the infants born in our institution with symptomatic CMV infection or referred for treatment during this period. Twenty-three of them were older than 1 year at the time of our analysis and constituted the study group. Their demographic and clinical data are presented in Table 1. There were 13 boys (57%) and 10 girls (43%) of median age 2.3 years (range 1.2–4.1 years). Eight (35%) had

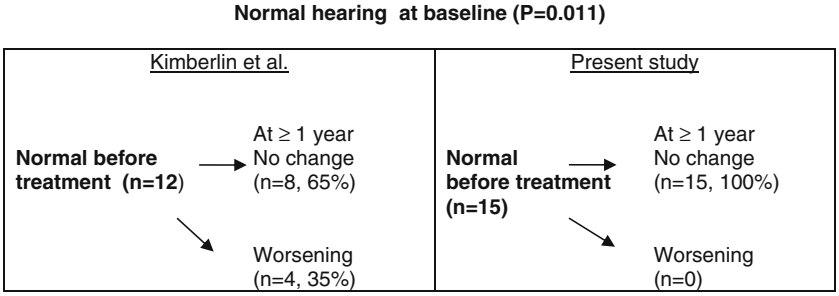


Fig. 2 Study outcomes according to BSER status before treatment, best ear analysis

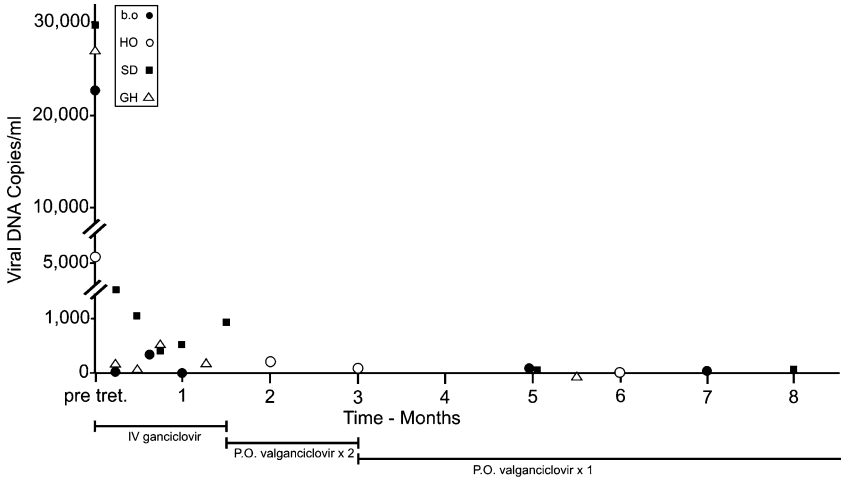


Fig. 3 Viral load of CMV before and after ganciclovir/valganciclovir treatment

intrauterine growth retardation, and only one was premature. Microcephaly was detected in 12 infants (52%). Thrombocyopenia (platelets <100,000 mm^3) was found in 5 (22%) and hepatitis (ALT >100 units/dl) in 4 (17%). Thirteen children (57%) had a hearing defect on initial evaluation, with involvement of 21 out of 46 ears (46%). Primary maternal CMV infection was documented in 14 cases, secondary in 4, and unknown in 5. In these five cases, no maternal serology was tested before or during pregnancy.

Four patients (17%) met only one of the 4 criteria for treatment, 13 (56%) met two criteria, 5 (22%) met 3, and 1 (5%) met all of them. The main abnormalities noted on brain ultrasound scan were lenticulostriated vasculopathy in 13 patients, calcifications in 10 patients, periventricular hyperechogenicity in 7, ventricular dilation in 4, and pseudocyst in 3. Most of the infants with LSV also had other ultrasonographic abnormalities (Table 2). Three infants with only LSV findings on US had other signs of CNS involvement: retinitis, microcephaly, and hearing deterioration.

The mean (±SD) duration of treatment was 9.7± 0.5 months. Nineteen patients (83%) completed the oral treatment at age 12 months. Of the remainder, the mothers stopped treatment at 5 months in one infant, at 7 months in one, and at 11 months in two. The four infants who did not finish the full course of treatment included one infant with severe psychomotor retardation (patient # 6), one with severe hearing deterioration who did not improve (patient # 13), and patients # 4 and 18, respectively.

Hearing assessment

Hearing was assessed in all the patients at birth, at 6 months, and at about 1 year (13±1 months). Most of the patients underwent later assessments as well. Thirteen children (57%) had hearing defects of various levels after birth compared to 8 (39%) at ≥1 year. The results of the last hearing assessment at age ≥1 year compared to baseline are presented in Table 3. Best ear was normal at birth in 65% of the infants compared to 87% at ≥1 year (p=0.365). By ears, 21 ears (46%) had hearing defects at the initial assessment compared to 11 (24%) at ≥1 year (p=0.166). In 26% of the affected ears, an improvement in hearing was noted. Of the remainder, 72% showed no change and 2% showed deterioration in hearing. No change in the BSER results was found in the studies performed after age 1 year compared to the studies done at about 1 year (Table 3).

Comparison of the outcome between our patients and those treated with the short-term protocol in Kimberlin et al's study [11] revealed no difference in baseline hearing between the groups: 35–40% of the infants in each group had a hearing defect, with a similar distribution by severity. However, in Kimberlin's study, 35% of the normal before treatment worsened at the age of ≥1 year. In our study, no change occurred at all in the 25 normal ears. The difference was statistically significant (p=0.001) (Fig. 1). Moreover, in our study, improvement was detected in 57% of the 21 ears impaired before treatment, compared to 39% in Kimberlin's study (p=0.38). Similar findings were calculated in the best-ear analyses (Fig. 2).

On analysis by ears, 76% of the long-term treatment group had normal hearing as opposed to 35% of the short-term treatment group (p<0.001). Among the affected patients in the present study, one had a cochlear implant at age 12 months, and one is using a hearing aid.

Follow-up of development and head growth

Formal developmental assessments were not performed as part of the follow-up, but developmental milestones were assessed during the neurological examination at every ambulatory visit. Four children (18%) had mental retardation, of whom three were born with microcephaly (head

circumference, 30.9, 31.0, and 31.8 cm at term) and showed significant ventricular dilatation on the first brain US examination, (bilateral in 2, unilateral in 1); one child was a late walker (full walking at 25 months) and was also born with microcephaly. Two of the children with mental retardation also have seizures and are being treated with anti-epileptic medications.

Besides these four children, another eight were born with microcephaly. All developed normally. The mean head circumference of all 23 infants was 32.6±1.9 cm at birth and 44.5±2.3 at age12±0.2 months. Three of the children born with microcephaly had a normal head circumference after 12 months (percentiles 10–50).

Viral load

In four children, blood was drawn for viral load assay before treatment, during intravenous treatment, and a few samples during oral treatment (Fig. 3). The viral load dropped from 5,000 to 30,000 viral DNA copies/ml before treatment to <50–500 viral DNA copies/ml in the first week of treatment. The decrease was sustained during intravenous therapy and persisted throughout oral treatment. In another 12 infants, viral load tested during the oral treatment period with single valganciclovir dose were under the cut-off of the assay. Viral load was not assessed after cessation of therapy.

Side effects

The main side effects of the treatment were neutropenia (12 patients) and central line infection (2 patients). Neutropenia was observed only during the first 3 months of treatment, mainly in the first 6 weeks of intravenous ganciclovir administration. In two infants (9%), the neutropenia was severe, defined as an absolute neutrophil count (ANC) of <500/mm³. In both of them, treatment was discontinued for 2–3 days until the ANC returned to normal (>1,500/mm³). Three patients (13%) experienced one episode each of moderate neutropenia (ANC 500–1,000/mm³), and seven patients (30%) had a total of 16 episodes of mild neutropenia (ANC 1,000–1,500/mm³). These cases were managed by repeated blood counts, with no change in treatment. The two patients with central line infections were treated with antibiotics, and new central lines were inserted.

Discussion

The present study employed a retrospective case-series design to further investigate the effect of treatment of congenital CMV infection with intravenous ganciclovir followed by long-term oral valganciclovir. The main advantage of the prolonged treatment was the persistent effect of the antiviral drug, with no deterioration in hearing after treatment was stopped at age 1. This finding was corroborated by comparing our results with historical control patients with CMV infection of similar age and baseline hearing damage who were treated for a short term with ganciclovir as described by Kimberlin et al. [11]. The results showed that the BSER at ≥1 year was better in the patients who received prolonged treatment. No ear with normal hearing at birth deteriorated at ≥1 year compared to 35% in the short treatment group (p=0.001) (Fig. 1).

In addition, our 18% rate of psychomotor retardation at age ≥1 year was considerably lower than the 55% reported in the past [18]. Although this comparison is problematic, as the definition of severely symptomatic congenital CMV may be completely different, the difference is remarkable. Michaels et al. [12] also found that neurological abnormalities were lessened with long-term treatment.

As expected, initial intravenous treatment resulted in a rapid decline in viral load of 2–3 logs by 1 week (Fig. 3). This extent of response is higher than that reported recently for oral valganciclovir treatment [10]. The dose of oral valganciclovir was based on the recommendation of the drug's manufacturer. Recently, two studies measuring valganciclovir plasma concentrations and pharmacokinetics in infants suggested that an oral dose of 15–16 mg/kg bid [8, 10] yields plasma concentrations comparable to those achieved with intravenous ganciclovir. We administered 17–18 mg/kg bid for 6 weeks followed by one daily suppressive dose. The dose was reduced after 12 weeks of full treatment (6 weeks intravenous ganciclovir and 6 weeks oral valganciclovir bid). Of note, CMV viral load which was monitored systematically in 4 children and occasionally during long-term follow-up in 12 additional children (data not shown) did not rebound during continued treatment with one daily dose, supporting the efficacy of this treatment regimen in suppressing viral replication in peripheral blood. By contrast, in the short treatment protocol, a rebound was noted within 2 weeks of stopping the drug [10].

The main side effect of intravenous and oral treatment was neutropenia. The only measure that was required in affected patients was to withhold treatment for a short period.

The main limitations of this study are the retrospective design, lack of a control group, small sample size, relatively short duration of follow-up, and no standardized developmental assessment. Nevertheless, our preliminary results suggest an advantage to prolonged antiviral therapy in patients with congenital CMV infection, with relatively well-tolerated side effects. A controlled, randomized study comparing the long- and short-term protocols is needed.

In conclusion, prolonged therapy of symptomatic congenital CMV infection with intravenous ganciclovir followed by oral valganciclovir is safe, and it appears to lead to a better auditory outcome than short-term treatment.

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Conflicts of interest All authors have no conflicts of interest.

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