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Assessment of mitochondrial toxicity in newborns and infants with congenital cytomegalovirus infection treated with valganciclovir

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ABSTRACT

Background Ganciclovir/valganciclovir is currently indicated during the first 6 months of life in symptomatic children with congenital cytomegalovirus (CMV) infection. However, this treatment may have the potential to induce mitochondrial toxicity due to off-target inhibition of DNA-polymerases. Similar anti-HIV drugs have been associated with mitochondrial toxicity but this has never been explored in CMV.

Objective To determine the potential mitochondrial toxicity profile at the genetic, functional and biogenesis level in peripheral blood mononuclear cells from a cohort of newborns and infants with symptomatic congenital CMV infection (treated with valganciclovir, untreated and uninfected controls).

Design Longitudinal, observational and controlled study.

Setting and patients Subjects were recruited at the tertiary referral Hospital Sant Joan de Déu and experiments were conducted at IDIBAPS-Hospital Clínic of Barcelona, Spain. CMV-infected newborns underwent comprehensive monthly clinical follow-up.

Methods Mitochondrial parameters, audiometry and neurological assessment were measured at baseline, 3–6 and 12 months after inclusion in the study. The Kruskal-Wallis test for k-independent samples and Friedman tests for repeated measurements were applied.

Results Complex IV, citrate synthase enzymatic activities and mtDNA remained preserved in congenital CMV-infected infants treated with valganciclovir compared with controls ($p>0.05$ in all cases).

Conclusions No evidence of mitochondrial toxicity was found in infants treated with valganciclovir for congenital CMV.

What is already known on this topic?

⇒ Valganciclovir treatment is currently used in congenital cytomegalovirus (CMV) infection. Mitochondrial toxicity derived from this treatment is unknown.

What this study adds?

⇒ This study found no evidence of mitochondrial toxicity in infants treated with valganciclovir for congenital CMV.

INTRODUCTION

Congenital cytomegalovirus (CMV)-infection is considered a rare disease (ORPHANET:294). It occurs via the placenta during pregnancy and represents the most common congenital infection in newborns.[1] It is estimated that 1%–4% of pregnant women who are seronegative for CMV become infected during pregnancy and 30%–40% of these women will transmit the infection to their children.[2 3] Although it is estimated that 90% of congenital CMV-infected infants remain free of symptoms,[4 5] this infection is the main cause of non-hereditary sensorineural hearing loss in infants and is also associated with severe neurodevelopmental disorders (cerebral palsy, mental retardation, seizures and impaired vision) and retinitis.[1 3 6–8]

Ganciclovir and valganciclovir are the main anti-CMV therapeutic options of choice. The first clinical trial on the administration of intravenous ganciclovir during 6 weeks in newborns with symptomatic congenital infection showed a slight improvement in hearing and neurodevelopmental outcomes at 6 and 12 months.[9] In contrast to ganciclovir, the prodrug valganciclovir can be administered orally. A more recent clinical trial with valganciclovir showed better long-term neurocognitive and audiological outcomes with a 6-month compared with a 6-week regimen.[10] These results support the current indication to treat newborns diagnosed with symptomatic congenital CMV infection with a 6-month regimen of oral valganciclovir. To date, the most common side effect of ganciclovir/valganciclovir treatment is neutropenia.[3 6 11]

Ganciclovir/valganciclovir are guanosine analogues and, thus, their mechanism of action is similar to that of the antiretrovirals: nucleoside reverse transcriptase inhibitors (NRTI). Ganciclovir/valganciclovir and NRTI act as substrates and inhibitors of viral DNA polymerases required for viral replication. Mitochondrial DNA (mtDNA) polymerase gamma in human cells is responsible for the synthesis of the mitochondrial genome. Thus, secondary inhibition of this enzyme may cause point mutations, deletions and depletion (loss of an entire copy of the mitochondrial genome) in

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Table 1A Number of subjects included in the study

	CTL	CMV-UT	CMV-VAL
N	n=8	n=18	n=16
Sex	6 M/2 F	10 M/8 F	9 M/7 F
Age	6 months (2–12 months)	3 months (1 week to 14 months)	3 months (21 weeks to 16 months)
Time on treatment	–	–	6 months (2 weeks to 12 months)

Clinical and epidemiological data of the subjects included in the study.
Values are reported as median (IQR). No significant differences were detected according to age (p=0.165).
CMV, cytomegalovirus; CTL, control; F, females; M, males; UT, untreated; VAL, valganciclovir.

the mtDNA molecules. These alterations in the mitochondrial genome, which codes for proteins of the mitochondrial respiratory chain (MRC), can lead to mitochondrial dysfunction, ultimately compromising cell function, tissue viability and even the organism exposed,[12–14] as described in previous literature in the case of NRTIs in vivo[15–18] and in vitro.[19 20] In fact, the range of clinical signs and symptoms that have been associated with mitochondrial dysfunction, of either congenital or acquired origin, is extensive and highly heterogeneous,[21] with hearing loss being among the most relevant.

We hypothesise that human cellular DNA polymerases (nuclear and mtDNA polymerases, including mtDNA polymerase gamma) may be inhibited by valganciclovir treatment, as previously reported in the case of other antiviral treatments with a similar mechanism of action, leading to mtDNA depletion and subsequent enzymatic dysfunction. To our knowledge, this is the first study on potential mitochondrial toxicity derived from the administration of valganciclovir.

METHODS

Study design

This was a controlled, observational, longitudinal, prospective study conducted in the Hospital Sant Joan de Déu, Barcelona from January 2018 to March 2021.

Patients

Parents or legal guardians provided informed consent. Sample recruitment was also approved at each centre and was performed with the collaboration of the Infectious Diseases Departments of both tertiary care hospitals: Hospital Sant Joan de Déu (Barcelona, Spain) and Hospital Clinic of Barcelona (Barcelona, Spain).

Inclusion/exclusion criteria and groups of study

This study included 34 CMV-infected children and 8 healthy infants classified as the control group. The exclusion criteria were a personal or family history of mitochondrial or neuromuscular disease or contact with drugs with potential toxicity for mitochondria (eg, aminoglycosides, linezolid or antipsychotics).

Symptomatic CMV-infected infants were treated with valganciclovir (n=16) (32 mg/kg/day, two times a day) for 6 months according to their presentation of clinical, neuroimaging and/or laboratory disorders related to CMV-infection.[5]

Table 1B Number of samples included in the study according to the different groups

	CTL	G0 (0–2 months)	G3 (2–4 months)	G6 (5–8 months)	G12 (9–16 months)
Total	n=8	n=15	n=20	n=18	n=23
CMV-UT	–	n=6	n=9	n=10	n=9
CMV-VAL	–	n=9	n=11	n=8	n=14

Note: The fact that not all the study subjects could be included longitudinally in all time points explains the heterogeneous numbers within the groups in tables 1A and 1B.
CTL, control; G, group; UT, untreated; VAL, valganciclovir.

CMV-infected infants underwent repeated measurements of clinical and mitochondrial parameters at 3, 6 and 12 months of age. The CMV-infected infants were divided into different groups depending on age: (i) GROUP 0: (age, 0–2 months; n=15), (ii) GROUP 3: (age, 2–4 months; n=20), (iii) GROUP 6: (age, 5–8 months; n=18) and (iv) GROUP 12: (age 9–16 months; (n=24). Eighteen infants were longitudinally followed at the different time points. The control group was made up of eight healthy infants. Clinical and epidemiological data of both patients and controls are shown in table 1.

Laboratory assays for mitochondrial function assessment

Experimental analyses of mitochondrial parameters were performed in the Mitochondrial Laboratory at the Cellex-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), at the Hospital Clínic of Barcelona, Spain.

Sample collection

Peripheral blood mononuclear cells (PBMC), lymphocytes and monocytes were obtained from 1 to 3 mL of venous blood. PBMCs were isolated by centrifugation, using the Ficoll gradient (Histopaque 1077, Sigma Diagnostics, St Louis, Missouri, USA).[22] Final pellets were collected and cryopreserved at −80°C for further experimental procedures.

Protein quantification

The bicinchoninic acid assay was used to calculate the total protein cell content following the manufacturer's instructions (PierceBCA-Protein Assay Kit #23225; Thermo-Scientific).

mtDNA quantification

A mitochondrial-DNA (mtDNA) depletion study was performed as described elsewhere.[19]

Mitochondrial function

Spectrophotometry was performed to assess complex IV (CIV)/cytochrome c oxidase (COX) enzyme activity and mitochondrial content by measuring citrate synthase (CS) enzyme activity. MRC enzyme activities were expressed as relative units, normalised for mitochondrial content, estimated by CS activity (COX/CS). A sample of pork muscle homogenate with known values of normality was assessed as an internal quality control of the technique. Experimental conditions for CIV and CS enzymatic activities are provided (online supplemental material).

Statistical analysis

Kolmogorov Smirnov tests were used to assess normality of the variables. Statistical analysis was performed using the SPSS v27. The non-parametric Kruskal Wallis test for k-independent samples was used for cross-sectional comparisons between groups. Samples obtained along the follow-up period were longitudinally compared (n=18) using the nonparametric Friedman test for repeated measurements. Statistical significance was set at p=0.05.

RESULTS

MtDNA content remained conserved among the different groups (figure 1). The sample size and the epidemiological and clinical data of the subjects included in the study are summarised in table 1. The patients included in the study were classified according to different age groups.

Out of 34 infants, 29 were diagnosed with CMV-infection at birth or prenatally and 5 were diagnosed retrospectively.

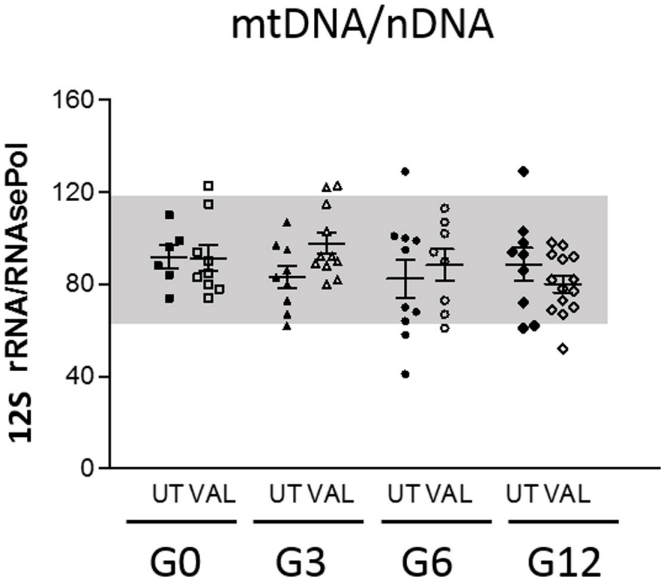

Figure 1 Mitochondrial DNA quantification (absolute values) estimated by mitochondrial DNA normalised by nuclear DNA (12SrRNA/RNAseP). Mitochondrial DNA was preserved among the different treatment groups and the different age groups. Black dots show untreated groups (UT) whereas clear dots show valganciclovir-treated groups (VAL). From left to right, the groups are divided depending on the age-dependent time period. The grey area indicates mitochondrial DNA reference values in the control group. MtDNA, mitochondrial DNA; nDNA, nuclear DNA; G0, group 0 including newborns from 0 to 2 months; G3, group 3 including newborns from 2 to 4 months; G6, group 6 including newborns from 5 to 8 months; G12, group 12 including newborns from 9 to 16 months; UT, untreated; VAL, valganciclovir. Figure created by CM coauthor.

Symptoms and signs of congenital infection were identified in 19 infants: severe neurological affectation (n=2), only sensorineural hearing loss (n=6), chorioretinitis (n=1), cholestatic hepatitis (n=1) and microcephaly, laboratory abnormalities (thrombocytopenia, CSF alterations or elevated ALT) and neuroimaging findings (n=9).

Sixteen infants (14 newborns) initiated treatment with valganciclovir based on the clinical disorders described. The remaining three symptomatic infants were not treated because they were diagnosed after 6 months of age. In three infants, the treatment was interrupted after 2–6 weeks due to haematological toxicity.

Quantitative data of all measured mitochondrial parameters are shown (table 2).

Mitochondrial enzymatic activities of CIV (figure 2) and CS (figure 3) did not show differences between groups.

MtDNA content (figure 4A) and CIV enzymatic activity (figure 4B) relative values to CS are shown.

Since mitochondrial genome quantification did not differ over time in the different groups (figure 5), alterations at a functional level were not expected to be different. Correlations between genetic, functional and biogenic parameters were analysed, with no statistically significant relationship between any of the parameters.

DISCUSSION

In this study, we explored the potential mitochondrial toxicity related to CMV-infection and 6 months of anti-CMV treatment with valganciclovir in infants at different timepoints during

Table 2 Analytical results of the genetic (mtDNA), functional (CIV) and biogenic (CS) assays from infants with congenital CMV infection and controls

	CTL	G0 (0–2 m)		G3 (2–4 m)		G6 (5–8 m)		G12 (9–16 m)	
		UT	VAL	UT	VAL	UT	VAL	UT	VAL
mtDNA (mtDNA/nDNA)	(60–144)	91.83 95% CI 78 to 105	91.33 95% CI 78 to 104	83.33 95% CI 72 to 94	97.82 95% CI 87 to 108	82.50 95% CI 63 to 101	88.38 95% CI 81 to 104	88.67 95% CI 72 to 105	80.07 95% CI 72 to 87
CIV (nmoles/min × mg protein)	(11–59)	41.5 95% CI 15 to 67	40.75 95% CI 26 to 55	38.33 95% CI 19 to 57	37.18 95% CI 23 to 50	28.50 95% CI 17 to 39	50.13 95% CI 20 to 80	38.67 95% CI 26 to 51	46.60 95% CI 32 to 60
CS (nmoles/min × mg protein)	(60–162)	113.7 95% CI 94 to 132	106.3 95% CI 96 to 116	104 95% CI 90 to 117	109.2 95% CI 89 to 129	101.2 95% CI 92 to 110	115.9 95% CI 93 to 137	104.4 95% CI 94 to 114	103.8 95% CI 96 to 111
mtDNA/CS	(0.8–2.8)	1.49 95% CI 1.1 to 1.8	1.69 95% CI 1.4 to 1.9	1.5 95% CI 1.2 to 1.7	1.56 95% CI 1.3 to 1.7	1.43 95% CI 1.1 to 1.7	1.50 95% CI 1.0 to 1.9	1.51 95% CI 1.3 to 1.7	1.40 95% CI 1.2 to 1.6
CIV/CS	(13–48)	21.17 95% CI 10 to 31	20.50 95% CI 16 to 24	25.22 95% CI 13 to 36	24.40 95% CI 16 to 32	20.80 95% CI 12 to 29	24.63 95% CI 16 to 33	26.11 95% CI 20 to 31	27.27 95% CI 23 to 31

P>0.05 in all cases.
mtDNA. mitochondrial DNA; CIV, complex IV of the mitochondrial respiratory chain; CS, citrate synthase; CTL, control; G, group; UT, untreated; VAL, valganciclovir.

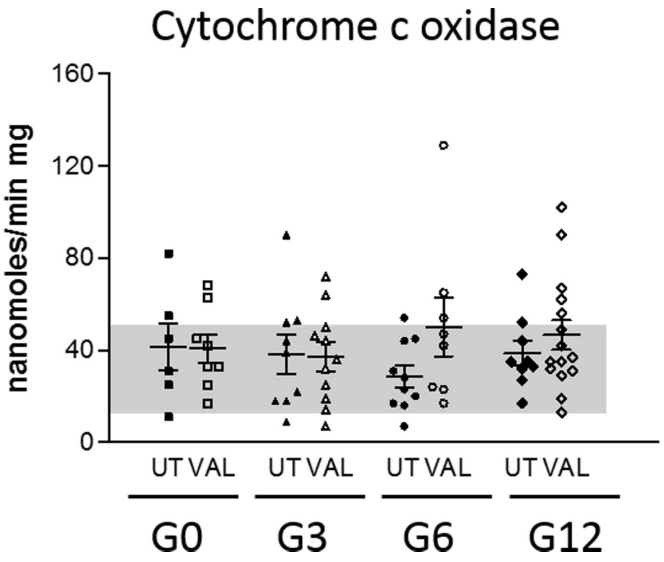

Figure 2 Mitochondrial cytochrome c oxidase or complex IV enzymatic activity (nmoles/min mg protein) suggestive of mitochondrial function at the level of cell respiration, estimated by nanomoles of consumed reduced cytochrome c per minute and milligram of protein. Mitochondrial enzymatic activity of complex IV of the mitochondrial respiratory chain remained unaltered among the different treatment and age groups. Black dots show untreated groups whereas clear dots show valganciclovir treated groups. From left to right, the groups are divided depending on the age-dependent time period. The grey area indicates the cytochrome c oxidase reference values in the control group. G0, group 0 including newborns from 0 to 2 months; G3, group 3 including newborns from 2 to 4 months; G6, group 6 including newborns from 5 to 8 months; G12, group 12 including newborns from 9 to 16 months; UT, untreated; VAL, valganciclovir. Figure created by CM coauthor.

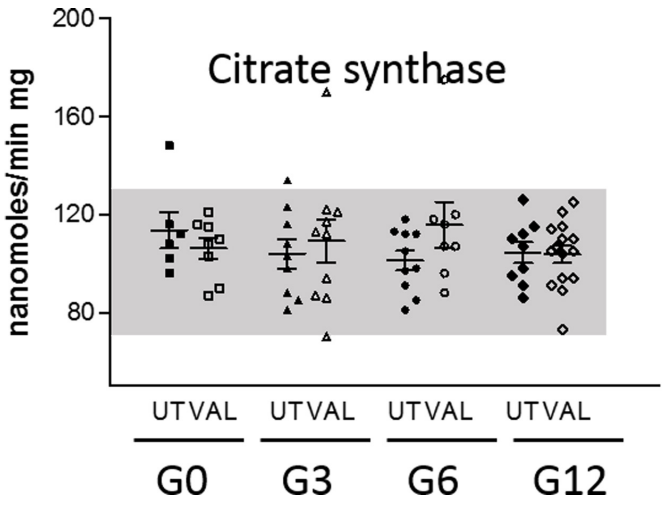

Figure 3 Citrate synthase enzymatic activity (nmoles/min mg protein) suggestive of mitochondrial mass, estimated by nanomoles of product per minute and milligram of protein. Mitochondrial mass was preserved among the different treatments and between age difference. Black dots show untreated groups whereas clear dots show valganciclovir treated groups. From left to right, the groups are divided depending on the age-dependent time period. The grey area indicates citrate synthase enzymatic reference values in the control group. G0, group 0 including newborns from 0 to 2 months; G3, group 3 including newborns from 2 to 4 months; G6, group 6 including newborns from 5 to 8 months; G12, group 12 including newborns from 9 to 16 months; UT, untreated; VAL, valganciclovir. Figure created by CM coauthor.

the first 2 years of life (0–2 months, 2–4 months, 5–8 months and 9–16 months). Molecular mitochondrial assessments were carried out at different levels including genetic, functional and biogenic processes to further decipher potential interactions and/or compensatory mechanisms. The rationale of the primary hypothesis of this study is that anti-CMV treatment with valganciclovir may be related to mitochondrial toxicity at a genetic and/or functional level mainly due to off-target inhibition of the mtDNA polymerase gamma. This assumption is based on a previous wide body of evidence reporting mtDNA depletion and mitochondrial dysfunction by other antiviral treatments, such as anti-HIV or anti-HCV NRTI therapies,[14] which present analogous mechanisms of action by blocking the viral DNA polymerases to avoid replication. NRTI-derived mitochondrial damage is not restricted to a specific level, but rather is generalised to the mitochondrial and functional levels. Thus, mtDNA depletion[23] and mitochondrial defects in the functional enzymatic activities of the MRC have been related to NRTI exposure.[17] Since the mechanism of action of valganciclovir anti-CMV treatment is similar to that of NRTI, it is conceivable that mitochondrial toxicity derived from valganciclovir could be present either at a genetic or functional level in newborns. While our study explored potential valganciclovir-mitochondrial interferences, mitochondrial safety was confirmed among the participants along the different time periods of age and follow-up timepoints.

The consequence of mitochondrial dysfunction is an imbalance in oxidation-reduction that leads to an accumulation of pyruvate, lactic acid, ketone bodies and alanine, producing heterogeneous spectrum of symptoms in the whole body.[5 21] The relevance of investigations looking for mitochondrial toxicity in infants treated for congenital CMV is important as the clinical manifestations of potential toxicity are not easily identifiable in symptomatic patients with hearing loss or neurological alterations. In addition, there is a growing trend to screen for CMV

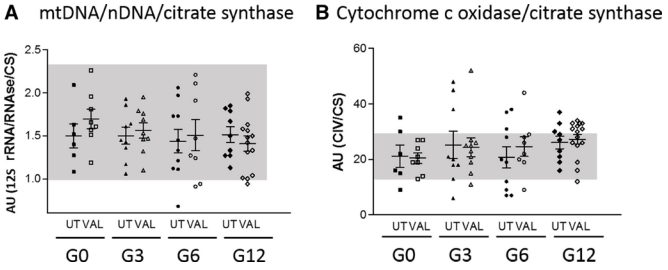

Figure 4 Mitochondrial DNA and cytochrome c oxidase relative values normalised by mitochondrial mass (citrate synthase). (A) The mitochondrial DNA relativised by mitochondrial mass remained preserved among the different study groups (either treatment or time period). (B) The relative values of enzymatic activity of cytochrome c oxidase or complex IV remained unaltered among the different study groups (either considering the treatment or time period). Black dots show untreated groups whereas clear dots show valganciclovir treated groups. From left to right the groups are divided depending on the age-dependent time period. The grey area indicates mitochondrial DNA and enzymatic reference values, relative to mitochondrial mass, in the control group. G0, group 0 including newborns from 0 to 2 months; G3, group 3 including newborns from 2 to 4 months; G6, group 6 including newborns from 5 to 8 months; G12, group 12 including newborns from 9 to 16 months; UT, untreated; VAL, valganciclovir. Figure created by CM co-author.

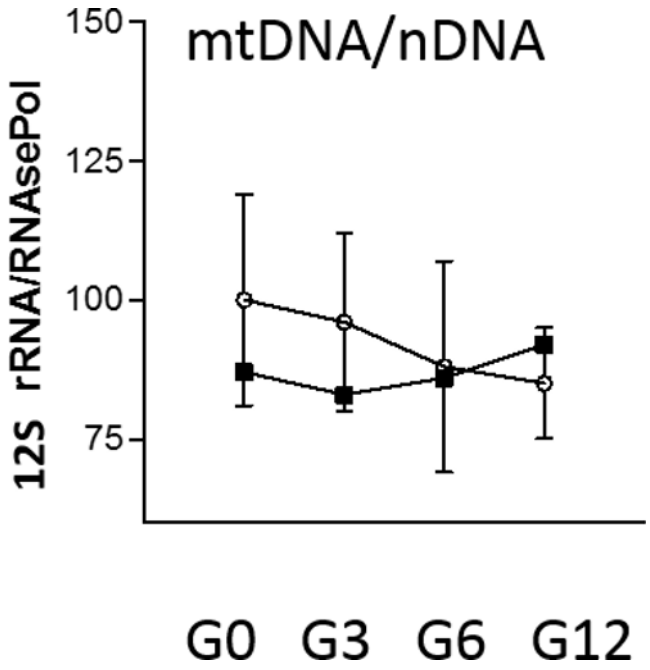

Figure 5 Mitochondrial DNA evolution over time. Black dots indicate untreated CMV-infected newborns whereas clear dots show valganciclovir treated CMV-infected newborns. Results expressed as mean±SD. Statistical analysis of related samples did not show differences over time by non-parametric Friedman tests in k-related samples in the different treatment groups (UT: $|^2 = 1.8$, df=3, p=0.615; VAL: $|^2 = 5.2$, df=3, p=0.157). G0, group 0 including newborns from 0 to 2 months; G3, group 3 including newborns from 2 to 4 months; G6, group 6 including newborns from 5 to 8 months; G12, group 12 including newborns from 9 to 16 months. Figure created by CM coauthor.

DNA in urine samples from newborns. The number of CMV-infected children receiving ganciclovir/valganciclovir treatment might be expected to grow substantially in the following years.

Moreover, the first months of life are a key stage in the development of the infants and any health issue at this point may have an impact on their future lives. We cannot rule out that the lack of differences may be due to the limited sample size or the source of the sample. However, longitudinal assessment with repeated measurements is expected to increase statistical power, and PBMC represents the main target tissues of CMV-tropism.

In some patients with CMV infection, high doses of ganciclovir/valganciclovir may be indicated, representing greater exposure of the infant to the drug.[24] Also, the treatment of infected infants requires exposure to these drugs over a longer period than that used in other indications (6 months).[24 25] This study found no evidence of mitochondrial toxicity in infants treated with valganciclovir for congenital CMV.

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Contributors AO-G and CM conducted all the experimental procedures. FC supervised the clinical and experimental data. GG, FJG-G and JC-S contributed to enzymatic activities. LV-R, JMG and ET contributed to mtDNA analyses. CF is the principal investigator and obtained financial support. AO-G, MR, AN-J and CF gathered the clinical data and recruited patients and controls. ES, AO-G and CM conducted statistical analyses. CM supervised the laboratory experiments. AO-G and CM wrote the manuscript. All coauthors approved the final version to be published. CM and CF authors share main last authorship as principal investigators. CM is the guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Ethics Committee of Hospital Sant Joan de Déu, Barcelona, Spain CEim NUMBER: PIC-145-17 (PI17/00359). Date of approval: 23/11/2017. Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1 Lanzieri TM, Dollard SC, Bialek SR, *et al*. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. *Int J Infect Dis* 2014;22:44–8.
2 Britt WJ. Cytomegalovirus. In: Wilson CB, Nizet V, Maldonado YA, *et al*, eds. *Remington and Klein's Infectious Diseases of the Fetus and Newborn Infant*. 724. 8th ed. Philadelphia: eElsevier Saundrs, 2016.
3 Rawlinson WD, Boppana SB, Fowler KB, *et al*. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect Dis* 2017;17:e177–88.
4 Baquero-Artigao F, Grupo de estudio de la infección congénita por citomegalovirus de la Sociedad Española de Infectología Pediátrica. [Consensus document from the Spanish Society of Paediatric Infectious Diseases (SEIP) on the diagnosis and treatment of congenital cytomegalovirus infection]. *An Pediatr* 2009;71:535–47.
5 Luck SE, Wieringa JW, Blázquez-Gamero D, *et al*. *Congenital cytomegalovirus a European expert consensus statement on diagnosis and management*. In: Pediatric Infectious Disease Journal, 2017.
6 Boppana SB, Ross SA, Fowler KB. Congenital cytomegalovirus infection: clinical outcome. *Clin Infect Dis* 2013;57 Suppl 4:S178–81.

7 Pinninti SG, Rodgers MD, Novak Z, *et al*. Clinical predictors of sensorineural hearing loss and cognitive outcome in infants with symptomatic congenital cytomegalovirus infection. *Pediatr Infect Dis J* 2016;35:924–6.
8 Marsico C, Kimberlin DW. Congenital cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. *Ital J Pediatr* 2017;43:38.
9 Kimberlin DW, Lin C-Y, Sánchez PJ, *et al*. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. *J Pediatr* 2003;143:16–25.
10 Kimberlin DW, Jester PM, Sánchez PJ, *et al*. Valganciclovir for symptomatic congenital cytomegalovirus disease. *N Engl J Med Overseas Ed* 2015;372:933–43.
11 Hamilton ST, van Zuylen W, Shand A, *et al*. Prevention of congenital cytomegalovirus complications by maternal and neonatal treatments: a systematic review. *Rev Med Virol* 2014;24:420–33.
12 Morén C, Noguera-Julián A, Garrabou G, *et al*. Mitochondrial disturbances in HIV pregnancies. *AIDS* 2015;29:5–12.
13 Noguera-Julian A, Morén C, Rovira N, *et al*. Decreased mitochondrial function among healthy infants exposed to antiretrovirals during gestation, delivery and the neonatal period. *Pediatr Infect Dis J* 2015;34:1349–54.
14 Romero-Cordero S, Noguera-Julian A, Cardellach F, *et al*. Mitochondrial changes associated with viral infectious diseases in the paediatric population. *Rev Med Virol* 2021;31.
15 Morén C, Rovira N, Noguera A, *et al*. 11 HIV and antiretroviral-mediated mitochondrial DNA depletion in children. *Mitochondrion* 2010;10:203.
16 Morén C, Noguera-Julian A, Garrabou G, *et al*. Mitochondrial evolution in HIV-infected children receiving first- or second-generation nucleoside analogues. *J Acquir Immune Defic Syndr* 2012;60:111–6.
17 Morén C, Garrabou G, Noguera-Julian A, *et al*. Study of oxidative, enzymatic mitochondrial respiratory chain function and apoptosis in perinatally HIV-infected pediatric patients. *Drug Chem Toxicol* 2013;36:496–500.
18 Bañó M, Morén C, Barroso S, *et al*. Mitochondrial toxicogenomics for antiretroviral management: HIV post-exposure prophylaxis in uninfected patients. *Front Genet* 2020;11.
19 Morén C, González-Casacuberta I, Álvarez-Fernández C, *et al*. HIV -1 promonocytic and lymphoid cell lines: an *in vitro* model of *in vivo* mitochondrial and apoptotic lesion. *J Cell Mol Med* 2017;21:402–9.
20 Morén C, Bañó M, González-Casacuberta I, *et al*. Mitochondrial and apoptotic *in vitro* modelling of differential HIV-1 progression and antiretroviral toxicity. *J Antimicrob Chemother* 2015;70:2330–6.
21 Noguera A, Fortuny C, Sanchez E, *et al*. Hyperlactatemia in human immunodeficiency virus-infected children receiving antiretroviral treatment. *Pediatr Infect Dis J* 2003;22:778–82.
22 Prilutskiĭ AS, Khodakovskiĭ AV, Maĭlian EA. [A method of separating mononuclears on a density gradient]. *Lab Delo* 1990;2:20-3.
23 Noguera A, Morén C, Rovira N, *et al*. Evolution of mitochondrial DNA content after planned interruption of HAART in HIV-infected pediatric patients. *AIDS Res Hum Retroviruses* 2010. ;;26:1015–8.
24 Leruez-Ville M, Ville Y. Optimum treatment of congenital cytomegalovirus infection. *Expert Rev Anti Infect Ther* 2016;14:479–88.
25 del Rosal T, Baquero-Artigao F, Blázquez D, *et al*. Treatment of symptomatic congenital cytomegalovirus infection beyond the neonatal period. *J Clin Virol* 2012;55:72–4.

SUPPLEMENTAL MATERIAL

MRC CIV enzymatic activity

Medium composition: 50 mM potassium phosphate pH 7.0, 100 µM reduced cytochrome c (SIGMA c-7752) and PBMC homogenate. Initial solution with 50 mM KP pH 7.0 and 100 µM cytochrome c was prepared in a ratio 0.805 ml buffer/mg cytochrome c). A 100% oxidised solution was prepared by adding potassium ferrocyanide in 1 ml of initial solution. A 100% reduced solution was prepared by adding sodium dythionite to 2 ml of initial solution. Absorbance of 100% oxidised solution at 550 nm was confirmed around 0.7 as quality control. Autozero of oxidised solution was set to measure absorbance of 100% reduced solution, which was considered as 100% reduced. Titration of initial solution was conducted by transferring aliquotes of the reduced solution to the initial cytochrome c solution and reading the absorbance until 90-95% of reduction was yielded. After initial air calibration at 550nm, 980 µl of 90-95% reduced solution was incubated at 37ºC for 5 minutes in the spectrophotometer. Reaction was initiated by adding 20 µl of cell suspension and absorbance was monitored every 15 seconds during 3 minutes, in a 6-cuvette carrousel simultaneously. (Units: nanomoles/min mg protein, cytochrome c molar extinction coefficient: $\sum$= 18,5 mM-1cm^{-1}).

MRC CS enzymatic activity

The reduced CoA (CoA-SH) formed in the reaction converts 5.5 'dithiobis 2 nitrobenzoic acid (DTNB) into 2-nitro-5-benzoic acid (TNB), which absorbs at 412 nm. (23). Medium composition: 100 µM DTNB 100 mM Tris HCl pH 8.1 300 µM acetyl coenzyme A, 500 µM oxaloacetate, 0,1 % triton X100 and cell suspension. After initial air calibration at 412 nm, 930 µl of medium and 20 µl ofand cell suspesion mixture werewas incubated at 37ºC in the spectrophotometer. Basal line was monitored every 15

seconds during 4 minutes, in a 6-cuvette carrousel simultaneously. Reaction was initiated by 50 µl of 10 mM oxaloacetate (in Tris HCl 100 mM pH 8.1) and measured every 15 seconds during 4 minutes. Basal activity was substracted from the specific activity. (Units: nanomoles/min mg protein, DTNB molar extinction coefficient: $\sum$=13,6 mM-1cm^{-1}).