DOI: 10.1111/j.1471-0528.2007.01308.x
www.blackwellpublishing.com/bjog

Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection

F Jacquemard,[a] M Yamamoto,[b] J-M Costa,[c] S Romand,[a] E Jaqz-Aigrain,[d] A Dejean,[b] F Daffos,[a] Y Ville[b]

[a] Service de Médecine fœtale, Institut de Puériculture de Paris, Paris, France [b] Service de Gynécologie Obstétrique, Centre Hospitalier Intercommunal de Poissy-St Germain, Universite Versailles St Quentin, Poissy, France [c] Service de Biologie Moléculaire Marcel Dassault, American Hospital of Paris, Neuilly, France [d] Service de Pharmacologie, Hôpital R. Debré, Paris, France
Correspondence: Prof. Y Ville, Service de Gynécologie Obstétrique, Centre Hospitalier Intercommunal de Poissy-St Germain, Universite Versailles St Quentin – Paris Ouest, 10 rue du Champ Gaillard, 78300 Poissy, France. Email yville@wanadoo.fr

Accepted 25 January 2007. Published OnlineEarly 6 July 2007.

Objectives To report early experience with treatment of intrauterine cytomegalovirus (CMV) infection using maternal oral administration of valaciclovir (VACV).

Design Observational study of fetuses infected with CMV with or without treatment with valaciclovir.

Population Pregnancies with confirmed fetal CMV infection were treated with oral VACV (8 g/day).

Main outcome measures Fetal viral load and drug concentration were monitored in amniotic fluid and in fetal blood. Data on the course and outcome of a group of untreated symptomatic fetuses infected with CMV are also reported.

Results Therapeutic concentrations were achieved in maternal and fetal bloods. The viral load in the fetal blood (VLFB) decreased significantly after 1–12 weeks of treatment (Wilcoxon paired test $P = 0.02$). Twenty pregnancies including 21 fetuses were treated at 28 weeks (median, range: 22–34) for 7 weeks (median, range: 1–12). Ten infants were developing normally at between 1 and 5 years of age. Two infants (both aged 2 years) had severe isolated unilateral deafness. One neonate presented with microcephaly and severe deafness but was also diagnosed with incontinentia pigmenti. Six out of seven cases that eventually required termination of pregnancy (TOP) had evidence of *in utero* progression of the disease with worsening cerebral lesions. One fetus died *in utero*. The outcome of 14/24 (58.3%) untreated symptomatic infected fetuses was poor with either TOP, intrauterine fetal demise or severe congenital infection disease of the neonate; the remaining ten infants were healthy at follow up.

Conclusion Maternal oral administration of VACV leads to therapeutic concentrations in the maternal and fetal compartments, with a decrease in VLFB. Our results suggest that in cases where TOP is declined, a randomised controlled trial to study this treatment option further is indicated.

Keywords cytomegalovirus, fetal therapy, foetus, infection, valaciclovir.

Please cite this paper as: Jacquemard F, Yamamoto M, Costa J, Romand S, Jaqz-Aigrain E, Dejean A, Daffos F, Ville Y. Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection. BJOG 2007;114:1113–1121.

Introduction

Cytomegalovirus (CMV) is the main cause of congenital viral infection, and urine and saliva are the main reservoirs of the virus in children.[1–3] In industrialised countries, approximately 50% of pregnant women are immune to CMV at the beginning of their pregnancy. Infection during pregnancy occurs in 1% of nonimmune and 5% of immune pregnant women, with vertical transmission rates of 30% and 0.2–8%, respectively.[1,3,4] It is estimated that 1% of all neonates are infected with CMV and that two-thirds of these cases are caused by primary maternal infection.[5,6] Among infected children, only 10% are symptomatic at birth. Cytomegalic inclusion disease (CID) is defined by the association of growth restriction, microcephaly, jaundice, hepatosplenomegaly, and thrombocytopenia.[7–11] Nearly 30% of symptomatic infants will die, and of the remainder, up to 60% will develop cerebral lesions with associated neurological handicap. Furthermore, about 15% of asymptomatic neonates will ultimately develop some degree of hearing loss and/or learning difficulty during or after the first year of life.[7] CMV-specific diagnosis is made by amplifying the viral genome by polymerase chain reaction

(PCR) in the amniotic fluid obtained by amniocentesis after 21 weeks.[6,8–11] Quantification of neonatal viraemia has been correlated with the risk of sequelae in symptomatic and asymptomatic newborns.[7] Association between the morbidity of the virus in infancy and hearing loss has also been demonstrated.[12] However, direct extrapolation of the neonatal syndrome to the fetus is problematic. While cerebral lesions seen with ultrasound can be assumed to carry a poor prognosis,[5] this assumption does not necessarily hold for ascites, hyperechogenic bowel, and hepatomegaly. By the same token, increased liver enzyme concentrations and thrombocytopenia in fetal blood obtained by fetal blood sampling (FBS) cannot be directly translated into defined adverse neonatal outcomes.[11,13,14] Given the potential for progressive *in utero* injury, however, intrauterine treatment may be justified in these fetuses as an alternative to termination of pregnancy (TOP) for couples wishing to continue with the pregnancy.

A number of antiviral drugs are active against CMV and are being used in the treatment of immunocompromised individuals. Ganciclovir and aciclovir (ACV) have proven to be effective in preventing CMV infection in kidney transplantation.[15] Ganciclovir is effective in neonates,[16,17] but its known teratogenic and haematopoietic adverse effects contraindicate its use in pregnancy. One pregnant woman with a renal transplant who presented with fetal CMV demonstrated by PCR in amniotic fluid was treated with oral ganciclovir and subsequently delivered a healthy newborn.[18] ACV with a drug oral regimen of 4 g/day is active against CMV, and its prodrug valaciclovir (VACV) at a dose of 8 g/day has been shown to be even more effective against herpes viruses[19–22] in individuals infected with HIV. Additionally, VACV has been shown to have fewer adverse effects than ACV.[19] Viral load seems to be correlated with the development of CMV disease in solid-organ transplant recipients, and treatment can prevent the occurrence of the disease in those with positive viraemia.[23,24] Compared with placebo or no treatment, prophylaxis with ACV, valganciclovir, or VACV significantly reduced the risks of CMV disease and associated mortality in recipients of solid-organ transplants.[25]

The aim of this pilot study was to demonstrate that treatment with VACV given orally to pregnant women is achievable in cases with confirmed fetal CMV infection, based on the demonstration of therapeutic concentrations and of a plausible effect on viral load in fetal blood (VLFB). The aim was not to demonstrate clinical efficacy of the drug in these cases of symptomatic intrauterine infection.

Population and methods

This study was given an Institutional Review Board (IRB) exemption by our institutional ethics committee on the basis of compassionate care, and all participants gave informed, written consent. The women were recruited in Poissy Hospital and in the Institut de Puériculture de Paris between 2003 and 2005. The women understood that the counselling they received was based on extrapolations from neonatal data.[13] We offered VACV therapy in cases of confirmed fetal CMV infection after primary maternal infection when the fetus showed biological and/or ultrasound markers of infection and when the parents had elected to continue with the pregnancy (in case 18, treatment was indicated because of raised concentrations of gamma-glutamyl-transpeptidase (GGT); cases 6 and 7 were twins, but inclusion criteria were found in case 6 only). No screening programmes exist in France, and cases were diagnosed when opportunistic maternal serology or ultrasound findings were suggestive of this diagnosis. Fetal CMV infection was confirmed by viral PCR testing of an amniotic fluid specimen collected by amniocentesis.[26] A symptomatic fetus was defined by the presence of one or more of the following ultrasound features: grade 3 hyperechogenic bowels,[27] ascites, growth restriction with an estimated fetal weight below the 10th percentile, ventriculomegaly with ventricular width of at least 10 mm at the level of the atrium, or any other brain anomaly.[28]

Pregnant women were given oral VACV in a dosage of 2 g taken four times per day from the day of enrolment into the study up to delivery or TOP. Women who had a history of hepatic disease, renal dysfunction, bone marrow suppression, or known ACV intolerance were not offered therapy.

The women clearly understood that VACV treatment was experimental, and no guarantee of efficacy was implied. We did inform women that treatment had the potential to reduce viral load in the fetuses and hence could potentially also reduce the morbidity of prolonged intrauterine infection. Women were informed of possible treatment-related morbidity with VACV including an increased risk of renal calculus, gastrointestinal intolerance, and cutaneous allergic reactions. All women enrolled in this study had the option of terminating the pregnancy in accordance with French law.

In these institutions, standard management of infected fetuses included FBS at diagnosis and 4–6 weeks later to determine the platelet count and GGT plasma concentration together with changes over time.[11,13,14] Fetal thrombocytopenias below 100 000/dl and/or elevated GGT were considered to be features of a symptomatic fetal infection. We obtained permission to use any excess of fetal blood for pharmacological and virological tests. Quantification of CMV DNA was performed by real-time PCR with a threshold of 250 copies/ml.[26] Changes in CMV DNA load and ACV concentration were measured in the amniotic fluid and in the fetal blood, before and during treatment, and the results were explained to the women. Statistical differences in viral loads were compared by Wilcoxon paired test.

Serial ultrasound examination was offered fortnightly for the early detection of growth restriction or further

abnormalities that may have altered the participants' decision making. Intrauterine magnetic resonance imaging of the fetal brain was also performed between 32 and 36 weeks of gestation in all cases. Postnatally, ganciclovir was offered only to infants with symptomatic congenital infection.[17]

ACV concentrations were determined by high-performance liquid chromatography,[29] as it is the final active form of VACV against the virus. Maternal ACV concentrations and pharmacokinetics were determined in the first ten cases to ensure that 8 g/day orally was a suitable regimen in pregnancy. Maternal plasma concentration before the morning dose, maximum plasma concentration, and area under the plasma concentration time curve were assessed. ACV concentrations were measured in maternal plasma, amniotic fluid, and fetal blood at the time of amniocentesis and FBS as described above. A change in DNA viral load of at least 1 log concentration was considered significant.

Although no attempt was made neither to match nor to compare outcomes of treated with untreated fetuses directly, outcomes were also collected from all cases of symptomatic CMV infection presenting for 12 months prior to the start of this study and from those who declined treatment during the study period. Diagnosis in this group was performed with PCR and in some cases with real-time PCR with viral load quantification. Only fetuses with confirmed CMV presence in the amniotic fluid were included.

Results

Twenty women with 21 symptomatic CMV-positive fetuses (one twin pregnancy) received oral VACV (8 g/day) in the second half of pregnancy for 1–12 weeks. They were treated at a median of 28 (range: 22–34) weeks for a median of 7 (range: 1–12) weeks.

The pharmacokinetics of ACV determined on the first ten cases are shown in Table 1. After maternal oral administration of VACV (8 g/day), drug levels were more than 20 μmol in both maternal blood and in the amniotic fluid in all cases but one. In fetal blood, mean maximum maternal plasma concentration was more than 20 μmol in five of ten cases. Both mean maternal and fetal blood plasma concentrations were lower than amniotic fluid concentration (Table 1). In one case (case 2), the mother elected to stop taking VACV 3 days before delivery at 38 weeks of gestation, and ACV concentrations were undetectable (<1 μmol) in maternal and cord bloods, amniotic fluid, and neonatal blood at the time of delivery. No maternal, fetal, or neonatal adverse effects were reported.

In case 18, microcephaly was suspected at 33 weeks of gestation, and intrauterine fetal death (IUFD) occurred after FBS for platelet count and liver enzymes concentrations. VACV had been administered for 7 weeks. Although thrombocytopenia had improved, autopsy confirmed severe disseminated viral infection.

DNA viral load in the amniotic fluid (VLAF) and VLFB, as well as duration of treatment and fetal/neonatal outcomes, are shown in Table 2. There were no significant changes between VLAF sampled before treatment and the VLAF measured at second sampling (median 7 weeks later), and it was not a good indicator of fetal outcome. Both in the fetuses that were subsequently found to be asymptomatic ($n = 10$) and in those in which progressive brain abnormalities were seen ($n = 5$), VLAF levels increased in cases 5 and 3, decreased in cases 1 and 0, and remained unchanged in cases 4 and 2, respectively. There was no correlation between VLAF and fetal outcome ($P = 0.2$, Fisher's exact test).

VLFB decreased significantly in eight of the ten asymptomatic cases (Wilcoxon paired test $P = 0.022$) (Figure 1). In two

Table 1. Pharmacokinetics after oral administration of VACV 8 g/day ($n = 10$) during 4–6 weeks

Area under the plasma concentration time curve (mg/ml/hour)	18.33–38.18			
	Mean	SD	Range	Statistical differences (Student's *t* test)
Maternal blood concentration before the morning dose (T0, μmol)	3.32	2.74	0.57–7.16	
Maximum plasma concentration (C Max) (μmol)	31.73	14.64	7.38–51.62	Higher than T0 ($P = 0.0004$)
Maternal blood concentration (C M) (μmol)	24.63	7.79	6.67–32.08	Higher than T0 ($P < 0.01$) and C FB ($P < 0.01$)
Fetal blood concentration (C FB) (μmol)	17.38	6.52	1.91–12.23	Lower than C AF ($P < 0.01$)
Amniotic fluid concentration (C AF) (μmol)	47.17	23.51	6.14–29.41	Greater than C M ($P < 0.01$)
C M/C FB	6.58	1.52		
Time interval between first maternal ingestion and fetal dosage by cordocentesis (hours)	3.55	0.51		
Clearance (l/hour)	68.78	25.35		

ACV concentrations are determined by high-performance liquid chromatography.[29]

Table 2. Virological and haematological parameters and fetal and neonatal outcomes in 21 fetuses of 20 pregnancies treated with VACV

Case	Gestational age at beginning and end of treatment (weeks)	VLAF (10^3 cp/ml) before/during treatment	VLFB (10^3 cp/ml) before/during treatment	Fetal platelets (×1000/dL) before/during treatment	Ultrasound features at diagnosis/over time	Outcome
1	31, 40	1700/47 000	13/7.7	103/184	Hepatosplenomegaly/ disappearance	Hepatitis at birth, unilateral deafness at 2 years
2	29, 38	40 000/277 000	290/3.2	166/230	Echogenic bowel	Healthy
3	22, 23	83/163	NA/NA	NA/NA	Echogenic bowel	TOP at parental demand
4	24, 26	37 000/13 000	215/7.7	80/NA	Normal/hepatomegaly and ascites	TOP at parental demand
5	25, 28	800/100 000	160/825	156/153	Mild ventriculomegaly that developed microcephaly and hepatomegaly	TOP for evidence of cerebral damage
6	29, 36	40/4400	375/NA	150/180	Brain perivascular calcifications	Healthy
7	29, 36	500/300	260/<250 cp	194/223	Normal/brain perivascular calcifications	Healthy
8	29, 38	1700/875	2.5/6	NA/NA	Echogenic bowel and meconium ileus/hepatomegaly	Healthy
9	29, 37	9/640	<250 cp/<250 cp	24/239	Normal/cerebral echogenic foci, cavitation, and calcifications	TOP at 36 weeks for evidence of cerebral damage
10	27, 30	3200/300	1.6/5.8	189/190	Echogenic bowel/cystic occipital horn lesions	TOP at 30 weeks for evidence of cerebral damage
11	23, 35	250/220	2.2/<250 cp	120/150	Echogenic bowel	Healthy at 2 years
12	23, 35	250/200	1.8/<250 cp	122/162	Echogenic bowel	Healthy at 2 years
13	30, 34	200/1600	1.6/<250 cp	124/147	Periventricular echogenicity and splenomegaly	Healthy at 2 years
14	24, 36	35/700 000	2.7/<250 cp	120/208	Stable microcephaly	Incontinentia pigment, microcephaly + deafness at 8 months
15	24, 25	1000/NA	3.6/NA	115/63	Progressive microcephaly, echogenic bowel and IUGR	TOP at 30 weeks for evidence of cerebral damage
16	27, 33	40/70	0.29/<250 cp	119/191	Echogenic bowel	Healthy at 2 years
17	32, 37	40/1600	18/<250 cp	157/175	Echogenic bowel	Healthy at 1 year
18	26, 33	5600/100	2/<250 cp	143/163	Normal/suspicion of microcephaly	Fetal demise after FBS
19	28, 36	490/2200	2.2/<250 cp	19/106	IUGR + brain perivascular calcifications + liver calcifications + cardiomyopathy + echogenic bowel	Unilateral deafness
20	34, 35	300/700	3.6/<250 cp	147/ NA	Echogenic bowel/multiple cerebral calcifications	TOP at 35 weeks for evidence of cerebral damage
21	31, 38	400/3000	4/380	109/147	Echogenic bowel	Healthy at 6 months

IUGR, intrauterine growth restriction; NA, not applicable.
DNA VLAF and VLFB, duration of treatment, and platelets count are shown.

cases of isolated unilateral deafness, the viral load decreased markedly in one case and remained unchanged in the other case. It also decreased in two cases with an adverse outcome not directly related to CMV infection (incontinentia pigmenti in one and IUFD after FBS in the other). Conversely, VLFB increased in one case that was asymptomatic (case 21). A decrease of VLFB by more than 1 log had a positive predictive value of 78% for a good outcome (defined as an asymptomatic infant with or without moderate unilateral deafness) ($P < 0.05$).

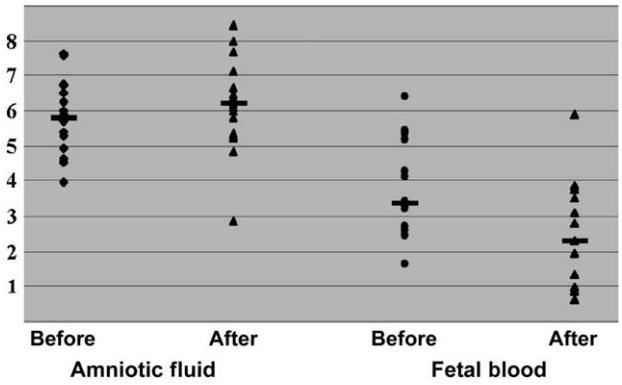

Figure 1. VLAF and VLFB before and after 4–6 weeks of treatment. Viral loads are expressed in 10 log (cp/ml). The bars represent median values. No difference was found in VLAF (Wilcoxon paired $P = 0.097$). VLFB decreased significantly (Wilcoxon paired $P = 0.022$).

In eight fetuses, the platelet count improved after therapy, and after delivery, six of these eight babies were noted to be healthy, and the other two presented with only isolated deafness. In one case (case 19), the fetal platelet count increased, and the only sequela was isolated unilateral deafness despite antenatal demonstration of growth restriction and brain calcification. Ten fetuses had elevated GGT, and in six of these, there was an improvement after therapy. These six neonates were shown to have a normal outcome.

All 21 fetuses had abnormal ultrasound features. Nine of them (cases 1, 2, 3, 4, 8, 11, 12, 16, 17, and 21) (Table 2) presented with extracerebral abnormalities (hyperechogenic bowel [$n = 7$] and hepatomegaly and splenomegaly [$n = 2$]) that resolved with time *in utero*; seven of these nine were asymptomatic at birth and are developing normally.

TOP was requested and eventually performed in five cases with progressive cerebral damage (cases 5, 9, 10, 15, and 20), confirmed on postmortem examination in all five cases.

Microcephaly (cases 5, 14, and 15) developed in three infants. In case 5, mild ventriculomegaly and perivascular calcifications preceded the development of microcephaly after 3 weeks of treatment. In case 14, ultrasound features remained unchanged for 5 weeks, and the otherwise unaffected neonate was diagnosed with incontinentia pigmenti, a rare X-linked dominant neurocutaneous syndrome with microcephaly and bilateral deafness included among its features. In case 15, intrauterine growth restriction, echogenic bowel, and hepatomegaly were subsequently followed by the development of microcephaly after only 1 week of treatment. TOP was requested, and cerebral lesions were confirmed on postmortem examination.

TOP was accepted after parents' decision in these cases with proven congenital infection based on the estimated severity of the disease in all cases but one (case 3). There were seven TOP performed at 25–36 weeks using a combination of feticide by injection of lidocaine in the umbilical vein under ultrasound guidance followed by maternal administration of mifepristone and misoprostol.[30] In six of seven cases, TOP was demanded at diagnosis of abnormalities on ultrasound, and autopsy confirmed the severity of the fetal infection in all these cases. In case 3, although no specific abnormality could be detected prenatally, the parents requested a TOP, as they could not cope with the uncertain outcome. Postmortem examination was declined.

Of the 13 infants born alive, normal clinical examination at the age of 6 months was found in ten infants with a 6–39 months follow up, as assessed by one paediatrician performing general and neurological examinations.

Three infants had neurosensory hearing loss as the sole clinical manifestation of congenital CMV infection, diagnosed at birth in two infants and at the age of 1 year in the third. One (case 14) of these infants also had incontinentia pigmenti as described above.

From 2002, 24 singleton pregnancies that presented at 19–38 weeks of pregnancy with a symptomatic intrauterine infection, but either presented before treatment was offered or declined intrauterine treatment with VACV, were compared with the 21 treated cases. The course of pregnancy and neonatal outcome are described in Table 3. A poor outcome was found in 14/24 (58%) including 12 TOP, 1 severe CID, and 1 IUFD. This was not statistically different from the 38% rate of poor outcome in fetuses treated with VACV (Fisher's exact test, $P = 0.42$).

Discussion

This is the first report on the use of VACV during pregnancy to treat known fetal CMV infection. Based on neonatal data,[3,7] we elected to offer therapy to women whose babies were believed to be at significant risk of adverse postnatal outcome. Prior to offering VACV, we confirmed that all fetuses were infected and symptomatic with ultrasonographic or biological features suggestive of infection and positive amniotic fluid viral PCR results (in case 18, treatment was indicated because of abnormalities of hepatic biology) (cases 6 and 7 were twins, but inclusion criteria were found only in case 6). We provided all potential participants with extensive counselling during which time we reviewed the likelihood of abnormal fetal and neonatal developments based on the presence of fetal signs. We also discussed the uncertainty surrounding both the extrapolation of neonatal data to the fetus and the unknown efficacy of VACV *in utero*. It was made clear to participants that although they may have initially elected to continue with an affected pregnancy, their options remained open. According to French law,[30] they could request TOP based on the results of serial ultrasound examinations and FBS. Treatment could also be stopped on request. All available information on the potential adverse effects of VACV against

Table 3. Virological and haematological parameters and fetal and neonatal outcomes in 24 untreated pregnancies

Case	GA at initial cordocentesis	VLFB (10^3 cp/ml) initial/second sample	Fetal platelets (1000/dL) initial/ second sample	Ultrasound features	Outcome
1	27	5/ND	247/358	Hepatomegaly	Healthy at 3 years
2	34	21.2/0.3	197/277	Echogenic bowel	Healthy at 2 years
3	31	ND	154/ND	Echogenic bowel	Healthy at 2 years
4	23	25 000/NA	238/ND	Hepatomegaly	IUFD
5	38	35/ND	ND	Ventriculomegaly, periventricular calcifications, ascites, hepatosplenomegaly	Severe CID, no long-term follow up
6	36	<250/ND	242/ND	Brain perivascular calcifications	Healthy at 4 years
7	35	ND	ND/305	Decrease of HC growth, calcifications, hepatomegaly	TOP at 37 weeks, confirmed severe brain damage on PM
8	23	55/15	149/ND	Echogenic bowel	TOP at 27 weeks (parental demand), moderate brain damage on PM
9	26	56/ND	82/ND	Echogenic bowel, intracranial calcifications, hepatomegaly, brain perivascular calcifications	TOP at 30 weeks, confirmed severe brain damage on PM
10	24	ND	128/225	Hydrocephaly, brain calcifications, germinative cysts	TOP at 34 weeks, confirmed severe brain damage on PM
11	27	0.9/65	180/ND	Echogenic bowel	Healthy at 6 months
12	26	2/2.2	130/ND	Echogenic bowel	Healthy at 4.5 months
13	25	5/3	235/ND	Echogenic bowel	Healthy at 2 years
14	28	ND	117/ND	Microcephaly, ventricular dilatation, gyri abnormalities, cerebellum abnormalities	TOP at 29 weeks, confirmed severe brain damage on PM
15	19	ND	19/ND	IUGR, oligohydramnios, echogenic bowel, enlarged placenta	TOP at 23 weeks, confirmed severe brain damage on PM
16	36	ND	223/NA	Echogenic bowel	Healthy at 6 months
17	30	ND	165/ND	Echogenic bowel	Healthy at 2 years
18	22	ND	90/ND	Decrease of HC growth, hydrocephaly, brain calcifications	TOP at 28 weeks, confirmed severe brain damage on PM
19	23	ND	44/ND	IUGR, echogenic bowel, pericardial effusion, oligohydramnios, decrease of HC growth, enlarged placenta	TOP at 26 weeks, confirmed severe brain damage on PM
20	21	17	88/ND	Periventricular calcifications, hepatosplenomegaly	TOP at 24 weeks, confirmed severe brain damage on PM
21	24	ND	137/ND	Echogenic bowel, germinative cysts	TOP at 27 weeks, moderate brain damage + hepatomegaly on PM
22	25	10/ND	177/ND	Hepatomegaly, echogenic bowel	TOP at 29 weeks, moderate brain damage on PM
23	26	330/1000	92/90	Echogenic bowel	TOP at 34 weeks, moderate brain damage
24	25	<250/ND	270/ND	Echogenic bowel	Healthy at 2 years

GA, gestational age; HC, head circumference; NA, not applicable; ND, not done; IUGR, intrauterine growth restriction; PM, postmortem examination. DNA VLFB and platelet count per decilitre of fetal blood (initial and second sample), ultrasound features and neonatal outcome or PM after TOP are shown. GA at the time of FBS is noticed.

both mother and fetus was also given. One iatrogenic fetal death occurred during FBS. While FBS as part of a systematic fetal evaluation is controversial, we strongly feel that non-specific biological features such as thrombocytopenia all the more if associated with ultrasound abnormalities were considered of sufficient prognostic importance to justify the additional risk.[3,7,13,31] Pharmacological monitoring of maternal and fetal concentrations of ACV was essential because of the lack of information on blood levels achieved with this dose regimen in pregnancy. We knew that in individuals with HIV co-infection treated with oral VACV in a dosage of 8 g/day, there is a 1.3 log reduction in plasma CMV viral load. Since this dose is associated with a significant decrease in the rate of infection-related visceral complications, it was important for us to establish therapeutic blood levels in the fetus.[20] This modest reduction in CMV viral load may translate into significant clinical benefits if CMV replication is kept below a critical threshold value identified in natural history studies.[32] ACV half maximal inhibitory concentration (IC50) values for CMV found *in vitro* are spuriously high,[33] so the IC50 grossly overestimates the amount of ACV required to inhibit CMV replication *in vivo*. Indeed, concentrations of ACV triphosphate within infected cells may be more than 40 times higher than that found in uninfected cells.[34] Certainly, the absence of adverse effects or teratogenicity reported here, albeit in small numbers, are compatible with its clinical use in pregnancy.[35] The regimen used aimed at ACV levels of at least 20 μmol in amniotic fluid and in maternal and fetal bloods,[35] but the area under the plasma concentration time curve was still lower than previous reports. Absorption and plasma levels in pregnant women are similar to those in non-pregnant women.[34,35] Kimberlin *et al.*[36] reported preliminary pharmacokinetics data from 20 pregnant women receiving suppressive therapy for herpes simplex infection. Peak ACV plasma concentrations after oral administration of VACV 500 mg three times a day were 13.9 ± 3 μmol, with steady-state levels of 13.5 ± 4 μmol. These concentrations are comparable with our data, taking into account differences in the dose regimens used. The ACV and VACV Registry has no reports of developmental abnormalities in more than 600 embryos and fetuses exposed across all trimesters of pregnancy.[17]

Our results demonstrate good placental transfer of ACV and clearly indicate that it is concentrated in the amniotic fluid, most probably because of active transfer across the placenta. However, the drug does not accumulate, as levels seem to drop very rapidly after cessation of treatment (as shown in case 2). After maternal oral administration of VACV, drug concentrations within the expected IC50 were seen in maternal and fetal bloods, in most cases without evidence of maternal or fetal intolerance.

CMV DNA levels in amniotic fluid did not show any consistent trend in relation to outcome. Monitoring VLAF does not appear to be of use for infection follow up. Furthermore, an increase in VLAF with advancing gestation has been previously described without relation to outcome.[11,37]

A decrease in VLFB correlated with a good outcome, even in cases where significant abnormalities were already present prior to beginning treatment. There is, however, some overlap between high and low viral load with good and poor outcomes at the end of treatment. However, these preliminary results suggest that DNA VLFB may be useful in monitoring the evolution of fetal infection. Among the 11 infants with a low VLFB after treatment, 6 of 11 (54%) were asymptomatic whereas 5 of 11 (46%) developed some significant damage. We can hypothesise that treatment was launched when the disease was at different stages even when biological and ultrasound features were similar. It is also very important to consider that individual ability of the virus may be determinant in the damage that it may cause. Furthermore, in infected neonates, diminishing or undetectable levels of viral DNA may be associated with an improved outcome if treatment can be started soon after the onset of fetal infection.[7]

Only 2 of 13 infected fetuses subsequently born alive had signs of congenital or neonatal sequelae clearly associated with congenital CMV infection. Enders *et al.*[38] reported on a series of 57 infected fetuses (where the TOP rate of 31% was similar to that in our study) and showed that 57% of liveborn infants (19/33) had CID. While this difference could be ascribed to selection bias, based on both the seriousness of the presumed fetal infection from our prenatal biological and ultrasound data and the available literature on untreated fetuses, we would have expected many more neonates to have demonstrated symptoms and signs at birth had they not received therapy.

For methodological reasons, it is not possible to assess whether treatment with VACV reduced the severity of disease by comparison with this untreated group. However, it is plausible that information about the possibility to administrate VACV could lead some parents to continue with the pregnancy despite the lack of proven effect regarding the potential reduction of disease. This could only be regarded as fair to them and their fetuses if they reject TOP or if they have the possibility to terminate the pregnancy later whenever more severe objective features of fetal damage develop before birth.

The aim of this pilot study was to prove that 8 g/day of VACV could provide therapeutical concentrations in the fetus. We also established that VACV given in such conditions is likely to provide significant reduction of VLFB although we did not study the natural history of viral load without treatment. We are not the first to report remarkable improvement in expected outcome after *in utero* therapy for confirmed CMV infection. Nigro *et al.*[39] have published the retrospective results of a nonrandomised clinical trial using intravenous CMV hyperimmune globulin for CMV maternal primary infection, suggesting a benefit of treatment. Our study and that of Nigro *et al.*[39] strongly indicate further research on

these two therapeutic modalities with well-designed randomised controlled trials in pregnant women with confirmed fetal CMV infection. The logistical requirements of such a study are daunting, and another option would be to offer treatment as soon as maternal primary infection has been identified. Such a study would require around 125 mothers with primary infection in each arm (assuming a 40% reduction in the fetal infection rate with 90% confidence intervals) and examination of amniotic fluid for CMV infection 6 weeks after seroconversion, this approach would require the prospective serial screening of 17 000 seronegative women. The potential benefit of reducing the rate of fetal transmission is the most cogent argument for multicentre collaboration. The risk–benefit issues around the design of such trials are those regarding screening for congenital infection in pregnancy,[40] but prevention of vertical transmission is more likely to be rewarding than attempting to treat severely infected fetuses. ■

References

1 Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *N Engl J Med* 2001;344:1366–71.

2 Ross SA, Boppana SB. Congenital cytomegalovirus infection: outcome and diagnosis. *Semin Pediatr Infect Dis* 2005;16:44–9.

3 Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. *J Clin Virol* 2006;35:216–20.

4 Ross SA, Fowler KB, Ashrith G, Stagno S, Britt WJ, Pass RF, *et al*. Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. *J Pediatr* 2006;148:332–6.

5 Gaytant MA, Steegers EA, Semmekrot BA, Merkus HM, Galama JM. Congenital cytomegalovirus infection: review of the epidemiology and outcome. *Obstet Gynecol Surv* 2002;57:245–56.

6 Revello MG, Gerna G. Pathogenesis and prenatal diagnosis of human cytomegalovirus infection. *J Clin Virol* 2004;29:71–83.

7 Lanari M, Lazzarotto T, Venturi V, Papa I, Gabrielli L, Guerra B, *et al*. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatrics* 2006;117:76–83.

8 Ville Y. The megalovirus. *Ultrasound Obstet Gynecol* 1998;12:151–3.

9 Nigro G, Mazzocco M, Anceschi MM, La Torre R, Antonelli G, Cosmi EV. Prenatal diagnosis of fetal cytomegalovirus infection after primary or recurrent maternal infection. *Obstet Gynecol* 1999;94:909–14.

10 Lazzarotto T, Varani S, Guerra B, Nicolosi A, Lanari M, Landini MP. Prenatal indicators of congenital cytomegalovirus infection. *J Pediatr* 2000;137:90–5.

11 Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. *Clin Microbiol Rev* 2002;15:680–715.

12 Boppana SB, Fowler KB, Pass RF, Rivera LB, Bradford RD, Lakeman FD, *et al*. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. *J Pediatr* 2005;146:817–23.

13 Istas AS, Demmler GJ, Dobbins JG, Stewart JA. Surveillance for congenital cytomegalovirus disease: a report from the National Congenital Cytomegalovirus Disease Registry. *Clin Infect Dis* 1995;20:665–70.

14 Jacquemard F, Dejean A, Daffos F, Ville Y. Correlations between ultrasound and fetal haematological parameters in 67 fetuses infected with cytomegalovirus (CMV). P09.01 *Ultrasound Obstet Gynecol* 2005;26:429.

15 Kalil A, Levitsky J, Lyden E, Stoner J, Freifeld A. Meta-analysis: the efficacy of strategies to prevent organ disease by cytomegalovirus in solid organ transplant recipients. *Ann Intern Med* 2005;143:870–80.

16 Nigro G, Scholz H, Bartmann U. Ganciclovir therapy for symptomatic congenital cytomegalovirus infection in infants: a two-regimen experience. *J Pediatr* 1994;124:318–22.

17 Kimberlin DW, Lin CY, Sanchez PJ, Demmler GJ, Dankner W, Shelton M, *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.

18 Puliyanda D, Silverman N, Lehman D, Vo A, Bunnapradist S, Radha RK, *et al*. Successful use of oral ganciclovir for the treatment of intrauterine cytomegalovirus infection in a renal allograft recipient. *Transpl Infect Dis* 2005;7:71–4.

19 Beutner KR. Valacyclovir: a review of its antiviral activity, pharmacokinetic properties, and clinical efficacy. *Antiviral Res* 1995;28:281–90.

20 Bell AR. Valacyclovir update. *Adv Exp Med Biol* 1999;458:149–57.

21 Emery VC, Sabin C, Feinberg JE, Grywacz M, Knight S, Griffiths PD. Quantitative effects of valacyclovir on the replication of cytomegalovirus (CMV) in persons with advanced human immunodeficiency virus disease: baseline CMV load dictates time to disease and survival. *J Infect Dis* 1999;180:695–701.

22 Lowance D, Neumayer HH, Legendre CM. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. *N Engl J Med* 1999;340:1462–70.

23 Strippoli GF, Hodson EM, Jones CJ, Craig JC. Pre-emptive treatment for cytomegalovirus viraemia to prevent cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev* 2006;CD005133.

24 Piiparinen H, Helantera I, Lappalainen M, Suni J, Koskinen P, Gronhagen-Riska C, *et al*. Quantitative PCR in the diagnosis of CMV infection and in the monitoring of viral load during the antiviral treatment in renal transplant patients. *J Med Virol* 2005;76:367–72.

25 Hodson EM, Jones CA, Webster AC, Strippoli GF, Barclay PG, Kable K, *et al*. Antiviral medications to prevent cytomegalovirus disease and early death in recipients of solid-organ transplants: a systematic review of randomised controlled trials. *Lancet* 2005;365:2105–15.

26 Picone O, Costa JM, Leruez-Ville M, Ernault P, Olivi M, Ville Y. Cytomegalovirus (CMV) glycoprotein B genotype and CMV DNA load in the amniotic fluid of infected fetuses. *Prenat Diagn* 2004;24:1001–6.

27 Penna L, Bower S. Hyperechogenic bowel in the second trimester fetus: a review. *Prenat Diagn* 2000;20:909–13.

28 Malinger G, Lev D, Zahalka N, Ben Aroia Z, Watemberg N, Kidron D, *et al*. Fetal cytomegalovirus infection of the brain: the spectrum of sonographic findings. *AJNR Am J Neuroradiol* 2003;24:28–32.

29 Boulieu R, Gallant C, Silberstein N. Determination of acyclovir in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997;693:233–6.

30 Senat MV, Fischer C, Bernard JP, Ville Y. The use of lidocaine for fetocide in late termination of pregnancy. *BJOG* 2003;110:296–300.

31 Boppana SB, Pass RF, Britt WS, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *Pediatr Infect Dis J* 1992;11:93–9.

32 Stagno S, Reynolds DW, Tsiantos A, Fuccillo DA, Long W, Alford CA. Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. *J Infect Dis* 1975;132:568–77.

33 Griffiths PD. Tomorrow's challenges for herpesvirus management: potential applications of valacyclovir. *J Infect Dis* 2002;186:S131–7.
34 Weller S, Blum MR, Doucette S. Pharmacokinetics of the acyclovir pro-drug, valacyclovir after escalating single- and multiple-dose administration to normal volunteers. *Clin Pharmacol Ther* 1993;54: 595–605.
35 Tyms AS, Scamans EM, Naim HM. The in vitro activity of acyclovir and related compounds against cytomegalovirus infections. *J Antimicrob Chemother* 1981;8:65–72.
36 Kimberlin DF, Weller S, Whitley RJ, Andrews WW, Hauth JC, Lakeman F, *et al*. Pharmacokinetics of oral valacyclovir and acyclovir in late pregnancy. *Am J Obstet Gynecol* 1998;179:846–51.
37 Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. *Obstet Gynecol* 2000;95:881–8.
38 Enders G, Bader U, Lindemann L, Schalasta G, Daiminger A. Diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. *Prenat Diagn* 2001;21:362–77.
39 Nigro G, Adler SP, La Torre R, Best AM. Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J Med* 2005; 353:1350–62.
40 Peckham C, Tookey P, Logan S, Giaquinto C. Screening options for prevention of congenital cytomegalovirus infection. *J Med Screen* 2001; 8:119–24.

