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Treatment of perinatal viral infections to improve neurologic outcomes

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Viral infections in the fetus or newborn often involve the central nervous system (CNS) and can lead to significant morbidity and mortality. Substantial progress has been made in identifying interventions decreasing adverse neurodevelopmental outcomes in this population. This review highlights progress in treatment of important viruses affecting the CNS in these susceptible hosts, focusing on herpes simplex virus (HSV), cytomegalovirus (CMV), human immunodeficiency virus (HIV), and enteroviruses. The observation that high-dose acyclovir improves mortality in neonatal HSV disease culminated decades of antiviral research for this disease. More recently, prolonged oral acyclovir was found to improve neurologic morbidity after neonatal HSV encephalitis. Ganciclovir, and more recently its oral prodrug valganciclovir, is effective in improving hearing and neurodevelopment after congenital CMV infection. Increasing evidence suggests early control of perinatal HIV infection has implications for neurocognitive functioning into school age. Lastly, the antiviral pleconaril has been studied for nearly two decades for treating severe enteroviral infections, with newer data supporting a role for this drug in neonates. Identifying common mechanisms for pathogenesis of viral CNS disease during this critical period of brain development is an important research goal, highlighted by the recent emergence of Zika virus as a potential cause of fetal neurodevelopmental abnormalities.

Improvement in neonatal mortality has been among the more noteworthy advances in child health over the last 50 y, yet for developmental reasons newborns remain more susceptible to severe disease from infection than older children and adults (1–3). Neurologic involvement is also more frequent, and improvements in survival of both premature and term newborns suggest that interventions which address morbidity of these diseases may take on increasing importance. Persisting neurodevelopmental deficits are associated with infection in the early newborn period, particularly in preterm infants (4). High rates of neurologic deficits are described in patients with perinatal insults, with more than 40% of survivors of herpes, encephalitis, or sepsis in the neonatal period having subsequent neurologic impairment (5). In developed countries, neurologic impairment in children is associated with a substantial and increasing demand for inpatient hospital resources (6).

Vaccines have greatly reduced the prevalence of certain viral pathogens in many populations, including measles, mumps, polio, rubella, and varicella (7–10), all of which can affect the central nervous system (CNS). However, for some medically important viruses for which vaccines are not yet available, antiviral treatment has been found to reduce both mortality and neurologic morbidity. Among these are herpes simplex virus (HSV), cytomegalovirus (CMV), human immunodeficiency virus (HIV), and potentially enteroviruses. This review will focus on studies from the past several decades which have defined the impact of antiviral interventions on neurologic outcomes from these perinatal viral infections involving the CNS (**Table 1**), and discuss additional research needs in this area.

ACYCLOVIR FOR NEONATAL HSV INFECTION

Disease consistent with HSV infection has been described as long ago as Hippocrates (11), and the first reported case of presumed neonatal HSV disease appeared in 1935 (12). Antiviral treatment with the thymidine analogue 5-iodo-2′-deoxyuridine was identified as effective for keratitis in the 1960's (13), and although systemic treatment was attempted in some newborns with apparent success (14), myelosuppressive toxicity and subsequent development of alternative agents ultimately limited its use (15). Through studies driven by Richard J. Whitley and the National Institute of Allergy and Infectious Diseases-sponsored Collaborative Antiviral Study Group (CASG) (16–18), the thymidine analogue acyclovir eventually emerged as the treatment of choice for neonatal HSV disease.

A seminal report from the CASG by Kimberlin *et al.* established the current standard practice of treating neonatal HSV disease involving the CNS or disseminated disease with 20 mg/kg/dose given every 8 h for 21 d (19). This study, conducted between 1989 and 1997, compared data from a trial using a "standard dose" of acyclovir (10 mg/kg/dose) (18) with "intermediate" and "high" doses (15 and 20 mg/kg/dose, respectively). Although designed as a phase II study to evaluate the

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Received 20 April 2016; accepted 2 August 2016; advance online publication 26 October 2016. doi:10.1038/pr.2016.191

Table 1. Key advances in improving neurologic and developmental outcomes of perinatal viral infections

Clinical syndrome	Clinical comparison	Outcome	Reference
Neonatal herpes simplex virus encephalitis	High-dose i.v. acyclovir vs. intermediate and standard doses	Improved mortality and neurologic morbidity	(19)
	Oral acyclovir vs. placebo for 6 mo after completion of initial i.v. course	Improved neurologic outcomes	(23)
Symptomatic congenital cytomegalovirus infection	i.v. ganciclovir vs. no treatment	Prevented deterioration of hearing	(40)
		Fewer developmental delays	(41)
	Oral valganciclovir for 6 mo vs. 6 wk	Improved hearing and developmental outcomes with longer course	(43)
Perinatal human immunodeficiency virus infection	Initiation of combination antiretroviral treatment before 3 mo of age vs. deferred	Improved neurodevelopmental scores at 1 y of age	(50)
	HIV viral load suppression vs. no suppression during infancy or early childhood	Improved neurodevelopmental scores at school age when viral load suppressed in early childhood	(51)
Neonatal enteroviral sepsis	Oral pleconaril vs. placebo for 7 d	Increased survival, faster time to culture and PCR negativity	(67)

HIV, human immunodeficiency virus; PCR, polymerase chain reaction.

safety of higher doses of the antiviral, the results demonstrated a significant survival advantage at 24 mo of age in newborns with disseminated disease treated with high-dose acyclovir when compared with the data from the prior study using a standard dose, though survival was not statistically different based on treatment dose for newborns classified as having CNS disease. Importantly, a greater percentage of patients who received the high-dose regimen were assessed to be developmentally normal at 12 mo of age than in the prior group treated with the standard dose, though again this result did not attain statistical significance. Neutropenia was identified as a fairly common adverse effect of acyclovir (21% of patients with localized disease), though it was transient and did not have apparent sequelae.

Despite the observed improvement in neurodevelopment with high-dose vs. standard dose acyclovir, more than two-thirds of patients with CNS disease had at least mild neurologic morbidity at 12 mo of age, with more than one-third of such patients classified as having severe morbidity (19). Although it was not uncommon for physicians to use oral suppressive acyclovir in newborn survivors of neonatal HSV disease, recurrent encephalitis while on suppressive treatment was reported (20), leading to uncertainty about whether this practice provided benefit on a population level (21,22). This question was ultimately answered in 2011, again by a CASG study, in which improved neurologic outcomes were demonstrated in patients with neonatal HSV and a history of CNS involvement, which were treated with oral acyclovir for 6 mo after their initial i.v. course (23). In a decade-long randomized trial, 45 infants from 19 institutions were enrolled to receive either suppressive oral acyclovir or placebo three times daily for 6 mo, with the primary neurodevelopmental outcome an assessment of Bayley Scales of Infant Development score at 12 mo of age. Of the 28 infants in whom this assessment was made, those patients randomized to acyclovir had statistically significantly higher mean scores (88.2 vs. 68.1, $P = 0.046$), and a higher percentage were reported to have normal neurologic outcomes (69% vs. 33%). This study provided the first controlled data showing that newborns with CNS disease due to HSV have ongoing neurologic injury even after treatment with i.v. acyclovir for 21 d, and that suppressive acyclovir treatment can decrease this injury. However, it is important to recognize that even with suppressive treatment, a significant proportion (~30%) of survivors of neonatal HSV involving the CNS have abnormal neurologic outcomes, suggesting that an improved understanding of the pathogenesis of neurologic injury in this population, and additional treatment interventions, remain important medical goals. Application of molecular diagnostic techniques as a means of providing prognostic information has not proved useful for identifying newborns at risk of abnormal neurologic outcomes as yet; Melvin *et al.* recently reported for HSV encephalitis in newborns that DNA copy numbers in cerebrospinal fluid or plasma did not correlate with neurodevelopmental outcomes (24). There are still not sufficient data to be certain how to use quantitative PCR to best guide prognosis or treatment in these infections.

GANCICLOVIR FOR CONGENITAL CMV INFECTION

CMV is the most common identified congenital infection in the developed world (25), and is the leading viral cause of sensorineural hearing loss and adverse neurodevelopmental outcomes (26). Either symptomatic or asymptomatic congenital infection may lead to morbidity (27–30), leading the Institute of Medicine to classify development of vaccines against CMV infection as a level I (most favorable) priority (31). Primary maternal CMV infection carries a 30–40% risk of vertical transmission, with 0.2–2% of secondary infections (reactivated latent infection or reinfection with a new strain in seropositive women) leading to fetal infection (32–35).

As with acyclovir, the CASG spearheaded the clinical evaluations of antiviral interventions to improve outcomes from symptomatic congenital CMV infection. In the early 1980's, ganciclovir was identified as an inhibitor of CMV replication *in vitro* and in animal models (36), ultimately leading to phase

I/II and phase II trials to establish safe dosing for newborns with symptomatic congenital infection (37–39). Importantly, the phase II trial also demonstrated virologic response and stabilization or improvement in hearing for a subset of infected babies (39), paving the way for a larger, randomized clinical trial to establish efficacy.

The challenging but pivotal CASG trial evaluating efficacy of i.v. ganciclovir for symptomatic congenital CMV infection enrolled 100 patients at 18 centers, and took 8 y to complete (40). In this trial, infants under 1 mo of age with confirmed CMV infection and CNS involvement (microcephaly, intracranial calcifications, abnormal cerebrospinal fluid for age, chorioretinitis, and/or hearing deficits) were randomized to 6 wk of i.v. ganciclovir or no treatment, with the primary outcome measure a comparison of hearing as assessed by brainstem auditory evoked response between baseline and 6 mo follow-up. The difficulty in conducting this study is underscored by the high loss to follow-up: only 42 of the 100 enrolled subjects completed the primary endpoint, with 43 patients completing the secondary endpoint comparing brainstem auditory evoked response at baseline to ≥12 mo follow-up. Despite the limitations of the poor follow-up, this study demonstrated improvement or stabilization in hearing in the "best ear" for 84% of subjects receiving ganciclovir as compared with 59% of controls receiving no treatment ($P = 0.06$), and no hearing deterioration in subjects receiving ganciclovir as compared with deterioration in 41% of controls ($P < 0.01$). There was also less deterioration of hearing in the treated group as compared with controls at 12-month follow-up. Neutropenia as a complication of treatment was common, with about half of participants requiring dose adjustments and one needing to be treated for gram negative septicemia.

This cohort of patients was also studied for developmental outcomes after randomization to i.v. ganciclovir vs. no treatment (41). Participants in the study were evaluated using the Denver II developmental test for attainment of developmental milestones at 6 wk, 6 mo, and 12 mo of age. Developmental testing was completed for most of the patients than in the prior study, with more than 70 patients evaluated at any individual time point and 60 patients completing testing at all three time points. Patients in the "no treatment" group were found to have delays in attaining significantly more milestones than those in the ganciclovir group at both 6 mo and 12 mo, an observation that also held for the individual components of the developmental assessment (personal/social, fine motor, gross motor, and language). To avoid the confounding contribution of hearing loss, the investigators also determined that the difference held when the language component was removed from the analyses. These observations support the conclusion that antiviral treatment of symptomatic congenital CMV infection results in a neurodevelopmental benefit. Importantly, however, the investigators noted that even the treatment group had delays at all three time points relative to population norms, so that ganciclovir treatment did not prevent delays in development for most patients.

While these data supported a role for ganciclovir in treatment of symptomatic congenital CMV infection, the risks of prolonged i.v. treatment and drug toxicity (neutropenia) made thorough discussion of risk and benefit critical in evaluating whether any individual patient should receive ganciclovir for cCMV (40,41). The development of the valyl ester of ganciclovir, valganciclovir, allowed for an oral alternative to be considered for this indication, eliminating the need for long-term i.v. access. After a favorable pharmacokinetic profile of valganciclovir oral solution was established in this age group (42), the CASG conducted a placebo-controlled study comparing a 6 wk course of valganciclovir to a 6 mo course of that medication in 96 newborns with symptomatic congenital CMV infection, with longitudinal follow-up out to 24 mo of age (43). Hearing at 6 mo did not differ between the two treatment groups, but hearing at 12 mo was more likely to be normal or improved in the patients who received the longer course of treatment, a benefit which was maintained at 24 mo. Neurodevelopmental outcomes, assessed in this study using the Bayley Scales of Infant and Toddler Development, were modestly improved in the longer duration treatment group as well. Neutropenia (occurring in 19% of participants) was a common adverse effect associated with the initial 6 wk of treatment when both groups were receiving drug, though this was a lower percentage than in the prior study with i.v. ganciclovir (63%) (40). Interestingly, during the subsequent 4.5 mo when one group continued to receive drug while the other received placebo, comparable percentages of patients in the two groups had grade 3 or 4 neutropenia, suggesting that congenital CMV may itself compromise neutrophil production. Together, these studies support the use of 6 mo of valganciclovir for treatment of symptomatic congenital CMV infection. However, as was observed in the studies of prolonged acyclovir in neonatal HSV, a significant proportion of patients had developmental scores below the normative means, supporting the need for ongoing research to understand the interactions between the viral pathogen and the developing nervous system to improve outcomes.

INFLUENCE OF CONTROLLING PERINATAL HIV INFECTION ON COGNITION

Peripartum antiretroviral (ARV) prophylaxis to decrease mother-to-child transmission of HIV represents one of the major public health successes of recent times; in the United States, vertical transmission has been reduced from ~25% with no treatment to <1% when maternal viral load is reduced below the limit of detection of sensitive PCR assays (44). However, when vertical transmission of HIV occurs, neurocognitive deficits in affected children are well-described even in the setting of suppressive ARV treatment (45).

Studies of neurocognitive outcomes after perinatal HIV infection which used HIV-exposed, uninfected subjects as controls, did not identify cognitive differences between children with HIV and HIV-exposed uninfected children, unless there was a history of an early AIDS-defining illness (46,47). It was not known from these reports whether early initiation of ARV treatment might provide a neurocognitive benefit: the developing brain may be more susceptible to injury after HIV

infection (48), suggesting that minimizing the effects of HIV replication during a developmental window might provide benefit for later cognitive measures.

A prospective cohort study in the Democratic Republic of Congo associated early access to care for HIV-infected children with longitudinal gains in cognitive development; the increase in the mean cognitive score 12 mo after entry into care of infected children between 18 and 71 mo of age was higher among those children presenting "early" for care (defined as prior to eligibility for highly-active ARV treatment, or HAART, using established clinical criteria to determine eligibility for HAART) when compared with patients eligible for HAART at the time of presentation (49). To test whether earlier initiation of ARV treatment would improve neurodevelopmental outcomes of HIV-infected infants, Laughton *et al.* studied South African infants involved in a study randomizing HIV-infected newborns to early ARV treatment (before 12 wk of age) vs. those in whom ARV treatment was delayed until clinical or immunological progression of HIV (50). The mean age of starting ARV treatment in the delayed group was 31.4 wk, and the mean time on ARV treatment before neurodevelopmental assessments were conducted was 40.9 wk in the early ARV group vs. 18.7 wk in the delayed group ($P < 0.01$). Neurodevelopmental scores were significantly higher at 11 mo of age for the 64 HIV-infected infants randomized to early ARV treatment when compared with 26 infants for whom ARV was delayed, supporting the ability of early ARV treatment to improve short-term neurodevelopmental outcomes in HIV-infected infants.

A recent study involving two US-based prospective cohorts of patients with either perinatal HIV infection or exposure reported by participants in the Pediatric HIV/AIDS Cohort Study (PHACS) showed that the benefits to neurodevelopment associated with early ARV treatment may persist into school age (51). Crowell *et al.* showed for 396 perinatally HIV-infected children enrolled in these cohorts that improved neurologic outcomes at school age (mean age of evaluation 9.6 y) were associated with HIV suppression during infancy or early childhood. Again, however, as seen in studies of neonatal HSV infection or congenital CMV infection, even in patients with improved outcomes associated with antiviral treatment, cognitive assessments were on average below population norms.

SEVERE ENTEROVIRAL INFECTION AND PLECONARIL

Enteroviruses are well known causes of CNS illness, with poliovirus perhaps the best known historical example (52). As in the case of decreasing perinatal transmission of HIV through ARV treatment, the near eradication of polio by vaccination efforts represents another significant achievement of modern global public health (53). Despite this success, recent years have seen outbreaks of enterovirus infections causing CNS disease, including enterovirus 71 (54,55) and enterovirus D68 (56,57). Although neurologic disease and neurodevelopmental sequelae in these recent outbreaks are more commonly reported in young children than newborns (55–59), newborns and infants are at higher risk of poor outcomes after enteroviral infection than older children or adults (60,61). There are no licensed antiviral agents for treatment of severe enteroviral disease.

Pleconaril was initially developed as an inhibitor of uncoating and attachment of picornaviruses, including rhinovirus and enterovirus, to host cell receptors (62,63). Administration of pleconaril to 38 patients of a range of ages with severe enterovirus infections under a compassionate use protocol led to clinical, virologic, and radiologic responses in the majority of individuals, including five of six neonates treated for enteroviral sepsis (64). A prior phase I study in newborns with presumed viral sepsis showed no apparent adverse effects, and established a dose for further clinical studies in this population (65).

The pharmacokinetics and efficacy of pleconaril in infants <1 y of age with meningitis due to enterovirus infection was evaluated in a double blind and placebo-controlled trial conducted through the CASG (66). This trial was unable to demonstrate virologic or clinical efficacy, which was attributed to the generally self-limited and clinically benign course of enteroviral meningitis in the population studied. However, in another CASG-organized study, a double blind and randomized controlled trial of pleconaril in newborns with enteroviral sepsis occurring prior to 15 d of life enrolled 61 subjects at 19 centers over more than 10 y period (67). The primary study endpoint was the percentage of patients with a positive viral culture from the oropharynx 5 d after initiating study drug, with secondary and tertiary endpoints including virologic, laboratory, and clinical measures. Patients treated with pleconaril had had negative viral cultures and PCR at multiple anatomic sites more quickly than the placebo group, and data suggested improved survival among those who received pleconaril. The potential for efficacy of pleconaril in this population supports further development of this medication for this indication. Although the study did not evaluate neurologic outcomes of patients, the high concentrations achieved by pleconaril in brain tissue (66) suggest that evaluation of this endpoint could be embedded into future studies with this drug or others with potential efficacy against enterovirus, if their development continues (68).

EMERGING VIRAL INFECTIONS WITH POTENTIAL TO AFFECT NEURODEVELOPMENT IN NEWBORNS

The recent Zika virus epidemic in Brazil, and the association between infection during pregnancy and brain anomalies including microcephaly (69), has attracted widespread attention in the United States and other countries which harbor the *Aedes* mosquito vector. Vector-borne arboviruses are important causes of viral CNS infection in developing countries (70,71), with newborn disease generally rarely reported. In the United States, congenital West Nile virus infection leading to significant neurologic damage was described soon after the epidemic started in the late 1990's (72), and CNS involvement in newborns has been described for chikungunya (73) and Japanese encephalitis virus (74,75). Perinatal infection with California group virus (LaCrosse subtype) has been

associated with subsequent adverse neurocognitive outcomes (76). Perinatal transmission of dengue appears to be uncommon, and the degree to which it is associated with severe outcomes in newborns is unclear (77–80). The global spread of vector-borne viruses, and their potential for CNS involvement, highlights the importance of improving our understanding of underlying pathophysiologic mechanisms which may compromise brain development when viral CNS infection may occur during particularly critical developmental periods.

COMMON THEMES IN PATHOGENESIS OF VIRAL CNS DISEASE IN THE NEWBORN

Differences in immunity have been identified between newborns and adults that may contribute to the enhanced susceptibility of newborns to infection (1–3). Identifying shared mechanisms that may be used by diverse viral pathogens to mediate CNS disease during critical periods of brain development could lead to interventions specifically modulating disease in this age group. For example, using models of HSV encephalitis, we have shown that there may be differences between newborn and adult animals in cellular tropism of virus within the CNS (81,82), in production of inflammatory mediators in brain tissue (82,83), and in intrinsic cellular antiviral responses in the CNS (84). This latter study in particular provides an important example of the need to distinguish newborns from older animals in pathogenesis studies: we found that autophagy during HSV infection the newborn brain was significantly less important than in adults for protection from mortality, suggesting that proposed pharmacologic strategies for HSV encephalitis to augment autophagy (85) might be protective in older but not younger brains.

Additional important common mechanistic themes emerge from considering perinatal CNS infection with these different viruses. Direct infection of neurons does not appear to be required for adverse cognitive outcomes, as neither CMV nor HIV primarily target neurons, and there may also be a contribution to disease in newborns from glial cell infection with HSV (81) and enterovirus (86). Results from murine models of neonatal HSV (83) and congenital CMV (87,88) infection suggest that host inflammation may induce at least some of the observed CNS pathology, as opposed to direct effects of virus infection and replication. However, although inflammatory responses may contribute to CNS disease in the young host, the clinical studies discussed in this review are evidence that persisting viral replication also leads to ongoing damage, as for at least the herpesviruses, prolonged treatment with antivirals improves neurodevelopmental outcomes (23,43). Infection of neural progenitors and immature neurons may be important to outcomes in the developing host, particularly cells originating from periventricular areas near the choroid plexus, with evidence of infection in these cells observed in model systems for HSV (82), CMV (89–91), HIV (92,93), and enteroviruses (94–96). There is also evidence that Zika virus can target human neural stem and progenitor cells in the fetal cortex, potentially explaining the defects in brain development associated with infection *in utero* (97). Further understanding of age-dependent differences in host-pathogen interactions may lead to interventions that target specific, developmentally-regulated pathways of host responses or viral replication.

The clinical trials of antiviral treatment for viral CNS infection in newborns highlighted in this review share an important further similarity: in general, studies of these relatively uncommon diseases in newborns are very difficult to conduct. The long duration required to enroll sufficient numbers to provide statistical power, and the long follow-up periods needed to assess certain developmental outcomes, frequently led to limitations in assessing outcome measures due to loss to follow-up (19,23,40), and one study even needed to be terminated prior to completing accrual (67). Longitudinal follow-up in these populations may be challenged by demographics, as risk factors for neonatal infection with many of the pathogens discussed include socioeconomic factors associated with difficulty in accessing health care. Multicenter studies are required to accrue sufficient numbers of patients, and large organizational structures such as the CASG, Comprehensive International Program for Research in AIDS (involved in the infant HIV neurodevelopmental study (50)), and PHACS network are critical to successful completion.

GLOBAL RESEARCH NEEDS FOR PERINATAL CNS INFECTIONS

Despite the advances in the treatment of perinatal CNS infection discussed in this review, the persisting neurologic deficits associated with such insults result in a significant and rising burden of disease globally (98), putting strain on health care resources of countries at all economic levels (6,99). All tiers of research—basic, translational, clinical, and population-level – need continued financial investment to facilitate development and implementation of effective interventions (100). Continued support for organizations capable of supporting multisite trials, such as the CASG, the Comprehensive International Program for Research in AIDS, and PHACS, is critical to ensuring the clinical study infrastructure needed to study new interventions to improve newborn outcomes. Bench studies of common and emerging viruses that can affect the developing CNS may promote a better understanding of shared mechanisms of pathogenesis and could identify additional interventions for treatment or prevention.

STATEMENT OF FINANCIAL SUPPORT

No financial assistance was received to support this study.

Disclosure: None.

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