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An animal model of neonatal cytomegalovirus infection

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Abstract

Cytomegalovirus (CMV) is the most common cause of congenital infection in the developed world and can lead to a life-threatening disease. We therefore developed an animal model to evaluate candidate anti-CMV drugs and to further define the pathogenesis of CMV infections. Newborn guinea pigs were infected by intraperitoneal administration of 10^6 pfu of a virulent salivary gland (SG) passaged guinea pig CMV (gpCMV) within 48 h of birth. Inoculation of animals produced 50% overall mortality. A lack of weight gain was also a hallmark of infection. By day 14 after inoculation the weight of gpCMV-infected animals was significantly less than controls (152.9 ± 45 g versus 254.7 ± 38.5 g, $P < 0.0001$). The most consistent isolation and highest titers of virus were found in the liver and spleen early while lung titers were maximal at day 10. A quantitative competitive PCR (qcPCR) assay confirmed the presence of a high CMV viral load in infected organs. Antiviral treatment with cyclic HPMPC (cHPMPC) for 7 days significantly reduced mortality (1/20 versus 14/20, $P < 0.001$) and viral replication but did not improve weight gain. This model should be useful for further evaluations of the pathogenesis of CMV infections and for evaluation of antiviral drugs and vaccines.

Keywords: Guinea pig cytomegalovirus; cHPMPC; Animal model; Congenital CMV; Neonatal CMV

1. Introduction

Cytomegalovirus (CMV) is the most common congenital infection in the developed world occurring in 1–2% of all live births; nearly 40,000 cases occur annually in the USA (Demmler, 1991; Gaytant et al., 2002). Approximately 10% of congenitally infected infants are symptomatic at birth with symptoms ranging from mild neurologic signs and transient hepatitis to a devastating disease with microcephaly and severe neurologic sequelae (Boppana et al., 1992; Bale et al., 2002). Death occurs in up to 30% of symptomatic infants and more than 90% of those who survive develop severe brain damage and/or profound hearing or visual problems (Alford and Britt, 1993). Further, even infants who are asymptomatic at birth can experience long-term progressive hearing deterioration (Hickson and Alcock, 1991; Dahle et al., 2000).

CMV infections of premature infants can also lead to a disseminated and life-threatening disease (Yeager et al., 1983; Ballard et al., 1979; de Cates et al., 1994; Vochem et al., 1998). While improvements in screening of blood products have reduced the problem of blood transfusion acquired CMV infection, transmission by breast milk remains an important risk factor for premature infants (Vochem et al., 1998; Hamprecht et al., 2001). Moreover, perinatally acquired CMV can be identified as the etiology in up to 20% of young infants hospitalized with pneumonitis (Stagno et al., 1980, 1981; Whitley et al., 1976; Hamprecht et al., 2001). We therefore sought to develop a model that would mimic perinatal and/or congenital CMV infection and be useful for antiviral evaluations.

A variety of antiviral therapies are available for CMV infection, although clinical experience with these agents is largely limited to immunocompromised patients (Schleiss, 2002). Ganciclovir (GCV) has been utilized for newborns with life-threatening CMV disease, with anecdotal reports of efficacy. Although use of GCV under these circumstances may be life-saving, it is less clear if antiviral therapy attenuates the neurodevelopmental sequelae of congenital CMV infection. A recently completed trial evaluating 6 weeks of intravenous GCV in congenitally infected symptomatic infants with evidence of CNS involvement suggested that antiviral therapy might prevent the development or progression of sensorineural hearing loss (SNHL) in some infants (Kimberlin et al., 2000). However, the long-term benefits of this therapy for SNHL, as well as the role of GCV in

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preventing or attenuating other forms of CNS injury, are unclear. Clearly, further development of CMV antivirals is needed.

Because CMV is species specific, preclinical evaluations of candidate therapies most often require the use of animal's CMV species. Murine and rat cytomegalovirus (mCMV and rCMV) models for the evaluation of antiviral agents have been well described (Kern, 1991; Stals et al., 1990). However, mCMV and rCMV do not cross the placenta, and there is limited information about the pathogenesis of infection in neonatal animals. In contrast to the CMVs of most small mammals, the guinea pig CMV (gpCMV) crosses the placenta, infecting the pup in utero (Bernstein and Bourne, 1999). A guinea pig model of congenital CMV infection has been used by us (Bourne et al., 2001; Bratcher et al., 1995) and others (Chatterjee et al., 2001; Harrison et al., 1995; Bia et al., 1984) to evaluate the effects of vaccines and passive antibody. This model is, however, less amenable to the evaluation of anti-CMV drugs because congenitally infected pups are usually stillborn or die shortly after birth. We therefore developed a guinea pig model using newborn guinea pigs expecting that infection would lead to dissemination and severe disease because we had previously shown that newborn guinea pigs are more susceptible to herpes simplex virus infections than adult animals (Mani et al., 1996). Previously, Griffith et al. (1985) used newborn guinea pigs (3–13 days of age) inoculated with a sublethal dose of gpCMV to assess the effect of maternally derived immunity. In this manuscript, we describe a lethal neonatal model of gpCMV infection and show its utility for evaluating an antiviral drug, cyclic HPMPC (cHPMPC) (Bischofberger et al., 1994; Bourne et al., 2000).

2. Material and methods

2.1. Virus

Guinea pig cytomegalovirus (gpCMV; strain-22122, American Type Culture Collection, Rockville, MD) was used to prepare a salivary gland (SG)-derived gpCMV stock of high virulence by sequential in vivo passage in male strain 2 guinea pigs as described previously (Harrison and Myers, 1988). SG gpCMV homogenates were clarified by centrifugation and stored frozen (−80 °C) in aliquots. A SG passage 10 work pool was used for these experiments.

2.2. Guinea pigs

Male strain 2 guinea pigs (Cincinnati Children's Hospital Research Foundation, Cincinnati, OH) were used for the production of the SG passaged virus used for challenge (Harrison and Myers, 1988). Hartley guinea pigs (Harlan, Indianapolis, IN) were obtained during the second/third trimester of pregnancy and tested to confirm that they were gpCMV seronegative as previously described (Bourne et al., 2001). Animals were housed under AAALAC approved facilities and all procedures were approved by the Institutional Animal Care and Use Committee.

2.3. Antiviral

Cyclic HPMPC (cHPMPC; 1-[((*S*)-2-hydroxy-2-oxo-1,4,2,-dioxaphosphorinan-5-yl)methyl]cytosine dihydrate) the cyclic congener of HPMPC (cidofovir), was supplied by Gilead Sciences Inc. (Foster City, CA) and was prepared for in vivo administration as described previously (Bravo et al., 1993).

2.4. Experimental design

Hartley newborn guinea pigs were inoculated within 48 h of birth with 0.5 ml of SG gpCMV or 0.5 ml of clarified SG homogenate from uninfected animals by intraperitoneal (i.p.) injection. For the initial evaluation of the model, animals were randomized by litters to receive virus or control tissue culture.

In experiment 1, 17 animals received 10^6 pfu SG gpCMV, 8 animals received 10^5 pfu SG gpCMV, and 7 served as controls. Animals were observed daily for signs of disease and mortality and weight gain was measured every other day for 20 days.

In experiment 2, 24 animal received 10^6 pfu SG gpCMV and 7 served as controls. Half of the infected animals were sacrificed on days 3 and 6 to evaluate virus replication in selected organs and the remaining 12 were followed as described above.

In experiment 3, 37 animals were inoculated with 10^6 pfu SG gpCMV and 6 served as controls. Four animals were sacrificed on days 6 and 12 were sacrificed on day 10 for virus replication studies. The remaining 21 animals were followed for death only.

For evaluation of cHPMPC, 40 newborn animals were randomized individually within litters into two treatment groups receiving either 5 mg/kg/day cHPMPC or placebo (0.9N sterile saline solution) once daily for 7 days by i.p. injection. This regimen has been shown to reduce the known nephrotoxicity associated with HPMPC, and to a lesser extent with cHPMPC (Bravo et al., 1993; Bourne et al., 2000). Treatment was initiated 24 h after viral inoculation. Animals were followed and weights and survival assessed for 20 days. The effect of antiviral therapy on viral replication in tissues was assessed in 24 additional pups treated with placebo or cHPMPC as detailed above. The spleen and liver of infected animals were harvested from animals on days 6 and 10 after viral inoculation and viral titers determined by plaque assay as described below.

2.5. Viral cultures

Guinea pig lung fibroblast (GPL) monolayers (American Type Culture Collection, cell line CCL-158, Rockville, MD) were grown and maintained with F-12 media (Invitrogen

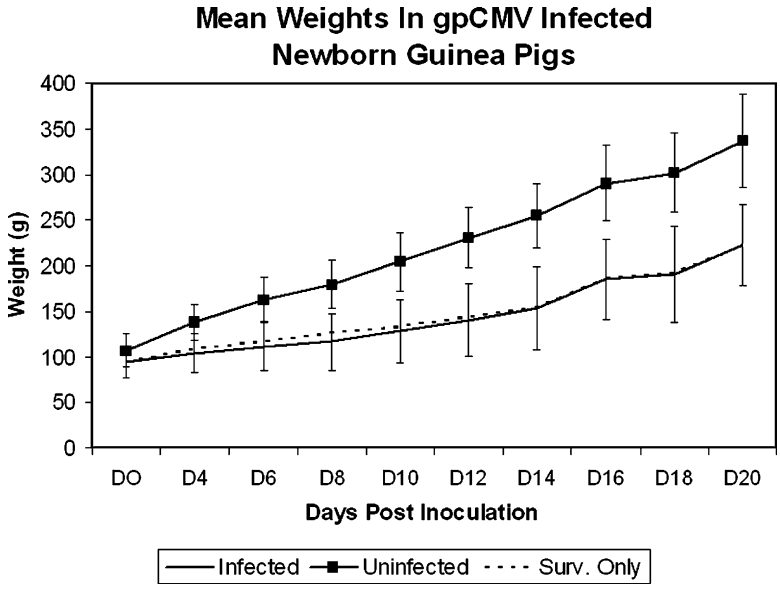


Fig. 1. Mean body weights in newborn guinea pigs infected ($n = 29$) with gpCMV within 48 h of birth and controls ($n = 14$). Weights of animals that survived ($n = 15$) in the gpCMV-infected group are also shown. Standard deviations are also shown.

Corporation, Grand Island, NY) supplemented with10% fetal bovine serum (Hyclone, Logan, UT) and penicillin/streptomycin 10,000 units/ml (Invitrogen Corporation). Animals were sacrificed on days 3, 6, or 10 post-inoculation (PI) and specimens from the liver, spleen, lung, and brain were homogenized (1:10 tissue/volume) using approximately 100 mg of tissue. Twelve-well plates with confluent GPL monolayers were used to inoculate serial dilutions of the clarified supernatant with 200 μl/well. Virus was quantified by counting plaques after staining with crystal violet. Samples with no identified plaques were assigned a titer of 10^2 pfu for the comparison of geometric mean viral titers in treated and untreated animals.

2.6. DNA extraction, PCR analyses, and histopathology

Tissue homogenates from day 10 samples were extracted using the Qiagen QIAamp DNA mini kit DNA extraction system according to the manufacturer's specifications. Eluted DNA (1% of sample) was subjected to quantitative competitive PCR (qcPCR) analysis. Briefly, the primer pair UL83F6 (5′-CGACGACGACGATGACGAAAAC-3′) and UL83B11 (5′-TCCTCGGTCTCAACGAAGGGTC-3′) amplifies a 248 base pair (bp) region, corresponding to Asp_{400} through Arg_{482} of the UL83 protein (Schleiss et al., 1999). This plasmid was modified by engineering a 91 bp internal deletion. The resultant clone served as an

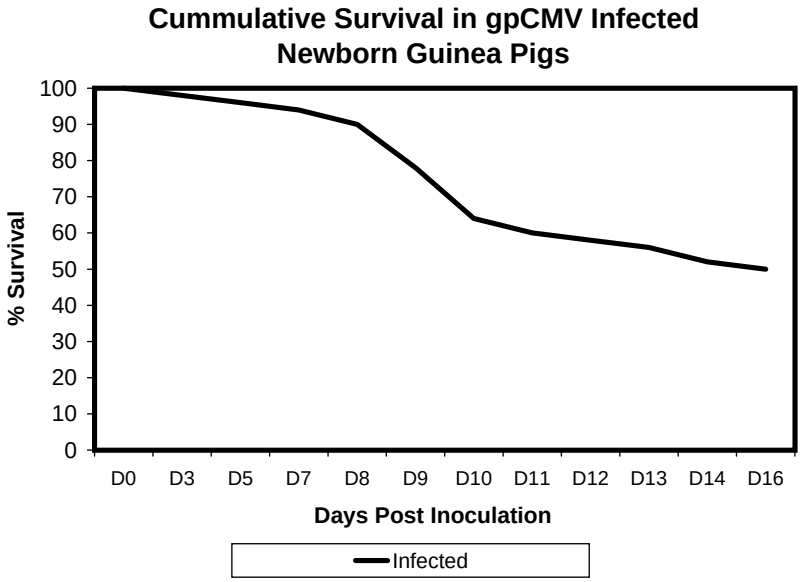


Fig. 2. Survival of gpCMV-infected guinea pigs ($N = 50$). The overall the mortality rate was 50%.

internal standard (IS) for qcPCR. A standard curve was generated by measuring the ratio of relative signal intensity of amplification products on ethidium bromide-stained gels for increasing amounts of full-length plasmid with IS. The signal intensity of the experimental standard was then compared to this standard curve to quantify the total copy number (gpCMV genome equivalents)/mg tissue extracted.

Selected tissues harvested from day 10 samples were also processed by fixation in 10% buffered formalin. Following fixation, tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and subjected to microscopy.

2.7. Statistics

Weights are shown as mean ± S.D. and compared by Student's *t* test. Mortality was compared by Fisher's exact test. All comparisons were two-tailed.

3. Results

3.1. Description of the model

Intraperitoneal (i.p.) administration of the virus was selected based on reports that challenge by this route produced

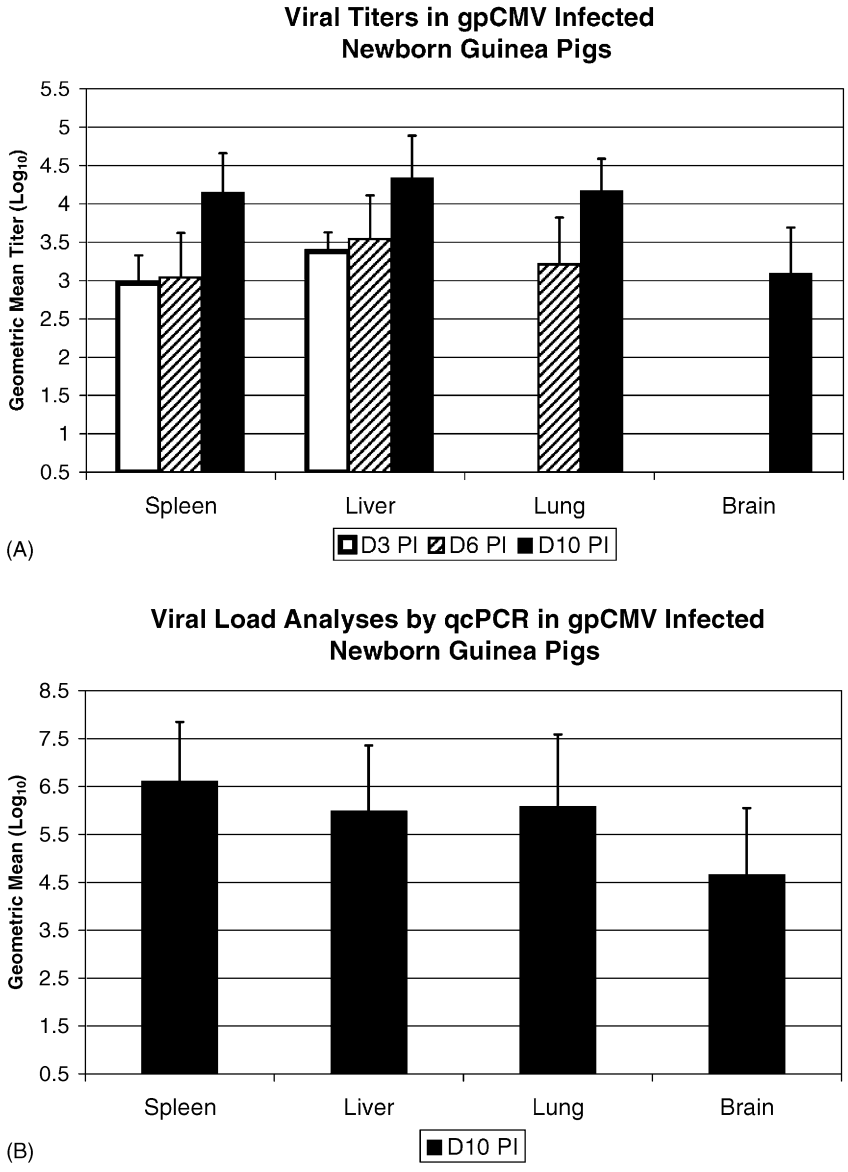


Fig. 3. (A) Geometric mean viral titers ($\log_{10}$) in organs harvested from infected newborn guinea pigs on day 3 ($n = 6$), day 6 ($n = 10$), and day 10 ($n = 12$) post-inoculation. Specimens of spleen, liver, lung, and brain were homogenized and plated on guinea pig lung monolayers and plaques counted to determine the titer per gram of tissue. (B) Mean viral load assessment by qcPCR (genome equivalents/mg) in tissues harvested from infected animals ($n = 10$), 10 days PI. Viral load is similarly distributed in liver, lung, and spleen but is lower in brain.

a more disseminated mCMV infection in mice (Shanley et al., 1993) and because preliminary evaluation of intranasal challenge showed that it did not produce a consistent infection (data not shown). The initial experiment indicated that 10^6 pfu of gpCMV was required to produce mortality of approximately 50% in newborn guinea pigs (Table 1).

Lack of weight gain was an important characteristic of the model. By day 6 PI, the difference in weights (Fig. 1) was significant comparing infected (111.5 ± 26.5) and control animals (163.0 ± 29.3 g, $P < 0.0001$). By day 14 differences were maximal for infected animals (152.9 ± 45.7) versus control animals (254.7 ± 38.5, $P < 0.0001$). Even animals that survived the infection had significantly delayed weight gain (Fig. 1). Thus, weight gain was an independent and more sensitive indicator of disease. Death occurred between days 3 and 16. The peak rate of mortality was seen between days 8 and 10, with a mean day of death of 9.8 days (Table 1 and Fig. 2). Mortality in the three natural history of infection studies was consistent, ranging from 42 to 53%.

3.2. Virology and histology

Virus was isolated from all of the tissues examined by culture (Fig. 3A). The most consistent recovery and the highest overall yield were obtained from the spleen and liver. Lung cultures were negative on day 3 PI. Virus was not recovered from brain cultures until day 10 PI.

Analyses of viral load by qcPCR assay in tissues extracted at day 10 PI confirmed that spleen and liver were targets for significant viral replication. The mean viral load by PCR was highest in spleen, although high quantities of viral DNA were observed in other organs, including brain (Fig. 3B).

Histopathological analyses indicated that gpCMV induced widespread inflammatory changes. In lung tissue, evidence for interstitial pneumonitis was present, including thickened alveolar septae, inflammatory infiltrates, and viral inclusions (Fig. 4A). Evidence of end-organ CMV disease was also noted in liver, spleen (Fig. 4B), and brain.

Table 1
Mortality following SG gpCMV infection of newborn guinea pigs

	Number	Dead (%)	Mean day of death
Experiment 1			
SG gpCMV[a]	17	9 (53)	11.0
SG 1:10	8	1 (12.5)	
Control	7	0	
Experiment 2			
SG gpCMV	12	5 (42)	11.2
Control	7	0	
Experiment 3			
SG gpCMV	21	11 (52)	8.3
Control	6	0	
Combined			
SG gpCMV	50	25 (50)	9.8
Control	20	0	

[a] Animals received either 10^6 pfu of SG gpCMV or a 1:10 dilution, 10^5 pfu SG gpCMV by intraperitoneal injection.

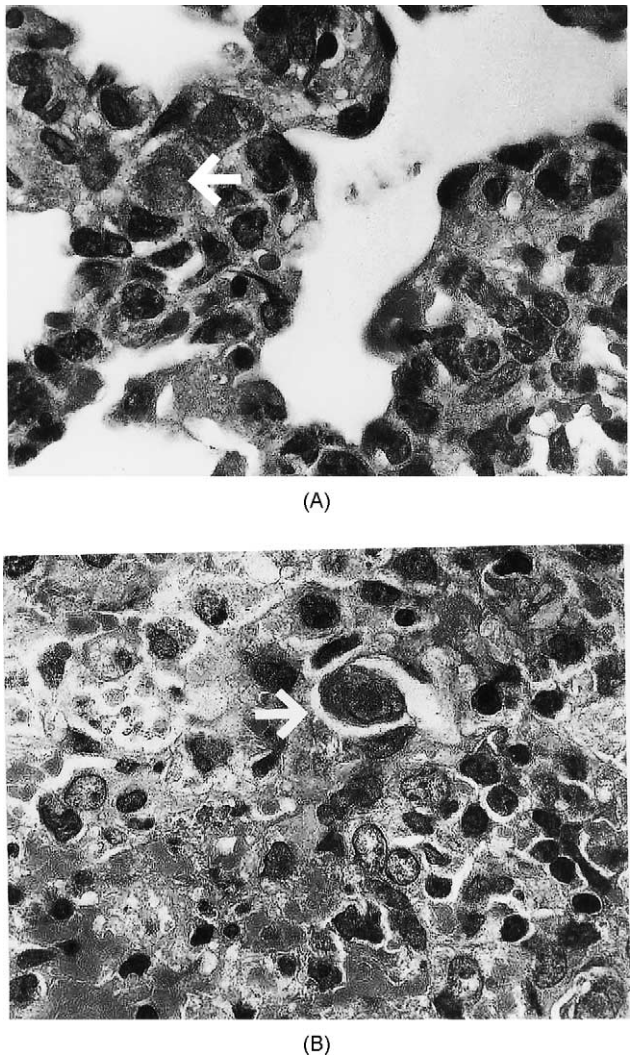
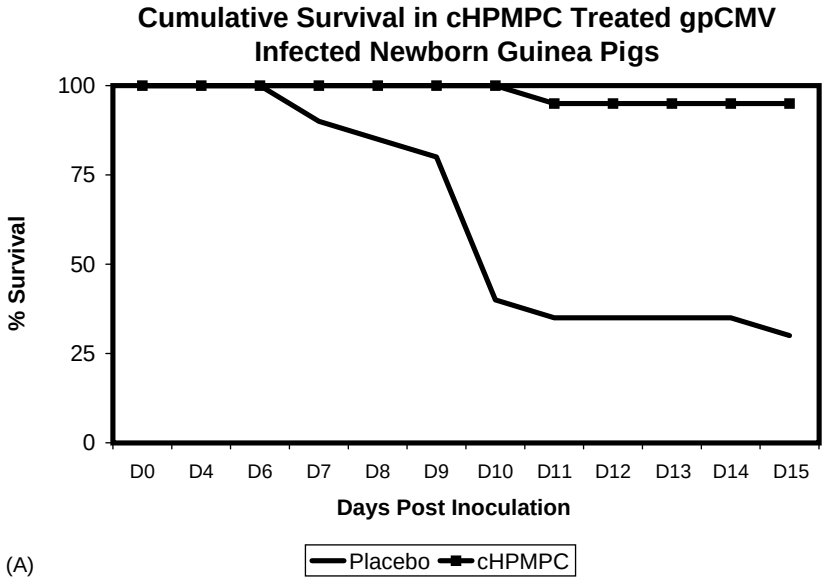


Fig. 4. Histopathology of disseminated gpCMV infection in newborn guinea pigs. (A) Photomicrograph of lung from animals sacrificed 10 days PI. Interstitial pneumonitis is present, with inflammatory cell infiltrate and viral inclusions (arrow) noted. (B) Spleen from day 10 animal. Follicular hyperplasia and inflammatory infiltrate noted. Also, noted is pathognomonic viral inclusion body (arrow).

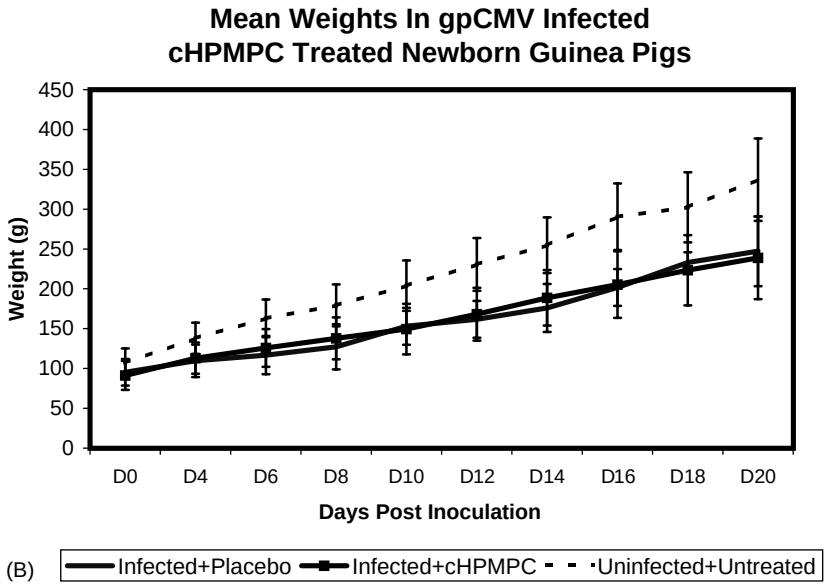
3.3. Evaluation of cHPMPC

cHPMPC therapy protected neonatal guinea pigs from death (1/20 versus 14/20 for controls, $P < 0.001$, Fig. 5A) but it did not appear to have an effect on weight gain (Fig. 5B). To further study the effect of cHPMPC treatment in this model, viral replication was evaluated in the spleen and liver of treated and control animals (Table 2). Significant differences were found in the viral replication in the liver on day 6 and in both the liver and spleen on day 10 further documenting the effectiveness of this therapy.

Because significant delays in weight gain were seen in each of the previous experiments and because both the



(A)



(B)

Fig. 5. Results of treatment of gpCMV-infected newborn guinea pigs with cHPMPC or saline solution begun 24 h after inoculation and continued for 7 days. (A) Percent survival of newborn pups. Although no significant differences were observed in weights among surviving pups, highly significant differences were noted in pup mortality ($P < 0.001$). (B) Mean body weight with standard deviation. The untreated uninfected control group from Fig. 1 is shown for comparison purposes only.

Table 2
Effect of cHPMPC therapy on viral replication in the spleen and liver

Group	Day	Number	Organ	Culture positive	Viral titer (S.D.)
Placebo	6	6	Spleen	4/6	3.4 (1.2)
			Liver	5/6	3.8 (0.9)*
cHPMPC	6	6	Spleen	2/6	2.6 (0.9)
			Liver	1/6	2.1 (0.2)*
Placebo	10	6	Spleen	5/6	4.2 (0.9)*
			Liver	6/6*	4.5 (1.2)
cHPMPC	10	6	Spleen	2/6	3.5 (0.1)*
			Liver	0/6*	

* $P < 0.05$ comparing placebo to cHPMPC.

treated and the untreated groups in the previous experiment had delayed weight gains compared to the uninfected groups in the previous experiments, we also examined whether cHPMPC therapy alone would interfere with weight gain. Ten uninfected newborn guinea pigs were divided into two groups to receive either cHPMPC at the same dose and schedule as the infected animals described above or placebo. As is seen in Fig. 6, cHPMPC therapy produced a delayed weight gain that was seen only after the drug had been discontinued. Thus, on day 14 and continuing until day 20 the weight of drug-treated animals was significantly lower ($P < 0.05$) than placebo controls.

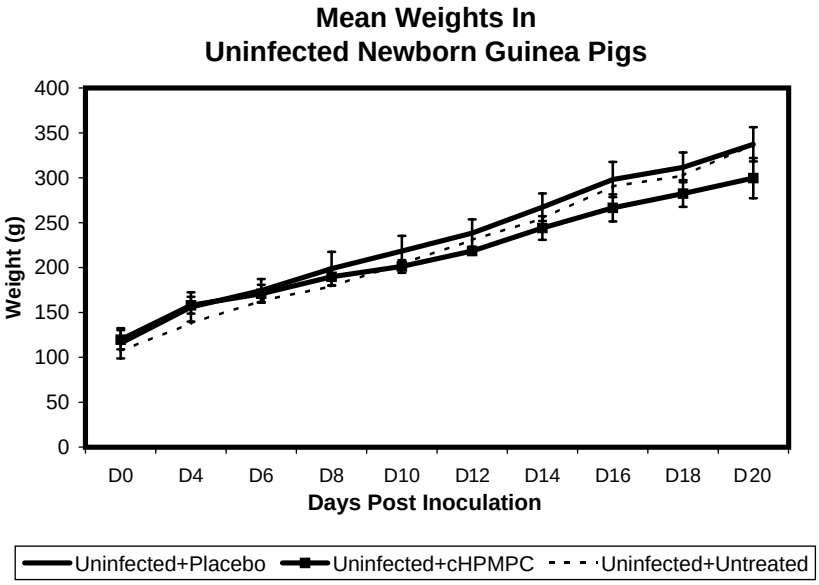


Fig. 6. Mean body weights of uninfected newborn guinea pigs treated with cHPMPC or saline solution for 7 days. The untreated uninfected control from Fig. 1 studies is shown for comparison purposes only. At day 20 PI, the weight in treated animals was significantly less than placebo-treated animals.

4. Discussion

As the leading infectious cause of mental retardation and deafness, the morbidity associated with congenital CMV infection is significant. The public health impact of congenital CMV is further emphasized by the fact that the development of effective vaccines and vaccination strategies are considered in the highest priority category by the Institute of Medicine (Stratton et al., 2001). While control of this disease may ultimately be achieved by immunization, this is unlikely to be accomplished in the near future. Therefore, the continued development of safe, effective anti-CMV drugs is of importance. Although most infants with congenital CMV infection are asymptomatic and may not be identified, antiviral treatment may be important in improving the outcome of severely infected and premature babies. To date, there are only a few reports on the use of antiviral in these infants.

Most recently the results of a study evaluating intravenous GCV indicated that antiviral therapy could improve the outcome of infants that were symptomatic at birth and had evidence of CNS involvement. In this trial, a 6-week course of intravenous GCV produced a reduction of CMV in the urine and stabilization or improvement of the hearing loss (Kimberlin et al., 2000; Whitley et al., 1997). The potential benefits of GCV, however, must be balanced against the toxicity of GCV. Ganciclovir is myelosuppressive, producing neutropenia and thrombocytopenia, potentially dose-limiting side-effects associated with long-term use in infants (Whitley et al., 1997). Further, administration of GCV in neonates requires long-term i.v. access. Thus, the development of effective antivirals that are orally bioavailable and that exhibit lower toxicity than GCV appears necessary in order for this strategy to be employed more widely. Because it is not possible to use the guinea pig model of congenital infection for the evaluation of new antivirals, we sought to develop a model that might mimic congenital infection but be better suited to antiviral evaluations.

Newborn guinea pigs have immature immune systems and are, therefore, more susceptible to other herpesvirus infections. For example, following intranasal inoculation of guinea pigs with herpes simplex virus type 2, virus disseminates more frequently and mortality is higher if animals are inoculated within 48 h of birth compared to 1 week of age while dissemination and death are only rare events in adult animals (Mani et al., 1996). If dissemination, morbidity, and mortality were also increased following CMV infection, a neonatal guinea pig model could prove useful in the study of the pathogenesis of congenital infection and as a model for intervention strategies. In addition, it would serve as a model for the severe outcome that can be seen following CMV infections of premature (Yeager et al., 1983) and healthy term infants (Kumar et al., 1984).

Our results indicate that infection of newborn guinea pigs with gpCMV leads to rapid dissemination of the infection to the liver and spleen with a later infection of the lungs. The finding of severe interstitial pneumonitis is similar to that reported in experimentally infected strain 2 guinea pigs (Bia et al., 1982), and is analogous to the pneumonitis observed in newborns with perinatally acquired HCMV disease (Ballard et al., 1979; Kumar et al., 1984), underscoring the relevance of this model to disease in infants. It is of additional relevance that the brain was an important target organ for infection in our model, with significant recovery of gpCMV by culture, and high viral load by qcPCR.

In the studies reported here, the mortality averaged 50% over three experiments with a mean day of death of about

10 days. The weight gain of animals was also significantly delayed providing another useful endpoint for antiviral evaluations as was previously seen by other investigators following gpCMV inoculation of young animals (Zheng et al., 1987). Our evaluations also showed that the anti-CMV drug cHPMPC was effective in reducing mortality and viral replication in this model. This finding substantiates the previous finding that cHPMPC had activity in an immunocompromised model of CMV infections (Bourne et al., 2000). Although therapy improved survival, it did not improve weight gain when compared to untreated animals.

One possible explanation for the lack of weight gain in cHPMPC-treated newborn animals is that treatment may be effective in preventing the most severe outcome of infection, death, but not more subtle outcomes, such as weight gain. Such an outcome may indicate that a drug is only marginally effective against CMV. Another explanation may be that the failure of cHPMPC to improve weight gain is due, at least in part, to drug toxicity. The evaluation of weight gain in treated but uninfected animals revealed that cHPMPC produced a significant decrease in weight gain but one that was more modest than that seen following cHPMPC treatment of CMV infection in newborn animals. This would suggest that the poor weight gain in CMV-infected and -treated animals was due to both a mild toxicity and less than optimal efficacy of the drug.

In summary, we have developed a model of neonatal gpCMV infection, and applied it to the study of antiviral therapies. Experimentally infected pups exhibit weight loss, extensive end-organ (including CNS) gpCMV infection, high viral loads, and mortality. These features of the model make this a uniquely valuable system for the study of antiviral therapies for neonatal CMV infections. The gpCMV neonatal infection model shares strong similarities to disseminated HCMV disease in human infants, including pneumonitis, hepatitis, and CNS injury. This system should prove useful for the evaluation of new anti-CMV antivirals, and may provide further insight into the pathogenesis and CNS manifestations of congenital and perinatal CMV infections.

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References

Alford, C.A., Britt, W.J., 1993. Cytomegalovirus. In: Roizman, B., Whitley, R.J., Lopez, C. (Eds.), The Human Herpesviruses. Raven Press, New York, pp. 227–255.

Bale, J.F., Miner, L., Petheram, S.J., 2002. Congenital cytomegalovirus infection. Curr. Treat. Options Neurol. 4, 225–230.

Ballard, R.B., Drew, W.L., Hufnagle, K.G., Riedel, P.A., 1979. Acquired cytomegalovirus infection in preterm infants. Am. J. Dis. Child. 133, 482–485.

Bernstein, D.I., Bourne, N., 1999. Animal models for cytomegalovirus infection: guinea-pig CMV. In: Zak, O., Sande, M.E. (Eds.), Handbook of Animal Models of Infection. Academic Press, San Diego, pp. 935–941.

Bia, F.J., Lucia, H.L., Fong, C.K., Tarsio, M., Hsuing, G.D., 1982. Effects of vaccination on cytomegalovirus-associated interstitial pneumonia in strain 2 guinea pigs. J. Infect. Dis. 145, 742–747.

Bia, F.J., Miller, S.A., Lucia, H.L., Griffith, B.P., Tarsio, M., Hsiung, G.D., 1984. Vaccination against transplacental cytomegalovirus transmission: vaccine reactivation and efficacy in guinea pigs. J. Infect. Dis. 149, 355–362.

Bischofberger, N., Hitchcock, M.J., Chen, M.S., Barkhimer, D.B., Cundy, K.C., Kent, K.M., Lacy, S.A., Lee, W.A., Li, Z.H., Mendel, D.B., 1994. 1-[((*S*)-2-Hydroxy-oxo-1,4,2,-dioxaphosphorinan-5-yl)methyl]cytosine, an intracellular prodrug for (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine with improved therapeutic index in vivo. Antimicrob. Agents Chemother. 38, 2387–2391.

Boppana, S.B., Pass, R.F., Britt, W.J., Stagno, S., Alford, C.A., 1992. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr. Infect. Dis. J. 11, 93–99.

Bourne, N., Bravo, F.J., Bernstein, D.I., 2000. Cyclic HPMPC is safe and effective against systemic guinea pig cytomegalovirus infection in immune compromised animals. Antiviral Res. 47, 103–109.

Bourne, N., Schleiss, M.R., Bravo, F.J., Bernstein, D.I., 2001. Preconception immunization with a cytomegalovirus (CMV) glycoprotein vaccine improves pregnancy outcome in a guinea pig model of congenital CMV infection. J. Infect. Dis. 183, 59–64.

Bratcher, D.F., Bourne, N., Bravo, F.J., Schleiss, M.R., Slaoui, M., Myers, M.G., Bernstein, D.I., 1995. Effect of passive antibody on congenital cytomegalovirus infection in guinea pigs. J. Infect. Dis. 172, 944–950.

Bravo, F.J., Stanberry, L.R., Kier, A.B., Vogt, P.E., Kern, E.R., 1993. Evaluation of HPMPC therapy for primary and recurrent genital herpes in mice and guinea pigs. Antiviral Res. 21, 59–72.

Chatterjee, A., Harrison, C.J., Britt, W.J., Bewtra, C., 2001. Modification of maternal and congenital cytomegalovirus infection by anti-glycoprotein B antibody transfer in guinea pigs. J. Infect. Dis. 183, 1547–1553.

Dahle, A.J., Fowler, K.B., Wright, J.D., Boppana, S.B., Britt, W.J., Pass, R.F., 2000. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J. Am. Acad. Audiol. 11, 283–290.

de Cates, C.R., Gray, J., Roberton, N.R.C., Walker, J., 1994. Acquisition of cytomegalovirus infection by premature infants. J. Infect. 28, 25–30.

Demmler, G.J., 1991. Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus diseases. Rev. Infect. Dis. 13, 315–329.

Gaytant, M.A., Steegers, E.A., Semmekrot, B.A., Merkus, H.M., Galama, J.M., 2002. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet. Gynecol. Surv. 57, 245–256.

Griffith, B.P., Lavallee, J.T., Jennings, T.A., Hsiung, G.D., 1985. Transmission of maternal cytomegalovirus-specific immunity in the guinea pig. Clin. Immunol. Immunopathol. 35, 169–181.

Hamprecht, K., Maschmann, J., Vochem, M., Dietz, K., Speer, C.P., Jahn, G., 2001. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet 357 (9255), 513–518.

Harrison, C.J., Myers, M.G., 1988. Peripheral blood mononuclear cell-mediated cytolytic activity during cytomegalovirus (CMV) infection of guinea pigs. J. Med. Virol. 25, 441–453.

Harrison, C.J., Britt, W.J., Chapman, N.M., et al., 1995. Reduced congenital cytomegalovirus (CMV) infection after maternal immunization with a guinea pig CMV glycoprotein before gestational primary infection in the guinea pig model. J. Infect. Dis. 172, 1212–1220.

Hickson, L.M., Alcock, D., 1991. Progressive hearing loss in children with congenital cytomegalovirus. J. Pediatr. Child. Health 27, 105–107.

Kern, E.R., 1991. Value of animal models to evaluate agents with potential activity against human cytomegalovirus. Transplant. Proc. 23, 152–155.

Kimberlin, D.W., Lin, C.Y., Sanchez, P., 2000. Ganciclovir treatment of symptomatic congenital CMV infections: results of a phase III randomized trial. In: 40th ICAAC Abstracts, Toronto, Canada (abstract # 1942).

Kumar, M.L., Nankervis, G., Cooper, A.R., Gold, E., 1984. Postnatally acquired cytomegalovirus infections in infants of CMV-excreting mothers. J. Pediatr. 104, 669–673.

Mani, C.S., Bravo, F.J., Stanberry, L.R., Myers, M.G., Bernstein, D.I., 1996. Effect of age and route of inoculation on outcome of neonatal herpes simplex virus infection in guinea pigs. J. Med. Virol. 48, 247–252.

Schleiss, M.R., 2002. Cytomegalovirus in immunocompromised persons. Curr. Treat. Options Infect. Dis. 4, 43–50.

Schleiss, M.R., McGregor, A., Jensen, N., Erdem, G., Atkan, L., 1999. Molecular characterization of the guinea pig cytomegalovirus UL83 (pp65) protein homolog. Virus Genes 19, 205–221.

Shanley, J.D., Biczak, L., Forman, S.J., 1993. Acute murine cytomegalovirus infection induces lethal hepatitis. J. Infect. Dis. 167, 264–269.

Stagno, S., Reynolds, D.W., Pass, R.F., Alford, C.A., 1980. Breast milk and the risk of cytomegalovirus infection. New Engl. J. Med. 302, 1073–1076.

Stagno, S., Brasfield, D.M., Brown, M.B., Cassell, G.H., Pifer, L.L., Whitley, R.J., Tiller, R.E., 1981. Infant pneumonitis associated with cytomegalovirus chlamydia pneumocystis and ureaplasma—a prospective study. Pediatrics 68, 322–329.

Stals, F.S., Bosman, F., van Boven, C.P., Bruggeman, C.A., 1990. An animal model for therapeutic intervention studies of CMV infection in the immunocompromised host. Arch. Virol. 114, 91–107.

Stratton, K.R., Durch, J.S., Lawrence, R.S., 2001. Vaccines for the 21st Century: A Tool For Decision Making. National Academy Press, Washington, DC.

Vochem, M., Hamprecht, K., Jahn, G., Speer, C.P., 1998. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatr. Infect. Dis. J. 17, 53–58.

Whitley, R.J., Brasfield, D., Reynolds, D.W., et al., 1976. Protracted pneumonitis in young infants associated with perinatally acquired cytomegalovirus infection. J. Pediatr. 89, 16–22.

Whitley, R.J., Cloud, G., Gruber, W., Storch, G.A., Demmler, G.J., Jacobs, R.F., Dankner, W., Spector, S.A., Starr, S., Pass, R.F., Stagno, S.A., Britt, W.J., Alford, C., Soong, S., Zhou, X.J., Sherrill, L., Fitzgerald, J.M., Sommadossi, J.P., 1997. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. National Institute of Allergy and Infectious Diseases Collaborative Study Group. J. Infect. Dis. 175, 1080–1086.

Yeager, A.S., Palumbo, P.E., Malachowsky, N., Ariagno, R.L., Stevenson, D.K., 1983. Sequelae of maternally derived cytomegalovirus infections in premature infants. J. Pediatr. 102, 918–922.

Zheng, Z.M., Lavalle, J.T., Bia, F.J., Griffith, B.P., 1987. Thymic hypoplasia, splenomegaly and immune depression in guinea pigs with neonatal cytomegalovirus infection. Dev. Comp. Immunol. 11, 407–418.