

# Cyclic HPMPC is safe and effective against systemic guinea pig cytomegalovirus infection in immune compromised animals

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## Abstract

Cidofovir (HPMPC) is licensed for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS but its use is limited by nephrotoxicity. We evaluated the safety and efficacy of 1-[[[(s)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine dihydrate (CHPMPC) the cyclic congener of cidofovir. Treatment was well tolerated both in normal guinea pigs and in animals immune compromised with cyclophosphamide. Further, blood chemistry analysis showed no adverse effects of CHPMPC treatment on kidney or liver function. In efficacy studies in immune compromised guinea pigs challenged with a virulent salivary gland passaged guinea pig CMV, CHPMPC treatment significantly reduced mortality resulting from disseminated virus infection. Quantitative culture showed that treatment also significantly reduced virus replication in the liver and spleen, but not the lungs of infected animals. The efficacy of CHPMPC combined with its improved safety profile appear to make it an attractive alternative to cidofovir for the treatment of herpesvirus infections. Further evaluation is warranted. © 2000 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Human cytomegalovirus (HCMV) is an important pathogen in immune compromised patient populations including neonates, organ and bone marrow transplant patients and people with AIDS (Demmler, 1999; Drew and Lalezari, 1999; Sia and Patel, 2000). The advent of highly active

antiretroviral therapy (HAART) has been beneficial in reducing the morbidity and mortality caused by HCMV disease, particularly retinitis, in AIDS patients (Macdonald et al., 1998; Drew and Lalezari, 1999; Whitcup, 2000). However, there are concerns that with time, treatment intolerance and antiviral resistance to HAART will increase, producing a concurrent increase in the incidence of serious HCMV disease in AIDS patients. Thus there is still a real need for safe and effective antiviral agents against HCMV.

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The cytidine nucleotide analogue cidofovir (HPMPC) has demonstrated antiviral activity against a wide range of herpesviruses (reviewed in Safrin et al., 1999) and is currently recommended for the treatment of HCMV retinitis in AIDS patients. However, cidofovir treatment has been associated with nephrotoxicity (Lalezari et al., 1995; Polis et al., 1995). The nephrotoxic effects can be reduced by extended dosing intervals, concurrent treatment with probenecid and hydration therapy (Lalezari et al., 1995, 1997) but toxicity remains a concern and has limited the clinical applications of this antiviral compound. 1-[(s)-2-hydroxy-2-oxo-1,4,2-dioxaphosphorinan-5-yl)methyl]cytosine dihydrate (CHPMPC) is the cyclic congener of cidofovir and is significantly less nephrotoxic than the parent compound in a number of animal species (Bischofberger et al., 1994; Hitchcock et al., 1995) but retains similar activity against many herpesviruses in vitro and is effective in murine models of cytomegalovirus and herpes simplex virus infections (Andrei et al., 1991; Bischofberger et al., 1994; Kern et al., 1995). In the studies described here we have examined the safety and efficacy of systemic CHPMPC treatment against disseminated infection produced by guinea pig cytomegalovirus (gpCMV) in immune compromised guinea pigs.

## 2. Materials and methods

### 2.1. Viruses and cells

Guinea pig CMV (gpCMV; strain 22122; American Type Culture Collection, Rockville, MD) was used to prepare the salivary gland-derived gpCMV stock of high virulence for challenge studies by sequential in vivo passages in male Strain-2 guinea pigs as previously described (Harrison and Myers, 1988). The salivary gland derived virus stock was stored frozen ( $-80^{\circ}\text{C}$ ) until used. Quantification of virus stocks, and, of virus load in infected tissue homogenates during antiviral studies was conducted by titration on guinea pig lung cells (cell line CCL 158, American Type Culture Collection) as previously described (Harrison and Myers, 1988).

### 2.2. Animals

Male Strain-2 guinea pigs used for the preparation of virulent salivary gland passaged virus stocks were obtained from Children's Hospital Research Foundation (Cincinnati, OH). Male Hartley guinea pigs weighing 300–400 g used in antiviral evaluation studies were obtained from Camm Research Institute (Wayne, NJ). All animals were housed under AAALAC approved conditions. Hartley animals were confirmed seronegative for gpCMV prior to use as previously described (Bratcher et al., 1995).

### 2.3. Immune compromised guinea pig model of CMV infection

Guinea pigs were administered an initial intraperitoneal injection of 100–200 mg/kg cyclophosphamide (CY) with a second dose of 50 mg/kg CY administered 7 days later. One day after the initial dose of CY animals were inoculated with 4.8–6.9  $\log_{10}$  pfu of clarified salivary gland passaged virus.

### 2.4. Antiviral treatment

CHPMPC was provided by Gilead Sciences Inc. (Foster city, CA) and stored refrigerated ( $4^{\circ}\text{C}$ ). For use in vivo CHPMPC was dissolved in sterile distilled water and the solution adjusted to pH 7.4 with sodium hydroxide. Two antiviral treatment regimens were evaluated with animals receiving either a single intraperitoneal injection of 5 mg/kg/day daily for seven days or two injections of 17.5 mg/kg administered on day 1 and 5 of the daily treatment schedule. Both treatment regimens resulted in a total dose of 35 mg/kg CHPMPC. In the initial study uninfected immune competent and immune compromised animals ( $n = 5/\text{group}$ ) were treated with CHPMPC without virus challenge to determine the safety of the treatment regimens. Blood samples were collected from all animals prior to the initiation of antiviral therapy and 1 day after the final daily treatment and assayed in the clinical laboratory (Children's Hospital Medical Center, Cincinnati, OH) for white blood cell counts, liver function (serum

Table 1  
Effect of CHPMPC treatment on blood chemistries in normal and immune compromised guinea pigs

Parameter (units)	Time relative to treatment	Group <sup>a</sup>				
		CY	CHPMPC (daily)	CHPMPC (twice)	CY + CHPMPC (daily)	CY + CHPMPC (twice)
White blood cells (K/ $\mu$ l)	pre	4.66	4.68	3.24	4.28	7.16
	post	2.18	7.38	7.10	1.72	2.80
Blood urea nitrogen (mg/dl)	pre	17.20	23.20	17.60	19.60	16.40
	post	21.20	18.00	17.40	23.00	18.00
Creatinine (mg/dl)	pre	0.42	0.48	0.42	0.38	0.46
	post	0.44	0.34	0.38	0.52	0.38
Serum glutamic pyruvic transaminase (IU/L)	pre	32.60	39.80	40.40	30.20	31.40
	post	39.40	42.20	38.40	22.80	26.60
Bilirubin (mg/dl)	pre	0.14	0.24	0.16	0.12	0.12
	post	0.24	0.30	0.10	0.12	0.18

<sup>a</sup> CY treatment was an initial dose of 100 mg/kg with a second dose of 50 mg/kg 7 days later. CHPMPC treatment was 5 mg/kg for 7 days (daily) or 17.5 mg/kg administered on days 1 and 5 (twice). Values are mean for five animals.

pyruvic transaminase and bilirubin) and kidney function (blood urea nitrogen and creatinine).

For efficacy studies antiviral treatment was initiated one day after virus inoculation. In some studies animals were sacrificed at various times post virus challenge and the effect of therapy on virus titers in spleen, liver and lung evaluated by quantitative plaque titration of tissue homogenates using the method of Harrison and Myers (1988).

### 2.5. Statistics

Incidence data were compared by Fisher's exact test. Virus titer data were compared by Student's *t*-test. All comparisons were two-tailed.

## 3. Results

### 3.1. Evaluation of safety

Prior to conducting studies to evaluate antiviral efficacy, we investigated the safety of two systemic CHPMPC treatment regimens in both normal and immune compromised guinea pigs without gpCMV infection. All of the CHPMPC treated

animals survived with no visible signs of toxicity. Results of blood chemistry analysis (Table 1) showed that as anticipated white blood cell counts were reduced in the groups treated with CY. However, CHPMPC treatment had no adverse effects on white blood cell counts when given alone or in combination with CY. In addition, toxicity was not detected in either liver (as measured by bilirubin and serum glutamic pyruvic transaminase concentrations) or kidney (as measured by blood urea nitrogen and creatinine concentrations).

### 3.2. Evaluation of efficacy

The outcomes of three studies to evaluate the efficacy of CHPMPC against CMV infection in immune compromised guinea pigs are shown in Table 2. In the first study each of the two antiviral treatment regimens provided significant protection against mortality (both  $P < 0.0001$ ). In the second study only the daily treatment regimen was evaluated. In this study mortality among control animals was unexpectedly low and we were unable to show a significant protective effect of treatment. Subsequent analysis showed that CY treatment failed to produce the previously seen reduction in white blood cell counts. Consequently, a third study was undertaken in which conditions were altered to be more stringent, the initial dose of CY was increased to 200 mg/kg and the virus inoculum increased from 4.8 to 6.9 log<sub>10</sub> pfu of salivary gland passaged virus. Under these conditions there was significantly less mortality in CHPMPC treated animals than in controls (Table 2;  $P < 0.005$ ). When the results of the three studies were combined 10 of 39 animals that received daily CHPMPC treatment died compared to 31 of 37 placebo treated animals ( $P < 0.0001$ ).

In addition to outcome studies, we also evaluated the effect of CHPMPC treatment on virus replication in three tissues in which the CMV disseminates (liver, lung and spleen) at two times post challenge. In the first study both the daily and two dose antiviral treatment regimens were examined while in the second only the daily treatment was evaluated. There were no significant differences comparing the two treatment regimens

Table 2  
Effect of CHPMPC treatment on mortality in CMV infected immune compromised guinea pigs

Group	<i>n</i>	Mortality (%)	<i>P</i> value
<i>Experiment 1</i>			
Placebo	15	13(87)	
CHPMPC (daily) <sup>a</sup>	16	1 (6)	<0.0001
CHPMPC (twice) <sup>b</sup>	16	0	<0.0001
<i>Experiment 2</i>			
Placebo	10	6 (60)	
CHPMPC (daily) <sup>a</sup>	11	4 (36)	NS
<i>Experiment 3</i>			
Placebo	12	12 (100)	
CHPMPC (daily) <sup>a</sup>	12	5 (42)	<0.005
<i>Combined</i>			
Placebo	37	31 (84)	
CHPMPC (daily) <sup>a</sup>	39	10 (26)	<0.0001

<sup>a</sup> Animals received a single intraperitoneal injection of 5 mg/kg daily for 7 days beginning one day after virus challenge.

<sup>b</sup> Animals received single intraperitoneal injection of 17.5 mg/kg on days 1 and 5 after virus challenge.

Table 3

Effect of CHPMPC treatment on disseminated CMV infection in immune compromised guinea pigs

Treatment	Liver		Lung		Spleen	
	Incidence	Titer <sup>b</sup>	Incidence <sup>a</sup>	Titer <sup>b</sup>	Incidence <sup>a</sup>	Titer <sup>b</sup>
<i>Day 7</i>						
Placebo	3/9	0.3 (0.5)	9/9	2.4 (0.7)	8/9	2.0 (1.0)
CHPMPC <sup>c</sup>	0/13	0	12/13	2.2 (0.9)	2/13 <sup>d</sup>	0.3 (0.5) <sup>e</sup>
<i>Day 10</i>						
Placebo	6/8	1.9 (1.3)	8/8	3.9 (0.8)	8/8	3.3 (0.9)
CHPMPC	6/13	0.8 (0.9) <sup>f</sup>	13/13	3.2 (0.9)	11/13	2.0 (1.4) <sup>f</sup>

<sup>a</sup> Animals from which virus was isolated by plaque titration/animals inoculated.<sup>b</sup> Mean Log<sub>10</sub> pfu/ml (SD).<sup>c</sup> Animals received a total dose of 35 mg/kg with treatment initiated on day 1 after virus challenge.<sup>d</sup>  $P < 0.005$ .<sup>e</sup>  $P < 0.001$ .<sup>f</sup>  $P < 0.05$ .

and the two studies, for this reason the results have been combined (Table 3). On day 7 post challenge, the last day of daily antiviral therapy, virus was not isolated by culture from livers of any CHPMPC treated animals, further the number of treated animals from which virus was isolated from spleens was significantly reduced compared to controls. On day 10 post challenge, after CHPMPC therapy had been completed, titers were increased in all organs compared to day 7. There was no significant reduction in the incidence of virus isolation from either liver or spleen of treated animals compared to controls on day 10. However in both tissues virus titers were significantly lower in treated animals than in controls ( $P < 0.05$  for both). In contrast to results in the liver and spleen, CHPMPC treatment failed to produce a significant impact on the incidence or titer of gpCMV in lung tissue at either time-point

#### 4. Discussion

Cidofovir (HPMPC) has proven effective in the treatment of a variety of experimental herpes virus infections including cutaneous, systemic and genital herpes simplex disease (Bronson et al., 1989; De Clercq and Holy, 1991; Bravo et al., 1993) and systemic cytomegalovirus infections (Li et al., 1990; Neyts et al., 1992). However, treated

animals experienced toxicity including kidney damage which was particularly severe in guinea pigs resulting in significant mortality (Li et al., 1990; Bravo et al., 1993). While it is currently approved for the treatment of CMV retinitis in AIDS patients, its clinical applications have been limited because nephrotoxicity has also been seen in patients (Lalezari et al., 1995; Polis et al., 1995). Cidofovir's nephrotoxicity results from damage to proximal tubules where the antiviral accumulates because it is actively transported across the basolateral membrane into the proximal tubular cells more rapidly than it is exported (Lalezari et al., 1995). In preclinical studies CHPMPC, the cyclic congener of cidofovir, has been shown to undergo more extensive tubular secretion than the parent compound (Cundy et al., 1996) with a corresponding reduction in nephrotoxicity observed in a number of animal species (Bischofberger et al., 1994; Hitchcock et al., 1995) and in humans (Cundy et al., 1999). In addition to reduced toxicity CHPMPC displays similar in vitro activity to the parent compound against a variety of herpesviruses including HCMV and HSV (Andrei et al., 1991; Bischofberger et al., 1994; Kern et al., 1995) because it acts as an intracellular prodrug being converted to cidofovir after entry into cells by the action of a cellular cyclic CMP phosphodiesterase (Mendel et al., 1997). CHPMPC is also effective in the treatment

of HSV and CMV infections in mice (Bischofberger et al., 1994; Kern et al., 1995). However, to date no efficacy studies have been conducted in guinea pigs which proved extremely sensitive to the toxic effects of the parent compound, even though Hitchcock et al. (1995) reported that by histopathology, CHPMPC was significantly less nephrotoxic than cidofovir in this species.

Outbred Hartley Guinea pigs inoculated with gpCMV experience transient splenomegaly, a self limited viremia and peripheral blood mononucleosis similar to that observed in humans (Griffith et al., 1981). Investigators have used weight loss, spleen index measurement and viral titers in blood and internal organs to measure the efficacy of antiviral agents in this model (Fong et al., 1987; Li et al., 1990). The severity of the disseminated gpCMV infection can be increased to include mortality by the use of immunosuppressive agents such as cyclosporin A or cyclophosphamide (Bia et al., 1985; Aquino-de Jesus and Griffith, 1989). This model is felt to more closely mimic the immune compromised human patient populations for which antivirals against CMV are needed. In the studies reported here we have extended previous histopathologic observations that showed that CHPMPC was less nephrotoxic than cidofovir, to show that by blood chemistry analysis CHPMPC was safe even in guinea pigs immune compromised by CY treatment. Further, treatment was effective in significantly reducing mortality and virus replication in organs affected by disseminated CMV infection in immune compromised animals, although the effects on virus replication were less than those seen in vivo studies with MCMV (Kern et al., 1995), possibly due to differences in susceptibility to CHPMPC between murine and guinea pig CMV's. These results provide further evidence that CHPMPC is an effective and less toxic alternative to cidofovir for the treatment of herpes virus infections. It is possible that the improved therapeutic index seen with this agent may make it attractive for clinical application against a number of herpesvirus infections. Further studies to more fully evaluate this antiviral are warranted.

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