Antiviral Research, 13 (1990) 237–252
Elsevier

AVR 00413

Activity of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) against guinea pig cytomegalovirus infection in cultured cells and in guinea pigs

S.B. Li[1], Z.H. Yang[1], J.S. Feng[1], C.K.Y. Fong[1,2], H.L. Lucia[3] and G.D. Hsiung[1,2]

[1]*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut,*[2]*Virology Laboratory, Veterans Administration Medical Center, West Haven, Connecticut and* [3]*Department of Pathology, University of Texas Medical Branch Galveston, Texas, U.S.A.*

(Received 25 August 1989; accepted 31 January 1990)

Summary

(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC, and two HPMPC-related nucleoside analogs, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine, HPMPA, and (2-phosphonylmethoxyethyl)guanine, PMEG, were evaluated for their antiviral activities against guinea pig cytomegalovirus (GPCMV) infection in guinea pig embryo (GPE) cells and human cytomegalovirus (HCMV) infection in human diploid fibroblast (MRC-5) cells. DHPG, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, was used for comparison. The antiviral activity of HPMPC against GPCMV infection in vivo and its toxicity to Hartley guinea pigs were also evaluated. The 50% antiviral effective doses (ED_{50}) of HPMPC, HPMPA, PMEG and DHPG against GPCMV infection in GPE cells were 0.22, 1.4, 0.07 and 62 μM, respectively; and against HCMV infection in MRC-5 cells, the ED_{50}s were 0.51, 0.72, 0.01 and 17.5 μM, respectively. Their cytotoxic doses (CyD_{50}) in GPE replicating cells were 84, 35, 1.4 and 700 μM, respectively and in MRC-5 cells were approximately 114, 31, 0.86 and 750 μM, respectively. Based on their calculated therapeutic indexes, HPMPC was the most potent and selective of the four compounds tested. In vivo, during acute infection, the spleen indexes of all infected animals that were treated with 1.25 to 5.0 mg/kg/day of HPMPC for 5 days were significantly reduced as compared with sham-treated animals. Virus infectivity titers in blood and various tissues of infected animals treated with HPMPC, 2.5 or

Correspondence to: G.D. Hsiung, Virology Laboratory 151 B, VA Medical Center, West Haven, CT 06516, U.S.A.

1.25 mg/kg/day were not significantly lower than those of the infected, sham-treated animals; with 5 mg/kg/day, infectivity titers in the blood, spleen, and salivary gland were significantly lower in HPMPC-treated than in sham-treated animals. However, HPMPC was toxic to guinea pigs especially at doses of 5 to 10 mg/kg/day. These data showed that HPMPC was highly active and selective in cultured guinea pig cells and human fibroblast cells against CMV infection but did not effectively inhibit GPCMV infection in guinea pigs at minimum toxic concentrations.

Cytomegalovirus infection; Antiviral agent; Nucleoside analog; HPMPC; Cultured cell; Guinea pig

Introduction

Human cytomegalovirus (HCMV) has been reported as one of the most frequent cause of mortality in AIDS patients (Quinnan et al., 1984) and in addition, it is one of the most important pathogen causing opportunistic infection in immunocompromised hosts, affecting particularly kidney, liver, heart, lung, and bone marrow transplant recipients and AIDS patients (Peterson et al., 1980; Meyers et al., 1982; Macher et al., 1983). So far, only 9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG, and phosphonoformate, (PFA), have been pursued for the treatment of patients with CMV infection (Mills, 1986). However, there are problems associated with the clinical use of these compounds. The collaborative DHPG treatment study group reported (Collaborative DHPG Treatment Study Group, 1986) that DHPG causes adverse reactions during the period of treatment, especially when high doses are used. Owing to the lack of effective treatment for HCMV infections, the search for more effective and less toxic antiviral drugs for the chemotherapy of HCMV infections is of a high priority. Recently, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC and HPMPC-related nucleoside analogs have been reported as potent and selective inhibitors against various DNA virus infections including HCMV (De Clercq et al., 1986; De Clercq et al., 1987; Votruba et al., 1987; Snoeck et al., 1988).

In the present study, we evaluated the inhibitory effect of HPMPC and two HPMPC-related nucleoside analogs on guinea pig cytomegalovirus (GPCMV) as well as HCMV infections in cultured cells. In addition, the efficacy of HPMPC against GPCMV infections in guinea pigs was investigated.

Materials and Methods

Cell cultures and virus assay

Primary GPE cells were prepared from 30- to 40-day-old embryos of Hartley guinea pigs as described previously (Hsiung et al., 1978). The cells were grown in

Eagle's minimum essential medium in Hanks' balanced salt solution (MEMH) containing 10% heat inactivated newborn bovine serum (NBS). When the cells became confluent, the growth medium was replaced with maintenance medium which consisted of Eagle's medium in Earle's balanced salt solution (MEME) supplemented with 2% NBS. GPE cells at passage levels one to three were used in the virus assay either by cytopathic effect (CPE) or plaque formation. GPCMV strain 22122 was propagated in GPE cells. The virus stock infectivity titer was in the range of 5 to 5.5 $\log_{10}$ $TCID_{50}$/ml (50% tissue culture infective dose). MRC-5 cells in 75 or 150 cm^2 flask were purchased from Viromed Laboratories, Minneapolis, Minn. HCMV strain AD169 was used for the preparation of virus stock which was propagated in MRC-5 cells. The virus titer was 5 to 5.5 $\log_{10}$ $TCID_{50}$/ml.

Chemicals

HPMPC and two HPMPC-related compounds, HPMPA and PMEG, were synthesized by procedures described previously (Bronson et al., 1989) and were kindly supplied by Bristol Meyers Co. They all have good aqueous solubility, and have excellent stability in powder and solution forms at 4°C. DHPG was supplied by Merck Sharp and Dohme Research Laboratories, Rahway, NJ. All compounds were dissolved in phosphate buffered saline (PBS) at concentrations of 1000, and/or 5000 μM, filtered through Millipore filter membranes with pore size of 0.2 μm, and stored at −20°C as stock solutions; they were diluted with MEME for in vitro assay. HPMPC was dissolved in PBS at concentrations of 10, 5, 2.5 and 1.25 mg/ml for in vivo studies.

Effect of HPMPC, HPMPA and PMEG on GPCMV and HCMV infection

For virus assay, confluent monolayers of GPE or MRC-5 cells in 24-well panels were infected with 0.1 ml GPCMV or HCMV containing approximately 70–100 PFU. After adsorption at 36°C for 1 h, infected cells were overlaid with MEME containing 10% NBS and 0.5% methylcellulose, and various concentrations of each compound. The infected cell cultures were incubated at 36°C in a CO_2 incubator for two to three weeks until plaques were visible in the control cultures. Plaques were enumerated after the cultures were fixed and stained with a solution of 10% formalin containing 1.3% crystal violet.

Cytotoxicity assays in uninfected replicating GPE or MRC-5 cells

The cytotoxic effect of compounds on uninfected GPE or MRC-5 cells was evaluated by measuring the ability of the compounds to reduce the growth rate of cells in the rapidly replicating stage. The MEME medium containing 10% NBS and various concentrations of each compound was added to duplicate 6-well panels of GPE or MRC-5 cells which were seeded 24 h previously; untreated cells were included as control. Compound-treated and untreated cells were maintained in the growing stage at 36°C for three days. Viable cells were counted daily with the aid

of trypan blue exclusion method. The concentrations of the compounds required to reduce the growth rate of cells by 50% (CyD_{50}) were calculated from the dose–response curves.

Direct virucidal effect

To test whether HPMPC has a direct virucidal effect on the virions when they are in the cell-free state, GPCMV or HCMV suspensions were mixed with HPMPC at a concentration of 5 μM, about 5-fold higher than the concentration that completely inhibited GPCMV or HCMV replication in GPE and MRC-5 cells, respectively. The virus–compound mixtures and virus suspension alone were incubated at 36°C for 1 h and their infectivity titers were determined by cytopathic effect (CPE) inhibition assay.

Effect of duration of treatment

Confluent monolayers of GPE cells were infected with GPCMV at a multiplicity of infection (MOI) of approximately one. After 2 h adsorption, unadsorbed virus was removed by washing with HBSS for three times, then maintenance medium containing 5 μM of HPMPC was either added to or removed from the GPCMV-infected cells at 3, 6, 12, 24, and 48 h postadsorption, and further incubated at 36°C in a CO_2 incubator. All infected cultures were harvested 72 h postinfection, frozen and thawed three times and stored at −70°C. Virus infectivity titers of these samples were assayed in GPE cell monolayers in 24-well panels by CPE method.

Ultrastructural studies

GPE cell monolayers grown in 6-well panels were infected with 0.4 ml of GPCMV (MOI of approximately one). Following virus adsorption for 1 h at 36°C, MEME medium containing 2% NBS and HPMPC at concentrations of 0.01, 0.1, 1, and 10 μM was added to the infected cell cultures and then incubated at 36°C in a CO_2 incubator. When approximately 75% of the cells in the virus infected HPMPC-free cell cultures showed CPE (about three days postinfection), all HPMPC-treated and untreated cultures were fixed, embedded, sectioned, stained, and examined under a Philips EM 300 electron microscope using procedures described previously (Fong et al., 1987).

Animal inoculation

Young female Hartley guinea pigs (280–320 g) were purchased from Camm Research Institute, Wayne, NJ. Prior to virus inoculation, blood samples were obtained by cardiac puncture from all animals and their sera were tested for the presence of neutralizing antibodies. For in vivo studies, GPCMV strain 22122 was passaged in salivary gland as described previously (Bia et al., 1979). Throughout these experiments, animals were infected intraperitoneally with 0.5 ml of salivary

gland-passaged GPCMV suspension which contained infectivity titers ranging from 3.2 to 5.2 $\log_{10}$ $TCID_{50}$.

In dose–response experiments for the determination of antiviral activity and toxic effect, animals (four to eight animals per group) were injected subcutaneously with HPMPC at concentrations of 10, 5, 2.5 and 1.25 mg/kg/day once daily, starting at 24 h after infection with GPCMV and continuing for five to eight days. The sham-treated animals were injected subcutaneously with sterile PBS using the same schedule. Another group of animals inoculated with virus alone was also included as control. Body weight of all animals was checked and recorded every day.

Specimen collection and virus isolation

Four of the animals used for drug toxicity test and antiviral dose–response experiments were sacrificed on day 10 and the other four on day 22 after drug treatment. Before animals were sacrificed, blood samples for differential leukocyte counts were obtained by intracardiac puncture. Differential leukocyte counts were determined on blood smears stained with Wright's stain. Virus isolation was done as previously described (Bia et al., 1979). Blood for virus isolation was collected in Alsever's solution (four parts blood to one part anticoagulant). Packed blood cells were diluted 10-fold serially with PBS and then inoculated into GPE fibroblast monolayer cell cultures in 24-well tissue culture panels. Spleen, lung, liver, and salivary gland were removed aseptically, finely minced, homogenized with tissue grinders, and suspended (10% weight/volume) in Hanks balanced salt solution (HBSS). Serial 10-fold dilutions of these tissue cell suspensions were prepared, and for each dilution of tissue suspension, 0.1 ml was inoculated into each of four duplicate wells with GPE cell monolayer cultures. Cell cultures were observed three to four weeks for the appearance of GPCMV-induced CPE. To confirm that the isolates were GPCMV, neutralization tests were performed in selected instances using specific anti-GPCMV serum made in rabbit. For histopathologic examination, portions of various organs were removed, fixed in Bouin's solution, embedded, processed and examined microscopically. Data analysis between virus-infected controls and drug-treated groups was performed using Student's *t*-test.

Results

Inhibitory effects of HPMPC and HPMPC-related compounds on GPCMV and HCMV plaque formation

Table 1 summarizes the comparative antiviral activities of the various compounds obtained from the plaque reduction assays. The ED_{50}s against GPCMV for compounds HPMPC, HPMPA, PMEG, and DHPG were 0.22, 1.4, 0.07, and 62 μM, respectively, and against HCMV were 0.51, 0.72, 0.01 and 17.5 μM, respectively. Thus, the relative potencies of the four antiviral compounds against both GPCMV and HCMV infections were consistent being PMEG>HPMPC> HPMPA>DHPG.

TABLE I

Comparative antiviral activities of HPMPC, HPMPA, PMEG and DHPG against both GPCMV and HCMV infections in cultured cells

Compounds	GPCMV			HCMV		
	ED_{50}[a] (μM)	CyD_{50}[b](μM)	Th. In.[c]	ED_{50} (μM)	CyD_{50} (μM)	Th. In.[c]
HPMPC	0.22	84	389	0.51	114	224
HPMPA	1.40	35	25	0.72	31	43
PMEG	0.07	1.4	19	0.01	0.86	86
DHPG	62.00	700	11	17.5	750	43

[a]ED_{50} = 50% effective dose (plaque reduction method).
[b]CyD_{50} = 50% cytotoxicity dose (reduction in the number of replicating cells).
[c]Therapeutic index = CyD_{50}/ED_{50}.

The cytotoxicity assay was conducted in uninfected replicating GPE or MRC-5 cells. The results are also summarized in Table 1. HPMPC reduced the GPE or MRC-5 viable cell counts by 50% (CyD_{50}) at a concentration of 84 and 114 μM, respectively; these concentrations were 389- and 224-fold higher than its ED_{50}s. HPMPC was the least toxic to replicating GPE or MRC-5 cells when compared with HPMPA and PMEG. Based on these data, the calculated therapeutic indexes for HPMPC, HPMPA, PMEG, and DHPG against GPCMV and HCMV were 389

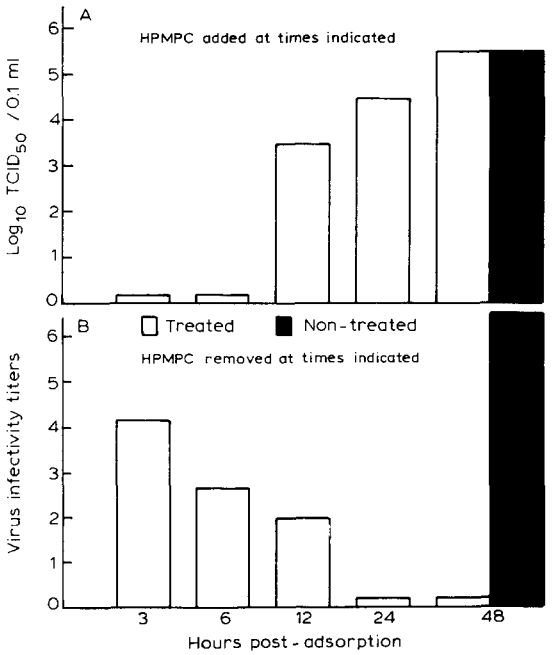


Fig. 1. Effect of HPMPC on GPCMV replication in cultured cells. Drug was added or removed at different times, 3, 6, 12, 24 and 48 h postinfection.

and 224, 25 and 43, 19 and 86, and 11 and 43, respectively. Among these four chemicals tested, it appears that HPMPC is the most selective inhibitor.

Direct virucidal effect

When suspensions of GPCMV or HCMV were mixed directly with HPMPC at a concentration of 5 μM, there were no significant differences in infectivity titers between the compound-treated and untreated virus suspensions (data not shown).

Effect of duration of treatment

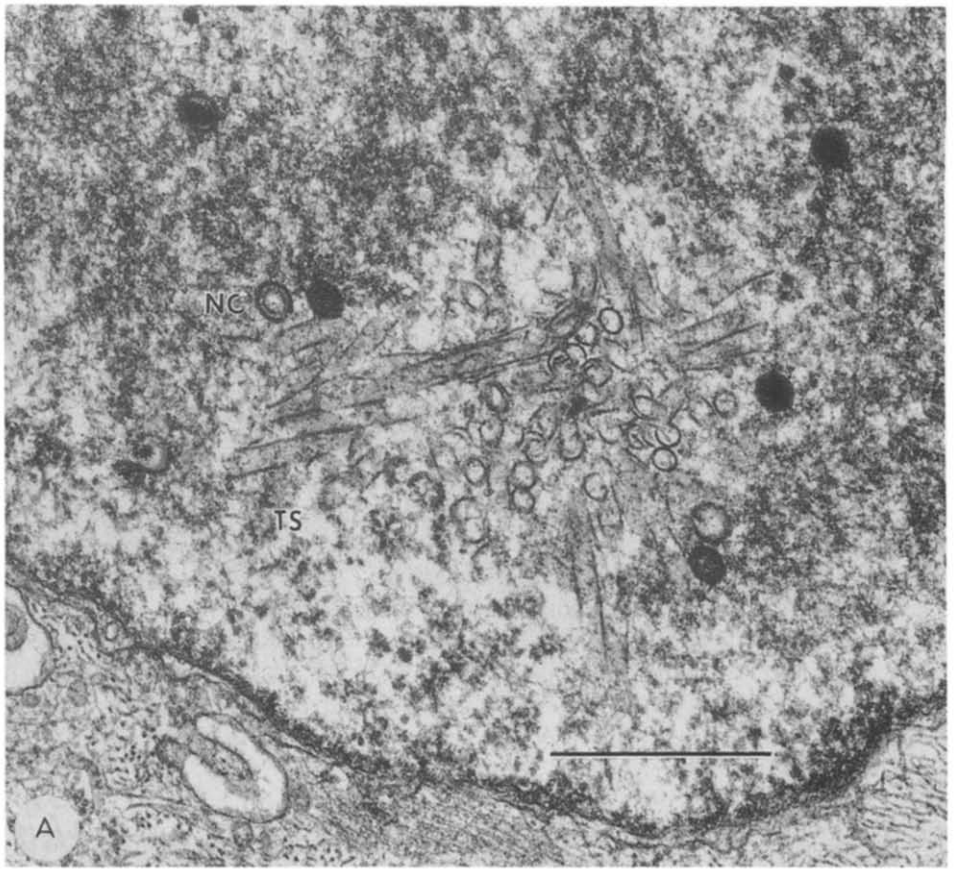
To determine the stage in the GPCMV replication in which HPMPC exerted its maximum activities, the drug addition-removal experiments were conducted. Fig. 1 shows the effect of HPMPC (5 μM) against GPCMV infection after adding or removing the compound at different times (3, 6, 12, 24 and 48 h postadsorption). When HPMPC was added at 6 h post-adsorption or earlier, GPCMV replication was completely inhibited, while at 12, and 24 h postadsorption, virus yield reductions were 2.0 and 1.0 $\log_{10}$ $TCID_{50}$, respectively. There was no reduction in the virus yield when HPMPC was added at 48 h postadsorption. When HPMPC was removed at 24 h postadsorption or later, GPCMV replication was completely inhibited, while removal of the compound at 12, 6, or 3 h postadsorption, virus yield reductions were 4, 3.3 and 1.8 log $TCID_{50}$, respectively. The data showed that HPMPC appeared to inhibit GPCMV replication in GPE cells at an early stage of the GPCMV replication cycle.

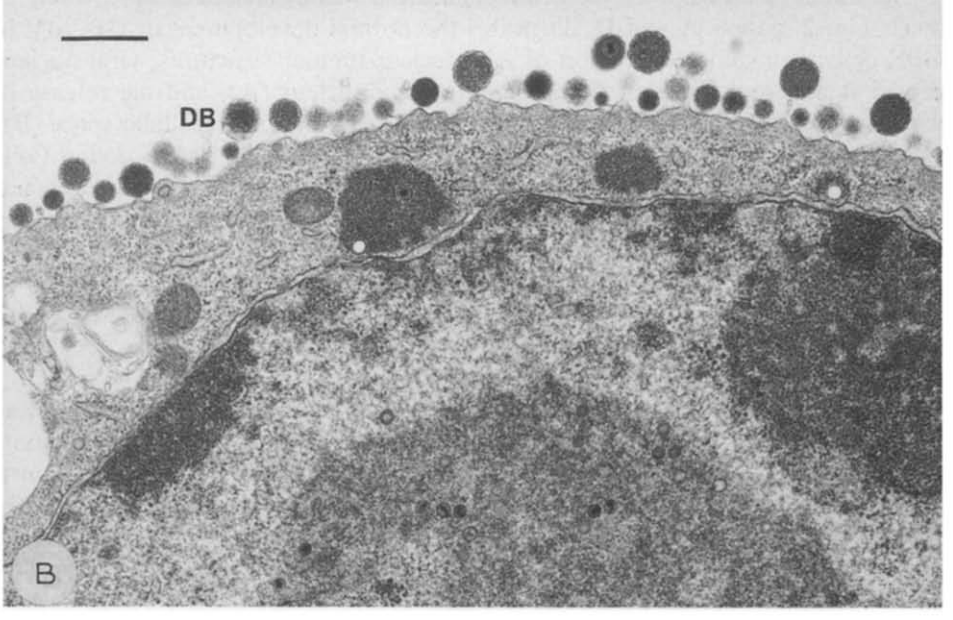
Effect of HPMPC on GPCMV ultrastructural development

The effect of HPMPC on GPCMV replication was evaluated at ultrastructural level. Fig. 2, panels A and B, illustrates the normal development of GPCMV in GPE cells; it includes formation of intranuclear tubular structures, viral nucleocapsid at different states of development in the nucleus (A), and the release of mature enveloped virus particles and dense bodies into the extracellular space (B). Infected cells usually became rounded and enlarged. When GPCMV-infected GPE cells were treated with 0.01 μM of HPMPC, intranuclear tubular structures and only a few nucleocapsids without dense cores were observed. Enveloped virions and dense bodies were not detected. At higher concentrations, 0.1, 1.0 and 10.00 μM of HPMPC, only tubular structures were observed (Fig. 2C).

Toxicity of HPMPC to guinea pigs with or without GPCMV infection

The effect of HPMPC treatment on body weight of uninfected guinea pigs was evaluated and the results are shown in Fig. 3. The average body weight of animals in each group treated with 1.25, 2.5, 5, or 10 mg/kg/day for five days are shown in Fig. 3. In the group treated with 10 mg/kg/day for five days, the average body weight continued to decrease during the 14 days of observation; six animals died


NC
TS
A


DB
B

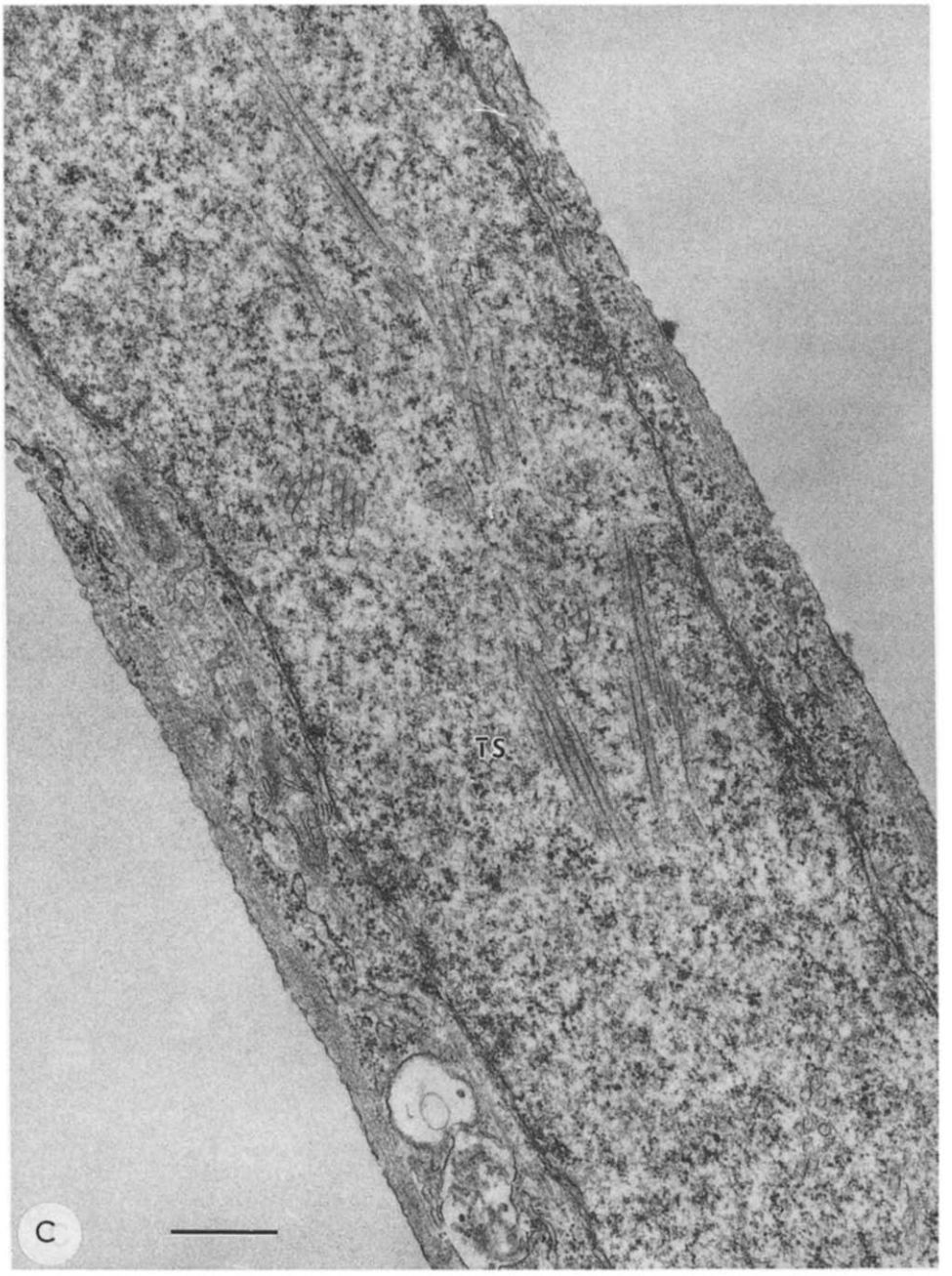


Fig. 2. GPCMV-infected GPE cell, 72 h postinfection. (A and B) without drug treatment: (A) nucleocapsids (NC) with or without dense cores and tubular structures (TS); (B) many dense bodies; (C) treated with 0.1 μM HPMPC, only tubular structures were seen. Bars = 0.5 μm.

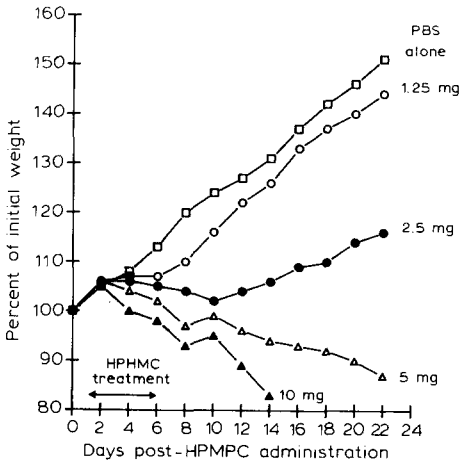


Fig. 3. Effect of various concentrations of HPMPC on body weight of uninfected guinea pigs. All drugs were administered subcutaneously, once daily for 5 days, four to eight animals per group.

between days eight and ten; the other two died on day 14. In the group treated with 5 mg/kg/day, one animal died on day 15 and their average body weight also decreased significantly during the period of observation. The average body weight of animals treated with 2.5 mg/kg/day for five days tapered during the first seven days and then slowly increased up to 115% of their initial average body weight with time. The average body weight of animals treated with 1.25 mg/kg/day for five days gained weight at a rate parallel to that of the sham-treated group. The

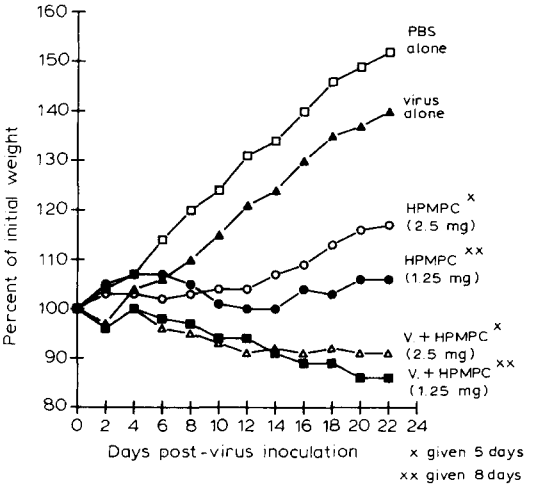


Fig. 4. Effect of HPMPC on the body weight of GPCMV-infected guinea pigs at dosages of 2.5 and 1.25 mg/kg/day, administered subcutaneously for 5 and 8 days, respectively, starting 24 h postinfection, four animals per group.

average body weight of the sham-treated group was up to 150% of their initial average body weight on day 22.

Fig. 4 shows the effect of HPMPC treatment on body weights of infected and uninfected guinea pigs treated with 1.25 mg/kg/day for eight days and 2.5 mg/kg/day for five days, PBS sham-treated and infected untreated controls. The body weight of infected, untreated animals (virus control) was increased up to 141% of their initial body weight on day 22, while uninfected animals treated with 1.25 mg/kg/day for eight days gained weight during the first six days after treatment, but subsequently decreased to 100% of their initial body weight, and then slowly increased again up to 106%. In contrast, body weights of infected animals treated with HPMPC at concentrations of 1.25 mg/kg/day for eight days or 2.5 mg/kg/day for five days tapered throughout the entire period of observation.

HPMPC inhibition of GPCMV replication in guinea pigs

Table 2 summarizes the results of GPCMV isolations from tissues of animals treated with different concentrations of HPMPC for five to eight days and then sacrificed on day 10 postinfection. Virus infectivity titers in blood, spleen, and salivary gland but not in the lung of infected animals treated with 5 mg/kg/day (Expt. 1, Group B) were significantly lower than sham-treated animals ($P<0.01$ or <0.05), especially in the salivary gland where the virus infectivity titer was 2.9 $\log_{10}$ $TCID_{50}$ lower than that of sham-treated control. Tissue cells obtained from animals that received 10 mg/kg/day of HPMPC were toxic to GPE cells even at a dilution of 10% tissue suspension; no virus was recovered in any of their tissues examined (data not shown). It was possible that there were very small amounts of virus which was beyond the sensitivity of the test. Virus infectivity titers in blood and various tissues of infected animals treated with 1.25 mg/kg/day for five days (Expt. 1, Group C) or eight days (Expt. 2, group F) or 2.5 mg/kg/day for five days (Expt. 2, Group E), were lower than those of the infected sham-treated animals, but there was no significant difference between treated and untreated groups ($P>0.05$), except in the salivary gland of group E.

Table 3 summarizes the effects of HPMPC on mononuclear cell count in peripheral blood and on the indexes of spleen, lung, and salivary gland on day 10 postinfection. There was significantly lower lymphocytosis in the group of infected animals treated with HPMPC at concentrations of 5.0 or 2.5 mg/kg/day for 5 days than in the group of untreated, infected animals ($P<0.01$ or <0.05). Indexes of spleen, lung and salivary gland in all the groups of animals treated with HPMPC were lower than those of the untreated-infected control; however, only spleen indexes were significantly decreased. The group of animals treated at a dosage of 1.25 mg/kg/day for eight days also showed significantly lower salivary gland index than the untreated control.

TABLE 2

Effect of HPMPC on virus infectivity titers in GPCMV-infected guinea pigs during acute infection (10 days post-infection)

Group	HPMPC dosage mg/kg/day (No. of days)	Treatment	Average virus infectivity titers[a] (Log $TCID_{50}$/0.1 ml Mean ± SD)			
			Blood	Spleen	Lung	Salivary gland
Expt. 1						
A	None	Virus control	1.43 ± 0.09	1.77 ± 0.52	1.57 ± 0.84	3.83 ± 0.47
B	5 (5)	Virus + HPMPC	0.5 ± 0	0.75 ± 0.43	0.8 ± 0.41	0.93 ± 0.38
		P value[b] (Between A and B)	$P < 0.01$	$P < 0.05$	$P > 0.05$	$P < 0.01$
C	1.25 (5)	Virus + HPMPC	1.33 ± 0.20	1.22 ± 0.60	1.13 ± 0.30	3.18 ± 0.34
		P value (Between A and C)	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Expt. 2						
D	None	Virus control	2.0 ± 0.5	2.40 ± 0.10	2.43 ± 0.26	2.95 ± 0.46
E	2.5 (5)	Virus + HPMPC	1.4 ± 0.10	1.95 ± 0.46	1.88 ± 0.54	2.25 ± 0.25
		P value (Between D and E)	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$
F	1.25 (8)	Virus + HPMPC	1.93 ± 0.13	1.98 ± 0.33	2.0 ± 0.41	3.15 ± 0.15
		P value (Between D and F)	$P > 0.05$	$P > 0.05$	$P > 0.05$	

[a]Each animal was inoculated intraperitoneally with GPCMV suspension containing 5.2 $\log_{10}$ $TCID_{50}$; 4 animals per group.
[b]Statistical analysis was performed using Student's *t*-test.

TABLE 3

Effect of HPMPC on tissue indexes and changes in mononuclear cells of guinea pigs infected with GPCMV during acute infection[a] (10 days post-infection)

Group	HPMPC dosage mg/kg/day (No. of days)	Treatment	Average tissue indexes[b] (Mean ± SD)			Mononuclear cells percentage of total leukocyte count (%)
			Spleen	Lung	Salivary gland	
Expt. 1						
A	None	PBS	17.16 ± 4.55	69.68 ± 2.92	19.22 ± 0.83	69.75 ± 6.18
B	None	Virus control	51.18 ± 6.3	89.45 ± 2.08	19.24 ± 1.66	84 ± 3.56
C	5 (5)	Virus + HPMPC	21.49 ± 4.89	88.18 ± 12.63	18.09 ± 3.23	68.25 ± 2.05
		P value[c] (Between B and C)	$P < 0.01$	$P > 0.05$	$P > 0.05$	$P < 0.01$
D	1.25 (5)	Virus + HPMPC	21.56 ± 4	74.5 ± 13.5	19.32 ± 1.23	81.25 ± 2.28
		P value (Between B and D)	$P < 0.01$	$P > 0.05$		$P > 0.05$
Expt. 2						
E	None	PBS	22.58 ± 11.20	68.73 ± 4.37	12.33 ± 1.06	69.00 ± 4.32
F	None	Virus control	70.07 ± 19.19	115.92 ± 22.90	18.71 ± 1.69	81.50 ± 6.6
G	2.5 (5)	Virus + HPMPC	30.34 ± 5.02	110.42 ± 13.75	18.24 ± 3.4	65.00 ± 7.97
		P value (Between F and G)	$P < 0.01$	$P > 0.05$	$P > 0.05$	$P < 0.05$
H	1.25 (8)	Virus + HPMPC	42.93 ± 7.57	98.60 ± 9.88	14.01 ± 2.96	74.75 ± 5.07
		P value (Between F and H)	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$

[a]Each guinea pig was inoculated intraperitoneally with GPCMV suspension, containing 5.2 $\log_{10}$ $TCID_{50}$, 4 animals per group.
[b]Tissue index: tissue weight/body weight $\times 10^4$.
[c]Statistical analysis was performed using Student's *t* test.

Histological findings

There were no significant differences in the extent of virus induced pathologic changes between the drug-treated (2.5 mg/kg/day) and control animals. Both groups of animals had interstitial pneumonia, inflammation of the kidneys, and inflammation of the salivary glands at day 10 after infection; virus-induced inclusions and inflammation of the salivary gland were noted at day 24 after infection. Drug treated animals, (5 mg/kg/day or higher) had marked drug-associated pathological changes to the kidneys and bone marrow. At day 10 after infection, the kidneys had necrosis of the proximal tubular epithelial cells, with acute inflammation, and an aberrant proliferative response of the remaining cells, resulting in megaloblastic epithelial cells. At day 24, the acute necrosis had disappeared, but the megaloblastic changes persisted. In addition, there was no interstitial fibrosis. In the bone marrow, drug treatment caused a decreased amount of erythropoiesis, and replacement of active marrow with fat. This was roughly twice as severe in the animals given 2.5 mg/kg compared with the animals receiving 1.25 mg/kg.

Discussion

Results of our study showed that HPMPC is a potent and selective agent against HCMV in MRC-5 cells as well as against GPCMV in GPE cells. Compared with DHPG, HPMPC was 35- and 5-fold more selective in GPE cells and MRC-5 cells, respectively. Snoeck et al. (1988) reported that HPMPC inhibits the replication of HCMV in cultured cells. In their in vitro studies, the inhibitory activity of HPMPC against HCMV infection in HEL cells was 10-fold greater than that of the DHPG; and they also reported that if HPMPC was added immediately postinfection, a 24- or 48-h incubation time sufficed to obtain a marked inhibitory effect on HCMV replication. Bronson et al. (1989) also reported that this new compound (HPMPC) is a highly potent and selective agent against HCMV, herpes simplex virus types 1 and 2, and murine cytomegalovirus (MCMV) in vitro. Our data confirmed their results; HPMPC has potent and selective activity against HCMV infection and also against GPCMV infection in vitro (Table 1). To inhibit GPCMV replication in GPE cells completely, HPMPC has to be added 6 h postadsorption or earlier, and needs to remain in contact with the infected cells for at least 24 h postadsorption. These observations are also in agreement with the data reported by Snoeck et al. (1988).

The efficacy of HPMPC against MCMV in vivo also showed some advantage in that it had low toxicity in mice; the highest dosage used was 200 mg/kg/day and all the mice survived (Bronson et al., 1989). In our experiments, however, HPMPC was highly toxic to guinea pigs which was unexpected. Some of the uninfected animals treated with 10 or 5 mg/kg/day for 5 days died during the period of 22 days of observation; and the body weight of animals treated with 2.5 mg/kg/day for 5 days still tapered during treatment. Infected animals treated with HPMPC at concentrations of 2.5, or 1.25 mg/kg/day lost weight throughout the entire period of observation, and their virus infectivity titers were not significantly suppressed. Thus,

we prolonged the treatment with a dosage of 1.25 mg/kg/day for eight days, but the virus infectivity titers still were not significantly reduced. Histopathological findings, similarly, showed marked drug-associated pathological changes to the kidneys and bone marrow of HPMPC-treated, infected animals, and virus-induced lesions were not significantly reduced.

There are many antiviral agents that have shown inhibitory activity in cultured cells but are either ineffective (Oxford et al., 1977) or very toxic when administered to animals, and also there may be examples of drugs that show little or no activity in cell cultures but are active in animal models and/or human patients. The reasons for these differences are manifold, and some were explained by Sidwell (Sidwell et al., 1979). In the present study, it was found that HPMPC was highly selective against GPCMV and HCMV infections in cultured GPE or MRC-5 cells. However, significant reduction of virus titers were obtained at higher dosage i.e. 5 mg/kg/day but did not effectively inhibit GPCMV infection in guinea pigs at minimum toxic concentration, i.e. 1.25 mg/kg/day.

Acknowledgement

This study was supported partially by the Research Contract AI-62519 from the National Institute of Allergy and Infectious Diseases, NIH, and VA Merit Award Research Fund. This is publication Number 105 from the Cooperative Antiviral Testing Group of the Antiviral Substance Program, Development and Applications Branch, National Institute of Allergy and Infectious Diseases. The authors are grateful to Dr V.F. Chan for the preparation and editing of the manuscript and Mrs Sigrid Klein for electron microscopy.

References

Bia, F.J., Hastings, K. and Hsiung, G.D. (1979) Cytomegalovirus infection in guinea pigs. III. Persistent viruria, blood transmission, and viral interference. J. Infect. Dis. 140, 914–920.

Bronson, J.J., Ghazzouli, I., Hitchcock, M.J.M., Webb, II, R.R. and Martin, J.C. (1989) Synthesis and antiviral activity of nucleotide analogue (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine. J. Med. Chem. 32, 1457–1463.

Collaborative DHPG Treatment Study Group (1986) Treatment of serious cytomegalovirus infections with 9-(1,3-dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. N. Engl. J. Med. 314, 801–805.

De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J. and Maudgal, P.C. (1986) A novel selective broad-spectrum anti-DNA virus agent. Nature (London) 323, 464–467.

De Clercq, E., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I. and Holy, A. (1987) Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Res. 8, 261–272.

Fong, C.K.Y., Cohen, S.D., McCormick, S. and Hsiung, G.D. (1987) Antiviral effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine against cytomegalovirus infection in a guinea pig model. Antiviral Res. 7, 11–23.

Hsiung, G.D., Choi, Y.C. and Bia, F.J. (1978) Cytomegalovirus infection in guinea pigs. I. Viremia during acute and primary and chronic persistent infection. J. Infect. Dis. 138, 191–196.

Macher, A.M.,. Reichert, C.M., Straus, S.E., Longo, D.L., Parrillo, J., Lane, H.C., Fauci, A.S., Rook, A.H., Manischewitz, J.F. and Quinnan, G.V., Jr. (1983) Death in the AIDS patients: role of cytomegalovirus. (Letter) N. Engl. J. Med. 309, 1454.

Meyers, J.D., Flournoy, N. and Thomas, E.D. (1982) Nonbacterial pneumonia after allogeneic marrow transplantation: a review of ten years experience. Rev. Infect. Dis. 4, 1119–1132.

Mills, J. (1986) New directions for clinical application and research. In: J. Mills and L. Corey (Eds), Antiviral Chemotherapy, pp. 195–203, Elsevier, New York.

Oxford, J.S. and Perrin, D.D. (1977) Influenza RNA transcriptase inhibitors; studies in vitro and in vivo. Ann. N.Y. Acad. Sci. 284, 613–623.

Peterson, P.K., Balfour, H.H. Jr., Marker, S.C., Fryd, D.S., Howard, R.J., Simmons, R.L. (1980) Cytomegalovirus diseases in renal allograft recipients: a prospective study of the clinical features, risk factors and impact on renal transplantation. Medicine (Baltimore) 59, 283–300.

Quinnan, G.V. Jr., Masur, H., Rook, A.H., Armstrong, G., Frederick, W.R., Epstein, J., Manischewitz, J.F., Macher, A.M., Jackson, L., Ames, J., Smith, H.A., Parker, M., Pearson, G.R., Parillo, J., Mitchell, C. and Straus, S.E. (1984) Herpesvirus infections in the acquired immune deficiency syndrome. J. Am. Med. Assoc. 252, 72–77.

Sidwell, R.W. (1979) Test systems for evaluating the antiviral activity of nucleoside analogues, In: R.T. Walker, E. De Clercq and F. Eckstein (Eds), Nucleoside Analogues, Chemistry, Biology and Medical Applications, 337 pp., Plenum Press, New York.

Snoeck, R., Sakuma, T., De Clercq, E., Rosenberg, I. and Holy, A. (1988) (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, a potent and selective inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 32, 1839–1844.

Votruba, I., Bernaerts, R., Sakuma, T., De Clercq, E., Merta, A., Rosenberg, I. and Holy, A. (1987) Intracellular phosphorylation of broad-spectrum anti-DNA virus agent (S)-9-(3-hydroxyphosphonylmethoxypropyl)adenine and inhibition of viral DNA synthesis. Mol. Pharmacol. 32, 524–529.