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Phosphonylmethoxyalkyl purine and pyrimidine derivatives for treatment of opportunistic cytomegalovirus and herpes simplex virus infections in murine AIDS

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Summary

Murine acquired immunodeficiency syndrome (MAIDS) was induced in C57BL/6 mice following infection with the LP-BM5 retrovirus complex. Infected mice developed splenomegaly, lymphadenopathy and loss of B- and T-cell functions 100 days after virus inoculation. Mice with AIDS were highly susceptible to opportunistic murine cytomegalovirus (MCMV) and herpes simplex virus (HSV-1) infections. The therapeutic activities of two phosphonylmethoxyalkyl derivatives, 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and (*S*)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine (HPMPC), were evaluated in MAIDS immunosuppressed mice infected with MCMV or HSV-1. MCMV infection resulted in extensive viral replication in lung, liver and spleen and death occurred five to twelve days post-infection. Treatment with either HPMPC or ganciclovir (DHPG) reduced mortality and viral replication in target organs; however, HPMPC was as effective as DHPG at one-fifth the DHPG dose. Moreover, when a single dose (100 mg/kg) of HPMPC was administered 24 h prior to MCMV infection, it suppressed virus replication at seven and 14 days post-infection, thus resulting in a significant prolongation of life. PMEA was effective against opportunistic HSV-1 infections, but appeared to be less effective than HPMPC against MCMV infections. These results indicate that MAIDS can be used as a model for evaluating antivirals in an

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immunocompromised host, and suggest that both PMEA and HPMPC may be useful in the treatment of opportunistic CMV and HSV-1 infections.

Murine acquired immunodeficiency syndrome, MAIDS; Opportunistic infection; HPMPC therapy; PMEA therapy

Introduction

Infection of adult C57BL/6 mice with LP-BM5 murine leukemia virus (MuLV) results in a chronic disease state referred to as murine acquired immunodeficiency syndrome (MAIDS; Mosier et al., 1985, 1987; Mosier, 1986). The virus causing this syndrome was originally obtained from the Duplan-Laterjet strain of radiation-induced leukemia virus which was derived from a nonthymic lymphoma in an X-irradiated C57BL/6 (B6) mouse (Laterjet and Duplan, 1962). The LP-BM5 complex consists of variable mixtures of C-type, B-tropic retroviruses, including ecotropic and mink-cell-focusing (MCF)-inducing viruses. The ecotropic component was once thought to be responsible for MAIDS; however, recent evidence now suggests that the etiologic agent of MAIDS is a defective virus (Hartley et al., 1989; Jolicouer et al., 1989).

While the initiation of disease requires the presence of $CD4^+$ lymphocytes (Yetter et al., 1988), MAIDS is characterized by functional alterations in B- and T-cell subsets ultimately leading to lymphoproliferation and hypergammaglobulinemia (Morse et al., 1989). These changes occur in a sequential fashion and eventually overwhelm normal immune functions. In some cases, LP-BM5 virus infection also results in B-cell lymphomas (Pitha et al., 1988; Cerny et al., 1990).

The profound immunodeficiency characteristically observed during the course of murine AIDS has many similarities to that observed in humans infected with the human immunodeficiency virus (HIV) (Fauci, 1988). Moreover, like HIV infections in humans, suppression of both cellular and humoral responses in MAIDS renders mice susceptible to otherwise innocuous or rare infections with viral, bacterial, and fungal agents (Gangemi et al., unpublished observations).

The role of cytomegalovirus (CMV) and other herpesviruses as co-factors in potentiating an underlying HIV infection is unclear; nonetheless, herpesviruses have been clearly identified as major opportunistic pathogens in AIDS (Thyms et al., 1989). For example, CMV causes life-threatening pneumonic infections which require immediate antiviral therapy (Skolnek et al., 1988), and while 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG, Ganciclovir) has been approved for the treatment of CMV pneumonitis and retinitis in AIDS patients, a significant number of therapeutic failures occur. These failures appear to result from CMV persistence and drug-induced myelosuppression (Rubin et al., 1989); thus, there is a need to develop less-toxic antivirals which

may be more effective in treating infections caused by CMV and other opportunistic pathogens. In this regard, a new generation of broad-spectrum acyclic nucleoside analogues (phosphonylmethoxyalkyl purine and pyrimidine derivatives) have been developed and evaluated for their antiherpetic activity (De Clercq et al., 1986, 1987; Holý and Rosenberg, 1987; Balzarini et al., 1989; Bronson et al., 1989a,b; Gangemi et al., 1989). Among this class of antivirals, 2 (phosphonylmethoxyethyl) adenine (PMEA) and (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) – have demonstrated remarkable in vitro and in vivo antiviral activity (i.e., anti-CMV and HSV) and intracellular stability (Snoeck et al., 1988; Bronson et al., 1989a,b; Balzarini et al., 1989).

Since human CMV is species-specific and generally does not cause productive infection in animals, murine CMV (MCMV), which shares many characteristics with CMV infections, has been used as a model for human disease (Hudson, 1979; Walker and Hudson, 1988; Kern, 1990; Staczek, 1990). In this model, the age of the mouse at the time of MCMV inoculation determines the outcome of infection; thus, young mice are more susceptible to acute MCMV infections that result in death than are adult mice, which usually survive infection (Mannini and Medearis, 1961; Selgrade and Osborn, 1974). Weanling mice have been used for the in vivo evaluation of antivirals in MCMV infections (Glasgow et al., 1982; Kern, 1988, 1990; Staczek, 1990) and, while the natural history of this virus infection appears to mimic that of human CMV, a lethal model in inbred adult mice has not been reported. This study describes a lethal MCMV and HSV-1 infection model in mice with MAIDS and documents the anti-herpetic activity of HPMPC and PMEA in immunocompromised mice.

Materials and Methods

Virus stocks

LP-BM5 virus and persistently infected SC-1 cell line (TC 2110) were obtained from Dr. Robert Yetter, Veterans Administration Hospital, Baltimore, MD. In most instances, infectious virus was obtained from the supernatant of TC 2110 cells co-cultivated with uninfected, rapidly dividing SC-1 cells as previously described by Gangemi et al. (1989). Otherwise, 3–4-week-old C57BL/6 mice (Harlan Sprague Dawley) were inoculated intraperitoneally (i.p.) with 1×10^5 focus-forming units (FFU) of the LP-BM5 retrovirus complex and sacrificed 30–45 days post-infection. Spleens were pooled and 10% homogenates (5×10^5 FFU/m) were used for infection.

MCMV (Smith strain, E. Kern, Birmingham, AL, U.S.A.), was propagated by passage in Swiss Webster mice (Charles River Breeding Laboratories). Three-week-old female mice were inoculated i.p. with a chronic dosage (2.3×10^5 PFU) of MCMV, and salivary glands were collected twelve days post infection. Virus stocks consisted of 10% salivary gland homogenates in MEM

supplemented with 10% fetal bovine serum and penicillin and streptomycin (Gibco). Virus titers in salivary gland homogenates were assayed on primary cultures of mouse embryo fibroblasts (MEF) and contained approximately 2×10^8 PFU/ml.

HSV-1 (strains McIntyre and VR-3) were propagated in Vero cells. Culture supernatants were harvested at 48 h post-infection when cytopathogenicity was observed. Virus stocks contained approximately 6×10^8 PFU/ml when assayed on Vero cells.

Supernatants from infected cells (LP-BM5 virus, MCMV and HSV-1) were clarified by centrifugation (1500 rpm × 15 min), assayed for mycoplasma contamination, and stored at −80°C in sealed ampoules.

MCMV plaque titrations

Spleen, liver, kidney, salivary glands, lungs, small intestine, adrenal glands and pancreas were aseptically collected from euthanized mice at selected time intervals (5, 7, 14, 25 days) post MCMV infection. Samples were weighed and 10% (weight/volume) tissue suspensions were prepared. The plaque titration assay used has been described previously (Kelsey et al., 1976). In brief, serial 10-fold dilutions of tissue homogenates were inoculated on monolayers of Swiss Webster MEF cells grown in 6-well plates (Costar, Cambridge, MA, U.S.A.). Virus adsorption was allowed to proceed for 60 min at 37°C in a humidified chamber containing 5% CO_2. The monolayers were overlaid with 0.4% Agarose in Dulbecco's Minimum Essential Medium (DMEM) and incubated at 37°C. Six days following infection (when evidence of virus-induced cytopathogenicity was evident), a solution of 1.5% neutral red in PBS was added (6-h incubation) and plaques were counted using an inverted microscope.

General protocol for HSV-1 and MCMV superinfection and drug evaluation

Mice were housed in microisolators in a BL-2 facility for approximately 100 days after infection with the LP-BM5 virus complex. A uniform level of immunosuppression was determined by assessment of splenomegaly, lymphadenopathy and failure of splenocytes to respond to mitogenic stimulation with Concanavalin A. For mitogenic assays, spleens from immunocompetent and immunosuppressed mice were aseptically removed and 10% tissue homogenates prepared. A procedure using tritiated thymidine incorporation was used to measure mitogenic stimulation (Aquino-de Jesus et al., 1989; Gangemi et al., 1989; Griffith et al., 1984).

Immunosuppressed mice were inoculated i.p. with a lethal dose of either the SW-2 strain of MCMV (1.9×10^6 PFU/ml) or the McIntyre strain of HSV-1 (1×10^5 PFU). In some instances, mice were also infected by intranasal instillation (1×10^5 PFU) of the VR-3 strain of HSV-1. Infection with this strain results in a pneumonitis and death due to interstitial involvement

(Nachtigal and Caulfield, 1984). Treatment protocols varied according to individual experiments, and each group consisted of at least ten animals. Mice were observed daily for 30 days and mortality was recorded. Mean survival times (MST) included dead plus surviving mice. The value given to a survivor was 30 days. In most experiments, drugs were administered beginning either 1 or 24 h after MCMV inoculation, and continued as described in the text for several days post-infection. Age-matched immunocompetent mice were used as controls in each study.

Drugs

9-(2-Phosphonylmethoxyethyl) adenine (PMEA) and (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) were provided through the Antiviral Substances Program, NIAID, NIH, Bethesda, MD, by Bristol-Myers Squibb Co. Wallingford, CT, U.S.A. The drugs were dissolved in sterile pyrogen-free Dulbecco's phosphate-buffered saline prior to use. Ganciclovir (DHPG; Syntex Laboratories, Palo Alto, CA, U.S.A.) was diluted in distilled water prior to i.p. administration.

Statistical analysis

The Student's unpaired *t*-test was used to test for statistical significance, unless otherwise indicated.

Results

Virus-induced immunosuppression of susceptible mice

Immunosuppression was induced in C57BL/6 mice following i.p. inoculation of 1×10^5 focus-forming units of the LP-BM5 retrovirus complex. The degree to which individual mice were immunocompromised varied during the first 60 days after infection; however, a more uniform level of immunosuppression was observed 100 days after infection. Loss of both humoral and cell mediated immunity were correlated with splenomegaly, failure of splenocytes to respond to Concanavalin A stimulation and generalized lymphadenopathy (data not shown).

Susceptibility of immunosuppressed mice to MCMV infection

Age-matched immunocompetent C57BL/6 as well as immunosuppressed mice (100 days post LP-BM5 infection), were inoculated with MCMV in order to assess susceptibility to acute infection. As indicated in Table 1, Expt. I, the mortality rate among immunosuppressed mice was 70%, while immunocompetent mice were fully resistant to CMV infection. Mice susceptible to MCMV

TABLE 1

PMEA and HPMPC treatment of MCMV infection in MAIDS

Experiment	% Mortality	MST[d]	*P* value
I[a]			
Immunosuppressed (untreated)	70	11.4	
Immunocompetent (untreated)	0	>21.0	<0.02
PMEA 100 mg/kg	20	17.8	<0.025
II[b]			
Immunosuppressed (untreated)	100	6.0	
Immunocompetent (untreated)	20	16.5	<0.02
HPMPC 50 mg/kg	20	20.7	<0.001
HPMPC 100 mg/kg	20	20.2	<0.001
III[c]			
Immunosuppressed (untreated)	80	5.4	
HPMPC 100 mg/kg	80	23.5	<0.05

[a] Immunosuppressed and age-matched immunocompetent mice were superinfected i.p. with 1.9×10^6 PFU of MCMV at day 100 post LP-BM5 infection and treated with PMEA (100 mg/kg) on the day of MCMV infection and again on days 2, 4, 6, 8, and 10 post-infection.
[b] LP-BM5 immunosuppressed mice and age-matched immunocompetent mice were superinfected i.p. with MCMV (1.6×10^7 PFU) and treated with HPMPC. Fifty mg/kg of HPMPC was administered i.p. 24 h after MCMV infection and again every four days for 2 weeks. Alternatively, 100 mg/kg was given 24 h after MCMV infection and again seven days later. Each experimental group consisted of ten mice.
[c] LP-BM5 immunosuppressed mice were treated with a single i.p. dose of 100 mg/kg of HPMPC, 24 h before superinfection with (1.6×10^7 PFU) MCMV.
[d] Mean survival time.

TABLE 2

Influence of HPMPC treatment on MCMV titers seven days after MCMV infection

Mice[b]	Virus titer ($\log_{10}$ PFU/gm of tissue)[a]				
	Lung	Spleen	Liver	Intestine	Kidney
Not treated	4.9	4.4	3.9	<2.0	3.3
	5.2	5.4	5.3	2.7	4.0
	5.5	5.3	5.3	3.5	4.9
Mean	5.2	5.0	4.8	2.7	4.06
Treated with HPMPC at 50 mg/kg	<2.0	<2.0	<2.0	<2.0	<2.0
	<2.0	<2.0	<2.0	<2.0	<2.0
	<2.0	<2.0	<2.0	<2.0	<2.0
Treated with HPMPC at 100 mg/kg	<2.0	<2.0	2.4	2.0	<2.0
	<2.0	2.7	2.4	<2.0	<2.0
	<2.0	<2.0	<2.0	<2.0	<2.0

[a] Organs were removed from three mice at day 7 post MCMV infection, 10% homogenates were prepared and plaque titrations were performed in MEF cells.
[b] All mice were immunosuppressed with the LP-BM5 retrovirus complex 100 days prior to infection with MCMV. HPMPC was administered i.p. either at 50 mg/kg one day after MCMV infection and again every four days for two weeks, or at 100 mg/kg one day after MCMV infection and again seven days later.

infection died between days 5 and 12 post-infection. Significant titers of infectious virus (2–6 $\log_{10}$ PFU/gm of tissue) were observed in liver, spleen and lung samples when assayed seven days after the infection. In contrast, virus titers in organs from immunocompetent mice were usually less than 100 PFU/gm of tissue.

HPMPC treatment of opportunistic MCMV infection

Treatment of MCMV-infected mice with HPMPC at 50 mg/kg (i.p.) on days 1, 5, 9 and 13 after infection prevented death in 80% of the mice (Table 1, Expt. II). Administration of HPMPC 100 mg/kg (i.p.) on days 1 and 8 after infection was equally effective in reducing the mortality rate ($P<0.001$). The reduction in mortality rate following treatment with HPMPC was accompanied by a reduction in infectious virus titers in the target organs. Virus titers in lung, liver, spleen, kidney and small intestine from mice treated with HPMPC at either 50 or 100 mg/kg were examined at seven and 14 days post MCMV infection (Tables 2 and 3). At 7 days post infection, mice which had received HPMPC at either 50 or 100 mg/kg revealed little if any virus in target organs. Also, at 14 days, mice which had received HPMPC had much less virus recoverable from their organs than untreated mice (Table 3).

Since as few as two injections of HPMPC at 100 mg/kg were effective in therapy of MCMV infection (Table 1, Expt. II), the effectiveness of a single prophylactic dose was determined. As illustrated in Table 1, Expt. III, a single dose of 100 mg/kg given 24 h before MCMV infection significantly increased the mean survival time (from 5.4 to 23.5) as compared with the placebo control

TABLE 3

Influence of HPMPC treatment on MCMV titers 14 days after MCMV infection

Mice[b]	Virus titer ($\log_{10}$ PFU/gm of tissue)[a]				
	Lung	Spleen	Liver	Intestine	Kidney
Not treated	4.4	<2.0	5.3	5.2	3.1
	5.5	<2.0	5.6	5.1	3.9
Mean	4.95	<2.0	5.45	5.15	3.5
Treated with HPMPC at 50 mg/kg	3.5	5.2	4.1	<2.0	<2.0
	3.0	3.1	3.1	<2.0	<2.0
	<2.0	<2.0	2.4	<2.0	<2.0
Mean	2.8	3.4	3.2	<2.0	<2.0
Treated with HPMPC at 100 mg/kg	<2.0	<2.0	<2.0	<2.0	<2.0
	2.4	<2.0	2.9	<2.0	<2.0
	<2.0	<2.0	<2.0	<2.0	<2.0

[a] Organs were removed from two to three mice at day 14 post MCMV infection, 10% homogenates were prepared and plaque titrations were performed in MEF cells.

[b] All mice were immunosuppressed with the LP-BM5 retrovirus complex 100 days prior to infection with MCMV. HPMPC was administered i.p. either at 50 mg/kg one day after MCMV infection and again every four days for two weeks, or at 100 mg/kg one day after MCMV infection and again seven days later.

TABLE 4

Influence of prophylactic (single) dose of HPMPC on MCMV titers seven days after MCMV infection

Mice[b]	Virus titer (log_{10} PFU/gm of tissue)[a]					
	Lung	Spleen	Liver	Intestine	Kidney	Pancreas
Not treated	6.0	4.6	6.6	<2.0	4.2	4.0
	5.0	5.3	6.6	<2.0	4.6	5.1
Mean	5.5	4.9	6.6	<2.0	4.4	4.5
Treated with HPMPC at 100 mg/kg	2.7	<2.0	<2.0	<2.0	<2.0	<2.0
	2.7	<2.0	2.7	<2.0	<2.0	<2.0
Mean	2.7	<2.0	2.35	<2.0	<2.0	<2.0

[a] Organs were removed from two mice at day 7 post MCMV infection, 10% homogenates were prepared and plaque titrations were performed in MEF cells.
[b] All mice were immunosuppressed with the LP-BM5 retrovirus complex 100 days prior to infection with MCMV. HPMPC was administered i.p. at 100 mg/kg one day before MCMV infection.

($P < 0.05$). In addition, target organs from treated mice were almost completely free of detectable virus at both seven and 14 days post-infection (Tables 4 and 5).

Comparison of HPMPC and DHPG for therapy of MCMV infection

To evaluate the therapeutic activity of HPMPC and DHPG against MCMV infection, equivalent doses of both drugs were administered i.p. 30 min after MCMV infection and again on days 2, 4, 6 and 8 after infection. When administered at 5 or 25 mg/kg, HPMPC was markedly more effective against MCMV infection in the LP-BM5 virus-infected mice than was DHPG at the same dose (Fig. 1). Moreover, except for a small amount of virus recovered from the liver, HPMPC treatment was able to completely block virus

TABLE 5

Influence of prophylactic (single) dose of HPMPC on MCMV titers 14 days after MCMV infection

Mice[b]	Virus titer (log_{10} PFU/gm of tissue)[a]					
	Lung	Spleen	Liver	Intestine	Kidney	Pancreas
Not treated	5.9	2.7	5.4	3.2	3.6	5.2
	4.1	<2.0	2.7	<2.0	2.7	5.4
Mean	5.0	2.3	4.0	2.6	3.1	5.3
Treated with HPMPC at 100 mg/kg	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Mean	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

[a] Organs were removed from two mice at day 14 post MCMV infection, 10% homogenates were prepared and plaque titrations were performed in MEF cells.
[b] All mice were immunosuppressed with the LP-BM5 retrovirus complex 100 days prior to infection with MCMV. HPMPC was administered i.p. at 100 mg/kg one day before MCMV infection.

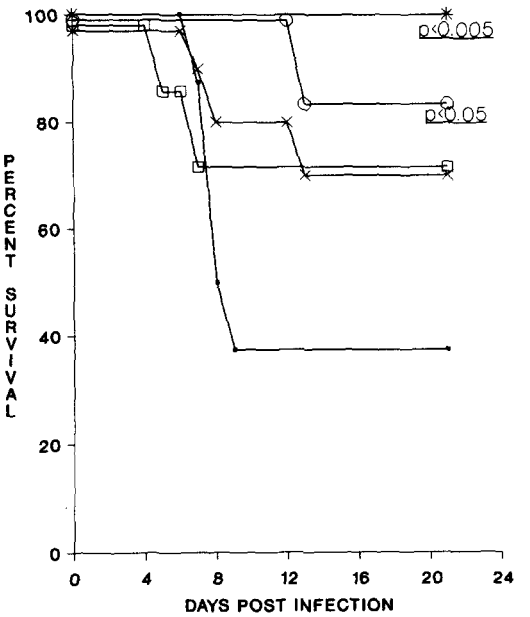

Fig. 1. Comparison of HPMPC and DHPG in MCMV infections. LP-BM5 immunocompromised mice were superinfected intraperitoneally with murine CMV (1.6×10^7 PFU) and treated with (*S*)-1-(3-Hydroxy-2-phosphonylmethoxy-propyl) cytosine (25 or 5 mg/kg) or Ganciclovir (25 or 5 mg/kg) beginning on the day of virus infection and again on days 2, 4, 6 and 8 post MCMV challenge. Each experimental group consisted of 10 mice. Symbols: ■, untreated; ○, HPMPC 5 mg/kg; *, HPMPC 25 mg/kg; □, DHPG 5 mg/kg; ×, DHPG 25 mg/kg

TABLE 6

Suppression of MCMV replication by HPMPC and DHPG

Treatment[b]	Virus titer (log_{10} PFU/gm of tissue)[a]					
	At 5 days post MCMV infection			At 25 days post MCMV infection		
	Lung	Spleen	Liver	Lung	Spleen	Liver
Untreated	5.3	6.3	7.1	–[c]	–[c]	–[c]
(±SD)	0.3	0.5	0.2			
HPMPC 5 mg/kg	<2.0	<2.0	<2.0	<2.0	<2.0	2.2
(±SD)	–	–	–	–	–	0.3
HPMPC 25 mg/kg	ND	ND	ND	<2.0	<2.0	2.5
(±SD)				–	–	0.6
DHPG 5 mg/kg	3.3	2.6	2.6	3.5	2.9	3.8
(±SD)	0.6	0.8	0.6	0.8	0.7	1.6
DHPG 25 mg/kg	ND	ND	ND	4.5	3.8	2.5
(±SD)				1.1	1.9	0.7

[a] Organs were removed at five and 25 days post MCMV infection, 10% homogenates were prepared and plaque titrations performed on MEF cells. Values at day 5 post-infection represent the mean of five mice.

[b] All mice were immunosuppressed with the LP-BM5 retrovirus complex 100 days prior to infection with MCMV. Drugs were administered i.p. on the day of MCMV inoculation and again on days 2, 4, 6 and 8 post-infection.

[c] Untreated mice died before day 25 post MCMV infection.

TABLE 7

PMEA treatment of HSV-1 infection in MAIDS

Experiment	% Mortality	MST	*P* value
I[a]			
Immunosuppressed (untreated)	80	13.1	
Immunocompetent (untreated)	0		<0.02
PMEA 100 mg/kg	44	17.2	
PMEA 10 mg/kg	44	15.5	
II[b]			
Immunosuppressed (untreated)	100	6.5	
Immunocompetent (untreated)	0		<0.001
PMEA 100 mg/kg	35	17.0	<0.001

[a] Immunocompromised and age-matched immunocompetent mice were infected i.p. with 1.6×10^6 PFU of HSV-1 (McIntyre) at day 100 post LP-BM5 infection and treated i.p. with PMEA (10 or 100 mg/kg/day) on the day of HSV-1 infection and again on days 2, 4, 6, 8 and 10 post-infection. Each experimental group consisted of ten mice.

[b] LP-BM5 immunosuppressed mice were superinfected with HSV-1 (VR3 strain) by intranasal instillation of 50 μl (1×10^5 PFU) of the virus and treated i.p. with HPMPC (100 mg/kg/day) on the day of HSV-1 infection and again on days 2, 4 and 6 post-infection. Each experimental group consisted of ten mice. MST: mean survival time.

replication in key target organs when tested at 5 days or 25 days after MCMV infection (Table 6).

PMEA treatment of opportunistic MCMV infection

Since HPMPC was remarkably active in MCMV infections, additional experiments were designed to examine the therapeutic activity of the purine derivative, PMEA. Like HPMPC, PMEA was also an effective therapy for MCMV infection. When administered 1 h after infection and again on days 2, 4, 6, 8 and 10, PMEA (100 mg/kg) caused a significant reduction in the mortality rate and enhanced mean survival time ($P<0.025$; Table 1, Expt. I).

Treatment of opportunistic HSV-1 infections in MAIDS

C57BL/6 mice immunosuppressed by infection with the LP-BM5 retrovirus complex were also susceptible to superinfection with HSV-1. Intraperitoneal infection with a neurotropic strain of HSV-1 (McIntyre) resulted in death due to encephalitis 8–15 days post-infection (data not shown). PMEA (at either 100 or 10 mg/kg), administered 1 h after HSV-1 infection and again on days 2, 4, 6, 8 and 10 after infection, prolonged the mean survival time and reduced the mortality rate (Table 7, Expt. I). Similar results were observed following PMEA treatment of a pneumonic HSV-1 infection (Table 7, Expt. II).

Discussion

Few studies have appeared in the literature in which the therapeutic potential of antivirals in an immunocompromised host has been evaluated. This omission is not unexpected, due to the difficulty in establishing an animal model in which the degree and nature of immunosuppression can be accurately controlled. Nonetheless, antiviral drug evaluation in such animals is essential since the efficacy of an antiviral drug in an host with a defective immune system may be quite different from the results in hosts with a fully functioning immune system. In this regard, MAIDS can be considered as a suitable model in which the degree of immunosuppression can be monitored based on the time following infection with the LP-BM5 retrovirus complex. These mice develop an increased sensitivity to infection with MCMV and HSV-1 (Gangemi et al., 1989) in which the natural history of the disease and the host immune response is similar to that observed in humans.

Murine CMV had been utilized as a model for human CMV infections, since many aspects of both CMV infections are similar (Hudson, 1979; Kern, 1988, 1990; Walker and Hudson, 1988; Staczek, 1990). Whereas the acute or persistent MCMV infection in normal mice is useful for drug evaluation, it would be desirable to have a model of CMV infection in an immunocompromised host in order to study the pathogenesis and treatment of opportunistic CMV infections. While treatment of mice with immunosuppressive agents such as hydrocortisone, cyclophosphamide, or gamma irradiation augment viral replication in the lung of MCMV-infected mice, consistent lethality was not observed (Quinnan et al., 1982; Shanley et al., 1982; Reddehase et al., 1985; Shanley and Pesanti, 1985; Staczek, 1990). We have shown that MCMV infection in the course of an acquired immunosuppressive disease (MAIDS) results in virus dissemination to the lungs, spleen, liver, small intestines and kidneys, and death, in more than 70% of the infected animals (Tables 1–3). This feature has allowed us to evaluate the effect of drug therapy on mortality and on virus replication. Our data clearly indicate that the two phosphonylmethoxyalkylpurine and pyrimidine derivatives, HPMPC and PMEA, are quite effective in the treatment of opportunistic MCMV infections. HPMPC was able to prevent mortality and suppress virus replication in key target organs when therapy was initiated either on the day of or 24 h following virus infection (Tables 1, 2 and 6). In addition, a single injection of HPMPC 24 h prior to MCMV infection was found to prolong mean survival time and to suppress virus replication. The prophylactic effect of HPMPC and its long-lasting therapeutic effect following infrequent dosing may be related to the remarkably long intracellular half-life of the drug (Bronson et al., 1989b; Balzarini et al., 1990). In this regard, while a single injection (100 mg/kg) of HPMPC 24 h prior to MCMV infection was able to prolong survival time and reduce viral replication in target organs, 80% of the infected mice eventually died. Thus, the virustatic activity of a single prophylactic dose of HPMPC was not able to control replication of persistent virus which remained well past the intracellular

half-life of this drug. The use of different therapeutic approaches and dosage modalities (5, 25, 50 and 100 mg/kg) in the evaluation of HPMPC extends previous observations on this drug's potent anti-MCMV activity and low toxicity in mice (Bronson et al., 1989).

The mechanism(s) by which HPMPC and PMEA inhibit MCMV or HSV-1 replication are subject to further study. The compounds are apparently targeted to the viral DNA polymerase (De Clercq, 1990); HPMPC has been shown to specifically block viral DNA synthesis in CMV-infected cells (Neyts et al., 1990) and PMEA acts as a chain terminator in the HIV-1 reverse transcriptase reaction (Balzarini et al., 1990). It should be pointed out that PMEA and HPMPC also have anti-proliferative properties and have been found to reduce the organomegaly observed in MAIDS (data not shown).

While DHPG is currently the drug of choice for the treatment of severe CMV infections, our current studies indicate that HPMPC appears to be more effective than DHPG in the treatment of MCMV infections in the MAIDS model. Thus, HPMPC at a dose of 5 mg/kg was more effective than DHPG at a dose of 25 mg/kg in prolonging the mean survival time and suppressing virus replication in target organs (Fig. 1 and Table 6). These results are encouraging, and indicate the need for clinical trials in which HPMPC is directly compared with DHPG for their efficacy in the therapy and/or prophylaxis of human CMV infections.

In conclusion, our investigations have demonstrated the usefulness of the MAIDS model in the evaluation of antivirals in an immunocompromised host, and suggest that both PMEA and HPMPC may be useful in the treatment of opportunistic CMV and HSV-1 infections.

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In conducting research using animals, the investigator(s) adhered to the 'Guide for the Care and Use of Laboratory Animals,' prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal , Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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