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Selective inhibition of cytomegaloviruses by 9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyloxy-methyl)guanine

[a]Dale L. Barnard, [a]John H. Huffman, [a]Robert W. Sidwell and [b]Elmer J. Reist

[a]*Utah State University, Logan, UT, USA and* [b]*SRI International, Menlo Park, CA, USA*

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Summary

9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyloxy-methyl)guanine (SR 3727A) was significantly inhibitory to strain AD169 of human cytomegalovirus (HCMV) utilizing plaque reduction and inhibition of intra- and extracellular virus yield in MRC-5 cells. The 50% effective concentrations (EC_{50}) ranged from 6–17 μM for three laboratory strains of HCMV, whereas the 50% cytotoxic doses were >4200 μM as determined by viable cell assay and inhibition of radiolabeled precursors into DNA, RNA and protein. EC_{50} values against ganciclovir-sensitive clinical isolates ranged from 8–47 μM. Against two ganciclovir-resistant strains of HCMV, EC_{50} values of SR 3727A were 84 and 320 μM; against murine CMV (MCMV); 17 μM and against guinea pig CMV, 56 μM. SR 3727A was most effective when infected cells were treated 24 h or less after virus adsorption. BALB/c mice infected intraperitoneally (i.p.) with a lethal dose of MCMV were treated i.p. with 31.3, 62.5, 125, or 250 mg/kg/day of SR 3727 twice daily for 5 days beginning 4 h pre-virus inoculation. All doses were well tolerated; the 125 and 250 mg/kg/day doses significantly prevented death. In a second experiment, SR 3727 at 125 mg/kg/day markedly reduced titers of recoverable virus from spleens, kidneys, and salivary glands harvested at varying times after virus inoculation.

Ganciclovir; Analog; Cytomegaloviruses; Antiviral; 3727A; 9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyloxymethyl)guanine

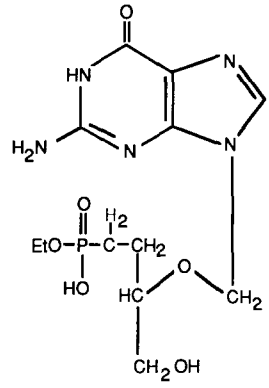
Correspondence to: D.L. Barnard, Utah State University, Logan, UT, USA.

Introduction

Human cytomegalovirus (HCMV) is a major cause of life-threatening infections in immunosuppressed patients including organ and tissue transplant patients, cancer patients, and those with AIDS (Grundy, 1990). Recognition of HCMV as major cause of morbidity and mortality in these patients has lead to an intensive search for novel, selective agents against HCMV. Most of the compounds tested have been nucleoside analogs which have targeted HCMV DNA polymerase. Several have been used for treatment of severe HCMV infections, including phosphonoformate (PFA, foscarnet) and ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG] (Balfour, 1990). Ganciclovir has been found to be one of the most potent inhibitors of HCMV (Balfour 1990). However, there are two major problems with prolonged ganciclovir therapy of HCMV infections. First, ganciclovir is ineffective against latent virus infections, and so patients must receive prolonged treatment to merely control the infection. This often results in severe bone marrow toxicity (Meyers, 1991). The second problem is that during treatment, virus resistance to ganciclovir often develops (Drew et al., 1991).

In response to the need for less toxic and more effective anti-HCMV compounds, several series of compounds, including HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (*S*)-HPMPC] (Neyts et al., 1990) and BHCG [(±)-(1α,2β,3α)-9-[2.3-bis(hydroxymethyl)cyclobutyl]guanine, SQ 33054] (Field et al., 1990), have been synthesized. HPMPC is a derivative of a class of compounds characterized by a stable phosphonoalkyl ether group and the absence of the usual glycosidic bond. It appears to be a potent and selective inhibitor of HCMV in vivo, causing a reduction in CMV titer and conversion to negative culture at dose levels of 3 and 10 mg/kg (Drew et al., 1993). However, it also showed nephrotoxicity which was dose-limiting after 2 doses at the 10 mg/kg level and was generally reversible after stopping therapy (Drew et al., 1993). BHCG is a derivative of oxetanocin-A; a recently isolated, naturally occurring nucleoside containing a novel 9-(2-oxetanyl)adenine linkage. Although BHCG is a broad spectrum antiviral, it appears to be as effective against HCMV as ganciclovir and is also effective against ganciclovir-resistant HCMV (Clement and Kern, 1991).

Recently, Reist et al. (1988) and Kim et al. (1991) synthesized a series of acyclic phosphonate analogs of ganciclovir and acyclovir [9-(2-hydroxy-2-propoxymethyl)guanine]. Some of the compounds synthesized by the former group lacked toxicity and were shown to have anti-HCMV activity equal to that of ganciclovir (Duke et al., 1986; Sidwell et al., 1988). We report here on the effects of a ganciclovir analog, 9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyloxymethyl)guanine (SR 3727A, Fig. 1) on in vitro cytomegalovirus infections and on the effects of an enantiomeric mixture, SR 3727, on in vivo cytomegalovirus infections.



SR 3727A

9-(3'-ethylphosphono-1'-hydroxymethyl-1'propyloxy-methyl)guanine

Fig. 1. Structural representation of the ethylphosphonate derivative of ganciclovir.

Materials and Methods

Compounds

SR 3727 [9-(3'-ethylphosphono-1'-hydroxymethyl-1'-propyloxymethyl)-guanine] and the purified active isomer, SR 3727A, were synthesized in the laboratory of Dr. Elmer Reist (SRI International; Menlo Park, CA). All animal studies were done with the enantiomeric mixture, SR 3727, because it was more readily available in sufficient quantities for in vivo testing. Ganciclovir was provided by Dr. Thomas Matthews (Syntex Research; Palo Alto, CA). Tritiated thymidine, uridine and leucine (20 Ci/mmol, 46 Ci/mmol, 38 Ci/mmol, respectively) were obtained from Amersham Life Sciences (Arlington Heights, IL).

Cells and virus

Human foreskin fibroblasts (Hs-68) and human lung fibroblasts (MRC-5) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and routinely grown in Dulbecco's minimal essential medium (DMEM) and basal minimal essential medium (BME), respectively; each medium was supplemented with 10% fetal bovine serum (FBS) and 0.035% $NaHCO_3$ without antibiotics. Guinea pig embryo cells (GPE) were obtained from Whitaker MA Bioproducts (Walkerville, MD) and grown in MEM with 10% FBS and 0.05% $NaHCO_3$. Mouse fibroblast cells (3T3) were purchased from ATCC. HCMV strains AD-169, Davis and Towne were obtained from ATCC. Two ganciclovir-resistant strains of HCMV, C8704 and C8805-37, and a ganciclovir-sensitive strain, C8708, were obtained from Dr. Karen Biron, Burroughs Wellcome (Research Park Triangle, NC). Ganciclovir-sensitive HCMV isolates EC, CH and LA were kindly provided by Dr. Earl Kern

(University of Alabama, Dept. Pediatrics). Guinea pig cytomegalovirus (GPCMV), strain 22122, was obtained from Dr. Brigette Griffith, Veterans Administration Medical Center (West Haven, CT) and mouse cytomegalovirus (MCMV), Smith strain, from Southern Research Institute (Birmingham, AL).

Antiviral activity

(a) Plaque reduction assay. Assays were done as described by Barnard et al. (1992) using MRC-5 cells and 100 plaque forming units (PFU) virus/well of the appropriate strain of HCMV. The plates were centrifuged at 2200 RPM for 30 min at room temperature to facilitate virus adsorption (Gleaves, 1984). Morphological changes due to compound cytotoxicity were graded as described below prior to fixing and staining of monolayers. Generally, the plaques were counted 6–7 days after infection. However, two of the clinical isolates (LA and CH) required 21 days to form plaques. EC_{50} and IC_{50} values were calculated by regression analysis.

(b) CPE inhibition assay. CPE inhibition assays were performed in 96-well flat bottomed microplates as described by Sidwell and Huffman (1971). Virus, either GPCMV or MCMV, was used at 100 $CCID_{50}$/well to infect guinea pig embryo cells or 3T3 mouse cells, respectively. Plates were incubated at 37°C and the cells examined periodically for evidence of viral cytopathic effect (CPE) and for morphological changes due to compound toxicity. Virus CPE was rated on a scale of 0–4, with a rating of 4 defined as all cells in the monolayer showing the particular cytopathic effect. Normally the assay was stopped and the plates scored when the control virus wells reached a rating of 4 (5–7 days). The 50% effective concentration (EC_{50}) was calculated by regression analysis using the means of the CPE ratings at each concentration of compound. Morphological changes due to compound cytotoxicity were graded on a scale of 0–5 with a grade of 5 being defined as complete (100%) cytotoxicity and 1, for example, defined as 20% of the monolayer showing cytopathic effect. The 50% cytotoxic dose (IC_{50}) was also calculated in a similar manner. A selective index (SI) was calculated for each compound using the following formula: $SI = (IC_{50})/(EC_{50})$.

(c) Virus yield assay. Growth medium from confluent monolayers of MRC-5 cells in 24 well plates was decanted and 1 ml of HCMV (AD-169) diluted to 100 PFU was added to each well. The plates were centrifuged at 2200 RPM for 30 min at room temperature to facilitate virus adsorption. The medium with virus was aspirated from each well and 0.8 ml of the appropriate drug dilution was placed in the test wells and 0.8 ml of medium without drug into 8 virus control wells and 4 cell control wells. This time of addition of virus was considered time 0.

On days 2, 4, 6, and 8 the supernatant virus was withdrawn from 2 wells at each drug dilution, from 2 virus control wells and from 1 cell control well. The supernatant fluids were pooled for each treatment and the virus titer was

assayed for each treatment by plaque assay as described above. The cell monolayers for each treatment were then scraped into 1 ml of medium without serum. The cell suspensions were then frozen and thawed to free the cell-associated virus and the lysates pooled for each treatment and the titers determined by plaque assay.

Cellular toxicity

(a) Biochemical cytotoxicity assay. The assay for quantitating the uptake of radiolabeled macromolecular precursors in the presence or absence of compound was done as described by Barnard et al. (1992). The effects of each test compound on the uptake of [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]leucine into acid-soluble and -insoluble fractions of late-log phase human diploid cells (Hs-68) were determined following an 18 h incubation period with drug and a subsequent 1 h pulse period with the appropriate macromolecular radiolabeled precursor in the presence of fresh antiviral compound. All statistical comparisons were made by analysis of variance. The experiment was done one time and each treatment was assayed in quadruplicate.

(b) Viable cell count assay. This assay was done as described by Barnard et al. (1992). Briefly, twelve-well tissue culture plates were seeded with MRC-5 cells and incubated for 5 h at 37°C prior to replacement of the growth medium with test medium (DMEM, 2% FBS, 0.1% $NaHCO_3$, 50 μg gentamicin/ml) containing test compounds at the desired concentrations. At the appropriate time periods, medium was aspirated from the wells treated with antiviral compound (duplicate) or from the untreated, control wells (triplicate) to allow counting of viable cells detached with trypsin using the trypan blue exclusion dye method (Freshney, 1983). The experiment was done twice and duplicate samples for each time period assayed counted three times.

Addition at different time intervals

Two equimolar concentrations of ganciclovir and SR 3727A, 0.88 mM and 0.28 mM, were added to HCMV (AD-169) infected cells (100 PFU/well) in 24-well tissue culture plates, either at time 0 (immediately following centripetal adsorption), or at 24, 48 or 72 h following centripetal adsorption. Drug remained on the wells for 24 h and was then replaced with drug-free overlay medium (1% agarose plus growth medium). Cultures were incubated for a total of 6 days at 37°C following adsorption. Plaque inhibition was determined as described above. The experiment was done one time and experimental treatments were assayed in duplicate.

Animal studies

(a) Lethal infection. One in vivo antiviral experiment was done with a enantiomeric mixture of SR 3727 to determine its antiviral efficacy and relative tolerance in animals. The compound was diluted in sterile saline. 4 week old BALB/C mice were infected with murine cytomegalovirus (10^6 $CCID_{50}$/mouse)

intraperitoneally (i.p.); SR 3727 was administered i.p. at four dosage levels twice daily for 5 days beginning 4 h before infection. Twenty infected mice treated with saline by the identical schedule served as virus controls. The animals were observed for death daily for 21 days. Five infected mice were not treated to serve as normal controls. Five toxicity control animals were run in parallel at each dosage of SR 3727. The toxicity and normal controls were weighed immediately before the initial treatment and again 18 h after the final treatment. They were also observed for death for 21 days.

(b) Sublethal infection. One experiment to determine SR 3727 treatment effects on virus titers in tissues was run with the same enantiomeric mixture of 3727 used above. In this assay, mice were infected with a sub-lethal dose of MCMV and then treated with 125 mg/kg/day SR 3727 4 h before virus infection and then twice daily for a total of 5 days. Controls as described for the lethal infection were again included. At days 3, 5, 7 and 9 post-infection, 5 mice from each infected, treated group were sacrificed to determine titers of virus found in various organs and to also monitor serum levels of liver enzymes.

TABLE 1

Relative sensitivities of various strains of human, murine, and guinea pig cytomegaloviruses to SR 3727A and ganciclovir

Virus strain	SR 3727A (μg/ml)			Ganciclovir (μg/ml)		
	IC_{50}[a]	EC_{50}[b]	TI[c]	IC_{50}	EC_{50}	TI
HCMV[d]						
AD-169	>4200	7	>600	>6000	8	>750
Davis	>4200	17	>250	>6000	12	>500
Towne	>4200	6	>750	>6000	4	>1500
C8704[e]	>4200	84	>50	>6000	60	>100
C8805[e]	>4200	320	>10	>6000	100	>60
C8708[f]	>4200	34	>120	>6000	4	>1500
EC[f]	>4200	8	>530	>6000	<4	>1500
LA[f]	>4200	25	>170	>6000	8	>750
CH[f]	>4200	47	>90	>6000	8	>750
MCMV[g]						
Smith	>4200	17	>250	>400	4	>100
GPCMV[h]						
22122	>4200	56	>80	1200	300	4

[a]The concentration of compound calculated by regression analysis to lay midway between those concentrations showing no evidence of cytotoxicity and those showing complete cytotoxicity, as observed by light microscopy.

[b]The concentration of drug that reduced plaque production or cytopathic effect by 50%.

[c]Therapeutic index (IC_{50}/EC_{50}).

[d]Plaque reduction assays done in MRC-5 cells.

[e]HCMV clinical isolates resistant to ganciclovir.

[f]HCMV clinical isolates sensitive to ganciclovir.

[g]CPE inhibition assay done in Swiss albino mouse fibroblasts (3T3 cells).

[h]CPE inhibition assay done in guinea pig embryo cells.

Titers of virus found in the kidney, spleen, salivary gland and liver homogenates were assayed by a CPE inhibition assay as described above. The liver homogenates proved to be toxic to the 3T3 cells used for virus assay. Therefore, liver damage was monitored by assaying serum levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) using a commercially available kit from Sigma Chemical Co. (St. Louis, MO).

Results

Antiviral activity

Plaque reduction and CPE inhibition. The effects of SR 3727A on various strains and isolates of cytomegalovirus (CMV) are shown in Table 1. When compared to ganciclovir, the compound had equivalent antiviral activity against the AD-169, Towne and Davis strains of HCMV. The analog was almost five times as inhibitory to guinea pig CMV (GPCMV) as was ganciclovir, and was also nearly as effective against mouse CMV (MCMV) as ganciclovir. SR 3727A was not as active against several ganciclovir-resistant clinical isolates when compared to ganciclovir.

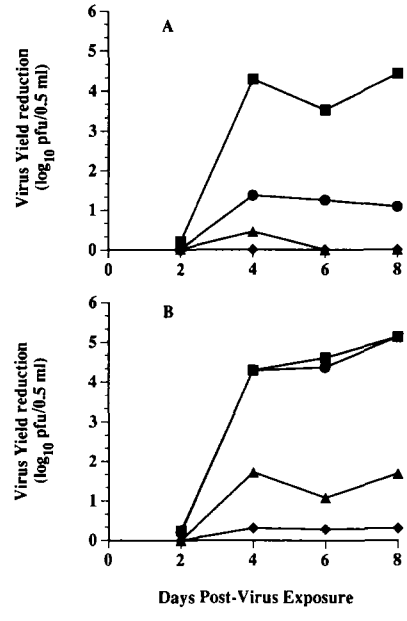


Fig. 2. Effects of various concentrations of SR 3727A and ganciclovir on HCMV yield. (A) represents the reduction in titer of cell-released virus as detected by plaque assay in the presence of SR 3727A. (B) represents the reduction in cell-released virus in the presence of ganciclovir. Infected cells were treated with compound immediately after adsorption. ■ 880 μM, ● 88 μM, ▲ 8.8 μM, ◆ 0.88 μM.

Virus yield reduction. Virus yield reduction assays showed that SR 3727A at 880 μM reduced dramatically the amount of virus released into the supernatant fluids, with the greatest reduction at 8 days post-exposure (Fig. 2). In comparison, ganciclovir greatly reduced the amount of virus at two concentrations, 880 μM and 88 μM; and unlike SR 3727A, the antiviral effect appeared to plateau at 4 days post-exposure. Cell associated virus was also significantly reduced at 880 μM by SR 3727A, but is effect plateaued at 4 days post-exposure. Again, two concentrations (880 and 88 μM) of ganciclovir considerably reduced cell-associated virus titers, and this reduction leveled off at 4 days post-exposure.

Cytotoxicity

Macromolecular synthesis. SR 3727A had no effect on macromolecular synthesis at the concentrations tested (2.8–2800 μM); i.e., an IC_{50} concentration was never attained. For SR 3727A any antiviral effect appeared to be independent of any inhibition of macromolecular synthesis (data not shown) which correlated well with the visual observations of toxicity (see Table 1). In contrast, ganciclovir did inhibit DNA synthesis with an EC_{50} of 2400 $\pm$ 200

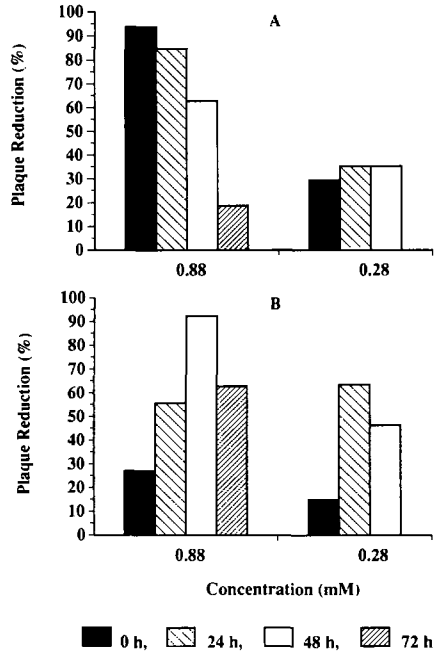


Fig. 3. Effect of the time of addition of SR 3727A (Panel A) and ganciclovir (Panel B) to virus-infected cells on the reduction of viral plaque formation. Two equimolar concentrations of ganciclovir and SR 3727A were added to HCMV-infected cells (100 PFU/well) either at time 0 (immediately following centripetal adsorption), or at 24, 48 or 72 h following centripetal adsorption. Drug remained on cells for 24 h and was replaced with drug-free overlay medium. ■ 0 h, 24 h, □ 48 h, 72 h.

μM, but this inhibition was far above any concentration inhibiting virus expression (see Table 1).

Cell growth cycle. The doubling time for MRC-5 cells in the absence of either SR 3727A or ganciclovir was approximately 24 h (data not shown). Prolonged exposure of actively growing cells to SR 3727A resulted in no significant reduction of cell growth and only a small delay in doubling time ($P > 0.05$). In comparison, ganciclovir at 3900 μM did inhibit cell growth at days 1 and 2 after plating and on day 1, a moderate inhibition in cell growth appeared at all ganciclovir doses. However, by day 3 cell yields were restored to control levels. An IC_{50} was never reached for either compound at the concentrations tested (2.8–3900 μM), confirming the data obtained by visual observations of toxicity and biochemical cytotoxicity assays.

Effect of time of addition of compounds

Experiments were done to determine the optimal time for therapeutic intervention during the course of infection in vitro (Fig. 3). Two equimolar, noncytotoxic concentrations of ganciclovir and SR 3727A were used. SR 3727A was much more effective in reducing plaque production than ganciclovir when present at early times of infection. At 48–72 h after virus adsorption, ganciclovir was more effective than SR 3727A. Ganciclovir exhibited its greatest activity when added later in the infection. The absolute reduction in plaque formation was nearly equal for both compounds, with both drugs reducing virus yield by greater than 93%. The time profile of inhibition also appeared to be independent of concentration, although reduction of virus yields were considerably less for both compounds when tested at 0.28 mM.

In vivo antiviral activity

Lethal infection. SR 3727 and its purified isomer SR 3727A showed very similar in vitro antiviral activity and cytotoxicity (data not shown, see Sidwell et al., 1988). Therefore, due to greater available amounts of SR 3727, SR 3727 was used in all animal experiments in this study. Neither SR 3727 at concentrations up to 250 mg/kg/day nor ganciclovir at 50 mg/kg/day caused any discernible toxic effects in the mice (Table 2). Significant ($P < 0.01$) increases in survivors were seen in the infected mice treated with SR 3727 at 125 and 250 mg/kg/day. Significant increases in mean survival time occurred in the infected mice treated with the two lowest doses of SR 3727. Ganciclovir exhibited the positive activity expected.

Sub-lethal infection. SR 3727, given at 125 mg/kg/day, significantly reduced virus titers in the kidney up to 7 days post-exposure and in the salivary glands up to 9 days post-exposure ($P < 0.05$). An even more dramatic reduction in virus recovered from the spleen was seen. Liver homogenates were toxic to the cells used for the virus assay, so virus reduction could not be quantitated in this tissue. Treatment with SR 3727 did prevent liver damage due to virus infection.

TABLE 2

Effect of i.p. treatment with SR 3727 on lethal murine cytomegalovirus infections[a]

Compound	Dosage (mg/kg/day)	Tox. control		Infected treated	
		Survivor /total	Percentage initial body wt. (g)[b]	Survivor /total	MST[c] (days)
SR 3727	250	5/5	102	10/10**	>21.0**
	125	5/5	105	9/10**	8.0
	62.5	5/5	102	2/10	6.6**
	31.3	5/5	101	0/10	5.6**
Ganciclovir	50	5/5	107	10/10**	>21.0**
Saline	–	–	–	0/20	3.9
Normals	–	5/5	105	–	

[a]Bid × 5 beginning 4 h previrus inoculation.
[b]Initial body weight at start of treatment divided by the mean weight 18 h following final treatment of toxicity control mice.
[c]Mean survival time of mice dying on or before day 21.
*$P<0.05$, **$P<0.01$.

However, the serum levels of two liver enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST) were near the levels seen with the normal, uninfected mice.

TABLE 3

Effect of i.p. treatment with SR 3727 on sublethal murine cytomegalovirus infections in mice[a]

Treatment	Days post-virus inoculation	Mean virus titer[b] (log_{10})			Mean serum enzyme level[c]	
		kidney	spleen	salivary gland	ALT[d]	AST[e]
SR 3727 (125 mg/kg/day)	3	0.0*	1.6**	0.0*	110	37.0**
	5	0.0*	0.6**	0.0*	103	29.0**
	7	0.0*	0.5**	2.4*	141	34.0
	9	0.0	0.0**	3.4*	113	37.0**
Saline	3	0.0	3.1	1.1	189	148
	5	0.6	3.6	1.4	479	301
	7	0.8	1.9	4.1	227	101
	9	1.7	2.5	7.5	655	407
Normals	3–9	0.0	0.0	0.0	60–150	18–37

[a]Bid × 5 beginning 4 h pre-virus inoculation.
[b]$n = 5$.
[c]Sigma-Fraenkel units/ml.
[d]Alanine amino transferase.
[e]Aspartate amino transferase.
*$P<0.05$, **$P<0.01$ (Compared to saline-treated controls at the same day).

Discussion

The ganciclovir analog, 9-(3′-ethylphosphono-1′-hydroxymethyl-1′-propyl-oxy-methyl)guanine (SR 3727), was previously described to be an inhibitor of HCMV in a brief report (Sidwell et al., 1988). In the present study, the in vitro antiviral effects of the purified isomer, SR 3727A, were further studied, with efficacy seen against standard strains of HCMV, MCMV and GPCMV. Ganciclovir-resistant HCMV appeared to be also resistant to SR 3727A indicating a similar mode of inhibition. However, SR 3727 may be a prodrug of the homophosphonate of ganciclovir which has been shown to inhibit HCMV polymerase in vitro (Duke et al., 1986). Although the homophosphonate of ganciclovir also inhibited ganciclovir-resistant virus (Sullivan, et al., 1993), SR 3727 did not, suggesting that SR 3727 may not be simply a prodrug of the homophosphonate of ganciclovir. The fact the ethyl ester bond appears to be relatively stable and is cleaved only slowly in vivo (data not published) also argues against SR 3727 as only a prodrug. This is supported by the data which show that the optimal time of addition of SR 3727 to inhibit virus replication is when it is used early in the virus infection. In contrast, ganciclovir as well as its homophosphonate derivative (data not published) optimally inhibited virus several days after the initiation of viral infection, in agreement with another study where ganciclovir was reported to significantly reduce HCMV yield even when administered several days after infection (Tyms et al., 1987). Alternatively, the ability to inhibit HCMV replication early in the infection when compared to ganciclovir may also have been a function of the chemical structure of SR 3727 which was designed to overcome the rate-limiting step of monophosphorylation (Reist et al., 1988). If SR 3727 is merely a prodrug of the homophosphonate of ganciclovir, it may have been more rapidly converted to the antivirally active triphosphate form of the homophosphonate than ganciclovir was to its triphosphate form (Duke et al., 1986). Another scenario is that the target of inhibition of SR 3727 could have also been the gene product of UL97, the viral enzyme that phosphorylates ganciclovir, if it is essential for viral replication (Sullivan et al., 1991). This could also explain the early inhibitory effects of SR 3727, especially if the protein kinase homologue is necessary early in the viral infection.

In virus yield reduction assays, ganciclovir was effective at lower concentrations than SR 3727A in inhibiting viral replication, although the plaque reduction assays indicated that the two compounds were effective against the clinical isolates and laboratory strains at very similar concentrations. This may have been so because plaque assays do not measure a reduction in plaque size and therefore, a reduction in virus yield; all plaques are given the same statistical weight regardless of quantitative differences in the virus yields from those plaques.

It is interesting to note that SR 3727A was not toxic in any of our assays, while ganciclovir did inhibit DNA synthesis at higher, non-therapeutic concentrations and delayed cell growth. This is consistent with the studies of

Smee et al. (1983) which showed an even more profound inhibitory effect on DNA synthesis in ganciclovir-treated Vero cells.

The enantiomeric mixture, SR 3727, was found to have significant in vivo antiviral activity against MCMV infections and was well tolerated by the animals at the concentrations used. Because of a shortage of available compound, the highest dose used was 250 mg/kg/day. We have not yet been able to determine the maximum tolerated dose of the compound. The results of this study suggest that SR 3727A is an effective cytomegalovirus inhibitor, both in vitro and in vivo, and may be a useful therapeutic agent for treatment of human cytomegalovirus infections.

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