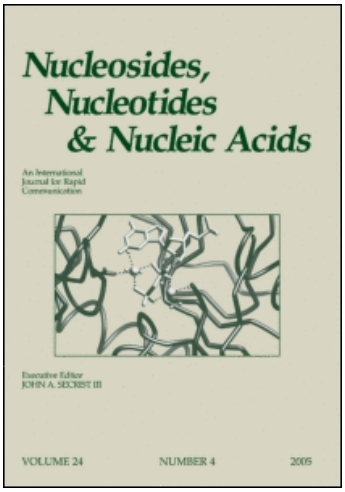

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STRUCTURE-ACTIVITY RELATIONSHIP OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR OR GANCICLOVIR AGAINST HUMAN CYTOMEGALOVIRUS IN MRC-5 CELLS

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Abstract: In vitro anti-HCMV activity of 22 phosphonic acid analogs of acyclovir or ganciclovir (DHPG) was determined in MRC-5 cells to establish structure-activity relationships.

Introduction: A need continues to exist for safe and effective therapies for human cytomegalovirus (HCMV). At the present time, two drugs have been approved in the United States for treatment of this important disease. The nucleoside analog, ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine] (DHPG) has been used for treating HCMV-induced pneumonia, retinitis and colitis[1,2], but its use is associated with suppression of bone marrow progenitor cells[3]. The pyrophosphate analog, foscarnet, approved for therapy of retinitis induced by HCMV, has been efficacious while being less toxic than DHPG[4]. Use of foscarnet, however, has been associated with nephrotoxicity often with proteinuria, hypocalcemia, hyperphosphatemia, anemia, gastrointestinal intolerance and neutropenia[4,5].

An approach to developing a drug which would be at least as effective as DHPG and foscarnet, yet have fewer toxic effects, has been to synthesize acyclonucleoside analogs of DHPG and the related antiviral, 9-[(2-hydroxyethoxy)methyl]guanine (ACV). Reist et al.[6] and Kim et al.[7] have synthesized a series of acyclic phosphonate analogs of these compounds. The materials developed by Reist and his associates have undergone extensive in

Dedicated to the memory of Dr. Roland K. Robins.

vitro evaluation against HCMV; a portion of those results were briefly described by Sidwell et al.[8] It has been reported by Prisbe et al.[9] and Duke et al.[10] that the 5'-mono-, 3',5'-bis (mono-), and 3',5'-cyclic monophosphate and 5'-homophosphonate forms of DHPG were strongly inhibitory to HCMV, and Duke et al.[10] have reported that the DHPG homophosphonate was also inhibitory to murine cytomegalovirus (MCMV) infections in mice. Barnard et al.[11] have recently described the in vitro HCMV- and in vivo MCMV-inhibitory activity of 9-(3'-ethylphosphono-1'-hydroxymethyl-1'propyloxymethyl)guanine.

The present report provides an overview of the structure-antiviral relationships of the complete series of acyclonucleosides of DHPG and ACV which have been synthesized by Reist and his associates as described in a companion article[12].

Materials and Methods: Cells. Continuous passaged human lung (MRC-5) cells were grown in basal medium Eagle (BME), 10% fetal bovine serum (FBS), 0.035% $NaHCO_3$, without antibiotics.

Virus. Stocks of human cytomegalovirus (HCMV), strain AD-169, obtained from the American Type Culture Collection (ATCC), Rockville, MD, were prepared in MRC-5 cells, stored at -86°C, and titered in MRC-5 cells prior to use in the experiments.

Compounds. The phosphonate analogs of ACV and DHPG were synthesized by Elmer J. Reist and associates at SRI International, Menlo Park, CA. Acyclovir was provided by Burroughs Wellcome Co. (Research Triangle Park, NC). DHPG was provided by Syntex Research (Mountain View, CA), or was synthesized at SRI International.

Antiviral Evaluation. Compounds were prepared in Dulbecco's modified Eagle medium (DMEM), 2% FBS, 0.1% $NaHCO_3$, 50μg gentamicin/ml (test medium). Virus was also diluted in this test medium. Growth medium was removed from confluent monolayers of MRC-5 cells in 24-well tissue culture plates and virus was allowed to adsorb to the cells during 30 minutes of centrifugation at 2200 rpm at room temperature. The virus inoculum was removed after centrifugation and compounds were placed on the cells which were incubated at 37°C until virus plaques had developed in the virus control wells. Media was removed and cells were stained by adding 0.2% crystal violet in 10% buffered formalin to allow virus plaques to be counted by use of a dissecting microscope. The 50% effective (plaque inhibitory) dose (ED50) was determined by regression analysis of the

plaque counts. The 50% cytotoxic dose (CD50) was determined by microscopic examination of cell anomalies in the treated cells. Cytotoxicity grades were assigned and regression analysis of these grades were used to calculate the CD50 value. A therapeutic index (TI) was calculated as CD50+ED50.

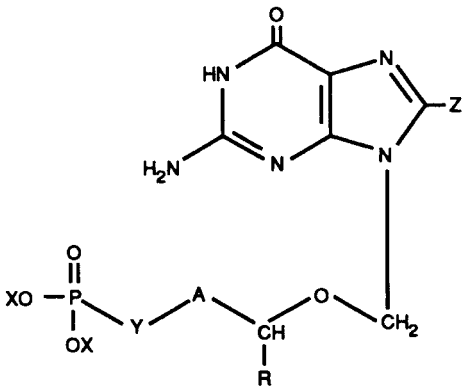
Results and Discussion: As seen in Table 1, the most active compound tested was the R-enantiomer of the phosphonate diacid of DHPG (SR3773). The equivalent mixture of the R- and S-enantiomers (SR3745A) was slightly less active, but still as active as DHPG itself (average ED50 of 8.6 μM); however, the S-enantiomer (SR3772) was much less active than any of these. All of these compounds, except SR3772, had CD50 values >3,000 μM. The isomer (SR3745B) of SR3745A had little antiviral activity and showed more cytotoxic activity than was seen with SR3745A.

The R-enantiomer (SR3775) of the cyclic phosphonate of DHPG, with an ED50 value of 11 μM, had somewhat less anti-HCMV activity than SR3745A. The racemic cyclic phosphonate (SR3759) was even less active, with an ED50 value of 15 μM. The CD50 value for each of the cyclic phosphonates of DHPG were >3,000 μM.

The phosphonate monoethylester of DHPG (SR3727A) was the next most active phosphonate, with an ED50 of 27 μM, while its isomer (SR3727B) was essentially inactive as an anti-HCMV compound. The CD50 values of these compounds were >2,800 μM.

The phosphonic diacid of acyclovir (SR3722), with an ED50 of 54 μM, and a CD50 >3,000 μM, followed SR3727A in antiviral efficacy. The next most potent phosphonate, following SR3722, was SR3723. As seen in Table 1, the phosphonate monoethylester of acyclovir was less active than was the diacid. This is consistent with the relative activities of the phosphonate monoethylester (SR3727A) and the diacid (SR3745A) of DHPG. Replacement of the ethylester of SR3723 with a butylester to give SR3754, caused a reduction of antiviral activity. Replacement of the hydrogen at position 8 of SR3723 with bromine to give SR3740 caused a further decrease of antiviral activity and an increase in cytotoxicity. Putting a methyl group in place of the hydrogen of SR3723 to give SR3742 essentially eliminated the anti-HCMV activity, but had little or no effect on the cytotoxicity. Increasing the side chain length of SR3723 by 4 carbons to give SR3724 eliminated antiviral activity but seemed to have no effect on cytotoxicity. Adding another ethyl

TABLE 1. Anti-HCMV activity of various phosphonate analogs of DHPG or ACV with the general structure shown.



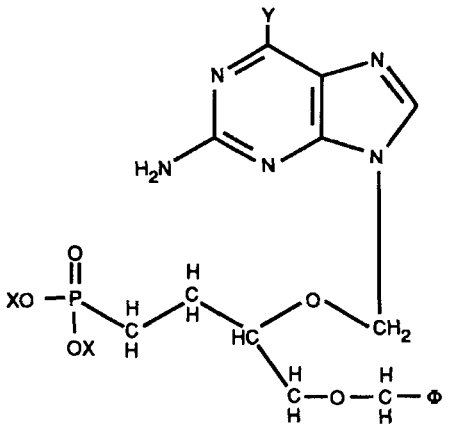
Compound Number	X	Y	R	Z	A	CD50 (μM)	ED50 (μM)	TI	Comments
3773	H,H	CH_2	CH_2OH	H	CH_2	>3,000	4.2	>714	R-enantiomer
3745A	H,H	CH_2	CH_2OH	H	CH_2	>3,000[1]	7.8	>385	mixture
3772	H,H	CH_2	CH_2OH	H	CH_2	1,023	66	16	S-enantiomer
3745B	H,H	$(CH_2)_2$	H	H	CHOH	1,686	549	3.1	
3727A	Et,H	CH_2	CH_2OH	H	CH_2	>2,800[1]	27	>104	
3727B	Et,H	$(CH_2)_2$	H	H	CHOH	>2,000	>2000	?	
3722	H,H	CH_2	H	H	CH_2	>3,300	54	>61	
3723	Et,H	CH_2	H	H	CH_2	>3,000[2]	139	>22	
3754	Bt,H	CH_2	H	H	CH_2	>2,900	292	>9.9	
3740	Et,H	CH_2	H	Br	CH_2	1,146[3]	312	3.7	
3742	Et,H	CH_2	CH_3	H	CH_2	>2,760	660	>4.2	
3724	Et,H	$(CH_2)_5$	H	H	CH_2	>2,580[2]	>2,580	?	
3725	Et,Et	CH_2	H	H	CH_2	>2,780	>2,780	?	

[1]Confirmed by viable cell count with trypan blue exclusion as measure of viability (cytotoxicity assays done in both log phase and stationary cells).

[2]Confirmed by viable cell count with trypan blue exclusion as measure of viability (cytotoxicity assays done in stationary cells only).

[3]Viable cell count gave a higher CD50 (>2,400 μM) in log phase cells than by visual grading in stationary cells (1,146 μM).

TABLE 2. Anti-HCMV activity of several phosphonate benzylether analogs of DHPG with the general structure shown.



Compound Number	X	Y	CD50 (μM)	ED50 (μM)	TI
3761	Et,Et	Cl	187	112	1.7
3762	Et,Et	OH	611	133	4.6
3763	H,H	OH	>2,362	479	>4.9
3774	H,H	Cl	>2,263	778	>2.9

group to make the phosphonate diethylester of ACV (SR3725) eliminated the antiviral activity but seemed to have no effect on cytotoxicity.

The anti-HCMV activity of ACV, with an ED50 of 101 μM, was between that of SR3722 and a series of phosphonates containing a benzylether moiety as shown in Table 2. The most active of these was SR3761, with an ED50 value of 112 μM, but this compound was also the most cytotoxic, with CD50 = 187 μM. The chlorine is not the entire reason for the cytotoxic activity since SR3774, with chlorine in the same position, had a CD50 >2,200 μM. In addition, SR3774 was essentially inactive as an HCMV inhibitor since the ED50 was 778 μM, indicating that the chlorine is not beneficial for antiviral activity. On the other hand, SR3762, with an ED50 of 133 μM, was as active as SR3761 but was less cytotoxic, with a CD50 of 611 μM. The next most active of this series of compounds was SR3763, with an ED50 of 479 μM and a CD50 of 2,362 μM. It is interesting that in this series of phosphonates, the diacids have higher ED50 values than do the diethylesters, which was just

the opposite of that seen without the benzylether moiety. A 7-substituted SR3761, designated SR3766, had no antiviral activity and was quite cytotoxic, with a CD50 of 112 μM.

We were surprised to find that replacement of the guanine of SR3773 with either adenine (SR3776) or with thymine (SR3777) caused a complete loss of anti-HCMV activity in this in vitro test system.

Summary: Twenty-two phosphonate analogs of DHPG or acyclovir were tested against HCMV in MRC-5 cells to determine structure-activity relationships. In general, the R-enantiomers were found to be much more active than the S-enantiomers but the chiral mixtures had activity quite near that of the R-enantiomers. The phosphonate diacids were more active than the phosphonate ethylesters except that this relative activity seemed to be reversed when a benzylether moiety was present. Substituting at the 7 position of guanine eliminated the antiviral activity and caused greater cytotoxicity. Replacement of the guanine moiety of the most active phosphonate with adenine or thymine eliminated the antiviral activity. The phosphonate analogs of DHPG and ACV appear to be good candidates for drugs to be used in the therapy of human cytomegalovirus disease.

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