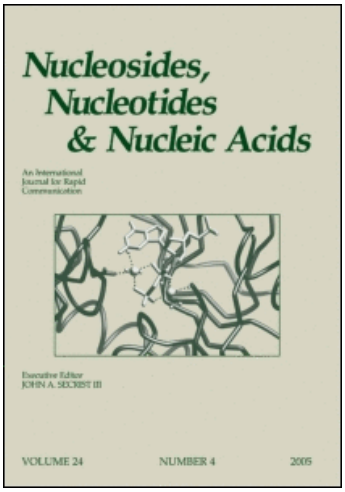

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Sidwell, Robert W. , Huffman, John H. , Barnard, Dale L. and Reist, Elmer J.(1989) 'EFFECT OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR AND GANCICLOVIR ON IN VITRO CYTOMEGALOVIRUS INFECTIONS', Nucleosides, Nucleotides and Nucleic Acids, 8: 5, 833 — 836
To link to this Article: DOI: 10.1080/07328318908054224
URL: http://dx.doi.org/10.1080/07328318908054224

NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 833-836 (1989)

EFFECT OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR AND GANCICLOVIR ON IN VITRO CYTOMEGALOVIRUS INFECTIONS

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Abstract: Eight phosphonic acid analogs of acyclovir (ACV) or ganciclovir (DHPG) inhibited human cytomegalovirus in vitro. Therapeutic indices were: phosphonate diacid of DHPG: 500; DHPG: 500; phosphonate monoethylester of DHPG: 258; phosphonate monoethylester of ACV: 94; cyclic phosphonate of DHPG: 64; ACV: 60; phosphonate monobutylester of ACV: 7.5; phosphonate monoethylester of deoxy DHPG: 4.6; 8-bromo ACV phosphonate monoethylester: >2; phosphonate monoethylester heptyl of ACV: 1. Types 1 and 2 herpesvirus (HSV-1, HSV-2) and varicella zoster virus (VZV) were poorly inhibited by these new compounds, suggesting highly specific anti-HCMV activity. None exhibited significant cytotoxic effects as measured by uptake of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine.

Introduction: A need exists for an effective drug for the treatment of human cytomegalovirus (HCMV) infection, which is a major problem in immunosuppressed patients, including those with acquired immunodeficiency syndrome (AIDS)[1,2]. One of the most effective inhibitors of HCMV now available is DHPG (9-[1,3-dihydroxy-2-propoxymethyl]guanine), which is the first consistent inhibitor of this virus[3]. Although used for treating clinical HCMV infections, DHPG is reported to have side effects which limit its acceptability[3,4]. A series of acyclonucleoside analogs of DHPG and of the related antiviral, ACV, were subsequently synthesized as described by Reist et al. in this volume in an attempt to develop an equally potent anti-HCMV compound which would have less toxic effects. The in vitro antiviral evaluation of 8 of these analogs is described in this report.

Materials and Methods: Viruses: Strain AD169 of HCMV, strain 22122 of guinea pig CMV (GPCMV), strain McCrae of HSV-1, strain E194 of HSV-2 and the Ellen strain of VZV were used. Each was used as a cell culture preparation.

Cells: Continuous passaged human lung (MRC-5) cells grown in basal medium Eagle (BME) with fetal bovine serum (FBS) and $NaHCO_3$ were used for HCMV studies. Guinea pig embryo (GPE) cells grown in minimum essential medium (MEM) with FBS and $NaHCO_3$ were used for GPCMV tests. Experiments with HSV-1, HSV-2 and VZV were run in monkey kidney cells (MA104) with MEM, FBS and $NaHCO_3$. Gentamicin (50 µg/ml) was included in all media when antiviral tests were run.

Compounds: All test compounds were synthesized by Reist and associates at SRI International, Menlo Park, CA (see Reist et al. this volume). Acyclovir was provided by Burroughs Wellcome Co. (Research Triangle Park, NC); DHPG was obtained from Syntex Research (Mountain View, CA).

Antiviral evaluation: Each compound was prepared in concentrations of 2000, 640, 200, 64, 20, 6.4, and 2 µg/ml in the appropriate cell culture medium. These were subsequently diluted 2-fold when added to cells in the antiviral test. Anti-HCMV experiments used a plaque reduction assay run in triplicate in 12-well tissue culture plates, with virus allowed to adsorb 1 hr, then replaced with an overlay of medium containing test drug and agarose which solidified over the cells. Plaques were counted after an 8-day incubation at 37 C. All other antiviral experiments used inhibition of viral cytopathogenic effect in 96-well microplates as we have previously described[5]. The 50% effective (virus inhibitory) dose (ED50) was determined for each compound, and the 50% cytotoxic dose (CD50) also determined by use of concomitantly run toxicity controls which were examined microscopically for cell anomalies. Antiviral activity for HCMV was expressed as therapeutic index (TI) measured as CD50 + ED50.

Radiolabel uptake studies: Effects on cellular DNA, RNA and protein synthesis were determined, respectively, by measuring uptake of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine in MRC-5 cells according to an earlier described procedure[6].

Results and Discussion: The phosphonate diacid of DHPG was equivalent to DHPG in its HCMV-inhibitory activity, followed closely by the phosphate monoethylester of DHPG and the cyclic phosphonate of DHPG

TABLE 1. IN VITRO ANTIVIRAL ACTIVITY OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR AND GANCICLOVIR

Compound	CD50[a]	Therapeutic Index				
		HCMV	GPCMV	HSV-1	HSV-2	VZV
Phosphonate diacid of DHPG	1000	500	50	3	2	–[b]
DHPG	~1000	500	4.3	500	100	-
Phosphonate monoethylester of DHPG	~1500	258	75	1	2.5	-
Phosphonate monoethylester of ACV	~1500	94	30	4.2	1.5	-
Cyclic phosphonate of DHPG	320	64	38	0.3	0.3	-
ACV	~1500	60	13	375	320	0
Phosphonate monobutylester of ACV	~1500	7.5	6.4	0	0	0
Phosphonate monoethylester of deoxy DHPG	~1500	4.7	1.3	0	0	0
8-Bromo ACV phosphonate monoethylester	~1500	≥2	10	0	1.4	0.8
Phosphonate monoethylester heptyl of ACV	1000	1	0	0	0	-

[a]50% cytotoxic dose determined in MRC-5 cells.
[b]Not determined.

TABLE 2. EFFECTS OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR AND GANCICLOVIR ON RADIOLABEL UPTAKE IN MRC-5 CELLS

Compound	50% Inhibitory Concentration (μg/ml)			
	HCMV	[^{3}H]Thymidine	[^{3}H]Uridine	[^{3}H]Leucine
DHPG	2.0	500	>1000	>1000
Phosphonate monoethylester of DHPG	5.8	>1000	>1000	>1000
Phosphonate monoethylester of ACV	16	>1000	~100	>1000
ACV	25	750	>1000	>1000
Phosphonate monobutylester of ACV	100	~1000	>1000	320
Phosphonate monoethylester of deoxy DHPG	320	>1000	>1000	>1000
8-Bromo ACV phosphonate monoethylester	≤500	750	>1000	>1000

(Table 1). Acyclovir was essentially equal to the latter compound in inhibiting HCMV. Other compounds considered to have reasonably strong activity vs HCMV included the phosphonate monoethylester of ACV and the cyclic phosphonate of DHPG. These latter materials also appeared to exceed ACV's anti-HCMV effects. The other materials shown in Table 1 were considered of lesser in vitro efficacy. With the exception of the phosphonate monoethylester heptyl analog of ACV, all of these materials were also active against GPCMV, but efficacy against HSV-1, HSV-2, and VZV, where tested, was minimal or not detectable.

Five of these new substances were also evaluated for effects on DNA, RNA and protein synthesis as measured by radiolabel uptake (Table 2). None were considered to be significantly cytotoxic by these parameters, and closely resembled both DHPG and ACV in this aspect. Also shown on the table are the ED50 values for HCMV to illustrate the major differences between antiviral activity and cytotoxic effects. These data suggest that these phosphonic acid analogs of ACV and DHPG are likely candidates for further evaluation as potential drugs for treatment of HCMV infections.

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Supported by: NIAID Contract NO1-AI-72643.