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Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines*

Erik De Clercq[1], Takashi Sakuma[1], Masanori Baba[1], Rudi Pauwels[1], Jan Balzarini[1], Ivan Rosenberg[2] and Antonin Holý[2]

[1]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium;* [2]*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

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Summary

Various 3-hydroxy-2-phosphonylmethoxypropyl (HPMP) and 2-phosphonylmethoxyethyl (PME) derivatives of purine [adenine (A), guanine (G), 2,6-diaminopurine (DAP), 2-monoaminopurine (MAP), hypoxanthine (HX)] and pyrimidine [cytosine (C), uracil (U), thymine (T)] have been evaluated for their antiviral properties. PMEDAP, (*S*)-HPMPA [and the cyclic phosphonate thereof, (*S*)-cHPMPA)], (*S*)-HPMPC, PMEG, PMEA, HPMPG and HPMPDAP proved to be effective inhibitors of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). (*S*)-HPMPA and (*S*)-cHPMPA were the most effective inhibitors of varicella-zoster virus (VZV), and (*S*)-HPMPC was the most effective inhibitor of cytomegalovirus (CMV). Against adenovirus (types 2, 3 and 4) and vaccinia virus again (*S*)-HPMPA and (*S*)-cHPMPA showed the greatest inhibitory activity. As a rule, the PME derivates were much less inhibitory to VZV, CMV, vaccinia and adenovirus than the HPMP derivatives. However, PMEA, PMEDAP and PMEMAP showed marked and selective activity against the human immunodeficiency virus (HIV). (*S*)-HPMPA was selected for further evaluation in animal model infections. It proved efficacious in the topical treatment of HSV-1 keratitis in rabbits and cutaneous HSV-1 infection in hairless mice, and in the systemic treatment of both HSV-1 and vaccinia virus infections in mice.

Phosphonylmethoxyalkylpurines; Phosphonylmethoxyalkylpyrimidines; (*S*)-HPMPA; PMEA; (*S*)-HPMPC; Adenovirus; Herpes simplex virus; Varicella-zoster virus; Cytomegalovirus

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Correspondence to: E. De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium.

Introduction

The majority of the anti-herpes agents which have been described to date, i.e. acyclovir [9-(2-hydroxyethoxymethyl)guanine], bromovinyldeoxyuridine [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine], 5-ethyl-2′-deoxyuridine, and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (also referred to as DHPG, NDG, BWU 759A or ganciclovir), depend to a large extent on phosphorylation by the virus-induced thymidine kinase (TK) and, hence, are not inhibitory or only weakly inhibitory to those viruses that do not induce such virus-specific TK, i.e. TK-deficient herpes simplex virus (TK^-HSV) and varicella-zoster virus (TK^-VZV) mutants, cytomegalovirus (CMV), adenoviruses and poxviruses. At first glance, phosphorylated nucleoside analogues may be considered suitable to circumvent the problem, but such nucleotides are of no avail as their phosphate group(s) may be cleaved off by esterases before they would be able to enter the cells. To prevent this cleavage the phosphorus atom should be attached to the nucleoside analogue via a P-C bond, thus forming a phosphonate linkage which is not apt to hydrolysis by esterases. To this end, new nucleotide analogues have been designed which contain a phosphonate linked to the alkyl side chain of various acyclic nucleoside derivatives. The prototype of this new class of compounds is (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine]. Conceptually this compound may be viewed as originated from the merger of two other antiviral agents, namely (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine] and PFA (phosphonoformate) (De Clercq, 1987a). (*S*)-DHPA has a relatively weak activity against several RNA and DNA viruses, whereas PFA is particularly active against herpes-, hepadna- and retroviruses. It was of interest, therefore, to evaluate whether the daughter compound, (*S*)-HPMPA, would also be endowed with antiviral properties.

Materials and Methods

Compounds

The phosphonylmethoxyalkyl derivatives of adenine, guanine, 2,6-diaminopurine (DAP), 2-monoaminopurine, hypoxanthine, cytosine, uracil and thymine were synthesized as described by Holý and Rosenberg (1987a,b).

Antiviral assays in vitro

Antiviral activity was determined as described previously for HSV-1, HSV-2, TK^-HSV-1, and vaccinia virus (De Clercq et al., 1980), for adenovirus (Baba et al., 1987a) and VZV (Baba et al., 1987b).

For determination of antiviral activity against CMV, human embryonic lung fibroblast (HEL) cells grown in 96-well microplates were infected with 20 PFU virus/well. After 2 h of incubation at 37°C, the infected cells were replenished with 0.1 ml of medium containing serial dilutions of the test compound. On day 7 the plaques were counted microscopically after staining the cells with Giemsa's solution. The minimum antiviral concentration was expressed as the dose required to inhibit virus-induced plaque formation by 50%.

Cytotoxicity measurements were based upon the inhibition of HEL cell growth: HEL cells were seeded at a rate of 3×10^3 cells per well into 96-well microplates and allowed to proliferate for 24 h in Eagle's minimum essential medium (MEM) containing 10% inactivated fetal calf serum. The medium was then replaced by MEM containing various concentrations of the test compound. After three days incubation at 37°C, when the cell monolayer was 70% confluent, the cell number was determined with the coulter counter. The minimum cytotoxic concentration was defined as the concentration required to reduce cell growth by 50%.

The activity against human immunodeficiency virus (HIV) was determined in MT-4 cells, as described previously (Pauwels et al., 1987a).

Antiviral assays in vivo

The animal model systems to determine the efficacy of (*S*)-HPMPA have been described previously: HSV-1 keratitis in rabbits (Maudgal et al., 1987), systemic (intraperitoneal) HSV-1 infection in mice (De Clercq and Rosenwirth, 1985), cutaneous HSV-1 infection in hairless mice (De Clercq, 1984), and pox tail lesion formation in mice inoculated intravenously with vaccinia virus (De Clercq and De Somer, 1968).

Results and Discussion

(*S*)-HPMPA turned out to be effective against a wide variety of DNA viruses, including adeno-, pox-, and all the major herpesviruses (Table 1) (De Clercq et al., 1986; Baba et al., 1987a,b; Lin et al., 1987; Osterhaus et al., 1987; De Clercq, 1987b; Gil-Fernández and De Clercq, 1987). These viruses were inhibited by (*S*)-HPMPA at concentrations far below the toxicity threshold for the host cells, as most clearly illustrated with a number of clinical VZV isolates which were inhibited by (*S*)-HPMPA at a mean 50% inhibitory concentration of 1.8 ng/ml, while the 50% inhibitory concentration for host cell DNA synthesis (as monitored by the incorporation of [^{3}H-*methyl*]thymidine) was 52 μg/ml (Baba et al., 1987b). Thus, in this assay system (*S*)-HPMPA achieved a selectivity index of 29000. The potent and selective antiviral activity of (*S*)-HPMPA has also been demonstrated with HSV-1, HSV-2, TK$^-$ mutants of HSV-1 and VZV, cytomegalovirus, vaccinia virus (De Clercq et al., 1986), adenovirus (types 1–8) (Baba et al., 1987a), Epstein-Barr virus (Lin et al., 1987), seal herpesvirus (Osterhaus et al., 1987) and African swine fever virus (Gil-Fernández and De Clercq, 1987). With the latter, as with VZV, a remarkably high selectivity index, i.e. 4 orders of magnitude, was attained. Retroviruses also appear to be sensitive to the antiviral action of this class of compounds, although (*S*)-HPMPA itself is less potent an inhibitor of human immunodeficiency virus (HIV) than are some of its congeners. It is likely that the activity spectrum of (*S*)-HPMPA extends to DNA virus families and types other than those listed in Table 1. In particular the hepadnaviridae and members of the poxviridae family should be examined in this regard. RNA viruses, with the exception of the retroviruses, are not inhibited by (*S*)-HPMPA, however (De Clercq et al., 1986).

TABLE 1

Antiviral activity spectrum of (*S*)-HPMPA.

Virus family	Virus type	Minimum inhibitory concentration (μg/ml)*	References
Adenoviridae	Adenovirus (AV) types 1–8	0.6–2.5	Baba et al. (1987a)
Herpetoviridae	Herpes simplex virus type 1 (HSV-1)	1–3	De Clercq et al. (1986)
	Herpes simplex virus type 2 (HSV-2)	1–3	De Clercq et al. (1986)
	Varicella-zoster virus (VZV)	0.002	Baba et al. (1987b)
	Cytomegalovirus (CMV)	0.15	Table 4
	Epstein-Barr virus (EBV)	0.02	Lin et al. (1987)
	Suid herpesvirus type 1 (SHV-1)	2	De Clercq et al. (1986)
	Bovid herpesvirus type 1 (BHV-1)	2	De Clercq et al. (1986)
	Equid herpesvirus type 1 (EHV-1)	0.2	De Clercq et al. (1986)
	Herpesvirus platyrrhinae (HVP)	2	De Clercq et al. (1986)
	Seal herpesvirus (SeHV)	<0.3	Osterhaus et al. (1987)
	TK^- (thymidine kinase-deficient) HSV-1	0.7	De Clercq (1987b)
	PAA^r (phosphonoacetate-resistant) HSV-1	0.3	De Clercq et al. (1986)
Iridoviridae	African swine fever virus (ASFV)	0.01	Gil-Fernández and De Clercq (1987)
Poxviridae	Vaccinia virus (VV)	0.3	De Clercq et al. (1986)
Retroviridae	Moloney murine sarcoma virus (MSV)	1	De Clercq et al. (1986)

*Required to inhibit virus-induced cytopathogenicity, plaque or focus formation by 50%.

The length of the phosphonylmethoxyalkyl side chain is of obvious importance in the antiviral activity of (*S*)-HPMPA. To ensure an optimal activity the alkyl moiety should be a 3-hydroxypropyl [or 2-(hydroxymethyl)-ethyl] with the phosphonylmethoxy group attached to the second carbon, as in (*S*)-HPMPA, although it may be shortened to ethyl, as in PMEA (Fig. 1). Further shortening to a methyl or lengthening to butyl are incompatible with antiviral activity. Likewise, the 2-phosphonylmethoxypropyl, 3-phosphonylmethoxypropyl, and 2-methoxy-3-phosphonylmethoxypropyl derivatives are inactive products. Theoretically, HPMPA can exist in 4 isomeric forms: (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine, (*R*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine, (*S*)-9-(2-hydroxy-3-phosphonylmethoxypropyl)adenine and (*R*)-9-(2-hydroxy-3-phosphonylmethoxypropyl)adenine. Of these 4 forms, only the first [which has been designated as (*S*)-HPMPA] is active as an antiviral agent. The cyclic phosphonate of (*S*)-HPMPA, (*S*)-cHPMPA (Fig. 1), is about equally active as (*S*)-HPMPA which suggests that the cyclic phosphonate may be readily hydrolyzed to yield the parent compound.

From (*S*)-HPMPA and PMEA as the prototype compounds various new congeners have been derived in which the adenine moiety was replaced by 2,6-diaminopurine, 2-aminopurine, guanine, hypoxanthine, cytosine, uracil or thymine (Fig. 2). The compounds were then compared for their antiviral activity in primary rabbit kidney cells, against HSV-1, HSV-2, TK^- HSV-1 and vaccinia virus (Table 2), and, in human embryonic lung cells, against VZV (Table 3), CMV (Table 4) and adenovirus (Table 5). As the salient features we noted that: the cyclic phos-

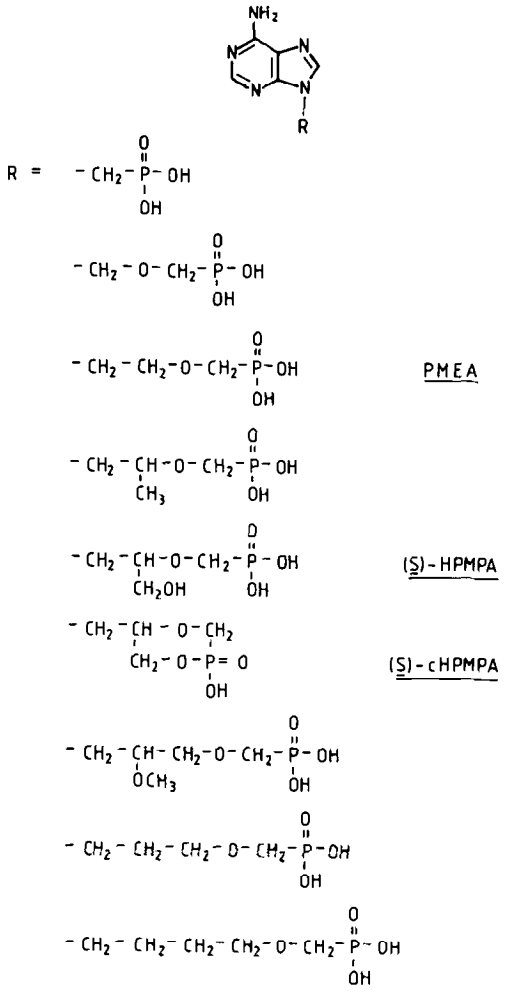

Fig. 1. 9-(Phosphonylmethoxyalkyl)adenine derivatives. PMEA: 9-(2-phosphonylmethoxyethyl)-adenine. (*S*)-HPMPA: (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine.

phonate (*S*)-cHPMPA was about equally active as (*S*)-HPMPA in all assay systems; the hypoxanthine and uracil derivatives (*S*)-HPMPHx, PMEHx and (*RS*)-HPMPU were virtually devoid of antiviral activity, and, similarly, (*S*)-HPMPT and PMEMAP were only weakly active as antiviral agents; the guanine derivative PMEG was quite active but was also the most toxic of the whole group; and (*RS*)-HPMPG, (*RS*)-HPMPDAP, (*S*)-HPMPC and PMEDAP, akin to (*S*)-HPMPA and PMEA, emerged as potent and selective antiviral agents (Tables 2–5).

In terms of anti-HSV potency, PMEDAP ranked first, followed by (*S*)-HPMPA,

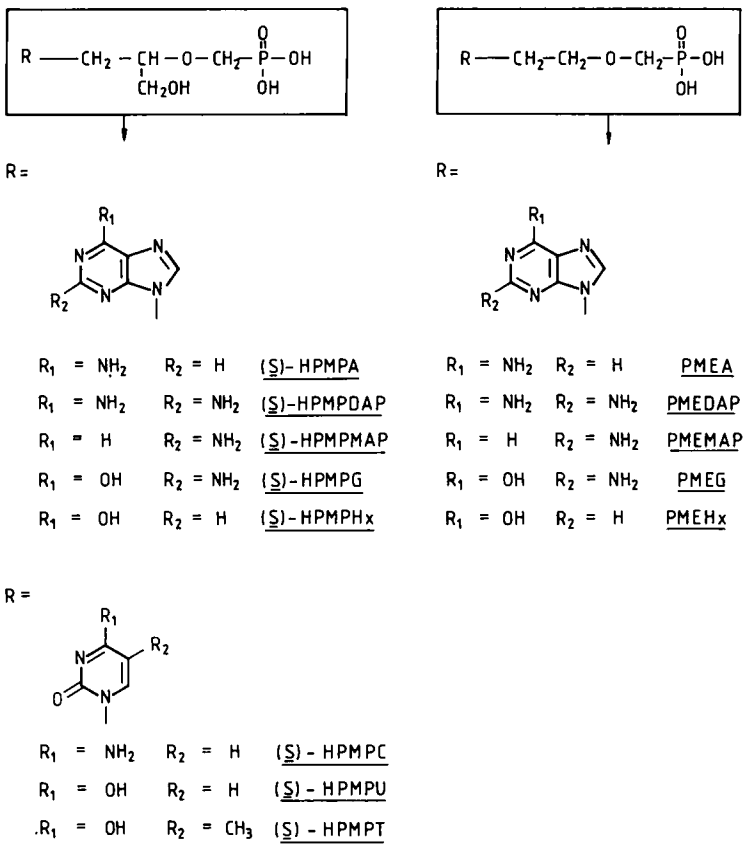

Fig. 2. 9-(3-Hydroxy-2-phosphonylmethoxypropyl)purine, 9-(2-phosphonylmethoxyethyl)purine and 1-(3-hydroxy-2-phosphonylmethoxypropyl)pyrimidine derivatives.

(*S*)-cHPMPA (*S*)-HPMPC, PMEG, PMEA, (*RS*)-HPMPG and (*RS*)-HPMPDAP (Table 2). (*S*)-HPMPA and (*S*)-cHPMPA proved superior to all the other compounds in both potency and selectivity against VZV (Table 3). With regard to the anti-CMV activity, (*S*)-HPMPC emerged as the most potent and most selective agent, closely followed by (*S*)-cHPMPA and (*S*)-HPMPA (Table 4). When compared to DHPG, currently the drug of choice for the treatment of CMV infections in immunodeficient patients, (*S*)-HPMPC was about 5- to 10-fold more potent and about 2- to 3-fold more selective in inhibiting the Davis and AD 169 strains of CMV. In terms of anti-adenovirus activity, again (*S*)-cHPMPA and (*S*)-HPMPA proved more potent and/or more selective than their congeners (Table 5).

When the phosphonylmethoxyalkyl purine and pyrimidine derivatives were evaluated for their inhibitory effect on HIV replication in MT-4 cell cultures, PMEDAP, PMEA and PMEMAP emerged as the most promising compounds. At a multiplicity of infection of 10 $CCID_{50}$ (50% cell culture infective dose)/well,

TABLE 2

Activity of (*S*)-HPMPA analogues against herpes simplex virus (HSV-1, HSV-2, TK⁻HSV-1) and vaccinia virus (VV) in primary rabbit kidney cells.

Compound	Minimum inhibitory concentration (μg/ml)*			
	HSV-1	HSV-2	TK⁻HSV-1	VV
(*S*)-HPMPA	2	4	2	0.7
(*S*)-cHPMPA	2	4	2	0.7
(*S*)-HPMPHx	> 400	> 400	> 400	> 400
(*RS*)-HPMPG	7	20	7	2
(*RS*)-HPMPDAP	10	20	10	2
(*S*)-HPMPC	4	10	2	4
(*S*)-HPMPT	70	70	> 400	300
(*RS*)-HPMPU	> 400	> 400	> 400	> 400
PMEA	7	7	7	150
PMEHx	> 400	> 400	> 400	> 400
PMEG	4	7	7	10
PMEDAP	2	0.7	1	20
PMEMAP	70	10	150	> 200

* Required to inhibit virus-induced cytopathogenicity by 50%; average values for three HSV-1 strains (KOS, F, McIntyre), three HSV-2 strains (G, 196, Lyons) and two TK⁻HSV-1 strains (B2006, VMW 1837).

PMEDAP, PMEA and PMEMAP effected 50% protection against HIV-induced cytopathogenicity at a concentration of 0.3 μg/ml, 0.6 μg/ml, and 14 μg/ml, respectively. Toxicity for the host cells (50% reduction in cell viability) was noted at

TABLE 3

Activity of (*S*)-HPMPA analogues against varicella-zoster virus (VZV) in human embryonic lung cells.

Compound	Minimum antiviral concentration (μg/ml)*	Minimum cytotoxic concentration (μg/ml)**	Selectivity index***
(*S*)-HPMPA	0.02	20	1000
(*S*)-cHPMPA	0.03	40	1333
(*S*)-HPMPHx	> 400	> 400	>< 1
(*RS*)-HPMPG	0.07	10	143
(*RS*)-HPMPDAP	1	> 200	> 200
(*S*)-HPMPC	0.25	50	200
(*S*)-HPMPT	70	> 400	> 6
(*RS*)-HPMPU	300	> 400	> 1.3
PMEA	10	100	10
PMEHx	> 400	> 400	>< 1
PMEG	0.05	2.5	50
PMEDAP	2	40	20
PMEMAP	150	> 400	> 2

* Required to inhibit virus-induced cytopathogenicity by 50%; average values for two TK⁻VZV strains (YS, Oka) and two TK⁻VZV strains (07-1, YSR).

** Required to inhibit growth of the host cells by 50%.

*** Ratio of minimum cytotoxic concentration to minimum antiviral concentration.

TABLE 4

Activity of (*S*)-HPMPA analogues against cytomegalovirus (CMV) in human embryonic lung cells.

Compound	Minimum antiviral concentration (μg/ml)*	Minimum cytotoxic concentration (μg/ml)**	Selectivity index***
(*S*)-HPMPA	0.15	20	133
(*S*)-cHPMPA	0.1	40	400
(*S*)-HPMPHx	100	> 400	> 4
(*RS*)-HPMPG	0.15	10	67
(*RS*)-HPMPDAP	2	> 200	> 100
(*S*)-HPMPC	0.08	50	625
(*S*)-HPMPT	100	> 400	> 4
(*RS*)-HPMPU	25	> 400	16
PMEA	25	100	4
PMEHx	200	> 400	> 2
PMEG	0.25	2.5	10
PMEDAP	10	40	4
PMEMAP	200	> 400	> 2

* Required to inhibit plaque formation by 50%; average values for two CMV strains (Davis, AD 169)

** Required to inhibit growth of the host cells by 50%.

*** Ratio of minimum cytotoxic concentration to minimum antiviral concentration.

a concentration of 6 μg/ml (PMEDAP), 21 μg/ml (PMEA) and > 396 μg/ml (PMEMAP). Thus, in their anti-HIV activity, PMEDAP, PMEA and PMEMAP achieved a selectivity index of 20, 35 and > 28, respectively.

TABLE 5

Activity of (*S*)-HPMPA analogues against adenovirus (AV) in human embryonic lung cells.

Compound	Minimum antiviral concentration (μg/ml)*	Minimum cytotoxic concentration (μg/ml)**	Selectivity index***
(*S*)-HPMPA	0.33	55	167
(*S*)-cHPMPA	0.37	> 125	> 338
(*S*)-HPMPHx	> 125	> 125	>< 1
(*RS*)-HPMPG	2.6	7	2.7
(*RS*)-HPMPDAP	2.8	125	45
(*S*)-HPMPC	3.4	> 125	> 37
(*S*)-HPMPT	> 125	> 125	>< 1
(*RS*)-HPMPU	> 125	> 125	>< 1
PMEA	> 125	> 125	>< 1
PMEHx	> 125	> 125	>< 1
PMEG	2.7	2.5	0.93
PMEDAP	> 125	64	< 0.5
PMEMAP	> 125	> 125	>< 1

* Required to inhibit virus-induced cytopathogenicity by 50%; average values for three AV types (2,3 and 4).

** Required to inhibit [^{3}H-*methyl*]thymidine incorporation into DNA of the host cells by 50%.

*** Ratio of minimum cytotoxic concentration to minimum antiviral concentration.

From a detailed analysis of the antiviral data it appears that the antiviral spectrum of the 'HPMP' series of compounds clearly differs from that of the 'PME' series. This is best illustrated by a direct comparison of the activity data for the 'HPMP' and 'PME' derivatives of adenine (A) and 2,6-diaminopurine (DAP). As a rule, the 'HPMP' compounds are much more active than the 'PME' compounds against vaccinia, adeno, cytomegalo and varicella-zoster virus; on the other hand, the 'PME' compounds are more active than the 'HPMP' compounds against human immunodeficiency virus. Against herpes simplex virus (HSV-1, HSV-2 and TK⁻HSV-1), the 'PME' compounds are about equally active as their 'HPMP' counterparts.

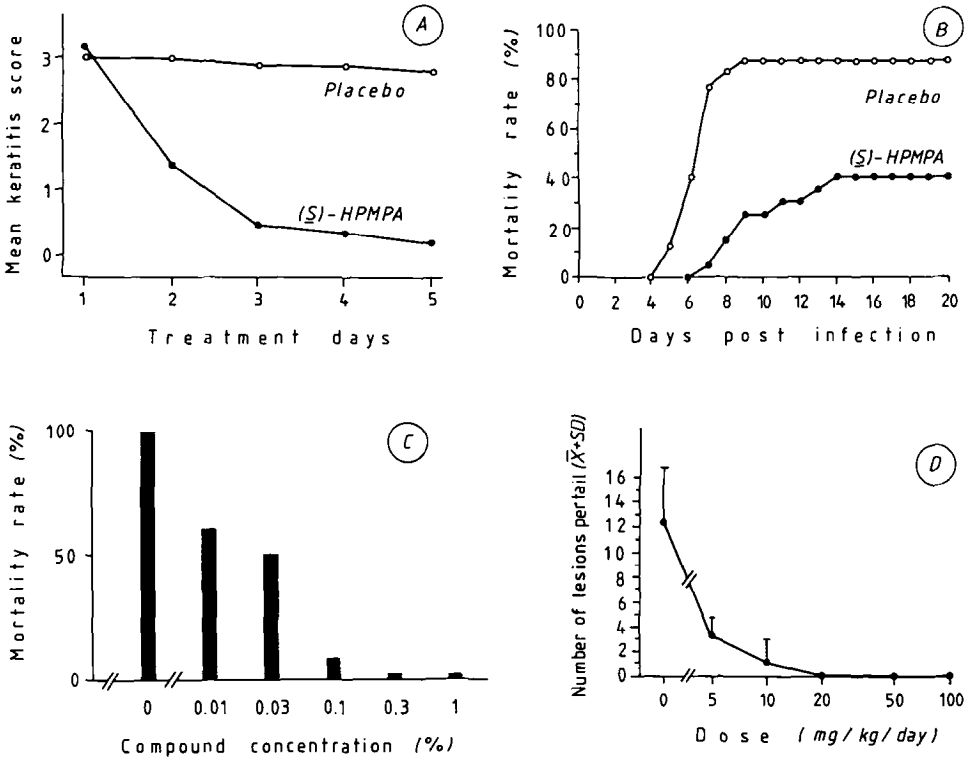

Fig. 3. Antiviral activity of (*S*)-HPMPA in four animal model infections. (A): Effect of (*S*)-HPMPA 0.2% eyedrops, as compared to placebo eyedrops on the course of epithelial keratitis caused by TK⁻HSV-1 (strain VMW 1837) in rabbits (Maudgal et al., 1987). Treatment was started on day 4 after virus inoculation. (B): Effect of (*S*)-HPMPA administered perorally at 250 mg/kg/day on the mortality rate of NMRI mice infected intraperitoneally with HSV-1 (strain KOS). The compound was given via gavage twice a day for 5 days, starting on the day of virus infection. (C): Effect of (*S*)-HPMPA applied topically at varying concentrations on the mortality rate of hairless (hr/hr) mice inoculated intracutaneously with HSV-1 (strain KOS). The compound was applied in dimethylsulfoxide four times a day for 5 days, starting immediately after virus infection. (D): Effect of (*S*)-HPMPA administered subcutaneously at varying doses on the development of tail lesions in NMRI mice inoculated intravenously with vaccinia virus. The compound was given twice a day for 5 days, starting on the day of virus infection; tail lesions were counted on day 7.

The mechanism of antiviral action of (*S*)-HPMPA, PMEA and their congeners remains subject of future studies. It is not evident to what extent the mode of action of the 'PME' compounds may differ from that of the 'HPMP' compounds. The fact that it is possible to obtain HSV mutants which are resistant to PMEA, yet susceptible to (*S*)-HPMPA [V. Vonka: personal communication (1987)], points to differences in the mode of action of these compounds. (*S*)-HPMPA is as such taken up by the cells and subsequently converted to its monophosphoryl [(*S*)-HPMPAp] and diphosphoryl [(*S*)-HPMPApp] derivatives by cellular enzymes (Votruba et al., 1987). (*S*)-HPMPA inhibits HSV-1 DNA synthesis at a concentration which is by several orders of magnitude lower than the concentration at which cellular DNA synthesis (in either virus-infected or uninfected cells) is inhibited (Votruba et al., 1987). Thus, (*S*)-HPMPA discriminates between viral and cellular DNA synthesis, and this may apparently contribute to its selective inhibitory activity against HSV, EBV (Lin et al., 1987) and other viruses as well.

In vivo, the antiviral activity of (*S*)-HPMPA has been demonstrated in a number of animal model infections under varying conditions. As varying parameters served the animal species (mice or rabbits), the virus type (HSV-1, TK^-HSV-1 or vaccinia virus), the route of virus infection (intracutaneous, intraperitoneal or intravenous inoculation, or instillation in the eye), and the route of drug administration (topical, subcutaneous or peroral). Irrespective of the experimental conditions used, (*S*)-HPMPA effected a marked protective effect against the manifestations of the disease: herpetic keratitis (Fig. 3A), mortality following either intraperitoneal (Fig. 3B) or intracutaneous (Fig. 3C) virus inoculation, and pox tail lesions (Fig. 3D). Where different doses of (*S*)-HPMPA were administered (Fig. 3C and D), the protective activity conferred was dose-dependent. Upon topical application a compound concentration as low as 0.1%, and upon systemic administration a dose of 5 mg/kg/day, sufficed to afford significant protection against intracutaneous HSV-1 infection (Fig. 3C) and intravenous vaccinia virus infection (Fig. 3D), respectively.

Conclusion

By virtue of their broad-spectrum activity against DNA viruses (i.e., herpesviruses) and retroviruses, (*S*)-HPMPA and its analogues offer a wealth of potential applications, particularly in the treatment of AIDS and AIDS-related complex (ARC), and the opportunistic virus infections associated with AIDS. AIDS patients are indeed predisposed to various opportunistic infections, i.e. due to HSV, VZV, CMV, EBV, AV, and VV (the latter upon vaccination), and, vice versa, such virus infections may trigger the expression of the HIV genome (Mosca et al., 1987), and, possibly, precipitate the manifestations of the AIDS disease. It would seem mandatory to have at hand one or more compounds which are effective against all these DNA viruses and retroviruses alike. The potency and selectivity shown by (*S*)-HPMPA and its analogues as inhibitors of HIV, AV, HSV, VZV, CMV, EBV and VV signal that these compounds may be of great value not only

in the treatment of the retrovirus infection itself but also in the treatment of (i) adenovirus infections, which are being recognized with increasing frequency in AIDS patients and which may be responsible for the colitis generally thought to be caused by CMV; (ii) TK^- HSV infections, which are resistant to the 'classical' antiherpetic drugs such as acyclovir and which have occasionally been identified in immunosuppressed patients; (iii) VZV infections, which may lead to atypical and often severe complications in AIDS patients; (iv) CMV infections, which may present as retinitis, colitis, pneumonitis or encephalitis, and only partially and temporarily respond to the drugs (DHPG, PFA) currently used to treat these infections; (v) EBV infections, which are, like adenovirus infections, diagnosed with increasing frequency among AIDS patients and which may be held responsible for oral hairy leukoplakia as well as a number of B-cell lymphoproliferative disorders in these patients; and (vi) vaccinia virus infections, which have since long been recognized as particularly dangerous and often fatal in immunodeficient patients, as has recently been proven again by the case of a military recruit with ARC who developed a lethal disseminated vaccinia virus infection following vaccination with the life vaccinia vaccine (Redfield et al., 1987).

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