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Efficacy of Phosphonylmethoxyalkyl Derivatives of Adenine in Experimental Herpes Simplex Virus and Vaccinia Virus Infections In Vivo

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The phosphonylmethoxyalkyl derivatives (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA], 9-(2-phosphonylmethoxyethyl)adenine (PMEA), and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) were evaluated for their in vivo efficacies in several animal model infections, i.e., mice infected intravenously with vaccinia virus and mice infected intracutaneously, intraperitoneally, or intracerebrally with herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) or thymidine kinase-deficient (TK^-) HSV-1. (*S*)-HPMPA inhibited the development of tail lesions caused by vaccinia virus if it was administered intraperitoneally or subcutaneously at a dosage as low as 5 mg/kg per day. All three compounds completely suppressed the development of skin lesions and the mortality associated therewith in hairless or athymic nude mice inoculated intracutaneously with HSV-1 or TK^- HSV-1, if they were administered topically at a concentration as low as 0.1%; when (*S*)-HPMPA was applied topically at a concentration of ≥0.3%, it completely abrogated mortality resulting from intracutaneous HSV-2 infection. Most dramatic were the effects shown by the compounds in mice inoculated intracerebrally with HSV-1, HSV-2, or TK^- HSV-1, in which all three compounds given intraperitoneally at a dose of 50 or 100 mg/kg per day effected a significant reduction in the mortality rate of HSV-1-infected mice. The mortality of mice infected intracerebrally with HSV-2 or TK^- HSV-1 was significantly reduced even when (*S*)-HPMPA was given at doses as low as 10 mg/kg per day. These data point to the great potential of the phosphonylmethoxyalkylpurines for both topical and parenteral treatment of HSV-1, HSV-2, and TK^- HSV-1 infections.

(*S*)-9-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA] (8) represents the prototype of a new class of compounds, the phosphonylmethoxyalkyl derivatives (11), which are endowed with a broad spectrum of activity against DNA viruses, including adenoviruses (4), herpesviruses (herpes simplex virus type 1 [HSV-1] and type 2 [HSV-2], varicella-zoster virus [VZV] [3], cytomegalovirus [CMV], Epstein-Barr virus [17], phocid herpesvirus type 1 [19], and various other herpesviruses of veterinary importance [8]), iridoviruses (African swine fever virus [12, 13]), and poxviruses (vaccinia virus [8, 11]). The phosphonylmethoxyethyl derivatives 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine(PMEDAP) are also potent inhibitors of retrovirus (i.e., human immunodeficiency virus) replication (20). Unlike acyclovir, (*S*)-HPMPA does not depend on the induction of a virus-specified thymidine kinase (TK) for its antiherpesvirus activity. Hence, (*S*)-HPMPA is active against TK-deficient (TK^-) mutants of HSV-1 which are resistant to acyclovir, bromovinyldeoxyuridine, and, in general, all antiherpesvirus agents that depend on phosphorylation by viral TK (6, 21). (*S*)-HPMPA is taken up as such by cells and phosphorylated intracellularly to its mono- and diphosphoryl derivatives (22). As has been demonstrated previously with HSV-1-infected cells (22), Epstein-Barr virus-infected cells (17), and African swine fever virus-infected cells (2), (*S*)-HPMPA achieves a marked inhibition of viral DNA synthesis at concentrations which are several orders of magnitude below those required to inhibit cellular DNA synthesis.

When administered topically as 0.2% eyedrops, (*S*)-HPMPA is highly effective against both TK^+ HSV-1 and TK^- HSV-1 keratitis in rabbits (18). The efficacy of (*S*)-HPMPA and its closely related analogs PMEA and PMEDAP (Fig. 1) was examined in various other experimental virus infections in mice, i.e., pox tail lesion formation following intravenous inoculation of vaccinia virus; systemic herpetic infection following intracutaneous or intraperitoneal (i.p.) inoculation of HSV-1, HSV-2, or TK^- HSV-1; and herpetic encephalitis following intracerebral inoculation of HSV-1, HSV-2, or TK^- HSV-1. Although direct inoculation of the virus into the brain can be considered as a stringent model for herpetic encephalitis, systemic (i.p.) treatment with (*S*)-HPMPA, PMEA, or PMEDAP proved remarkably efficacious in this model.

MATERIALS AND METHODS

Compounds. (*S*)-HPMPA and PMEA (sodium salts) were synthesized as described previously (15, 16). PMEDAP was prepared from 2,6-diaminopurine following the procedure described for PMEA (16), i.e., by treatment of the sodium salt of the base with diethyl 2-chloroethoxymethylphosphonate, followed by bromotrimethylsilane reaction of the intermediary diethyl ester of PMEDAP. The product was isolated by ion-exchange chromatography as a free acid (crystallized from water) and transformed to the water-soluble sodium salt. All compounds were homogeneous by high-pressure liquid chromatography and paper chromatography. As a rule, stock solutions of the compounds were prepared in phosphate-buffered saline. The stock solutions were further diluted in phosphate-buffered saline, if the compounds were administered i.p. or subcutaneously (s.c.), or in drinking water, if given perorally (p.o.), whereupon the compounds

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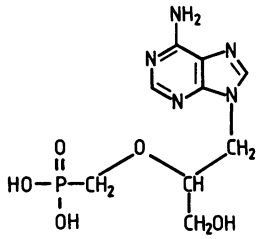
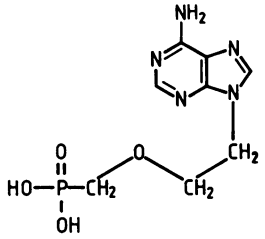
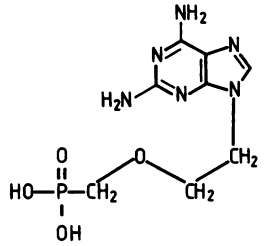


(S) - HPMPA



PMEA



PMEDAP

FIG. 1. Structural formulas of (*S*)-HPMPA, PMEA, and PMEDAP.

were administered twice daily in 0.2-ml volumes or four times daily (if applied topically). If intended for topical use, the compounds were dissolved in dimethyl sulfoxide (DMSO) at the indicated concentrations and applied topically four times a day.

Mice. The animals used throughout the experiments were 25-day-old NMRI (Naval Medical Research Institute) mice (weight, 11 to 13 g), 25- to 30-day-old hairless (*hr/hr*) mice (weight, 15 to 20 g), or 25- to 30-day-old athymic nude (*nu/nu*) mice (weight, 15 to 20 g). The NMRI mice were randomly bred. The *hr/hr* mice were bred by backcross and intercross of the homozygous parents. The *nu/nu* mice were bred by breeding scheme IV of Giovanella and Stehlin (14). All the mice were obtained from the Animal Production Center (Proefdierencentrum) of the Katholieke Universiteit, Leuven, Belgium. The mice were housed under conventional conditions in groups of 5 (*hr/hr* and *nu/nu* mice) or 10 (NMRI mice) and were given food and drinking water ad libitum. Throughout all experiments male or female mice were used at random.

Viruses. The origin of the virus strains has been described previously: for HSV-1 (KOS), TK^- HSV-1 (B2006), HSV-2 (G), HSV-2 (196), see reference 7; for TK^- HSV-1 (VMW-1837), see references 6 and 21. The latter variant was isolated from an immunosuppressed patient with a chronic HSV-1 infection that had become resistant to acyclovir treatment (21) and actually consisted of 92% TK^- and 8% TK^+, as demonstrated by plaque autoradiography (J. Christophers, Manchester, United Kingdom). All HSV stocks were prepared in primary rabbit kidney cells. The following virus stocks had the indicated titers: HSV-1 (KOS), $10^{6.7}$ PFU/ml; HSV-2 (196), $10^{6.0}$ PFU/ml; TK^- HSV-1 (B2006), $10^{7.1}$ PFU/ml. All titers were determined by plaque formation in Vero cell cultures. The titers based on the 50% cell culture infective dose ($CCID_{50}$), which were determined in primary rabbit kidney cells, were as follows: HSV-1 (KOS), $10^{7.0}$ $CCID_{50}$/ml; HSV-2 (196), $10^{5.5}$ $CCID_{50}$/ml; TK^- HSV-1 (B2006), $10^{7.0}$ $CCID_{50}$/ml; and TK^- HSV-1 (VMW-1837), $10^{6.3}$ $CCID_{50}$/ml. The vaccinia virus stock used for the animal experiments originated from calf lymph and was provided by the Rijksentstofinrichting (Brussels, Belgium). Its titer was 1.5×10^8 PFU/ml, and it was stored at 4°C.

Virus yield reduction. Primary rabbit kidney cell monolayers were infected with HSV-1 (KOS), HSV-2 (G), TK^- HSV-1 (B2006), or vaccinia virus at 3×10^4 PFU per 0.5 ml per petri dish and, after 1 h of adsorption of the virus, were incubated in the presence of various concentrations of the test compounds. Virus yield was measured at 48 h after virus infection. To this end, the cell cultures were frozen at −70°C and thawed, and the cell homogenates were assayed for virus content by plaque formation in Vero (green monkey kidney) cells.

Pox tail lesion model. NMRI mice (weight, 11 to 13 g) were inoculated intravenously (in a tail vein) with 0.2 ml of a virus dilution containing approximately 5×10^4 PFU/ml. The mice were treated for 5 days, starting 1 h after virus infection, with (*S*)-HPMPA administered i.p., s.c., or p.o. at the indicated doses. Pox tail lesions were enumerated 7 days after infection.

Intracutaneous HSV infection. Hairless mice (weight, 15 to 20 g) were inoculated intracutaneously (in the lumbosacral area by scratching the skin with the aid of a scarificator) with either HSV-1 (KOS) at $10^{4.7}$ PFU/0.05 ml per mouse or HSV-2 (196) at $10^{3.7}$ PFU/0.05 ml per mouse. The mice were treated for 5 days, starting 1 h after virus infection, with the test compounds, which were applied topically at the indicated concentrations in DMSO (four times daily). The mice were monitored daily for the development of skin lesions, paralysis of the hind legs, and mortality. The mortality rate recorded at 20 days after infection was used to assess the efficacy of the compounds in this model.

Intraperitoneal HSV infection. NMRI mice (weight, 11 to 13 g) were inoculated i.p. with HSV-1 (KOS) at 10^3 PFU/0.2 ml per mouse. The mice were treated for 5 days, starting 1 h after virus infection, with the test compounds administered p.o. at the indicated doses. The mortality rate was recorded at 20 days after infection.

Intracerebral HSV-1, HSV-2, or TK^- HSV-1 infection. NMRI mice (weight, 11 to 13 g) were inoculated intracerebrally with HSV-1 (KOS) at 1.5 PFU/0.02 ml per mouse, HSV-2 (196) at 0.6 PFU/0.02 ml per mouse, or TK^- HSV-1 (B2006) at 75 $CCID_{50}$/0.02 ml per mouse. The mice were treated for 5 days, starting 1 h after virus infection, with the test compounds administered i.p. at the indicated doses. Mortality was monitored for 20 days postinfection.

Intracutaneous TK^- HSV-1 infection in nude mice. Athymic nude mice (weight, 15 to 20 g) were inoculated intracutaneously in the lumbosacral area with TK^- HSV-1 (VMW-1837) at 10^5 $CCID_{50}$/0.05 ml per mouse. The mice were treated for 5 days, starting 1 h after virus infection, with the test compounds, which were applied topically at the indicated concentrations in DMSO (four times daily). The mice

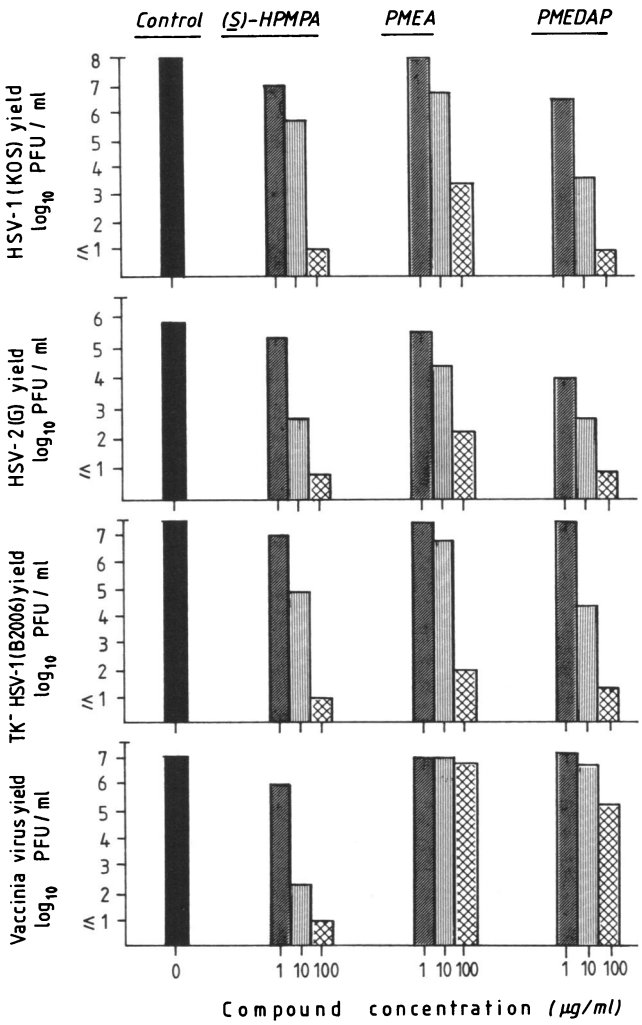


FIG. 2. Inhibitory effects of (*S*)-HPMPA, PMEA, and PMEDAP on the multiplication of HSV-1 (KOS), HSV-2 (G), TK⁻ HSV-1 (B2006), and vaccinia virus in primary rabbit kidney cells.

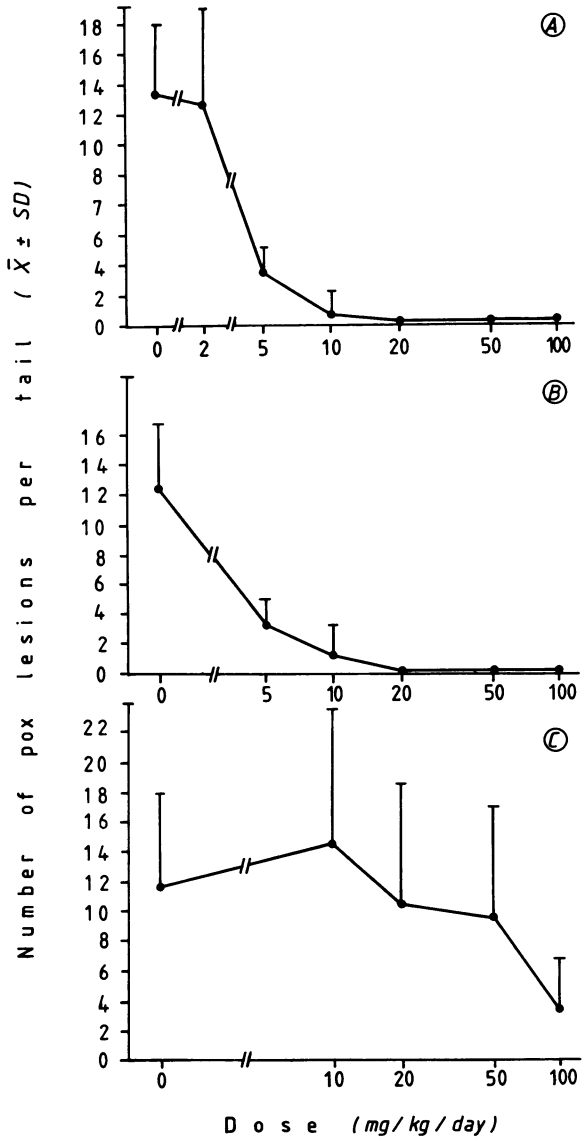


FIG. 3. Inhibitory effects of (*S*)-HPMPA on tail lesion formation in NMRI mice inoculated intravenously with vaccinia virus. (*S*)-HPMPA was administered i.p. (A), s.c. (B), or p.o. (C) at the indicated doses. There were 10 mice per group.

were monitored daily for the development of skin lesions, paralysis of the hind legs, and mortality over a 20-day period. The number of mice that ultimately developed skin lesions over this period was recorded.

Statistical analysis. The statistical significance of the results obtained in the pox tail lesion model was assessed by Student's *t* test, whereas in all other models, in which the endpoint was based on mortality or the number of mice with herpetic skin lesions, the statistical significance of the results was assessed by the χ^2 test (with the Yates correction).

RESULTS

Within a dose range of 1 to 100 μg/ml, (*S*)-HPMPA, PMEA, and PMEDAP brought about a dose-dependent reduction in the yield of HSV-1 and HSV-2 in primary rabbit kidney cells when added to the cells immediately after virus adsorption (Fig. 2). The TK⁻ HSV-1 yield was reduced to a similar extent as the TK⁺ HSV-1 yield, and PMEA proved slightly less inhibitory to HSV replication than its two congeners, (*S*)-HPMPA and PMEDAP. Of the three compounds, only (*S*)-HPMPA effected a significant reduction in vaccinia virus yield (Fig. 2).

(*S*)-HPMPA was further evaluated for its efficacy in the vaccinia virus model in vivo (Fig. 3). It achieved a significant ($P < 0.005$) reduction in the number of pox tail lesions if administered i.p. (Fig. 3A) or s.c. (Fig. 3B) at a dose of 5, 10, 20, 50, or 100 mg/kg per day. When administered p.o., however, (*S*)-HPMPA only caused a significant reduction in the number of pox tail lesions at a dose of 100 mg/kg per day (Fig. 3C). In keeping with the in vitro results, PMEA was not efficacious in the vaccinia virus model in vivo at doses up to 100 mg/kg per day; PMEDAP was active only if administered i.p. at doses of 50 and 100 mg/kg per day (average pox lesion number per tail reduced from 31.8 to 22.6 [$P < 0.01$] and 8.3 [$P < 0.005$], respectively) (data not shown).

In hairless mice inoculated intracutaneously with HSV-1, (*S*)-HPMPA, PMEA, and PMEDAP brought about a significant reduction in the mortality rate (>50% [$P < 0.05$]) if they were applied topically at a concentration of 0.03, 0.1,

TABLE 1. Inhibitory effects of (*S*)-HPMPA, PMEA, and PMEDAP on the mortality rate of hairless mice inoculated intracutaneously with HSV-1 (KOS) or HSV-2 (196)

Compound concn (% [wt/vol])[a]	Mortality rate (%)[b]					
	HSV-1 (KOS)			HSV-2 (196)		
	(*S*)-HPMPA	PMEA	PMEDAP	(*S*)-HPMPA	PMEA	PMEDAP
0	100	100	100	100	100	100
0.01	60	80	40	ND[c]	ND	ND
0.03	50	40	30	90	100	100
0.1	10	10	0	40	100	90
0.3	0	0	0	0	100	60
1	0	0	0	0	70	40
3	ND	ND	ND	0	50	20

[a] With DMSO as the vehicle.
[b] There were 10 mice per group.
[c] ND, Not determined.

0.3, or 1% (Table 1); even at a concentration of 0.01%, PMEDAP still achieved a significant reduction in mortality. In hairless mice inoculated intracutaneously with HSV-2, (*S*)-HPMPA achieved a significant reduction in the mortality rate (≥50% [$P < 0.05$]) if it was applied topically at a concentration of 0.1, 0.3, 1, or 3% (Table 1); PMEDAP did so only at concentrations of 1 and 3%, and PMEA reached the level of significant protection only at a 3% concentration. The reference compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BrVdUrd) was ineffective in this model infection, even if it was applied at a concentration of 10% (data not shown).

When (*S*)-HPMPA, PMEA, and PMEDAP were compared for their protective activity following oral administration to mice infected i.p. with HSV-1, a significant reduction in mortality rate was achieved only by (*S*)-HPMPA and PMEDAP at a dose of 250 mg/kg per day (mortality rate reduced from 36 of 40 to 8 of 20 mice [$P < 0.001$] and from 34 of 35 to 9 of 20 mice [$P < 0.001$], respectively). A similar protective effect (50% reduction in mortality rate) was observed with BrVdUrd given orally at 250 mg/kg per day. When given orally at a dose of 50 or 10 mg/kg per day, (*S*)-HPMPA and PMEDAP did not achieve an appreciable reduction in the mortality rate of mice infected i.p. with HSV-1. In this model infection, PMEA was ineffective over the whole dosage range used (10, 50, or 250 mg/kg per day administered orally).

In the herpetic encephalitis model in which HSV-1 was inoculated intracerebrally, all three compounds administered i.p. brought about a significant reduction in the mortality rate (Fig. 4). At a dose of 100 mg/kg per day, (*S*)-HPMPA reduced the mortality rate from 95 to 40% ($P < 0.001$), PMEA reduced it from 100 to 40% ($P < 0.001$), and PMEDAP reduced it from 100 to 45% ($P < 0.001$). Even at a dose of 50 mg/kg per day, these compounds caused a significant reduction in mortality (from 95 to 60% [$P < 0.025$), from 100 to 70% [$P < 0.05$], and from 100 to 70% [$P < 0.05$], respectively). When evaluated in the HSV-1 encephalitis model, BrVdUrd at a dose of 250 mg/kg per day afforded little protection: a reduction in the mortality rate from 49 of 50 mice (98%) to 25 of 30 mice (83%) (data not shown).

(*S*)-HPMPA was highly efficacious in the HSV-2 encephalitis model. Following i.p. administration at a dose of 100, 50, or 10 mg/kg per day, it reduced the mortality of mice inoculated intracerebrally with HSV-2 from 90% to 30, 15,

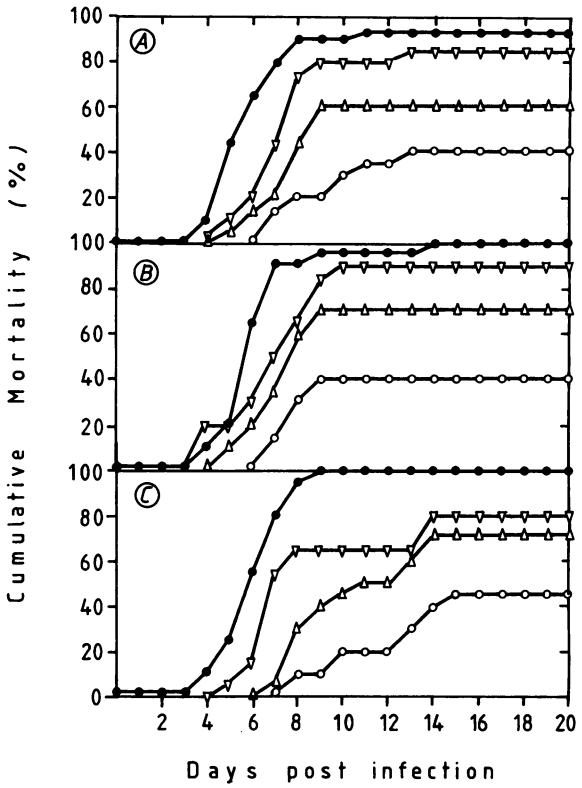


FIG. 4. Inhibitory effects of (*S*)-HPMPA (A), PMEA (B), and PMEDAP (C) on the mortality of NMRI mice inoculated intracerebrally with HSV-1 (KOS). The compounds were administered i.p. at the following doses: 0 mg/kg per day (●), 10 mg/kg per day (▽), 50 mg/kg per day (△), and 100 mg/kg per day (○). There were 20 mice per group.

and 25%, respectively ($P < 0.001$) (Fig. 5A). On the contrary, PMEA did not offer much protection in this model when it was used at a dose of 100, 50, or 10 mg/kg per day (Fig. 5B). PMEDAP was almost as effective as (*S*)-HPMPA. At a dose of 50 or 100 mg/kg per day, it reduced the mortality rate from 95 to 30% ($P < 0.001$), and at 10 mg/kg per day it reduced the mortality rate to 65% ($P < 0.025$) (Fig. 5C). BrVdUrd, which was evaluated in parallel with (*S*)-HPMPA, PMEA, and PMEDAP in the HSV-2 encephalitis model, did not cause an appreciable reduction in the mortality rate when it was administered i.p. at a dose of 250 mg/kg per day (data not shown).

Development of herpetic skin lesions in athymic nude mice infected intracutaneously with the TK^- HSV-1 variant VMW-1837 was significantly suppressed (≥50% reduction in the number of mice with skin lesions [$P < 0.05$]) if they were treated topically with (*S*)-HPMPA, PMEA, or PMEDAP at a concentration of 0.03, 0.1, 0.3, or 1%; even at 0.01% PMEDAP protected 50% of the mice against the development of these skin lesions (Table 2). In marked contrast with the phosphonylmethoxyalkyl derivatives, BrVdUrd and vidarabine at concentrations up to 10% did not prevent the development of skin lesions in *nu/nu* mice infected intracutaneously with the TK^- HSV-1 variant VMW-1837 (data not shown).

(*S*)-HPMPA proved highly effective in the encephalitis model based on intracerebral inoculation of the TK^- HSV-1 B2006 mutant. Following i.p. administration at 100, 50, or 10 mg/kg per day it reduced the mortality rate from 90 to 50% (*P*

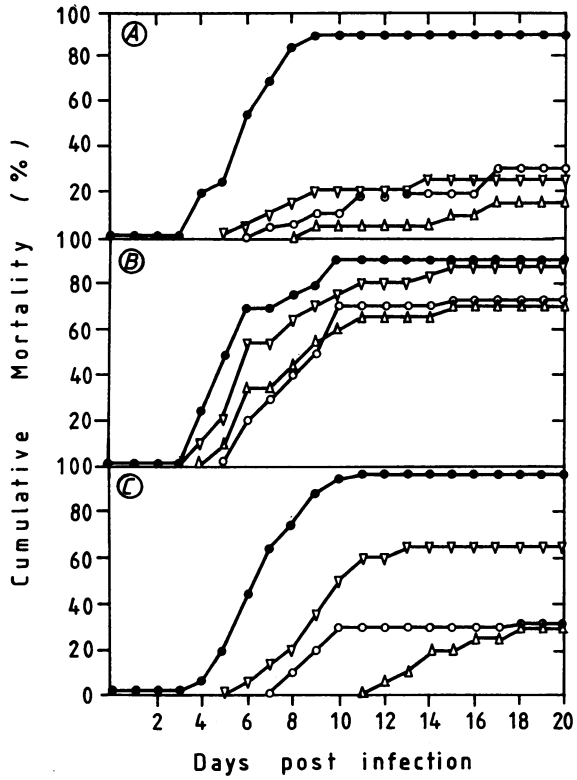


FIG. 5. Inhibitory effects of (S)-HPMPA (A), PMEA (B), and PMEDAP (C) on the mortality of NMRI mice inoculated intracerebrally with HSV-2 (196). The compounds were administered i.p. at the following doses: 0 mg/kg per day (●), 10 mg/kg per day (▽), 50 mg/kg per day (△), and 100 mg/kg per day (○). There were 20 mice per group.

< 0.005), 20% ($P < 0.001$), and 15% ($P < 0.001$), respectively (Fig. 6). PMEA at 50 mg/kg per day reduced the mortality rate from 90 to 50% ($P < 0.005$), while at 10 mg/kg per day it reduced the mortality rate to 60% (not significant). Also, PMEDAP proved effective in reducing the mortality rate of mice inoculated intracerebrally with TK$^-$ HSV-1 B2006: from 90 to 30% ($P < 0.001$) at a dose of 100 or 50 mg/kg per day and to 50% ($P < 0.005$) at a dose of 10 mg/kg per day (data not shown). BrVdUrd administered i.p. at a dose of 250 mg/kg per day did not afford any protection in the TK$^-$ HSV-1 B2006 encephalitis model (data not shown).

DISCUSSION

The salient feature emerging from this study was the potent activity demonstrated by (S)-HPMPA, PMEA, and PMEDAP against HSV-1, HSV-2, and TK$^-$ HSV-1 infections following either topical or i.p. administration. Also, (S)-HPMPA was active against vaccinia virus infection, whereas its phosphonylmethoxyethyl counterparts PMEA and PMEDAP were not. Following i.p. or s.c. administration, (S)-HPMPA blocked the development of vaccinia virus tail lesions at a dosage of 5 mg/kg per day or higher, and at the highest doses that were evaluated (100 mg/kg per day) no toxicity for the host was noted (Fig. 3). These observations make (S)-HPMPA a valuable candidate compound for the treatment of complications that may result from vaccination with (recombinant) vaccinia virus, i.e., in immunosuppressed patients.

TABLE 2. Inhibitory effects of (S)-HPMPA, PMEA, and PMEDAP on the development of skin lesions in athymic nude mice inoculated intracutaneously with TK$^-$ HSV-1 (VMW-1837)

Compound concn (% [wt/vol])[a]	% of mice with skin lesions[b]		
	(S)-HPMPA	PMEA	PMEDAP
0	100	100	100
0.01	90	90	50
0.03	40	10	0
0.1	0	0	0
0.3	0	0	0
1	0	0	0

[a] With DMSO as the vehicle.
[b] There were 10 mice per group.

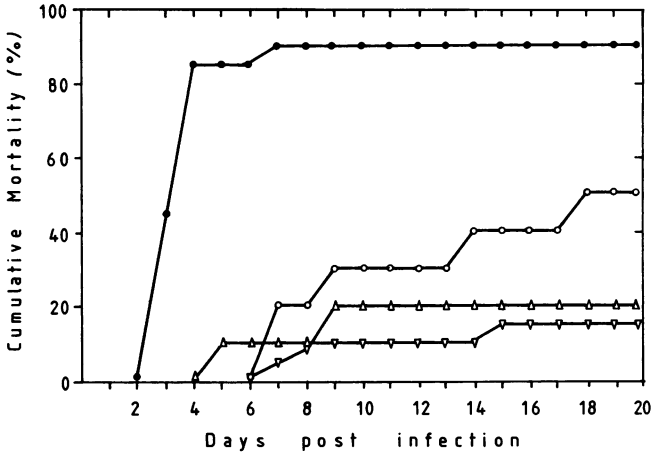


FIG. 6. Inhibitory effect of (S)-HPMPA on the mortality of NMRI mice inoculated intracerebrally with TK$^-$ HSV-1 (B2006). (S)-HPMPA was administered i.p. at the following doses: 0 mg/kg per day (●), 10 mg/kg per day (▽), 50 mg/kg per day (△), and 100 mg/kg per day (○). There were 20 mice per group.

(S)-HPMPA was approximately 20 times more active against vaccinia virus infection when administered by the parenteral (i.p. and s.c.) route than by the oral route (Fig. 3). Similarly, (S)-HPMPA and PMEDAP were effective against HSV infection at doses of 10 mg/kg per day given i.p. (Fig. 4 through 6), whereas following p.o. administration, the dosage had to be increased to 250 mg/kg per day to obtain an equivalent reduction in the mortality rate. These results point to the limited bioavailability of (S)-HPMPA and its congeners following oral administration.

(S)-HPMPA, PMEA, and PMEDAP were highly effective against cutaneous TK$^+$ and TK$^-$ HSV-1 infections [and (S)-HPMPA was also effective against cutaneous HSV-2 infection] following topical application; here, a concentration of 0.03 or 0.1% sufficed to block the infection and the symptoms associated with it (Tables 1 and 2). No other compound has ever been reported to suppress cutaneous HSV infection, whether it is caused by HSV-1, HSV-2, or TK$^-$ HSV strains, at such a low concentration. Hence, (S)-HPMPA, PMEA, and PMEDAP would seem ideally suited for further development as therapeutic modalities in the topical treatment of HSV and other virus infections. It would be worthwhile to examine their efficacies under the same conditions in which other established antiviral drugs such as acyclovir and foscarnet have been found to be effective, i.e., the guinea pig model for cutaneous HSV-1 lesions with treatment started 48 h after infection (1).

In the present study treatment was uniformly initiated 1 h after infection. This allowed a comparative study of the

efficacy of the different compounds in the different animal model systems in which they were explored. (*S*)-HPMPA and its cytosine counterpart (*S*)-HPMPC have also been examined for their efficacy following delayed treatment. (*S*)-HPMPA was found to be effective in suppressing the formation of human immunodeficiency virus type 1 lesions in guinea pigs if topical treatment (5%) was initiated 24 h postinfection (M. J. M. Hitchock, I. Ghazzouli, Y. H. Tsai, C. A. Bartelli, R. R. Webb, and J. C. Martin, Antiviral Res. **9**:82, 1988). (*S*)-HPMPC proved to be active against murine CMV when systemic treatment (11 or 33 mg/kg per day) was begun 24 or 48 h after infection (I. Ghazzouli, R. R. Webb, J. J. Bronson, C. A. Bartelli, C. Franco, H. Yang, R. Salvagno, K. Woods, P. E. Vogt, E. Kern, and J. Martin, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 732, 1988). These observations point to the therapeutic potential of the phosphonylmethoxyalkylpurines and -pyrimidines.

The fact that (*S*)-HPMPA, PMEDAP, and (to a lesser extent) PMEA showed marked activity against intracerebral HSV-1, HSV-2, or TK$^-$ HSV-1 infection (Fig. 4 through 6), which is a stringent model for herpetic encephalitis, suggests that these compounds must be able to cross the blood-brain barrier. Preliminary findings with ^{3}H-labeled PMEA injected intravenously into NMRI mice indicate that the compound is actually taken up by the brain (L. Naesens, J. Balzarini, and E. De Clercq, unpublished data). (*S*)-HPMPA, PMEDAP, and PMEA can thus be considered valuable candiates for the treatment of HSV encephalitis. They should also be considered for use in the treatment of VZV and CMV infections, as VZV and CMV are equally, if not more, sensitive to inhibition by (*S*)-HPMPA in vitro than are HSV-1, HSV-2, and TK$^-$ HSV-1 (8, 11).

With the exception of the intracutaneous TK$^-$ HSV-1 infection in athymic nude mice and intracerebral TK$^-$ HSV-1 infection in ordinary mice, most of the other animal model infections used to demonstrate the in vivo efficacy of (*S*)-HPMPA, PMEA, and PMEDAP, i.e., the vaccinia virus tail lesion model (9), intracutaneous HSV-1 or HSV-2 infection of hairless mice (5, 10), and intracerebral HSV-1 infection (10), have been described previously. From a comparative analysis of the data presented here and those obtained previously in the same animal model systems (5, 9, 10), it appears that (*S*)-HPMPA (Fig. 3) is more efficacious than ribavirin, 5-iodo-2′-deoxyuridine, and 5-ethyl-2′-deoxyuridine in the systemic (i.p. or s.c.) treatment of vaccinia virus infection (9); (*S*)-HPMPA, PMEA, and PMEDAP (Table 1) are much more effective than various other drugs, such as acyclovir and BrVdUrd, in the topical treatment of intracutaneous HSV-1 infection (5, 10); and (*S*)-HPMPA, PMEA, and PMEDAP (Fig. 4) are also more efficacious than acyclovir and BrVdUrd in the systemic (i.p.) treatment of intracerebral HSV-1 infection (10). In only one particular condition, namely, p.o. treatment of (i.p.) HSV-1 infection, another compound, i.e., 5-(2-chloroethyl)-2′-deoxyuridine (10), may seem more efficacious than the phosphonylmethoxyalkyl derivatives.

The finding that (*S*)-HPMPA and its congeners are markedly active against intracutaneous TK$^-$ HSV-1 infection (Table 2), intracerebral TK$^-$ HSV-1 infection (Fig. 6), and TK$^-$ HSV-1 keratitis (18) points to their potential for the treatment of HSV infections that have become resistant to treatment with the conventional antiherpes drugs (i.e., acyclovir). In fact, the TK$^-$ HSV-1 variant used for the intracutaneous infection of athymic nude mice was derived from an immunocompromised patient with a chronic HSV-1 infection that no longer responded to acyclovir treatment (21). Treatment of such patients with (*S*)-HPMPA may be successful in abrogating the HSV infection, as suggested by the results obtained in athymic nude mice.

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