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Intracellular Phosphorylation of Broad-Spectrum Anti-DNA Virus Agent (*S*)-9-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine and Inhibition of Viral DNA Synthesis

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SUMMARY

The acyclic nucleotide analogue (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine [(*S*)-HPMPA], which contains a phosphonate-substituted aliphatic chain, is a potent and selective inhibitor of the replication of various DNA viruses, including herpes simplex virus type 1 (HSV-1). We have synthesized radiolabeled (*S*)-[U-^{14}C-adenine]HPMPA and investigated its metabolism by HSV-1-infected and mock-infected cells. The drug is as such taken up by the cells and subsequently converted to its monophosphoryl [(*S*)-HPMPAp] and diphosphoryl [(*S*)-HPMPApp] derivatives by cellular enzymes. It is incorporated to a very low extent into DNA of both mock-infected and HSV-1-infected Vero cells. (*S*)-HPMPA inhibits HSV-1 DNA synthesis at a concentration that is several orders of magnitude lower than the concentration required for inhibition of cellular DNA synthesis. Thus the selectivity of (*S*)-HPMPA as an antiviral agent cannot be attributed to a differential phosphorylation by virus-infected or uninfected cells but resides in a specific inhibitory effect on viral DNA synthesis. The exact basis for the latter effect is under investigation.

Since the discovery of ACV as a potent and selective inhibitor of herpes simplex virus replication, many new acyclic nucleosides have been synthesized (1, 2). Foremost among these acyclic nucleoside analogues is 2′NDG (DHPG), which is significantly more effective *in vivo* compared with ACV (3). The mechanism of action of 2′NDG is similar to that of ACV. It is preferentially phosphorylated to its monophosphate form by the HSV-encoded TK and then converted to the triphosphate by cellular enzymes. In this form, 2′NDG acts as a potent inhibitor of the HSV DNA polymerase (4).

Several mutants of HSV have been characterized that are either deficient or altered in their TK activity; such mutants are, as a rule, resistant to ACV and DHPG (5). To bypass the phosphorylation activation step, and hence ensure activity against those viruses that do not encode for a specific TK, many phosphonate and cyclic phosphate derivatives of acyclic nucleosides have been synthesized and found active as antiviral agents (6–10). Recently, the synthesis and antiviral activity of the novel phosphonate derivative (*S*)-HPMPA has been described (11).

(*S*)-HPMPA (I: see Scheme 1) inhibits the replication of a broad variety of DNA viruses and is also active against retroviruses (11). (*S*)-HPMPA has been found inhibitory to all herpes viruses that have been investigated for their sensitivity to the compound. In contrast with acyclovir, (*S*)-HPMPA is equally active against TK positive (TK$^+$) and TK negative (TK$^-$) strains of HSV and varicella zoster virus. (*S*)-HPMPA is also effective *in vivo* against TK$^-$HSV-1 keratitis in rabbits (12).

The present study was aimed at gaining better insight in the mechanism of action of (*S*)-HPMPA and its metabolism by the cells. The phosphorylation pattern of (*S*)-HPMPA was examined in both mock-infected and HSV-1-infected cells, and its interaction with DNA synthesis was investigated in intact cells.

Materials and Methods

Compounds. (*S*)-HPMPA sodium salt was synthesized from (*S*)-DHPA, as briefly described by De Clercq *et al.* (11). The purity of the

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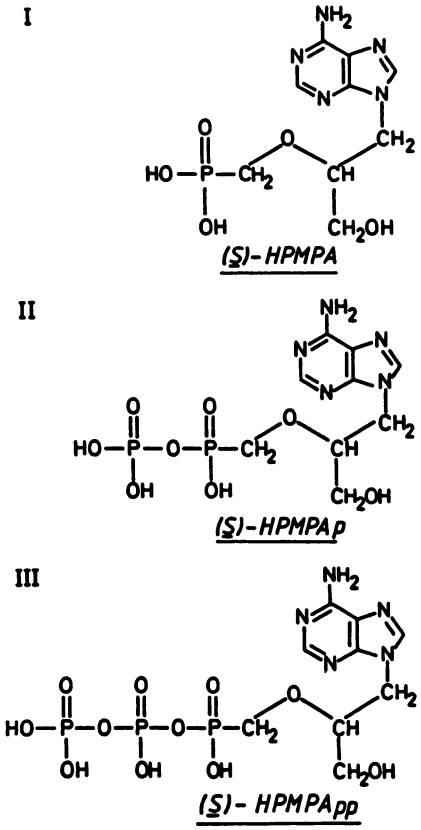
ABBREVIATIONS: (*S*)-HPMPA, (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine; (*S*)-HPMPAp, monophosphoryl derivative of (*S*)-HPMPA; (*S*)-HPMPApp, diphosphoryl derivative of (*S*)-HPMPA; HSV-1, herpes simplex virus type 1; HEL, human embryonic lung; TK, thymidine kinase; ACV, acyclovir [9-(2-hydroxy-ethoxymethyl)guanine];2′NDG,2′nor-2′-deoxyguanosine; DHPG, 9-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-guanine; MIC, minimum inhibitory concentration; PFU, plaque forming units; (*S*)-DHPA, (*S*)-9-(2,3-dihydroxypropyl)adenine; HPLC, high-performance liquid chromatography; TLC, thin layer chromatography.



Scheme 1. Structural formulas of (*S*)-HPMPA (I), its monophosphoryl derivative (*S*)-HPMPAp (II), and its diphosphoryl derivative (*S*)-HPMPApp (III).

compound was confirmed by HPLC analysis [Separon SIX C18 column, 200 × 4 mm, in 5% (vol/vol) methanol in 0.05 M triethylammonium hydrogen carbonate]. (*S*)-HPMPA was converted to its diphosphoryl derivative [(*S*)-HPMPApp] (III: see Scheme 1), according to a modification of the procedure originally described by Moffatt (13), that is by reacting the tri-*n*-butylammonium salt of diphosphoric acid in dimethylsulfoxide with (*S*)-HPMPA morpholidate. (*S*)-HPMPApp was isolated by ion-exchange chromatography and stored as the sodium salt. The purity was confirmed by HPLC analysis as described above.

(*S*)-[^{14}C]HPMPA (133 μCi/μmol) was prepared from (*S*)-[U-^{14}C] adenine-DHPA following the same procedure as described for the nonlabeled material. (*S*)-[U-^{14}C]adenine-DHPA was purchased from the Institute for Research, Production and Use of Isotopes, Prague, and isolated by preparative HPLC under the same conditions as described above.

Cells. Vero cells and HEL cells were grown in Eagle's minimum essential medium (Flow Laboratories) supplemented with 10% fetal calf serum and 2 mM L-glutamine (Flow Laboratories) and 0.075% $NaHCO_3$. Murine leukemia L1210 cells were obtained from female DBA/2 mice 8 days after intraperitoneal inoculation of 10^5 cells/mouse.

The origin of HSV-1 (KOS) has been described (14).

Antiviral activity of (*S*)-HPMPA. HEL cells or Vero cells were grown to confluency in 96-well microtiter plates and infected with HSV-1(KOS) at 100 $CCID_{50}$/well, 1 $CCID_{50}$ being the infective dose for 50% of the cell cultures. After 1 hr adsorption at 37°C, the virus was removed, and the cells were further incubated in the presence of various concentrations of (*S*)-HPMPA. Viral cytopathogenicity was recorded as soon as it reached 100% in the control virus-infected but untreated cell cultures. The antiviral activity of (*S*)-HPMPA was expressed as the MIC, that is the concentration of compound required to reduce virus-induced cytopathogenicity by 50% (14).

Analysis of acid-soluble fractions. Vero cells or HEL cells were grown to confluency in 60-mm Falcon Petri dishes and either mock-infected or infected with HSV-1(KOS) at 1 PFU/cell or 0.1 PFU/cell, respectively. After 1 hr adsorption, virus or mock medium was removed, and cells were further incubated for 6 hr in the presence of (*S*)-[^{14}C] HPMPA (15 nmols/ml/Petri dish). Cells were washed three times with ice-cold PBS, collected, and centrifuged for 5 min at low speed. The pellet was resuspended in 5% trichloroacetic acid, and the samples were placed on ice. After 10 min, the acid-precipitable material was removed by centrifugation in an Eppendorf 5412 centrifuge. Residual trichloroacetic acid was then extracted from the supernatants by shaking the samples during 30 min in the presence of 0.4 vol freon and 0.1 vol tri-*n*-octylamine, as described previously (15).

After centrifugation, the upper phase, containing the acid-soluble material, was evaporated in a Savant speed vac concentrator. The material was redissolved in 10 μl water, of which 5 μl samples were spotted on PEI-cellulose TLC plates and chromatographed for 30 min with 4 M LiCl/1 M acetic acid (1:4) as the solvent. After drying, the plates were cut into 5-mm pieces and evaluated for radioactivity. Radioactivity peaks were identified by comparing the R_F values of the (*S*)-[^{14}C]HPMPA metabolites with the R_F values of the reference compounds.

Preparation of a cell-free extract from L1210 cells. L1210 cells [17 g (wet wt)] were washed with PBS, resuspended in 11 ml buffer containing 10 mM Tris-HCl (pH 7.4) and 2 mM 2-mercaptoethanol and sonicated with a Measuring and Scientific Equipment Ltd. sonifier (150 W, 60 sec, amplitude 7 at 22 kc) at 0°C. After centrifugation at 30,000 × *g* for 20 min and again at 100,000 × *g* for 100 min, the resulting supernatant (100,000 × *g* supernatant) was applied onto a Sephadex PD-10 column equilibrated in a buffer containing 20 mM Hepes (pH 8.0) and 2 mM 2-mercaptoethanol.

L1210 kinase assay. The standard reaction mix contained, in a volume of 50 μl, 0.8 mM ribonucleoside 5′-triphosphate, 2 mM creatine phosphate, 0.15 mg/ml creatine phosphokinase, 1 mM $MgCl_2$, 1 mM dithiothreitol, 20 mM Hepes-HCl (pH 8.0), and 3 mg/ml of the 100,000 × *g* supernatant described above. After the appropriate incubation time, aliquots were withdrawn from the reaction mix, spotted on PEI-cellulose TLC plates, and chromatographed with 4 M LiCl/1 M acetic acid (1:4) as the solvent. After drying, the plates were cut into pieces and evaluated for radioactivity.

Cesium chloride density gradient analysis of viral and cellular DNA. Vero or HEL cells were grown to confluency in 60-mm Falcon Petri dishes and either mock-infected or infected with HSV-1(KOS) at 0.1 PFU/cell. After 1-hr adsorption, virus or mock medium was removed, and the cells were washed three times with phosphate-free Eagle's minimum essential medium (Gibco) supplemented with 3% fetal calf serum, 2 mM L-glutamine, and 0.075% $NaHCO_3$. The cells were incubated 24 hr in 2 ml of this medium containing 25 μCi [^{32}P] orthophosphate (carrier free) and varying concentrations of (*S*)-HPMPA. Cells were then processed as has been described (16).

Vero cells that had been either mock-infected or infected with HSV-1(KOS) at a multiplicity of infection of 0.1 PFU/cell and further incubated in the presence of 5 μM (*S*)-[^{14}C]HPMPA were also processed for CsCl density gradient analysis as previously described (16).

Results

Antiviral activity of (*S*)-HPMPA. It was first ascertained that (*S*)-HPMPA inhibited the replication of HSV-1 in both Vero and HEL cells. However, (*S*)-HPMPA was about 1000 times more active against HSV-1(KOS) in HEL cells (MIC: 0.02 μM) than in Vero cells (MIC: 22 μM). The metabolism and mechanism of action of (*S*)-HPMPA was examined in both cell systems.

Phosphorylation pattern of (*S*)-[^{14}C]HPMPA. Upon exposure of mock-infected and HSV-1(KOS)-infected cells to (*S*)-[^{14}C]HPMPA for 6 hr, three radioactivity peaks were detected by TLC of the acid-soluble fractions of the cell lysates of either Vero cells (Fig. 1) or HEL cells (Fig. 2). Based on the R_F values

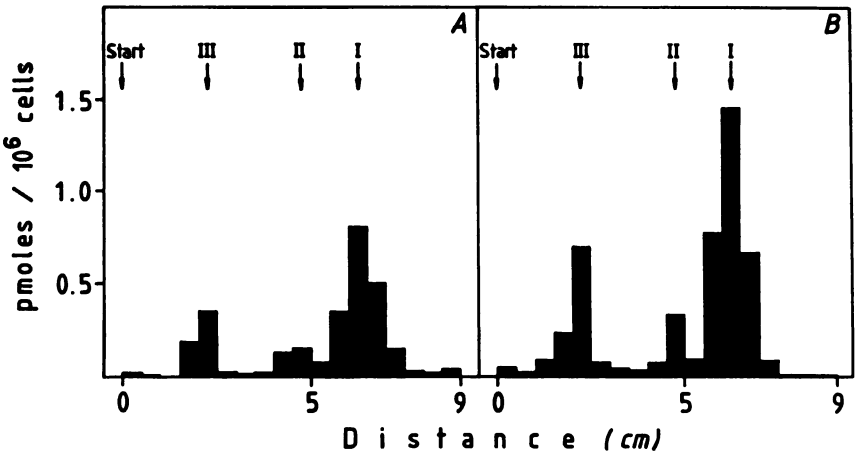


Fig. 1. TLC of the acid-soluble fractions of either mock-infected (*panel A*) or HSV-1(KOS)-infected (*panel B*) Vero cells, which were incubated for 6 hr in the presence of (*S*)-[^{14}C]HPMPA at 15 μM. *Arrows*, positions of the metabolites depicted in Scheme I.

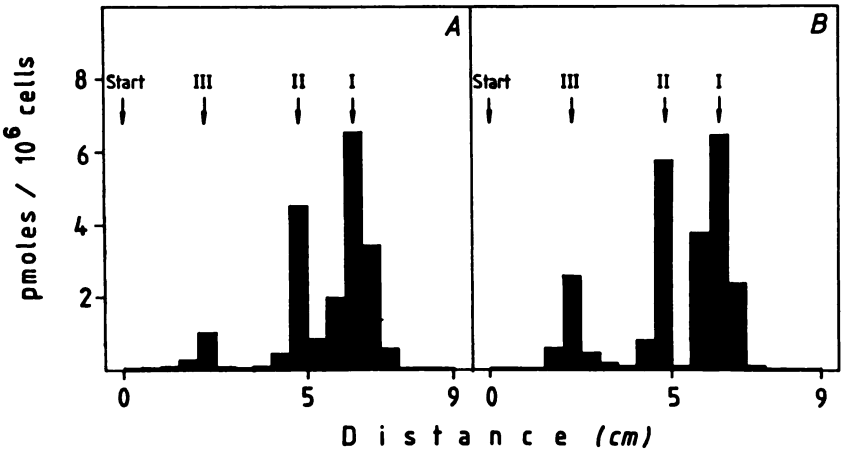


Fig. 2. TLC of acid-soluble fractions of either mock-infected (*panel A*) or HSV-1(KOS)-infected (*panel B*) HEL cells, which were incubated for 6 hr in the presence of (*S*)-[^{14}C]HPMPA at 15 μM. *Arrows*, positions of the metabolites depicted in Scheme I.

of the comigrating reference compounds, the radioactivity peaks were identified as (*S*)-[^{14}C]HPMPA (Scheme 1, formula I), (*S*)-[^{14}C]HPMPAp (Scheme 1, formula II), and (*S*)-[^{14}C]HPMPApp (Scheme 1, formula III). No deamination products of (*S*)-HPMPA were found.

The intracellular concentrations achieved by (*S*)-[^{14}C]HPMPA, (*S*)-[^{14}C]HPMPAp, and (*S*)-[^{14}C]HPMPApp in HEL cells were higher than those attained in Vero cells, which is in line with the stronger antiviral activity of (*S*)-HPMPA in HEL than in Vero cells. In either cell type (HEL or Vero cells), however, the phosphorylation pattern was very similar for mock-infected and HSV-1(KOS)-infected cells. These data suggest that (*S*)-HPMPA is as such taken up by the cells and then converted to its monophosphoryl and diphosphoryl derivatives by cellular nucleotide kinases.

This hypothesis was further supported by nucleotide kinase experiments with cell-free extracts of L1210 cells, a murine leukemia cell line that produces high levels of nucleotide kinases. The reaction was followed by using ribonucleoside 5′-triphosphates as phosphate donors in the presence of an ATP regenerating system. The data presented in Table 1 demonstrate that (*S*)-HPMPA is effectively phosphorylated in the presence of ATP and an ATP regenerating system (creatine phosphate/creatine phosphokinase) to form mainly the diphosphoryl derivative III [(*S*)-HPMPApp]. This compound was identified by comparison of its R_F value after chromatography on PEI-cellulose with the R_F value of the comigrating reference compound III [(*S*)-HPMPApp].

To ascertain that the phosphorylation did not occur at the

TABLE 1

(*S*)-[^{14}C]HPMPA as substrate for nucleotide kinases from L1210 cell extracts

Assay conditions are described in Materials and Methods. Extent of phosphorylation after 60-min incubation is expressed as percentage of the original amount of (*S*)-[^{14}C]HPMPA (data taken from a representative experiment).

Phosphate donor	Product (%)		
	(*S*)-HPMPA (I)	(*S*)-HPMPAp (II)	(*S*)-HPMPApp (III)
ATP	43	5	52
GTP	77	7	16
UTP	69	5	26
CTP	76	2	22

3′-hydroxyl group of (*S*)-HPMPA, product III (Table 1) and the reference material (*S*)-HPMPApp were submitted to treatment with 0.33 M KOH at 37°C for 16 hr. In both cases, the reaction gave rise to (*S*)-HPMPA solely, a result that could only be expected if the phosphate groups were attached to the phosphonate (as in formula III of Scheme 1) and not to the 3′-hydroxyl group.

The transformation of (*S*)-HPMPA to (*S*)-HPMPApp also occurred with GTP, UTP, and CTP as phosphate donor, albeit less efficiently as with ATP as phosphate donor (Table 1). Formation of (*S*)-HPMPAp (formula II in Scheme 1) as an intermediate product was observed with all four phosphate donors.

Inhibition of viral DNA synthesis by (*S*)-HPMPA. Viral and cellular DNA synthesis, based on [^{32}P]orthophosphate incorporation into DNA, was measured in HSV-1(KOS)-

infected or mock-infected Vero or HEL cells, incubated in the presence of varying concentrations of (*S*)-HPMPA.

As shown in Fig. 3, inhibition of viral DNA synthesis in HSV-1(KOS)-infected Vero cells exposed to (*S*)-HPMPA increased proportionally with concentrations of (*S*)-HPMPA within the concentration range of 0.5–50 μM, whereas cellular DNA synthesis was unaffected at compound concentrations up to 50 μM in both mock-infected and HSV-1(KOS)-infected cells. Only at a concentration of 500 μM (*S*)-HPMPA was cellular DNA synthesis reduced to about 50% of its control value; at this concentration, viral DNA synthesis was completely suppressed.

In HSV-1(KOS)-infected HEL cells the inhibitory effect of (*S*)-HPMPA on viral DNA synthesis was even more pronounced. Here, viral DNA synthesis was almost completely suppressed at a concentration of only 0.05 μM, whereas no inhibition of cellular DNA synthesis occurred even at (*S*)-HPMPA concentrations up to 50 μM (Fig. 4).

[^{32}P]Orthophosphate incorporation into viral DNA was reduced to background radioactivity in HEL cells at a compound concentration of 0.5 μM, whereas in Vero cells (*S*)-HPMPA had to be added at 500 μM to achieve a complete suppression of viral DNA synthesis [Figs. 3 (panel J) and 4 (panel C)]. This difference in the inhibitory effects of (*S*)-HPMPA on viral DNA synthesis in HEL and Vero cells closely reflects differences in the MIC values of (*S*)-HPMPA for inhibition of HSV-1 cytopathogenicity in these cell lines.

Incorporation of (*S*)-[^{14}C]HPMPA into DNA of mock-infected and HSV-1(KOS)-infected Vero cells. When Vero cells were incubated in the presence of 5 μM (*S*)-[^{14}C]HPMPA for 24 hr, acid-precipitable [^{14}C]radioactivity was recovered from CsCl gradients of both mock-infected and HSV-1(KOS)-infected cells (Fig. 5). The major radioactivity peak had a

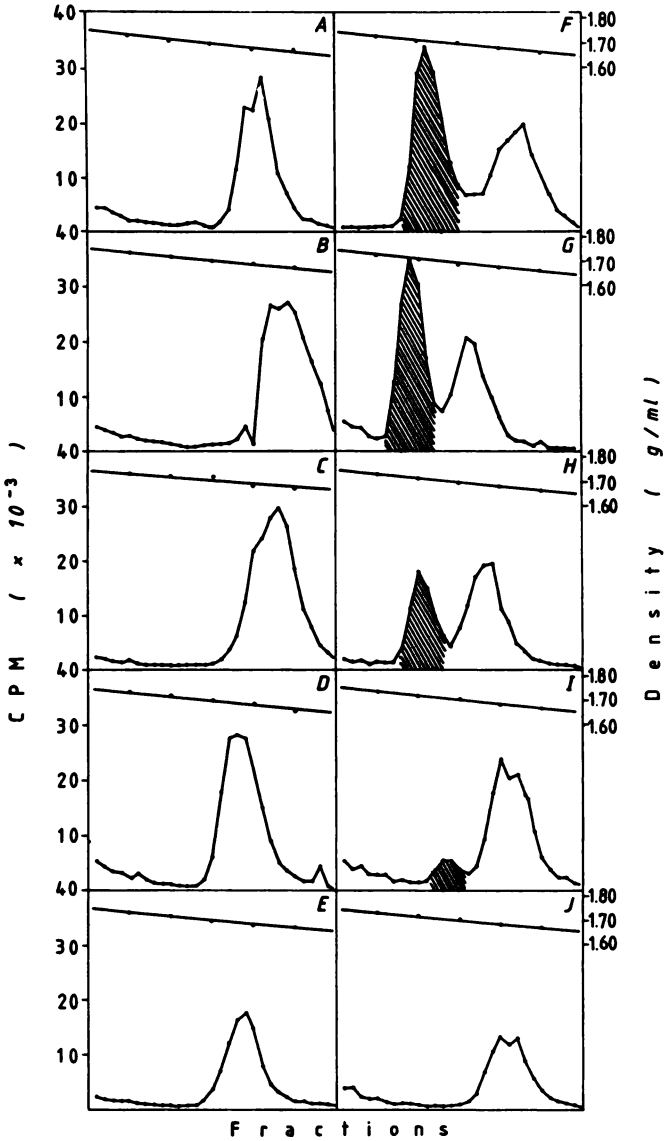


Fig. 3. CsCl equilibrium gradient analysis of DNA from either mock-infected (*panels A–E*) or HSV-1(KOS)-infected (*panels F–J*) Vero cells incubated 24 hr in the presence of 25 μCi [^{32}P]orthophosphate together with (*S*)-HPMPA at 0 μM (*panels A* and *F*), 0.5 μM (*panels B* and *G*), 5 μM (*panels C* and *H*), 50 μM (*panels D* and I), or 500 μM (*panels E* and *J*). *Shadowed areas* correspond to viral DNA.

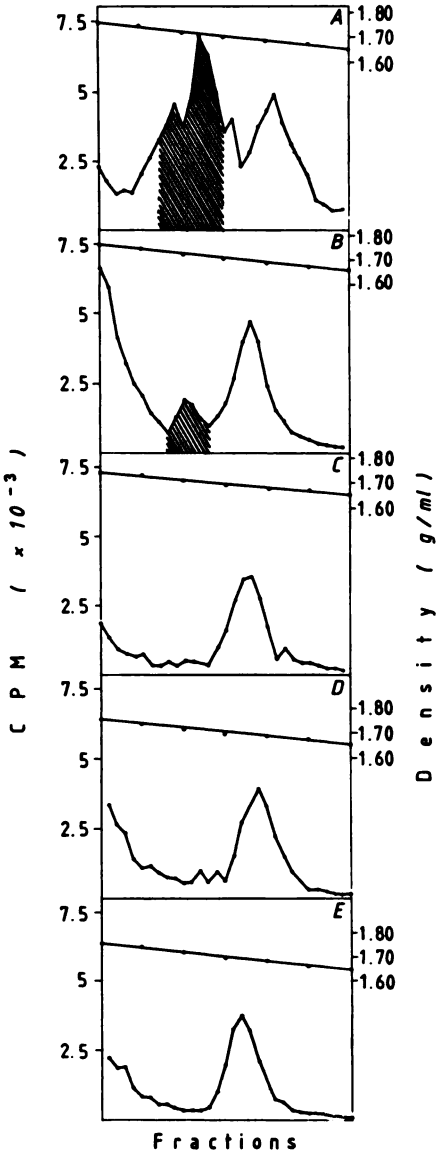


Fig. 4. CsCl equilibrium gradient analysis of DNA from HSV-1(KOS)-infected HEL cells incubated 24 hr in the presence of 25 μCi [^{32}P] orthophosphate together with (*S*)-HPMPA at 0 μM (*panel A*), 0.05 μM (*panel B*), 0.5 μM (*panel C*), 5 μM (*panel D*), and 50 μM (*panel E*). *Shadowed areas* correspond to viral DNA.

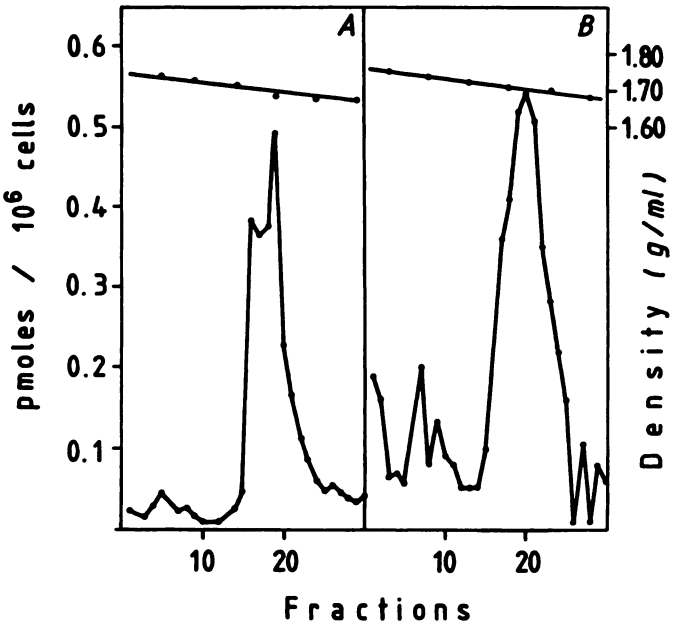


Fig. 5. CsCl equilibrium gradient analysis of DNA from either mock-infected (*panel A*) or HSV-1(KOS)-infected (*panel B*) Vero cells incubated 24 hr in the presence of 5 μM (*S*)-[^{14}C]HPMPA.

buoyant density of 1.695 g/ml that corresponds to the density of cellular DNA. (*S*)-[^{14}C]HPMPA was incorporated to a similar extent into cellular DNA of mock- and virus-infected cells.

Incorporation of (*S*)-[^{14}C]HPMPA into viral DNA of HSV-1(KOS)-infected Vero cells could not be clearly demonstrated at a compound concentration of 5 μM [Fig. 5 (panel B)]. Note that the inhibitory effect of (*S*)-[^{14}C]HPMPA on viral DNA synthesis [50% at a concentration of 5 μM: Fig. 3 (panel H)] may counteract its incorporation into the DNA at this drug concentration.

No incorporation of (*S*)-[^{14}C]HPMPA into DNA of mock-infected or HSV-1(KOS)-infected HEL cells could be demonstrated. It is not surprising that no incorporation of (*S*)-[^{14}C]HPMPA into viral DNA could be visualized, because viral DNA synthesis is completely shut off at a (*S*)-HPMPA concentration of 5 μM in HSV-1(KOS)-infected HEL cells [Fig. 4 (panel D)]. Moreover, [^{32}P]orthophosphate incorporation into cellular DNA is about four to five times lower for HEL than for Vero cells, which indicates a lower DNA replication rate in HEL cells and may explain the lack of detection of incorporation of (*S*)-[^{14}C]HPMPA into HEL cell DNA.

Additional CsCl gradient analyses were conducted with extracts from Vero or HEL cells exposed to concentrations of (*S*)-[^{14}C]HPMPA lower than 5 μM. At a concentration of 1 μM (*S*)-[^{14}C]HPMPA, no incorporation into HSV-1 DNA could be demonstrated (data not shown). At this concentration the incorporation of (*S*)-[^{14}C]HPMPA into cellular DNA was about 5-fold lower than that noted with a concentration of 5 μM (*S*)-[^{14}C]HPMPA: that is two to three times higher than background radioactivity (data not shown).

To establish the nature of the [^{14}C]radioactivity, the acid-precipitable material of HSV-1(KOS)-infected Vero cells incubated in the presence of either 5 μM [*methyl*-^{3}H]dThd or 5 μM (*S*)-[^{14}C]HPMPA was incubated overnight in 1 N perchloric acid at 56°C. The samples were neutralized with K_2CO_3, and 5-μl aliquots were chromatographed on PEI-cellulose plates in *n*-propanol/water (70:30). After drying, the plates were cut into 0.5-cm pieces and examined for radioactivity. For the [*methyl*-^{3}H]dThd samples all radioactivity comigrated with thymine, whereas for the (*S*)-[^{14}C]HPMPA-treated samples all radioactivity remained at the start (data not shown). For the latter samples, no radioactivity was associated with either 2′-deoxyadenosine or adenine. The [^{14}C]radioactivity remaining at the start may be associated with di-, oligo-, or polymers containing (*S*)-[^{14}C]HPMPA and resisting acid hydrolysis.

Discussion

Our experiments clearly indicate that (*S*)-HPMPA, which is a potent and selective inhibitor of HSV replication (11), is as such taken up by the cells and subsequently phosphorylated to its mono- and diphosphoryl derivatives. This was demonstrated by TLC analysis of acid-soluble fractions from both HEL and Vero cells, whether they had been mock-infected or infected with HSV-1(KOS). There was no significant difference in the phosphorylation pattern of (*S*)-HPMPA between mock-infected and HSV-1(KOS)-infected cells. Additional evidence for the phosphorylation of (*S*)-HPMPA by cellular nucleotide kinases was obtained from experiments with a 100,000 × *g* supernatant from L1210 cells. The major metabolite detected was the diphosphoryl derivative III (cf. Scheme 1) that actually corresponds to the nucleoside 5′-triphosphate. Hypoxanthine derivatives, which could have originated from the deamination of (*S*)-HPMPA or its metabolites, were not detected.

(*S*)-HPMPA strongly and selectively inhibited viral DNA synthesis, as monitored by [^{32}P]orthophosphate incorporation into DNA of HSV-1(KOS)-infected cells, the extent of inhibition being dependent on the cell line used: in HEL cells [^{32}P]orthophosphate incorporation into viral DNA was reduced to background radioactivity at a (*S*)-HPMPA concentration of 0.5 μM, whereas in Vero cells complete suppression of viral DNA synthesis was achieved only at a concentration of 500 μM. In neither HEL nor Vero cells, (*S*)-HPMPA was inhibitory to cellular DNA synthesis unless its concentration was several orders of magnitude in excess of that effecting inhibition of viral DNA synthesis. (*S*)-HPMPA did not inhibit cellular DNA synthesis within virus-infected cells to a greater extent than cellular DNA synthesis within mock-infected cells, thus indicating that by the virus-infected cells no specific metabolite was formed that interfered with DNA synthesis.

(*S*)-HPMPA inhibits the replication of HSV-1 in HEL cells at a 1,000-fold lower concentration (MIC: 0.02 μM) than in Vero cells (MIC: 22 μM). That antiherpes compounds may vary considerably in antiviral potency, depending on the nature of the host cell, is a well-recognized phenomenon (17). The differences in the antiviral activity of (*S*)-HPMPA in Vero and HEL cells seem to be closely related to differences in the extent of inhibition of viral DNA synthesis (Figs. 3 and 4): the concentration of (*S*)-HPMPA required to achieve a significant (~80%) reduction of HSV-1 DNA synthesis in Vero cells was 50 μM, whereas in HEL cells a similar reduction in viral DNA synthesis was achieved at a 1000-fold lower concentration (0.05 μM). The differences in the inhibitory potency of (*S*)-HPMPA on viral DNA synthesis in HEL and Vero cells could only partially be explained by differences in the extent of phosphorylation of (*S*)-HPMPA by these cell lines. The latter did not exceed a factor of 10-fold (Figs. 1 and 2), which does not explain the 1000-fold difference observed in antiviral potency.

(*S*)-[^{14}C]HPMPA is incorporated to a very small extent into

cellular DNA of both mock-infected and HSV-1(KOS)-infected Vero cells. Incorporation of the compound into viral DNA could not be demonstrated, probably because the compound inhibited viral DNA synthesis at the concentrations required for establishing its incorporation. In which form (*S*)-HPMPA is actually incorporated into cellular DNA and whether it is incorporated internally *via* internucleotide linkage or externally at the 3′-terminal remain subjects of further investigation, where (*S*)-[^{14}C]HPMPA of the highest possible specific radioactivity should be employed.

In conclusion, our study has shown that (*S*)-HPMPA, the recently discovered broad-spectrum anti-DNA virus agent, is phosphorylated intracellularly to its mono- and diphosphoryl derivatives. This phosphorylation occurs equally well in virus- and mock-infected cells. Yet the compound is significantly more inhibitory to viral DNA synthesis than cellular DNA synthesis. Its selective antiviral activity must therefore reside in a specific inhibitory effect on viral DNA synthesis rather than a specific phosphorylation by the virus-infected cells.

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