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(*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) inhibits HIV-1 replication in epithelial cells, but not T-lymphocytes

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Abstract

PMEA [9-(2-phosphonylmethoxyethyl)adenine] inhibited both HSV-1 and HIV-1 replication in MT-2 and HeLa–CD4 cells. (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC) inhibited both these viruses in the epithelioid HeLa–CD4 cells, but did not inhibit either virus in the T-lymphocytic MT-2 cells. PMEA and HPMPC are metabolized to their diphosphorylated forms within cells, which then inhibit viral polymerases. We therefore compared the metabolism of PMEA and HPMPC in MT-2 and HeLa–CD4 cells. PMEApp formation was efficient in both the cell types, whereas HPMPCpp levels were $\sim$3–10 fold lower in MT-2 cells, compared to HeLa–CD4 cells. These results indicate that HPMPC can inhibt HIV replication in the appropriate cell types, and show that differences in their metabolism cannot account entirely for the lack of antiviral efficacy of HPMPC in MT-2 cells.

Keywords: HIV-1; Epithelial cell; T-lymphocyte

1. Introduction

Acyclic nucleoside phosphonates (ANP) are broad-spectrum antiviral agents with activity against a number of DNA viruses including herpesviruses, and retroviruses including the human immunodeficiency virus (HIV), the etiologic agent of AIDS (Bronson et al., 1990; Balzarini, 1994). ANPs which vary in the nucleoside base, or in the alkyl side-chain, have been synthesized and shown to display substantial differences in spectra of their activity. Of these, two compounds PMEA [9-(2-phosphonylmethoxyethyl) adenine]

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(Balzarini, 1994) and HPMPC ((*S*)-1-[3-hydroxy - 2 - (phosphonylmethoxy)propyl] cytosine) (De Clercq, 1993) are being evaluated for the treatment of HIV and cytomegalovirus (CMV) infections, respectively.

HPMPC has been more extensively tested in the clinic, and its efficacy in the control of symptomatic CMV infections in AIDS patients has recently been demonstrated (Lalezari et al., 1995a,b; Kirsch et al., 1995). A notable feature of HPMPC is that it confers a long lasting antiviral activity both in vitro and in vivo. A single application of HPMPC after virus adsorption can establish an antiviral effect that can last >7 days in cell culture, or animal models. This long-lasting antiviral effects of HPMPC has been attributed to the persistence of its active intracellular derivatives (De Clercq, 1993).

PMEA has been shown to inhibit both retroviruses and herpesviruses, whereas HPMPC has been shown to be effective against various herpesviruses, but not retroviruses (e.g. HIV-1). The reason for the differences in the antiviral selectivity of these closely related analogs is not clear. HPMPCpp, the biologically active metabolite of HPMPC, is a potent inhibitor of HSV-1 polymerase (Ki $\sim 0.86\ \mu$M, Ho et al., 1992). HPMPCpp also inhibits HIV-1 reverse transcriptase in vitro (Ki $\sim 8.16\ \mu$M, Mendel et al., 1994), at levels comparable to those reported for the inhibition of CMV polymerase (Ki $\sim 6.6\ \mu$M, Xiong et al., 1995). The different viruses vary in their tropism. The herpes viruses replicate in a variety of tissues while HIV is primarily lymphocyte-tropic. The antiviral susceptibility assays against herpes viruses are usually performed in various epithelial or fibroblast cell lines, while peripheral blood mononuclear cells or T-lymphocytic cell lines are used with HIV-1. Here we have investigated whether cell type-specific differences in the formation of HPMPCpp may be responsible for the restricted antiviral spectrum of HPMPC. Using HeLa–CD4 cells—an epithelial cell line engineered to express the cell surface receptor for HIV-1 which is susceptible to both HIV-1 and HSV-1—we show that cell-type specific differences in the metabolism of ANPs is an important, but not the sole determinant of their antiviral spectrum.

2. Antiviral effects of HPMPC and PMEA

The effect of HPMPC on HSV replication was monitored by plaque reduction assays on HeLa cell monolayer cultures (Srinivas et al., 1990), or by its ability to protect MT-2 cells from virus-induced cytopathicity (Gong et al., 1993). The effect of HPMPC on HIV replication was monitored using virus yield reduction assays (Gong et al., 1994). The HIV stocks were titrated for cytopathic effects in MT-2 cells. MT-2 cells were infected at a multiplicity of 0.01, and the virus-infected cells were seeded at a concentration of 0.2×10^6 cells/ml in media containing varying concentrations of HPMPC or PMEA. After 5 days incubation, the p24 antigen levels in the culture supernates were determined by p24 antigen capture assay (Coulter, Hialieh, FL). HeLa cells were seeded in 96-well plates at a density of 5000 cells/well, and infected with an HIV-1 inoculum containing 100 $TCID_{50}$ units. After 1 h adsorption, the cells were washed and incubated with media containing different concentrations of the drugs. After 5 days, the amount of virus produced was determined by p24 antigen capture assay. The drug concentrations yielding half-maximal inhibition in p24 antigen production activity, compared to drug-free controls, was calculated using a non-linear curve fitting program (Enzfitter, Elsevier-BioSoft, Evanston, IL).

The effect of HPMPC on the replication of HIV-1 and HSV-1 in different cell types are summarized in Table 1. HPMPC did not inhibit either HSV or HIV replication in the T-lymphocytic MT-2 cell line, unlike PMEA which inhibited both viruses in MT-2 cells. Surprisingly, HPMPC inhibited both HSV-1 and HIV-1 in CD4 + HeLa

Table 1
Antiviral activity of HPMPC and PMEA in different cell types

Cell	HPMPC		PMEA	
	HIV	HSV	HIV	HSV
ED_{50} (μM)				
MT-2	>50	>50	1 ± 1.5	2.6 ± 1.5
HeLa-T4	0.9 ± 0.3	2.5 ± 1.5	2.7 ± 0.3	2.5 ± 1

Each value represents a mean of three or more experiments.

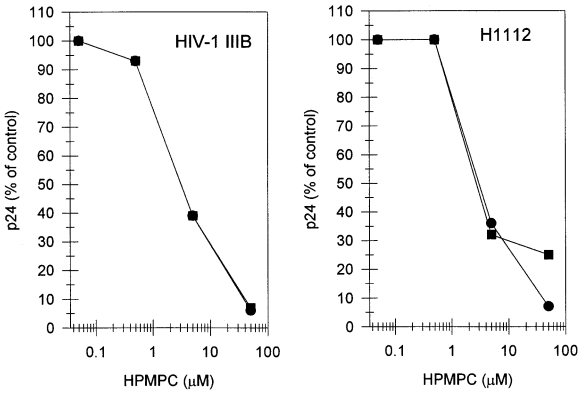

Fig. 1. Inhibition HIV-1 replication by HPMPC in CD4+ HeLa cells. CD4+ HeLa cells were infected with HIV-1_{IIIB} or H112 and maintained in presence of varying concentrations of HPMPC. The culture supernates were assayed for p24 antigen after 5 days. The results of two independent experiments are shown.

cells, and the ED_{50} values against the two viruses were comparable. HIV-1_{IIIB} is a laboratory adapted virus strain which has been passaged extensively in vitro. Therefore, we also tested the effect of HPMPC on a different clinical isolate H112. HPMPC was equally effective against both HIV-1_{IIIB} and H-112 (Fig. 1). Furthermore, the effect of HPMPC against the different HIV isolates are comparable to those reported with other inhibitors, such as ddI (Mayers et al., 1994).

The 50% inhibitory concentrations (IC_{50}) of PMEA was 160 and 300 μM, respectively for MT-2 and HeLa–CD4 cells. HPMPC was far less toxic, no cytostatic effect against either MT-2 or HeLa–CD4 was observed at concentrations up to 500 μM HPMPC ($IC_{50} > 500$ μM). Thus, the antiviral activity of the compounds, or the cell-type specific differences were not due to the cytotoxic effects of these compounds. Our results suggest that HIV-1 is not intrinsically resistant to HPMPC, and we therefore investigated whether a differential metabolism is responsible for the cell types-specific differences in the antiviral spectrum of HPMPC.

3. Metabolism of PMEA and HPMPC in HeLa–CD4 and MT-2 cells

The metabolism of PMEA and HPMPC was investigated according to previously described procedures (Srinivas et al., 1993; Connelly et al., 1993; Aduma et al., 1995). Briefly, [^{3}H]PMEA (11 Ci/mmol) and [^{3}H]S-HPMPC (21 Ci/mmol) were obtained from Moravek (Brea, CA). The radiochemical purity of the compounds was first ascertained by analytical reverse phase HPLC. Exponential cultures of HeLa–CD4 or MT-2 cells were incubated with medium containing 10mM [^{3}H]PMEA or [^{3}H]HPMPC at 37°C for 6 or 24 h. Labeling medium was removed from HeLa–CD4 cells by aspiration, and the cells were washed extensively in ice-cold PBS (pH 7.4) prior to extraction, whereas MT-2 cells were centrifuged through a layer of Nyosil oil. The cells were extracted with 500 ml of ice-cold 70% methanol in 15 mM Tris pH 7.4 for 30 min on ice. The methanol extracts were clarified by centrifugation at 14 000 rpm for 5 min in an Eppendorf microcentrifuge and the supernatant was stored at −20°C until further analysis.

PMEA and HPMPC metabolites in the labeled cell extracts were analyzed by HPLC on a Whatman Partisil 10 SAX column. A linear gradient of 5–600 mM ammonium phosphate buffer pH 4.0 at a flow rate of 1.5 ml/min was used for the elution of PMEA and its metabolites. HPMPC metabolites were analyzed using a gradient of 15–700 mM ammonium phosphate buffer pH 3.5, at a flow rate of 1.5 ml/min. The radioactive peaks were identified by their comparison to the elution profiles of the authentic standards.

The intracellular concentrations of HPMPC or PMEA, and their metabolites, in MT-2 and HeLa–CD4 cells are summarized in Table 2. The levels of HPMPC (and PMEA) was similar between MT-2 and HeLA–CD4 cells suggesting a similar uptake of these compounds. These results were not surprising since the ANPs are internalized by fluid-phase endocytosis in both T-lymphocytes and epithelial cells (Connelly et al., 1993; Palu et al., 1991).

PMEA was efficiently metabolized by both MT-2 and HeLa–CD4 cells. PMEApp accounted

Table 2
Metabolism of HPMPC and PMEA in HeLa-CD4 and MT-2 cells

Cells	Time (h)	Picomole/10^6 cells				
		HPMPC	HPMPCp-choline	HPMPCp	HPMPCpp	Total
MT-2	6	0.65	0.06	0.003	0.02	0.733
	24	0.93	0.22	0.02	0.14	1.31
HeLa-CD4	6	0.72	1.04	0.04	0.21	2.01
	24	0.87	0.62	0.07	0.34	1.90
		PMEA	PMEAp	PMEApp		Total
MT-2	6	1.27	0.34	0.30		1.91
	24	1.32	0.19	0.34		1.85
HeLa-CD4	6	0.90	0.14	0.10		1.14
	24	1.35	0.33	0.62		2.30

The intracellular levels of various ANP metabolites in HeLa-CD4 or MT-2 cells incubated with [^{3}H]PMEA or [^{3}H]HPMPC was determined by analytical HPLC. Two or more times more sets of experiments were performed, and the results of a representative experiment is shown. The variation between different experiments did not exceed 15%.

for $\sim$18–27% of all drug-related metabolites in MT-2 and HeLa–CD4 cells respectively. Peak levels of PMEApp were attained by 6 h in MT-2 cells, whereas the diphosphates continued to increase up to 24 h in HeLa–CD4 cells. These results are consistent with the observed antiviral efficacy of PMEA in both MT-2 and HeLa–CD4 cells.

HPMPC was also metabolized to the diphosphate derivative by both MT-2 and HeLa–CD4 cells, despite the lack of antiviral effects in MT-2 cells. Interestingly, MT-2 cells accumulated HPMPC metabolites more slowly than in HeLa–CD4 cells. After 6 h, the intracellular levels of HPMPCp, HPMPCpp and HPMPCp–choline were over ten-fold lower in MT-2 cells than those seen with HeLa–CD4 cells. Moreover, HPMPCpp, the biologically active metabolite, was barely detected in MT-2 cells at this time point. HPMPC metabolites accumulated in MT-2 cells over time, and by 24 h the intracellular levels of the different metabolites were only two- to three-fold lower than those seen with HeLa–CD4. These results, therefore, show that the lymphoid cells have a low capacity to phosphorylate HPMPC to its active metabolites; however, this reduced capacity does not entirely explain the $>$25–50-fold reduction of antiviral effects HPMPC in MT-2 cells.

Additional factors may contribute to the differences in the antiviral spectra of these compounds. For example, the antiviral efficacy of dideoxynucleoside analogs is inversely correlated with the intracellular pools of corresponding deoxynucleotide triphosphate (dNTP) analogs. Infections with HSV result in increases in cellular dNTP pools via virus-induced ribonucleotide reductase. HSV thymidine kinase also affects cellular dNTP pool size. The affinity of different ANPpp to different viral polymerases vary greatly. Compared to HPMPCpp, PMEApp is a more efficient inhibitor of both HIV-1 RT (Ki $\sim$8.16 vs. 1.68 μM), as well as HSV-1 polymerase (0.86 vs. 0.02 μM) (Foster and Cheng, 1991; De Clercq, 1993; Mendel et al., 1994; Bischofberger, N., Personal Communication). Finally, the dynamics of viral infection may also influence the extent of their susceptibility to a given compound.

The therapeutic implications of the anti-HIV effects of HPMPC in non-lymphocytic cells is not known. Various epithelia are infectable with HIV in vivo, and play an important role in HIV transmission (Phillips et al., 1994). Given the documented efficacy of HPMPC in epithelial cells, it may have a use in prevention of mucosal transmission of HIV (and associated herpes viruses), both during heterosexual transmission and during

perinatal transmission. Studies in AIDS patients already receiving Cidofovir for management of CMV infection may provide insights on the possible anti-HIV effects of HPMPC in vivo in selected cell types.

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