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COMMENTARY

BROAD-SPECTRUM ANTI-DNA VIRUS AND ANTI-RETROVIRUS ACTIVITY OF PHOSPHONYLMETHOXYALKYLPURINES AND -PYRIMIDINES

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The acyclic guanosine and adenosine analogues 9-(2-hydroxyethoxymethyl)guanine (acyclovir) and (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA) were the first acyclic nucleoside analogues shown to be effective inhibitors of virus replication: acyclovir as a selective inhibitor of herpes simplex virus replication [1, 2] and DHPA as a broad-spectrum antiviral agent active against a number of DNA and RNA viruses [3]. Later on, various derivatives of acyclovir and DHPA were synthesized [4, 5]. These compounds behave either like acyclovir, in that their antiviral activity is restricted to those viruses [i.e. herpes simplex virus (HSV), varicella zoster virus (VZV)] that encode for a specific viral thymidine kinase (TK) [6], or like DHPA, in that their antiviral activity appears to be mediated by inhibition of *S*-adenosylhomocysteine hydrolase, a key enzyme involved in transmethylation reactions (including those that are required for the maturation of viral mRNA) [7].

Those viruses that do not encode for a viral-specific TK [such as human cytomegalovirus (CMV)] or induce a deficient TK (such as TK^- HSV) are resistant to the antiviral action of acyclovir and all other nucleoside analogues that depend on the viral TK for their intracellular phosphorylation to the active nucleotide forms. To circumvent this problem, one might envisage the use of nucleotide analogues in which the phosphate group has already been attached to the nucleoside. However, such nucleotides are degraded rapidly by esterases and thus behave similarly to their parent nucleosides. If, however, a phosphonate is attached to an acyclic nucleoside analogue such as DHPA, the resulting acyclic nucleotide analogue (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) is very stable and resists degradation by esterases.

ACYCLIC NUCLEOSIDE PHOSPHONATE ANALOGUES

HPMPA can be regarded as a hybrid molecule between DHPA and phosphonoacetic acid (PAA) (Fig. 1). PAA and its derivative phosphonoformic acid (PFA) have long [8] since been recognized as broad-spectrum antiviral agents that are active against herpesviruses (i.e. HSV, VZV, CMV), hepadnaviruses [i.e. hepatitis B virus (HBV)] and retroviruses [i.e. human immunodeficiency virus (HIV)]. This antiviral activity spectrum is preserved when the phosphonomethyl group of PAA is transferred to DHPA or the truncated form thereof [9-(2-hydroxyethyl)adenine (HEA)], thus resulting in the formation of HPMPA and 9-(2-phosphonylmethoxyethyl)adenine (PMEA), respectively (Fig. 1). In fact, HPMPA and PMEA are much more potent in their antiviral action than PAA or PFA. They offer substantial promise as broad-spectrum antiviral agents for the treatment of various DNA virus and retrovirus infections.

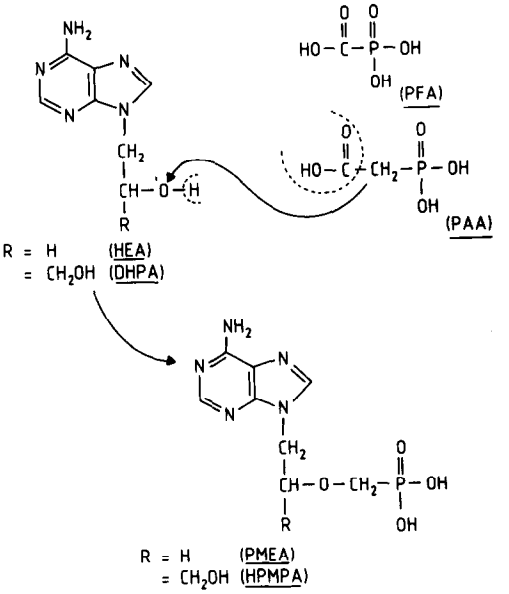

Fig. 1. PMEA [9-(2-phosphonylmethoxyethyl)adenine] and HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine]: hybrid molecules between phosphonoacetic acid (PAA) and 9-(2-hydroxyethyl)adenine (HEA) or (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA), respectively.

The antiviral properties of HPMPA and PMEA were first described in 1986 by De Clercq *et al.* [9]. PMEA was mentioned as being as active as HPMPA against HSV (including TK^- HSV), but less active than HPMPA against other herpesviruses such as VZV and CMV. Later [10], it was recognized that

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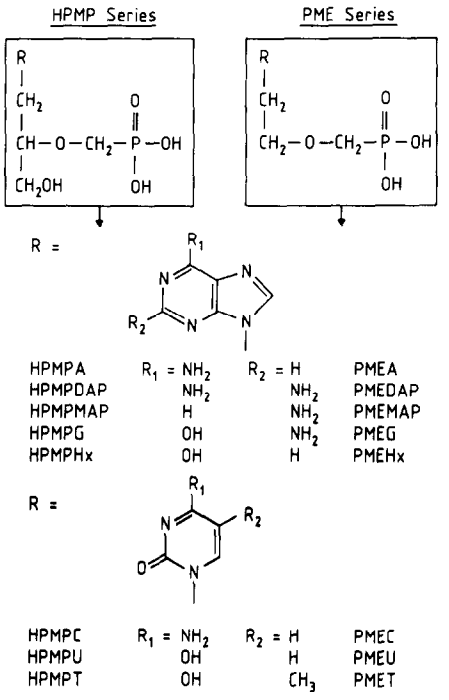

Fig. 2. Division of phosphonylmethoxyalkylpurines and -pyrimidines into two different classes: (*S*)-3-hydroxy-2-phosphonylmethoxypropyl (HPMP) derivatives and 2-phosphonylmethoxyethyl (PME) derivatives.

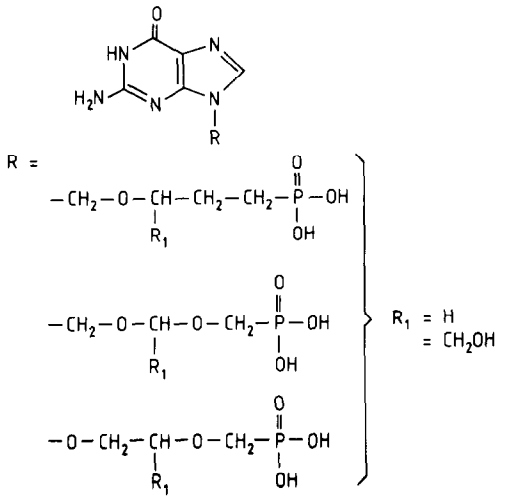

Fig. 3. Phosphonylalkoxymethyl, phosphonylmethoxyalkoxymethyl and phosphonylmethoxyalkoxy derivatives of guanine. These compounds can be divided again into two classes, depending on whether R_1 = H or CH_2OH.

HPMPA and PMEA actually belong to two different classes of phosphonylmethoxyalkyl derivatives, namely (*S*)-3-hydroxy-2-phosphonylmethoxypropyl (HPMP) and 2-phosphonylmethoxyethyl (PME) derivatives, respectively (Fig. 2). Within both series of compounds, all representative purine and pyrimidine derivatives have been synthesized [11], and it has become increasingly clear that the HPMP and PME series of compounds show remarkable differences in their activity spectrum, as described below.

In addition to the phosphonylmethoxyalkyl (HPMP, PME) derivatives of purines and pyrimidines (Fig. 2), various phosphonate derivatives of acyclovir and ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)-guanine (DHPG)] have been reported as antiviral agents active against HSV (including TK^- HSV) and CMV [12–15]. These compounds belong to one of the following categories: phosphonylalkoxymethyl analogues [12, 13], phosphonylmethoxyalkoxymethyl analogues [14] or phosphonylmethoxyalkoxy analogues [15] (Fig. 3). The compounds with R_1 = H are acyclovir derivatives, whereas compounds with $R_1 = CH_2OH$ are ganciclovir derivatives. Too little is known (or reported) about the activity spectrum or mode of action of these three categories of compounds, but, based on the analogy with the phosphonylmethoxyalkyl derivatives, it is tempting to speculate that the phosphonylalkoxymethyl, phosphonylmethoxyalkoxymethyl and phosphonylmethoxyalkoxy derivatives with R_1 = H (Fig. 3) behave like the PME series (Fig. 2), whereas their counterparts with $R_1 = CH_2OH$ (Fig. 3) would, in turn, behave like the HPMP series (Fig. 2).

ANTIVIRAL ACTIVITY SPECTRUM

The HPMP and PME derivatives that have been studied most intensively are HPMPA, HPMPG, HPMPC, PMEA, PMEG and PMEDAP (Fig. 2). HPMPA has been the lead compound of the HPMP series [9], and it has proved active against several adenovirus (AV) serotypes [9, 16], vacinia virus (VV) [9], herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), TK^- mutants of HSV-1 [9], VZV [9, 17], CMV [9, 18], Epstein–Barr virus (EBV) [19], suid herpesvirus type 1 (SHV-1, pseudorabies virus or Aujeszky's disease virus) [9], bovid herpesvirus type 1 (BHV-1, infectious bovine rhinotracheitis virus) [9], equid herpesvirus type 1 (EHV-1, equine abortion virus) [9, 20], herpesvirus platyrrhinae (HVP) [9], phocid herpesvirus type 1 (PHV, seal herpesvirus) [9, 21], duck hepatitis B virus (DHBV) [22], human hepatitis B virus (HBV) [23], and African swine fever virus (ASFV) [9, 24, 25] (Table 1). However, HPMPA is not particularly active against retroviruses (i.e. HIV) [26].

To the extent that they have been examined, HPMPC and HPMPG seem to have an activity spectrum similar to that of HPMPA [10, 18, 26–29]. However, the PME derivatives PMEA and PMEDAP show an activity spectrum that only partially overlaps with that of the HPMP derivatives. Like HPMPA and HPMPC, PMEA and PMEDAP are also active against herpeto-, hepadna-, and iridoviruses (Table 1), but, unlike HPMPA and HPMPC, they do not inhibit adeno- or poxviruses [10]. While losing part of their activity spectrum at the DNA virus side, PMEA and PMEDAP gain marked activity against retroviruses, i.e. human immunodeficiency virus

Table 1. Antiviral activity spectrum of the HPMP and PME series of phosphonylmethoxyalkyl derivatives

Virus*	HPMP series	PME series
Papovaviridae (HPV)	?	+
Adenoviridae (AV)	+	−
Poxviridae (VV)	+	−
Herpetoviridae (HSV-1, HSV-2, VZV, CMV, EBV, SHV-1, BHV-1, EHV-1, HVP, PHV)	+	+
Hepadnaviridae (DHBV, HBV)	+	+
Iridoviridae (ASFV)	+	+
Retroviridae (HIV-1, HIV-2, SIV, FIV, FLV, SRV, MLV, MSV, LP-BM5)	−	+

* For abbreviations and references, see text.

type 1 (HIV-1) [26], human immunodeficiency virus type 2 (HIV-2) [30], simian immunodeficiency virus (SIV) [30], feline immunodeficiency virus (FIV) [30, 31], feline leukemia virus (FLV) [32], simian AIDS-related virus (SRV) [30], murine leukemia virus (MLV) [33], murine sarcoma virus (MSV) [26, 30, 34, 35] and murine AIDS (LP-BM5) virus [36] (Table 1). PMEG has proved inhibitory to human papillomavirus (HPV) infection [37], but it has not been ascertained whether other phosphonylmethoxyalkyl derivatives are also inhibitory to HPV. It is not known whether the phosphonylmethoxyalkylpurines and -pyrimidines are effective against human parvovirus (i.e. B19) infections. Also, their activity against human herpes virus type 6 (HHV-6) remains to be established. In analogy with the other herpesviruses, HHV-6 may be expected to be sensitive to both the HPMP and PME series. Except for the retroviruses, no other RNA viruses have proved sensitive to either HPMPA or PMEA.

It is noteworthy that both the HPMP and PME series are active against the TK^- HSV and VZV mutants that are resistant to the "classical" antiherpes drugs such as acyclovir [9, 38]. Acyclovir-resistant HSV infections appear to be increasing in frequency in patients with AIDS [39], and such infections may well be amenable to therapy with any of the acyclic nucleoside phosphonate analogues. Ganciclovir-resistant CMV strains have been isolated from immunocompromised patients treated with ganciclovir for an intercurrent CMV infection [40]. If this resistance is based upon an impaired intracellular phosphorylation of the drug, as suggested [41], such ganciclovir-resistant CMV variants may be expected to be sensitive to the acyclic nucleoside phosphonate analogues, since the latter follow a phosphorylation pattern different from that of ganciclovir (see below).

The fact that the PME set of compounds (i.e. PMEA, PMEDAP) are effective against both retro- and herpesviruses (Table 1) makes them particularly attractive for the chemotherapy of AIDS, where they could be used for the treatment of both the underlying retroviral disease and the opportunistic herpesvirus (HSV, VZV, CMV, EBV) infections. This is a distinct advantage over currently available antiviral drugs such as zidovudine (azidothymidine, retrovir), which is active against HIV, but not CMV, and ganciclovir, which is active against CMV, but not HIV. If for the treatment of CMV infections in AIDS patients, ganciclovir is superimposed on zidovudine, severe to life-threatening hematologic toxicity results [42]. Such patients may constitute an appropriate study group for the clinical evaluation of PMEA or any of its congeners.

MOLECULAR BASIS FOR SELECTIVITY

The acyclic nucleoside phosphonate analogues inhibit virus replication at concentrations which are lower by several orders of magnitude than the concentrations at which they inhibit host cell growth. This selectivity is also reflected by the marked difference in the concentrations that are required to inhibit viral DNA synthesis as compared to cellular DNA synthesis. HPMPA inhibits HSV-1 DNA synthesis in HEL (human embryonic lung) cells at a concentration of only 0.05 μM whereas cellular DNA synthesis is not affected even at a concentration of 50 μM [43]. Also, HPMPA and PMEA inhibit EBV DNA synthesis in Raji cells [19], and ASFV DNA synthesis in Vero cells [44] at concentrations which do not affect host cell DNA synthesis. Following a 96-hr exposure of CMV-infected HEL cells to HPMPC, the compound effected a 50% reduction in CMV DNA synthesis at a concentration of 0.1 μg/mL, while to achieve an equivalent reduction in cellular DNA synthesis, the HPMPC concentration had to be raised to 100 μg/mL (Fig. 4).

The 50% inhibitory concentration (IC_{50}) of HPMPC for CMV DNA synthesis (0.1 μg/mL) [45] is identical to its IC_{50} for CMV replication (plaque formation) [18]. Likewise, the IC_{50} of HPMPC for cell growth (100 μg/mL) [18] is identical to its IC_{50} for cell DNA synthesis [45]. Thus, the selectivity index of HPMPC against CMV is 1000, whether this selectivity index is based upon the ratio of the IC_{50} for cell growth to the IC_{50} for virus replication or the ratio of the IC_{50} for cellular DNA synthesis to the IC_{50} for viral DNA synthesis. It follows that the selective antiviral action of HPMPC and its congeners is reflected by, and

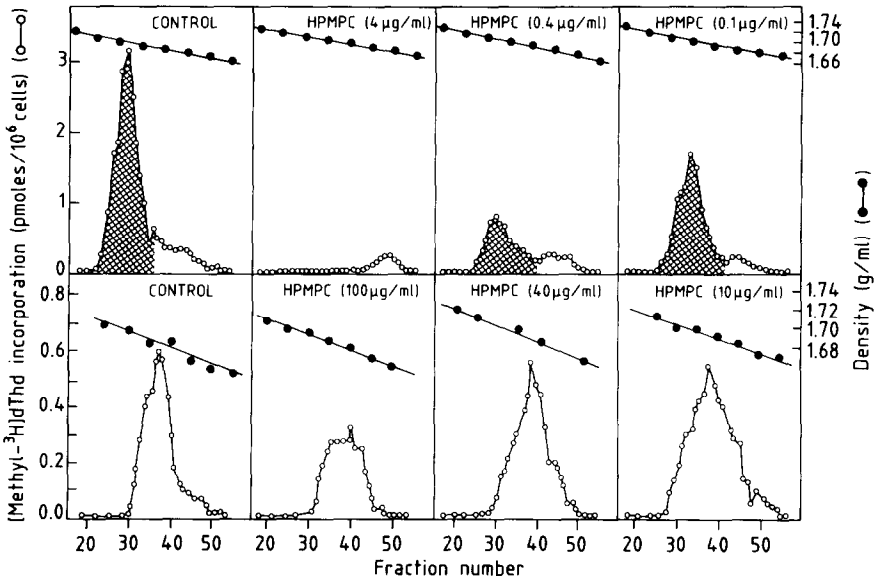

Fig. 4. Differential inhibitory effects of HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] on CMV DNA synthesis and host cell DNA synthesis. Upper panels: HEL (human embryonic lung) cells infected with CMV (strain AD-169) at a multiplicity of infection (M.O.I.) of 0.2. Viral DNA is shadowed. Lower panels: mock-infected HEL cells. The cells were exposed for 96 hr to various concentrations of HPMPC (as indicated), and DNA was analyzed by CsCl equilibrium gradient ultracentrifugation.

probably resides in, a selective inhibition of viral DNA synthesis.

MECHANISM OF ACTION

HPMPA and PMEA are taken up as such by the cells through a process resembling endocytosis, as it is temperature dependent and inhibited by endocytosis inhibitors such as sodium azide and cytochalasin B [46]. Following their uptake by the cells, the HPMP and PME derivatives are phosphorylated by cellular enzymes to monophosphorylphosphonates (i.e. HPMPAp, PMEAp) and diphosphorylphosphonates (i.e. HPMPApp, PMEApp) [43, 47]. Thus, for their intracellular phosphorylation HPMPA and PMEA do not depend on the virus-encoded TK, and this explains why these compounds are equally effective against TK^- and TK^+ HSV and VZV strains.

Which cellular enzymes are responsible for the phosphorylation of the acyclic nucleoside phosphonates? AMP kinase is unable to phosphorylate PMEA. However, 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase is able to directly convert PMEA to PMEApp, albeit at a higher K_m and lower V_{max} than noted for the conversion of AMP to ATP [47]. Although AMP is converted to ATP by PRPP synthetase, this is not the predominant route for the intracellular synthesis of ATP. Yet, the PRPP synthetase reaction may well be the predominant intracellular route leading to PMEApp (Fig. 5), and may thus play an important role in the antiviral activity of PMEA [48].

PMEApp is a potent inhibitor of HIV reverse transcriptase, and, being an alternative substrate to dATP, PMEApp acts as a chain terminator in the RNA-directed DNA polymerization reaction [48]. Also, avian myeloblastosis virus (AMV) reverse transcriptase has proved sensitive to the inhibitory effects of PMEApp and its congeners (in order of decreasing activity: PMEDAPpp > PMEApp > PMEGpp ≫ PMECpp ≫ PMETpp > PMEUpp) [49].

The diphosphates of the PME derivatives have also proved inhibitory to HIV-1 DNA polymerase, their order of decreasing activity being PMEADAPpp ≫ PMEGpp > PMEApp > PMETpp ≫ PMECpp ≫ PMEUpp [50]. In comparison with PMEApp ($K_i = 0.1\ \mu M$) and PMEDAPpp ($K_i = 0.03\ \mu M$), HPMPApp is only a weak inhibitor of HSV-1 DNA polymerase ($K_i = 1.4\ \mu M$) [50]. On the contrary, HPMPApp is a more efficient inhibitor of HIV-1-induced ribonucleotide reductase than are PMEApp and PMEDAPpp (IC_{50}: 0.9, 8 and 19 μM, respectively) [51].

HPMPApp has also proved inhibitory to adenovirus DNA replication in a reconstituted cell-free DNA replication system [52]. This inhibitory effect is strongly enhanced in the presence of the adenovirus DNA binding protein (DBP), probably because the increased processivity of the DNA polymerization reaction in the presence of DBP leads to increased drug sensitivity.

Taking together all the data that have been obtained for HPMPA, PMEA and their congeners [43, 47–52], one may postulate that these compounds, in their active (diphosphoryl) form, are targeted at the viral DNA polymerase (or retroviral reverse

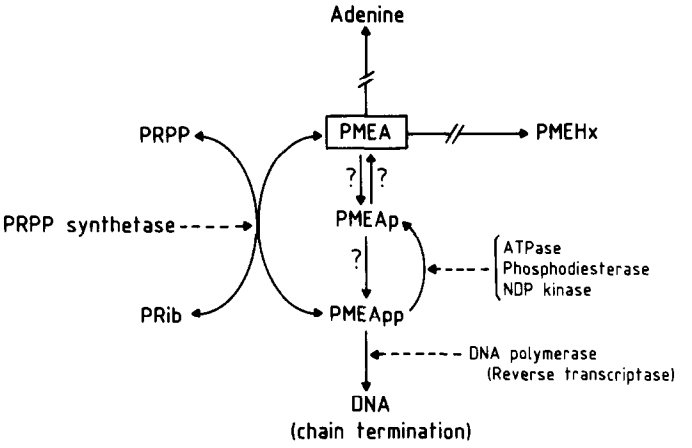

Fig. 5. Putative scheme for the intracellular metabolism and mechanism of anti-HIV action of PMEA [47]. While not an efficient substrate for purine nucleotide kinase(s), purine nucleoside phosphorylase(s) or adenosine (or AMP) deaminases, PMEA could be recognized as substrate by PRPP synthetase and be converted directly to PMEApp, which, on the one hand, could be phosphorylated by several enzymes (ATPase, phosphodiesterase, NDP kinase) to PMEAp, and, on the other hand, serve as a competitive inhibitor/alternative substrate of the reverse transcript reaction, and, if incorporated, act as a chain terminator.

transcriptase). The role of the HIV-1 ribonucleotide reductase [51] in the anti-HSV activity of HPMPA and PMEA remains unclear. A PMEA-resistant HSV-1 strain has been isolated (following 33 passages of the HSV-1 KOS strain in the presence of increasing concentrations of the drug) that was more susceptible to HPMPA than the original virus [53]. Yet, the ribonucleotide reductase isolated from the mutant PMEA-resistant HSV-1 strain was insensitive to both PMEApp and HPMPApp [51].

Assuming that both the HPMP and PME derivatives, following their intracellular conversion to the diphosphates, are eventually targeted at the viral DNA polymerase (or retroviral reverse transcriptase), how could this then explain the differences in the antiviral spectrum of these two series of compounds (the PME series being active against retroviruses whereas the HPMP series is not)? These differences may be related to the fact that when acting as alternative substrates (i.e. PMEApp and HPMPApp instead of dATP) of the DNA polymerase (or reverse transcriptase), the HPMP congeners may still allow further chain elongation [because of the presence of the additional CH_2OH group (Fig. 2)], whereas the PME congeners, lacking this group (Fig. 2), have no other option but to act as chain terminators when incorporated into DNA.

As, on the one hand, PMEA is strongly inhibitory to retroviruses (i.e. HIV) whereas HPMPA is not, and, on the other hand, PMEA is a chain terminator whereas HPMPA can also be incorporated into the interior of the DNA chain, chain termination appears to be a prerequisite for the anti-HIV activity of those nucleotide analogues that interact with the reverse transcriptase (Fig. 5). The same conclusion has been reached for azidothymidine 5'-triphosphate (N_3dTTP): whereas it was originally hypothesized that either competitive inhibition, or chain termination, or a combination of both may be important to the anti-HIV activity of N_3dTTP [54], it was later ascertained that chain termination was the decisive determinant [55].

ANIMAL MODELS

Pronounced efficacy has been demonstrated with both the HPMP derivatives (i.e. HPMPA, HPMPC) and the PME derivatives (i.e. PMEA, PMEDAP) in a large variety of experimental virus infections in animal models: intracutaneous HSV-1 or HSV-2 infection in hairless mice [9, 56, 57], intracutaneous HSV-1 infection in guinea pigs [28], intracutaneous TK^- HSV-1 infection in athymic-nude mice [56, 57], intraperitoneal HSV-1 or HSV-2 infection in mice [9, 28, 56–58], intracerebral HSV-1, HSV-2 or TK^- HSV-1 infection in mice [56, 57], intranasal HSV-1 or HSV-2 infection in mice [59], intracorneal HSV-1 or TK^- HSV-1 infection in rabbits [60, 61], simian varicella virus (SVV) infection in African green monkeys [62], intraperitoneal murine CMV infection in mice [29, 33], intranasal EHV-1 infection in mice [20], and intravenous VV infection in mice [56]. In addition, PMEA and PMEDAP have shown marked efficacy in a variety of retrovirus models, i.e. SIV infection in rhesus monkeys [30], FIV infection in cats [31], FLV infection in cats [32], MLV infection in mice [33], MSV infection in mice [34, 35, 63], and LP-BM5 (murine AIDS) virus infection in mice [36]. Furthermore, PMEG has proved effective against HPV-induced transformation of human foreskin cells grafted to athymic mice [37]. All these experimental model infections are reminiscent of the clinically important herpes-, pox-, retro-, and papovavirus infections in humans (Table 2), and, thus, the acyclic nucleoside phosphonate analogues could be

Table 2. Clinical manifestations of herpes-, pox-, papova- and retrovirus infections, in which the HPMP and PME derivatives should be effective according to animal data*

Clinical manifestation	HPMP series	PME series
Herpes labialis	+	+
Genital herpes	+	+
Herpetic keratitis	+	+
Herpetic encephalitis	+	+
Herpetic gingivostomatitis	+	+
Systemic HSV infections	+	+
VZV infections	+	
CMV infections	+	+
VV infections	+	
HPV infections		+
HIV infections		+

* For abbreviations and references, see text.

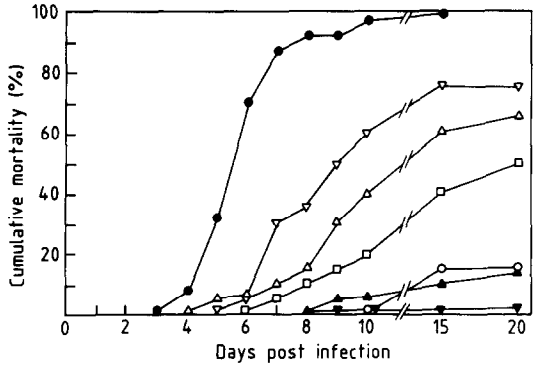

Fig. 6. Inhibitory effects of intraperitoneal administration of HPMPC on the mortality of mice inoculated intracerebrally with HSV-2 (strain 196) [57]. The compound was administered twice a day for 5 days (starting 1 hr after virus infection) at a dose of 0 mg/kg/day (control) (●), 5 mg/kg/day (▽), 10 mg/kg/day (△), 50 mg/kg/day (□), 100 mg/kg/day (○), 200 mg/kg/day (▲) or 400 mg/kg/day (▼). There were 20 mice per group (30 mice for the control group).

considered as particularly promising for the treatment of such infections.

In fact, in all virus infection models in which the efficacy of HPMPA, HPMPC, PMEA and PMEDAP was compared with that of the established antiviral drugs (acyclovir, ganciclovir, zidovudine), the acyclic nucleoside phosphonate derivatives proved much more efficacious, i.e. PMEA is superior to zidovudine (azidothymidine) in the treatment of MSV infections in mice [34, 63], HPMPC is superior to ganciclovir (DHPG) in the treatment of CMV infections in mice [29], and HPMPC by far outweighs acyclovir in the topical and systemic treatment of various HSV-1 and HSV-2 infections [57]. This includes intracerebral HSV-2 infection, which can be considered as a stringent model for herpetic encephalitis. Figure 6 illustrates the inhibitory effects of intraperitoneal HPMPC administration on the mortality of mice inoculated intracerebrally with HSV-2 [57]. Even at a dose of 5 mg/kg/day, HPMPC achieved a significant reduction in the mortality rate. This protective effect increased with increasing doses of HPMPC. If treated with HPMPC at 400 mg/kg/day, all mice survived the infection. Even at this dosage, the compound was not toxic to the mice. Acyclovir, if tested in parallel with HPMPC, was toxic at a dose of 400 mg/kg/day; at lower doses (200 or 100 mg/kg/day) it did not affect significantly the mortality of mice infected intracerebrally with HSV-2 [57].

Of particular importance is the fact that the acyclic nucleoside phosphonate analogues, in keeping with their *in vitro* activity against TK^- HSV *in vitro* [9, 10, 38], have also proved effective in suppressing the manifestations of TK^- HSV infection *in vivo* [56, 57]. TK^- HSV is resistant to acyclovir, and so is the disease [64]. Hence, TK^- HSV infections that no longer respond to acyclovir treatment may be successfully treated with HPMPC or any other phosphonate analogue. It should be pointed out that PFA itself is effective in the treatment of acyclovir-resistant TK^- HSV infections, as first demonstrated in a patient with chronic lymphocytic leukemia (CLL) [64], and later confirmed in patients with AIDS [65–67].

PROLONGED ANTIVIRAL ACTION

A remarkable feature of the phosphonylmethoxyalkyl derivatives, whether belonging to the HPMP or PME series, is that they generate a prolonged antiviral action. When CMV-infected cells were exposed for 6 hr post-infection to HPMPC, viral DNA synthesis was suppressed over a period of at least 7 days (Fig. 7). This contrasts with the antiviral action of the "classical" antiviral drugs such as ganciclovir (DHPG), which does not persist for such a long time [45]. A prolonged antiviral response has also been demonstrated *in vivo*, i.e. with HPMPC in the topical treatment of HSV-1 keratitis in rabbits [61], with HPMPC in the topical treatment of cutaneous HSV-1 infection in hairless mice [57], with HPMPC in the (subcutaneous) treatment of systemic (intraperitoneal) HSV-1 infection in mice [57], with HPMPC in the systemic (subcutaneous) treatment of HSV-2 encephalitis in mice [57], with HPMPC in the systemic (intravenous) treatment of SVV infection in monkeys [62], and with PMEA in the systemic (intraperitoneal) treatment of MSV infection in newborn mice [68].

For the treatment of HSV-1 keratitis, one eyedrop (0.2% HPMPC) per day suffices to accomplish complete healing within 5 days [61]. This is quite surprising, since antiviral [i.e. idoxuridine (IDU) or trifluridine (trifluorothymidine, TFT)] eyedrops must normally be given hourly (during the day) to afford any healing effect.

The prolonged antiviral activity conferred by the acyclic nucleoside phosphonate analogues makes an infrequent dosing (i.e. twice or once a week, or even just once) possible. In fact, a single dose of HPMPC, administered at either 1 hr, 2 days or 4 days after infection, sufficed to protect mice against an otherwise lethal infection with HSV-1 or HSV-2 (Table 3). Also, Bronson *et al.* [69] have shown that

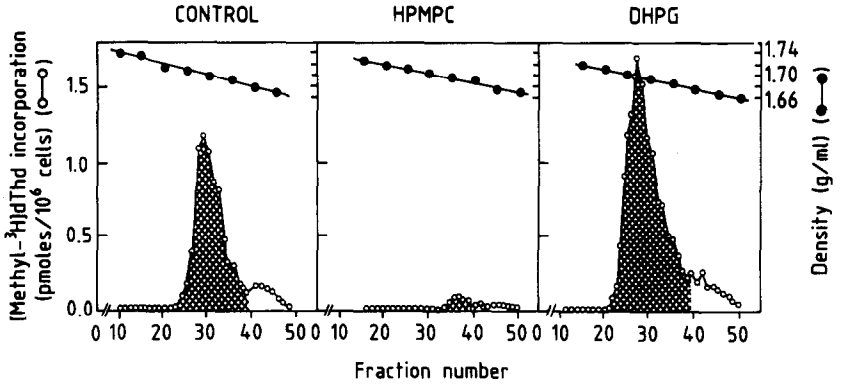

Fig. 7. Long-lasting antiviral action of HPMPC, as monitored by CMV DNA synthesis in HEL (human embryonic lung) cells. The cells were infected with CMV (strain AD-169) at a multiplicity of infection (M.O.I.) of 0.2 and either not treated (control) or treated during the initial 6-hr post-infection period with HPMPC (4 μg/mL) or DHPG (4 μg/mL). DNA was analyzed at 7 days post-infection by CsCl equilibrium gradient ultracentrifugation.

Table 3. Effects of single doses of HPMPC or acyclovir on the mortality rate of HSV-infected mice

Dose (mg/kg)	Time of administration*	Mortality rate (%) HPMPC	Acyclovir
(A) Mice infected intraperitoneally with HSV-1 (strain KOS)			
0 (control)		100	100
20	day 0	50	90
100	day 0	10	90
500	day 0	0	100
100	day 2	0	90
500	day 2	0	100
500	day 4	30	100
(B) Mice infected intracerebrally with HSV-2 (strain 196)			
0 (control)		97	97
20	day 0	20	100
100	day 0	20	100
500	day 0	0	100
100	day 2	50	90
500	day 2	0	100
500	day 4	60	100

* At day 20 post-infection. There were 10 mice per group (20 mice for control group A and 30 mice for control group B). When the compound was administered on day 0, it was 1 hr after infection.

a single dose regimen of HPMPC is effective in an HSV-2 infection model even if treatment is delayed until 4 days post-infection. This, again, is in sharp contrast with the "classical" antiviral drugs such as acyclovir which are totally ineffective when their administration is limited to single dosing (Table 3).

What could be the reason for the long-lasting antiviral action of HPMPC and its congeners? Apparently, the active metabolites (diphosphoryl derivatives) of the acyclic nucleoside phosphonates, i.e. HPMPCpp and PMEApp, have a long intracellular half-life (about 16–18 hr) [48, 69] (for HPMPC, a phosphate choline adduct has been detected intracellularly that decays with a half-life of 48 hr [69]). Thus, although the acyclic nucleoside phosphonates penetrate the cells rather slowly, once inside they may be "trapped" in the form of their active metabolites. The persistence of these active metabolites may then explain the prolonged antiviral action ensuing from a short-pulse treatment.

CONCLUSION

Phosphonylmethoxyalkylpurines and -pyrimidines such as HPMPC, HPMPA and PMEA can be considered as promising candidate drugs for the treatment of various DNA virus and retrovirus infections. This includes several DNA virus (i.e. adenovirus, Epstein–Barr virus, hepatitis B virus, acyclovir-resistant herpes simplex virus, ganciclovir-resistant cytomegalovirus) infections for which there is currently no satisfactory treatment.

In contrast with the currently available antiviral drugs (i.e. acyclovir, ganciclovir, zidovudine) whose activity range is limited to either herpes simplex virus, cytomegalovirus or retroviruses (human immunodeficiency virus), the phosphonylmethoxyalkyl derivatives have a broad-spectrum anti-DNA virus activity. For the 2-phosphonylmethoxyethyl (PME) derivatives, such as PMEA, the activity spectrum extends to retroviruses. This makes PMEA an attractive candidate drug for AIDS patients, where it may be useful for the therapy of both the opportunistic herpesvirus infections as well as the underlying retroviral disease.

A particular feature of all acyclic nucleoside phosphonates, whether belonging to the HPMP series (i.e. HPMPC) or the PME series (i.e. PMEA), is their prolonged antiviral action, lasting for several days, 1 week or even longer. Thus, it is possible to sustain an antiviral response with infrequent dosing or even a single dose of the compound, even if applied topically (on the skin or as eyedrops) or if used systemically (subcutaneously) in the treatment

of such severe HSV infections as herpetic encephalitis.

The main target for the antiviral action of the acyclic nucleoside phosphonates appears to be the viral DNA polymerase (or retroviral reverse transcriptase); and the main reason for the long-lasting antiviral action may well be the persistence of the active metabolites (diphosphoryl derivatives) of the acyclic nucleoside phosphonates within the cell. Yet, an important issue that remains to be addressed concerns the molecular basis for their high selectivity: why are these compounds so much more inhibitory to viral DNA synthesis than cellular DNA synthesis?

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