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Mechanism of action of acyclic nucleoside phosphonates against herpes virus replication

Abstract—Foremost among the acyclic nucleoside phosphonates currently pursued for their potential in the treatment of herpes and retrovirus infections are (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA). These compounds are as such taken up by the cells and then phosphorylated by cellular enzymes to their diphosphoryl derivatives HPMPCpp and PMEApp. The main target for the antiviral action of HPMPCpp and PMEApp is the viral DNA polymerase. Whereas PMEApp has been shown to interact as a DNA chain terminator with both retro- and herpes viruses, the mechanism by which HPMPCpp inhibits herpes viral DNA synthesis remains the subject of further study.

Most nucleoside analogues with anti-herpes virus activity [acyclovir, ganciclovir, (*E*)-5-(bromovinyl)-2′-deoxyuridine (BVDU) and its arabinofuranosyl counterpart BVaraU] are selectively phosphorylated to the monophosphates (acyclovir, ganciclovir) or diphosphates (BVDU, BVaraU) by the virus-encoded TK*. These mono- or diphosphates are further phosphorylated by host enzymes to the triphosphate forms which are then capable of blocking viral DNA synthesis. However, some herpes viruses [i.e. human cytomegalovirus (HCMV)] do not encode a specific TK, and those that do so can become drug resistant due to an acquired deficiency in the enzyme (TK^- strains of HSV and VZV). Therefore, strategies have to be worked out so as to circumvent this viral TK dependence of anti-herpes virus agents.

The use of phosphorylated nucleosides is of no avail as their phosphate group(s) may be cleaved off by esterases before they would be able to enter the cells. Thus, acyclic nucleotide phosphonate analogues (parent compound, HPMPA [1] (Fig. 1) were designed, which contain a phosphonylmethyl ether group [O–CH_2–P(O)$(OH)_2$] instead of the phosphate methyl moiety [CH_2–O–P(O)$(OH)_2$]. In contrast with the regular nucleotides, where the C–O–P linkage is readily hydrolysed by esterases, the C–P linkage in the acyclic nucleoside phosphonate derivatives is resistant to such cleavage. Several acyclic nucleoside phosphonate analogues were found active against a wide array of human and animal herpes viruses including HCMV and TK^- strains of HSV and VZV [1–3]. Of this class of compounds, HPMPC emerged as one of the most potent inhibitors of herpes virus (i.e. cytomegalovirus) replication described so far [4, 5].

Structurally, HPMPA and HPMPC are very similar to PMEA which can be regarded as a truncated derivative of HPMPA in that it lacks the hydroxymethyl appendage on the acyclic side chain (Fig. 1). PMEA is the parent compound of a group of potent anti-retroviral agents active against human immunodeficiency virus (HIV) [6]. Moreover, PMEA and its diaminopurine derivative PMEDAP, show significant anti-herpes virus activity [2, 7, 8]. Compounds such as PMEA and PMEDAP that are effective against both retro- and herpes viruses seem particularly attractive for the chemotherapy of acquired immunodeficiency syndrome (AIDS) as they could be used for the treatment of both the underlying retroviral disease and the opportunistic herpes virus infection.

A remarkable feature of nucleoside phosphonate

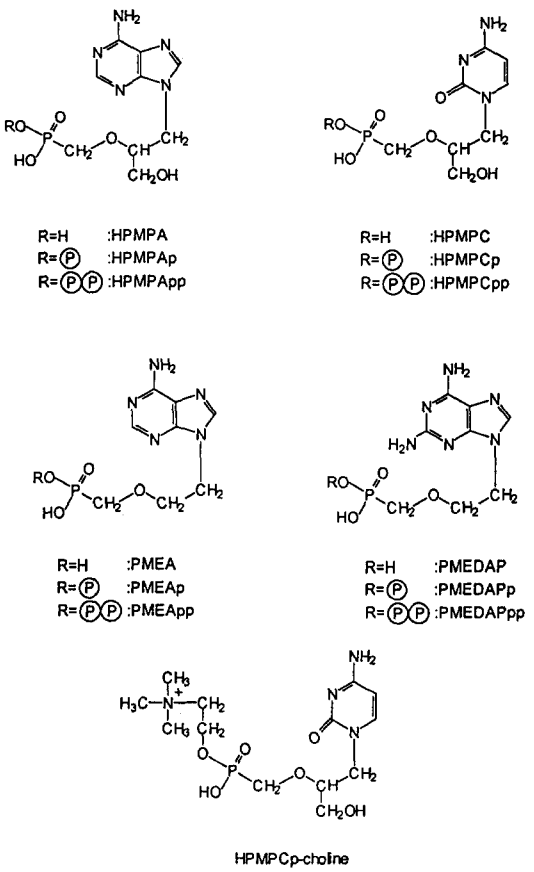

Fig. 1. Formulae of HPMPA, HPMPC, PMEA, PMEDAP and their metabolites.

* Abbreviations: HPMPA, (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine; HPMPC, (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PMEDAP, 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine; HPMPAp, HPMPCp, PMEAp, PMEDAPp, monophosphoryl derivatives of HPMPA, HPMPC, PMEA and PMEDAP; HPMPApp, HPMPCpp, PMEApp, PMEDAPpp, diphosphoryl derivatives of HPMPA, HPMPC, PMEA and PMEDAP; PRPP, 5-phosphoribosyl-1-pyrophosphate; PR, ribose-5-phosphate; HSV, herpes simplex virus; VZV, varicella-zoster virus; (H)CMV, (human) cytomegalovirus; TK, thymidine kinase; TK^-, thymidine kinase deficient.

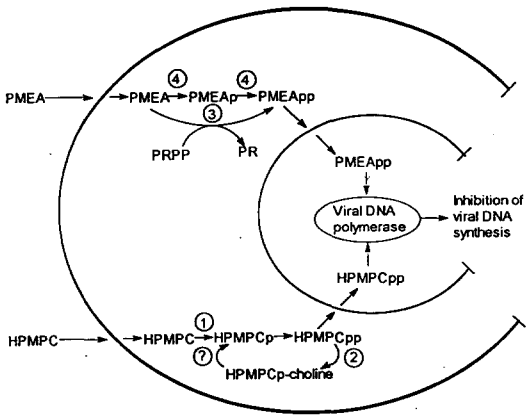

Fig. 2. Scheme of the intracellular metabolism and mechanism of anti-herpes virus activity of the nucleoside phosphonate analogues HPMPC and PMEA. ①, Pyrimidine nucleoside monophosphate kinase; ②, CTP:phosphorylcholine cytidyltransferase. ③, 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase; ④, AMP (dAMP) kinase.

analogues, whether belonging to the HPMP or the PME series is that they generate a long-lasting antiviral effect. Even a short incubation period (a few hours) of the cells with the drugs, either before or shortly after the infection, results in efficient protection (for several days) against viral replication. This contrasts with the antiviral action of other antiviral drugs such as ganciclovir, acyclovir and foscarnet, which do not persist for such a long time. For HPMPC, this long-lasting antiviral effect has been demonstrated in several animal models for herpes virus infections [3, 9–11; for review see Ref. 12]. This sustained antiviral effect permits infrequent and/or prophylactic administration, which are attractive features for a compound that has to be used in the therapy and prevention of persistent herpes virus infections.

The replication of herpes viruses proceeds in the cell nucleus; thus, the biologically active metabolites of the nucleoside phosphonates have to enter the nucleus in order to inhibit the viral replication machinery. A first barrier that has to be overcome is the cell membrane (Fig. 2). Phosphonates, unlike nucleosides (which enter the cells via facilitated diffusion), are negatively charged molecules and may not easily be taken up by the cells, because of electrostatic repulsion between the drug and the exterior of the cell membrane. Nevertheless these compounds must gain access to the intracellular compartment to achieve their antiviral activity. Balzarini *et al.* [13] detected relatively small amounts of PMEA (and its metabolites) in cells that had been incubated with relatively high extracellular concentrations of the compound. The influx of PMEA in cells is sodium dependent, requires energy and is not affected by inhibitors of nucleoside transport [14, 15]. Other acyclic nucleoside phosphonate analogues (such as HPMPA and HPMPC) were shown to inhibit the influx of PMEA [14]. These results may indicate that PMEA and other nucleoside phosphonates enter cells via a carrier system functionally distinct from those mediating nucleoside transport. There is as yet no information on the intracytoplasmatic and/or intranuclear distribution of the nucleoside phosphonates and their metabolites (Fig. 2).

HPMPA is equally well phosphorylated in mock-infected as in HSV-1-infected VERO cells. Two metabolites were identified, HPMPAp and HPMPApp [16]. AMP kinase from murine L1210 cells was shown to phosphorylate the (*S*)-enantiomer but not the (*R*)-enantiomer of HPMPA and thus may be held responsible for the differential antiviral effects that have been observed with these enantiomers [17]. HPMPA can also be recognized as substrate by PRPP synthetase, which converts HPMPA directly to HPMPApp and does not discriminate between the two enantiomers [18].

Also no differences in the metabolism of HPMPC were observed between non-infected and HSV-infected cells. Three metabolites of HPMPC were identified: HPMPCp, HPMPCpp and HPMPCp-choline (which is analogous to CDP-choline) [19, 20] (Figs 1 and 2). The anti-herpes virus activity of HPMPC is not readily reversed upon addition of deoxycytidine or cytidine to the cell cultures, suggesting that HPMPC, unlike other deoxycytidine analogues (i.e. FIAC, AraC), does not depend on deoxycytidine kinase for its phosphorylation [21]. The synthesis of HPMPCp was found to be catalysed by pyrimidine nucleoside monophosphate kinase and HPMPCp-choline is formed from HPMPCpp and choline phosphate through the action of CTP:phosphorylcholine cytidyltransferase [20].

PMEA was reported to be directly phosphorylated to its diphosphorylated derivative PMEApp by PRPP synthetase, albeit at a higher K_m and lower V_{max} than noted for the conversion of AMP to ATP [18]. Merta *et al.* [17] reported that also AMP kinase (from murine L1210 cells) is able to phosphorylate PMEA.

Although the nucleoside phosphonates and their metabolites egress to some extent from the cells to the extracellular medium, they persist for a long time inside the cell, which may explain their long-lasting antiviral activity [13, 19, 20]. In particular the HPMPC metabolite, HPMPCp-choline, could function as an intracellular reservoir of HPMPC after removal of extracellular compound.

HPMPA was shown to inhibit HSV-1 DNA synthesis in human embryonic lung cells [16] and EBV DNA synthesis in Raji cells [22] at concentrations that do not affect host cell DNA synthesis. HPMPApp specifically inhibits HSV- and HCMV-induced DNA polymerases [7, 23]. HPMPApp has also been demonstrated to inhibit HSV-1-induced ribonucleotide reductase [24]. However, the role of HSV-1 ribonucleotide reductase inhibition in the anti-HSV activity of HPMPA remains unclear. When this enzyme was isolated from a PMEA-resistant HSV-1 strain that was more susceptible to HPMPA than the original virus, it proved insensitive to HPMPApp [24, 25]. Also, since HCMV does not encode a functional ribonucleotide reductase, the anti-HCMV activity of HPMPA may not result from inhibition of this enzyme. The anti-HCMV activity of HPMPC results from a selective inhibition of viral DNA synthesis, which is inhibited by 50% at concentrations that are 1000-fold lower than the concentrations required to inhibit cellular DNA synthesis by 50% [5]. HPMPCpp was demonstrated to inhibit HSV-1 and HSV-2 DNA polymerase at a lower K_i than DNA polymerase-α [19]. PMEApp and PMEDAPpp were shown to inhibit the HSV- and HCMV-induced DNA polymerases [7, 23, 26]. The inhibition of HSV DNA polymerase by PMEApp appears to involve chain termination after incorporation of PMEA into the growing DNA [26].

In conclusion, several phosphonylmethoxyalkylpurines and -pyrimidines can be considered as candidate drugs for the treatment of various herpes virus infections (including acyclovir-resistant HSV and VZV and ganciclovir-resistant CMV) as well as retrovirus infections. A particular feature of this class of compounds is their sustained antiviral effect. The main target of anti-herpes virus action appears to be the viral DNA polymerase in the cell nucleus. At present both HPMPC and PMEA are undergoing clinical evaluation for the treatment of herpes virus (i.e. HCMV) and retrovirus (i.e. HIV) infections.

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