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Review

The antiviral activity and mechanism of action of (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) nucleosides

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ABSTRACT

One class of compounds that has shown promise as antiviral agents are the (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) nucleosides, members of the broader class of acyclic nucleoside phosphonates. These HPMP nucleosides are nucleotide analogs and have been shown to be effective inhibitors of a wide range of DNA viruses. Prodrugs of these compounds, which achieve higher levels of the active metabolites within the cell, have an expanded activity spectrum that also includes RNA viruses and retroviruses. Because they are analogs of natural nucleotide substrates, HPMP nucleosides are predicted to target polymerases (DNA polymerases, RNA polymerases and reverse transcriptases), resulting in the inhibition of viral genome replication. Previous work using the replicative enzymes of different viruses including human cytomegalovirus (HCMV) and vaccinia virus DNA polymerases and human immunodeficiency virus type 1 (HIV-1) reverse transcriptase has shown that the activated forms of these compounds are substrates for viral polymerases and that incorporation of these compounds into either the primer strand or the template strand inhibits, but does not necessarily terminate, further nucleic acid synthesis. The activity of these compounds against other viruses that do not encode their own polymerases, like polyoma viruses and papilloma viruses, suggests that host cell DNA polymerases are also targeted. This complex mechanism of action and broad activity spectrum has implications for the development of resistance and host cell genome replication, and suggests these compounds may be effective against other viruses such as influenza virus, respiratory syncytial virus and Dengue virus. This class of nucleotide analogs also points to a potential avenue for the development of newer antivirals.

Contents

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1. Acyclic nucleoside phosphonates

The acyclic nucleoside phosphonates are a class of antiviral agents that are nucleotide, rather than nucleoside, analogs. As a group, these compounds have shown activity against a range of DNA viruses, RNA viruses, and retroviruses (De Clercq and Holy, 2005; De Clercq et al., 1986; Wyles et al., 2009). The structure of these compounds consists of an acyclic nucleoside moiety attached to a phosphonate group through a phosphate–carbon–oxygen bond. This type of bond is not cleaved by cellular esterases, making these compounds more stable than those that contain the phosphate–oxygen–carbon bond of a phosphate group (De Clercq, 1997; De Clercq and Holy, 2005). The phosphonate group on this class of compounds acts as a phosphate mimic, making these antiviral agents analogs of deoxynucleoside monophosphates (dNMPs). As a result of this structure, only two phosphorylation steps are required to activate these compounds to their active diphosphoryl metabolites, which then act as analogs of deoxynucleoside triphosphates (dNTPs).

Several acyclic nucleoside phosphonates have been described, and these generally fall into three categories: the phosphonomethoxyethyl (PME) purine and pyrimidine derivatives, the phosphonomethoxypropyl (PMP) purine and pyrimidine derivatives, and the (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) purine and pyrimidine derivatives (Fig. 1). The PME and PMP derivatives, including 9-(2-phosphonomethoxyethyl)adenine (PMEA, adefovir) and 9-(2-phosphonomethoxypropyl)adenine (PMPA, tenofovir), are obligate chain terminators because they lack a hydroxyl group that can act as a 3′-hydroxyl as found in the natural dNTP structure. In contrast, the HPMP derivatives do possess a 3′-hydroxyl group in the correct position and can theoretically be incorporated into DNA and extended (De Clercq and Holy, 2005; De Clercq and Neyts, 2009).

2. HPMP nucleosides

The first of the acyclic nucleoside phosphonates to be described was HPMP-adenine (HPMPA) (De Clercq et al., 1986) (Fig. 2). This compound originated as a merger of two other antiviral agents, phosphonoformate and (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] (De Clercq and Holy, 2005; De Clercq et al., 1987). Other HPMP nucleosides have subsequently been developed (reviewed in De Clercq, 2007, 2011; De Clercq and Holy, 2005); each of these compounds has the same backbone structure and differ only in the base moiety. This review will focus on those HPMP nucleosides that possess the natural bases adenine, cytosine, guanine and thymine: HPMPA, HPMP-cytosine (HPMPC, cidofovir), HPMP-guanine (HPMPG) and HPMP-thymine (HPMPT), analogs of dAMP, dCMP, dGMP and dTMP, respectively (Fig. 2). We have focused on these compounds as they exhibit a more interesting and complex mode of action than do the chain terminating drugs.

2.1. Uptake and metabolism of HPMP nucleosides

Much of the work on the uptake and metabolism of the HPMP nucleosides has been performed using the two most well studied compounds, HPMPA and HPMPC. Both of these analogs are taken up into cells by fluid-phase endocytosis (Connelly et al., 1993; Palú et al., 1991), where they are converted to various products including their monophosphoryl and diphosphoryl derivatives; the latter are the active metabolites (Fig. 3A). Cellular enzymes catalyze the metabolism of these compounds, as shown by data demonstrating that the phosphorylation pattern of HPMPA is similar in uninfected cells and in cells infected with herpes simplex virus type 1 (HSV-1) (Votruba et al., 1987), and the production of HPMPC metabolites is unchanged by infection with HSV-1 (Ho et al., 1992), murine cytomegalovirus (MCMV) (Okleberry et al., 1997) or with cowpox virus (Smee et al., 2002). The fact that cellular enzymes are used to catalyze these steps means that viral enzymes are not required, thus expanding the range of potential viral targets to include kinase-deficient and mutant viruses.

The metabolites formed after the uptake of HPMPA are HPMPA monophosphate (HPMPAp) and HPMPA diphosphate (HPMPApp) (Votruba et al., 1987). AMP(dAMP) kinase has been shown to catalyze the two-step phosphorylation of HPMPA to HPMPApp (Merta et al., 1992). Creatine kinase is also able to convert HPMPAp to HPMPApp (Merta et al., 1992).

In contrast to HPMPA, the intracellular metabolism of HPMPC yields three major products: HPMPC monophosphate (HPMPCp), HPMPC diphosphate (HPMPCpp), and HPMPC phosphocholine (HPMPCp-choline; Fig. 3B) (Aduma et al., 1995; Ho et al., 1992). Pyrimidine nucleoside monophosphate kinase has been shown to catalyze the first phosphorylation step, and one of three enzymes, pyruvate kinase, creatine kinase or nucleoside diphosphate kinase, catalyze the second step (Cihlar and Chen, 1996). Interestingly, Cihlar and Chen (1996) showed that human cytomegalovirus (HCMV) infection resulted in an elevation of the intracellular levels of HPMPCp and HPMPCpp relative to uninfected cells. These ele-

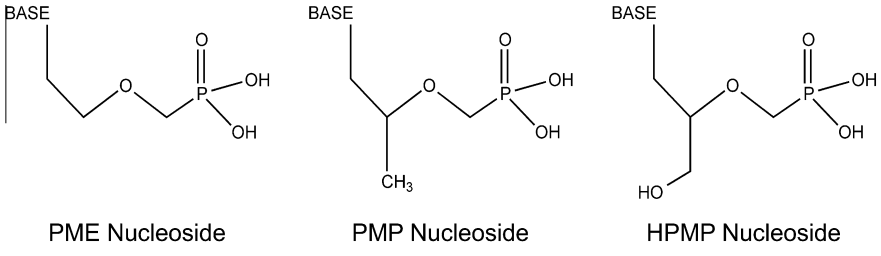

Fig. 1. Categories of acyclic nucleoside phosphonates. The phosphonomethoxyethyl (PME) nucleosides and the phosphonomethoxypropyl (PMP) nucleosides lack a hydroxyl group equivalent to the 3′-hydroxyl of a natural nucleoside and as such are obligate chain terminators. In contrast, the (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) nucleosides do possess a hydroxyl group in the appropriate position.

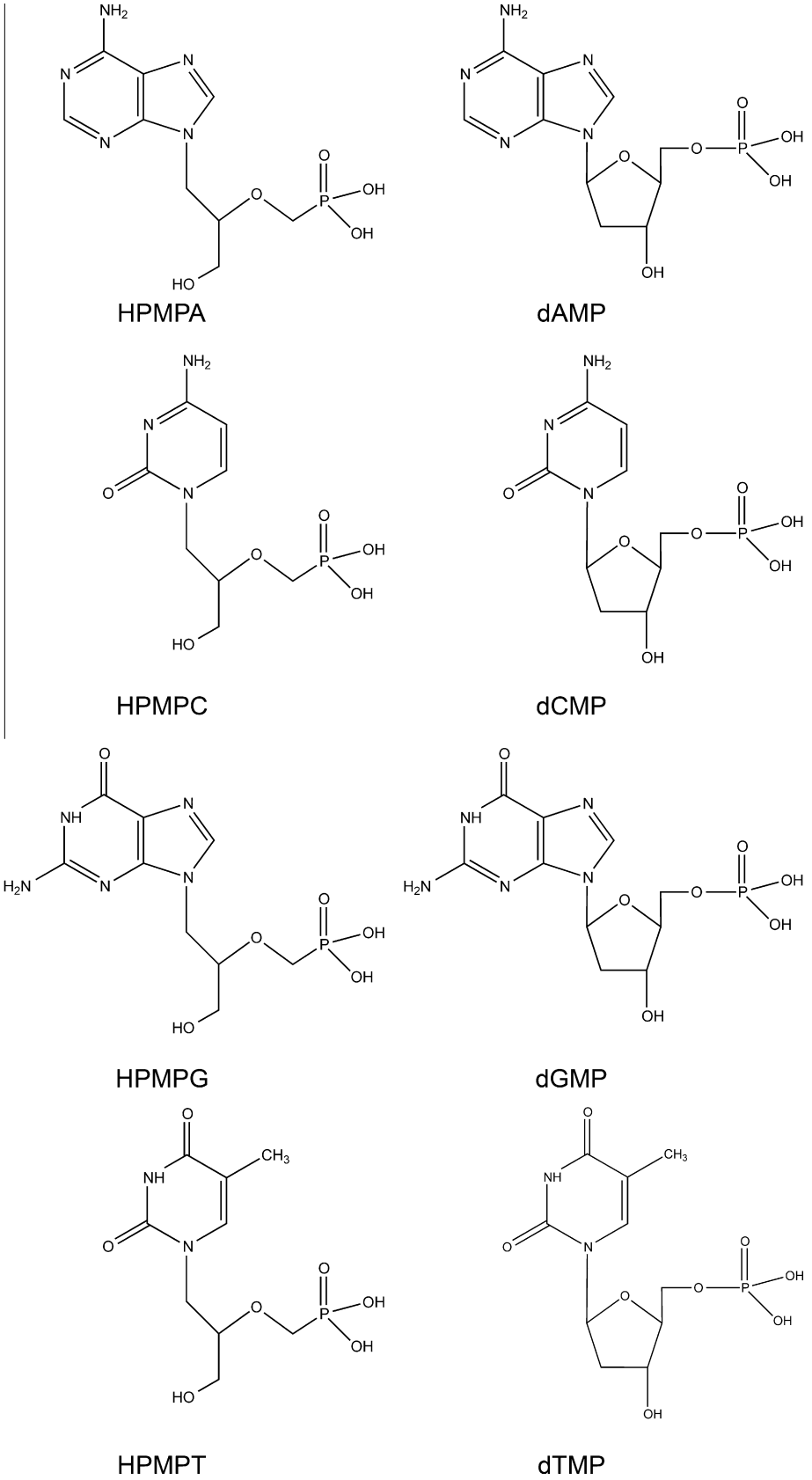

Fig. 2. Structures of the HPMP nucleosides. HPMPA, HPMPC, HPMPG and HPMPT are analogs of the natural nucleotides dAMP, dCMP, dGMP and dTMP, respectively. The acyclic backbone structures of these compounds are identical; only the structures of the base moieties differ.

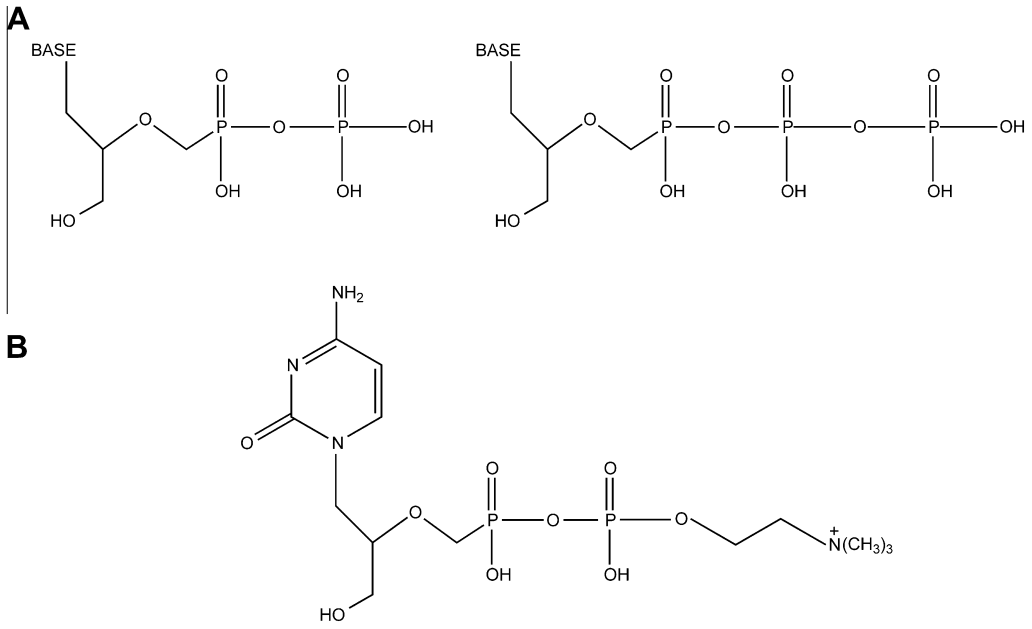

Fig. 3. Metabolites of HPMP nucleosides. (A) Structures of the monophosphoryl (left) and diphosphoryl (right) derivatives of the HPMP nucleosides. The HPMP nucleoside diphosphates are the active intracellular compounds. (B) Structure of HPMPC phosphocholine (HPMPCp-choline), a third metabolite formed upon treatment of cells with HPMPC.

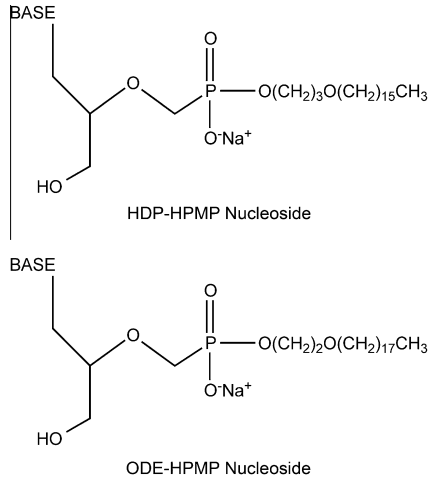

Fig. 4. Alkoxyalkyl ester derivatives of HPMP nucleosides. Structures of the hexadecyloxypropyl (HDP; top) and octadecyloxyethyl (ODE; bottom) esters are shown.

vated levels were due to higher activities of the cellular kinases in infected cells, rather than the induction of virus-encoded enzymes capable of phosphorylating HPMPC (Cihlar and Chen, 1996).

The formation of HPMPCp-choline, an analog of the phospholipid synthesis intermediate cytidine 5′-diphosphocholine (Cihlar et al., 2001), from HPMPCpp is catalyzed by choline phosphate cytidyltransferase (Cundy, 1999). HPMPCp-choline has a half-life of greater than 48 h (Ho et al., 1992) and acts as a slow-release reservoir for HPMPCpp. The production of this metabolite is thought to be the source of the prolonged antiviral state (>7 days) induced by HPMPC pretreatment of cells (Aduma et al., 1995). This effect is presumably also responsible for the infrequent dosing that can be used to treat established virus infections (Bronson et al., 1990; Flores-Aguilar et al., 1994; Quenelle et al., 2003; Smee et al., 2000). In comparison, the antiviral state induced by pretreatment of cells with HPMPA is much shorter (24–72 h); the latter compound is not metabolized to a long-lasting reservoir like HPMPCp-choline (Aduma et al., 1995).

Although an analysis has not been performed of the metabolites produced upon treatment of cells with HPMPG, Terry et al. (1988) showed that chemically-synthesized (*R,S*)-HPMPG could be enzymatically activated to HPMPGpp. Guanylate kinase catalyzes the conversion of HPMPG to HPMPG monophosphate (HPMPGp), and nucleoside 5′-diphosphate kinase catalyzes the second phosphorylation step to HPMPG diphosphate (HPMPGpp).

A series of alkoxyalkyl ester prodrugs of the HPMP nucleosides designed to resemble natural lipids have also been developed (Painter and Hostetler, 2004); these conjugates include the hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE) esters of these compounds (Fig. 4). Treatment of cells with the HPMPA or HPMPC prodrugs results in greater intracellular levels of the respective diphosphoryl derivatives compared to treatment with the unmodified compounds (Aldern et al., 2003; Magee et al., 2008). This increase in active metabolite concentration appears to be a result of a rapid cellular uptake of the prodrugs based on their interaction with the cell membrane relative to slow uptake of the parent compound by endocytosis (Aldern et al., 2003). As shown with HDP-HPMPC (CMX001), the prodrug forms a large pool in the cell membrane that is first metabolized by cellular phospholipase C and phosphodiesterases to release the parent compound, which is then further metabolized to the diphosphoryl derivative (Aldern et al., 2003).

2.2. Antiviral activity spectrum

Collectively, the HPMP nucleosides inhibit the replication of a number of virus families in cell culture, including the DNA viruses herpesviruses [HSV-1, herpes simplex virus type 2 (HSV-2), CMV, Epstein-Barr virus (EBV), varicella zoster virus, human herpesvirus 6 (HHV6), human herpesvirus 7, human herpesvirus 8] (Table 1), poxviruses (vaccinia virus, cowpox virus, ectromelia virus, monkeypox virus, orf virus, variola virus, camelpox virus) (Table 2), adenoviruses, asfarviruses [African swine fever virus (ASFV)], hep-

Table 1
Inhibition of herpesviruses by HPMP nucleosides.

Virus[a]	Antiviral activity				Selected references
	HPMPA	HPMPC	HPMPG	HPMPT	
HSV-1	+	+	+	+/−[b]	Balzarini et al. (1993), De Clercq et al. (1986, 1987), (1987), Terry et al. (1988)
HSV-2	+	+	+	+/−	Balzarini et al. (1993), De Clercq et al. (1986, 1987), Terry et al. (1988), Yu et al. (1992)
CMV	+	+	+	+/−	Balzarini et al. (1993), De Clercq et al. (1986, 1987), Shigeta et al. (1991), Snoeck et al. (1988), Terry et al. (1988), Yu et al. (1992)
EBV	+	+	Unk[c]	Unk	Lin et al. (1991)
VZV	+	+	+	+/−	Andrei et al. (1995), Balzarini et al. (1993), De Clercq et al. (1986), De Clercq et al. (1987), Terry et al. (1988)
HHV6	+	+	+	Unk	Naesens et al. (2006), Reymen et al. (1995), Yoshida et al. (1998)
HHV7		+			Yoshida et al. (1998), Zhang et al. (1999)
HHV8	+	+	Unk	Unk	Neyts and De Clercq (1997)

[a] Virus abbreviations are HSV-1 (herpes simplex virus type 1), HSV-2 (herpes simplex virus type 2), CMV (cytomegalovirus), EBV (Epstein-Barr virus), VZV (varicella zoster virus), HHV6 (human herpes virus 6), HHV7 (human herpes virus 7), HHV8 (human herpes virus 8).
[b] Weak or no activity.
[c] Unknown or not tested.

Table 2
Inhibition of poxviruses by HPMP nucleosides.

Virus	Antiviral activity				Selected references
	HPMPA	HPMPC	HPMPG	HPMPT	
Vaccinia	+	+	+	+/−[a]	Baker et al. (2003), Balzarini et al. (1993), De Clercq et al. (1986), De Clercq et al. (1987), Quenelle et al. (2007), Smee et al. (2002), Terry et al. (1988)
Cowpox	+	+	Unk[b]	Unk	Baker et al. (2003), Quenelle et al. (2007), Smee et al. (2002)
Ectromelia		+	Unk	Unk	Buller et al. (2004)
Monkeypox	+	+	Unk	Unk	Baker et al. (2003), Smee et al. (2002)
Orf	+	+	Unk	Unk	Dal Pozzo et al. (2007)
Variola		+	Unk	Unk	Baker et al. (2003)
Camelpox	+	+	Unk	Unk	Duraffour et al. (2007), Smee et al. (2002)
Molluscum contagiosum					
Unk	+	Unk	Unk		Bachmeyer et al. (2009), Baxter and Highet (2004), Briand et al. (2008), Davies et al. (1999), Ibarra et al. (2000), Lin et al. (1991), Meadows et al. (1997), Toro et al. (2000), Zabawski and Cockerell (1999)

[a] Weak or no activity.
[b] Unknown or not tested.

adnaviruses [human hepatitis B virus (HBV), duck hepatitis B virus (DHBV)], and polyomaviruses (BK virus, murine polyomavirus, SV40), and the simple retrovirus murine sarcoma virus (Table 3). Further, HPMPC inhibits the proliferation of human papillomavirus infected cells (Andrei et al., 1998a; Johnson and Gangemi, 1999) and although activity has not been demonstrated in vitro, this compound has also been used to treat human molluscum contagiosum infections (see references in Table 2). In contrast, these compounds do not have activity (or lack selective activity) against RNA viruses and human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) (Balzarini et al., 1993; De Clercq et al., 1986; Pauwels et al., 1988; Yu et al., 1992), although Srinivas et al. (1997) demonstrated that HPMPC could inhibit HIV-1 in HeLa-CD4 cells, but not in the MT2 lymphocyte cell line. Interestingly, the related compound HPMP-uracil (HPMPU), has no activity against HSV-1, HSV-2, CMV, VZV, vaccinia virus, adenovirus or ASFV (De Clercq et al., 1987; Gil-Fernandez et al., 1987).

The alkoxyalkyl ester derivatives of the HPMP nucleosides described in Section 2.1 exhibit increased antiviral activity relative to the parent compounds (Beadle et al., 2006; Buller et al., 2004; Dal Pozzo et al., 2007; Hartline et al., 2005; Keith et al., 2004; Kern et al., 2002; Lebeau et al., 2006; Morrey et al., 2009; Quenelle et al., 2007, 2004; Valiaeva et al., 2009). This increased activity is thought to be due to the increased levels of active diphosphoryl metabolites produced upon treatment of cells with these prodrugs (Aldern et al., 2003). A further advantage of these prodrugs is that they expand the antiviral activity spectrum of these compounds to HIV-1 (Hostetler et al., 2006; Magee et al., 2011), hepatitis C virus (Wyles et al., 2009) and JC virus (Jiang et al., 2010).

2.3. Inhibition of viral and cellular enzymes

The active diphosphoryl metabolites of the HPMP nucleosides are analogs of dNTPs and as such, are predicted to target DNA polymerases. Indeed, these compounds inhibit both viral and cellular DNA polymerases. This activity has not been well studied with a great diversity of isolated enzymes, due to the greater difficulty of purifying DNA polymerases and of obtaining the biochemically active diphosphoryl compounds, as well as the greater complexity of the assays. The HCMV and vaccinia virus DNA polymerases have been the best-studied of the purified virus enzymes and consequently we will emphasize the results of these studies in the discussions that follow.

2.3.1. HPMPApp

HPMPApp, an analog of deoxyadenosine triphosphate (dATP), has been shown to inhibit the DNA replication of HSV-1 (Votruba et al., 1987), EBV (Lin et al., 1991), vaccinia virus (Magee et al., 2008), adenovirus (Mul et al., 1989), HBV (Yokota et al., 1991), and DHBV (Yokota et al., 1990). HPMPApp is a competitive inhibitor of DNA polymerases with respect to dATP and exhibits some selectively for viral DNA polymerases over cellular DNA polymer-

Table 3
Inhibition of other viruses by HPMP nucleosides.

Virus[a]	Antiviral activity				Selected references
	HPMPA	HPMPC	HPMPG	HPMPT	
Adenoviruses	+	+	+	+/−[b]	De Clercq et al. (1986, 1987), Gordon et al. (1991), Hartline et al. (2005), Naesens et al. (2005)
Asfarvirus					
ASFV	+	+	+	+/−	De Clercq et al. (1986), Gil-Fernandez et al. (1987)
Hepadnaviruses					
HBV	+	+	Unk[c]	Unk	Heijtink et al. (1994), Yokota et al. (1991)
DHBV	+	+	Unk	Unk	Yokota et al. (1990)
Polyomaviruses					
BK Virus	+	+	Unk	Unk	Topalis et al. (2011)
Murine Polyomavirus	+	+	Unk	Unk	Andrei et al. (1997)
SV40	+	+	Unk	Unk	Andrei et al. (1997)
Retroviruses					
MSV	+	Unk	+	–	De Clercq et al. (1986), Pauwels et al. (1988)
HIV-1	+/−	+/−	+/−	Unk	Balzarini et al. (1993), Hostetler et al. (2006), Pauwels et al. (1988), Srinivas et al. (1997), Yu et al. (1992)
HIV-2	+/−	Unk	+/−	Unk	Balzarini et al. (1993)

[a] Virus abbreviations are ASFV (African swine fever virus) HBV (human hepatitis B virus), DHBV (duck hepatitis B virus), MSV (murine sarcoma virus), HIV-1 (human immunodeficiency virus type 1), HIV-2 (human immunodeficiency virus type 2).
[b] Weak or no activity.
[c] Unknown or not tested.

ases (Kramata et al., 1996; Merta et al., 1990; Mul et al., 1989). This selectivity is illustrated by the respective $K_{i(HPMPApp)}/K_{m(dATP)}$ values for HSV-1 DNA polymerase, adenovirus DNA polymerase, human DNA polymerase α, rat DNA polymerase α, human DNA polymerase β, rat DNA polymerase δ, and rat DNA polymerase ε: 1.9, 0.58, 3.1, 2.3, >170, 0.25 and 0.07, respectively (Kramata et al., 1996; Merta et al., 1990; Mul et al., 1989). The low K_i/K_m values (with the exception of human DNA polymerase β) obtained with the cellular enzymes are most likely the cause of the cytotoxic effects of HPMPA seen at higher drug doses (Bronson et al., 1989; Vesely et al., 1990).

Although we did not calculate a K_i value for HPMPApp and vaccinia virus DNA polymerase, we demonstrated that this compound is an efficient substrate for the viral enzyme. The K_m of HPMPApp was calculated to be 3.8 ± 0.8 μM, relative to a value of 4.6 ± 0.5 μM for dATP (Magee et al., 2008). The V_{max} for the two substrates were similar, with values of 2.1 ± 0.1 and 2.0 ± 0.07 pmol/min, respectively (Magee et al., 2008).

2.3.2. HPMPCpp

The deoxycytidine triphosphate (dCTP) analog HPMPCpp also inhibits viral DNA replication, as shown for vaccinia virus (Magee et al., 2008), EBV (Lin et al., 1991) and HBV (Yokota et al., 1991). This active compound is a competitive inhibitor of DNA polymerases with respect to dCTP (Cherrington et al., 1994; Ho et al., 1992; Xiong et al., 1996), and interestingly, has greater selectivity for viral enzymes relative to cellular DNA polymerases than is seen with HPMPApp. HPMPCpp is a poor inhibitor of human DNA polymerases β and γ ($K_{i(HPMPCpp)}/K_{m(dCTP)}$ values are 121 and 1424, respectively) (Cherrington et al., 1994), a moderate inhibitor of human DNA polymerase α and HCMV DNA polymerase ($K_{i(HPMPCpp)}/K_{m(dCTP)}$ values are 10.8 and 9.2, respectively) (Ho et al., 1992; Xiong et al., 1996), and a strong inhibitor of HSV-1 and HSV-2 DNA polymerases ($K_{i(HPMPCpp)}/K_{m(dCTP)}$ values are 2.8 and 3.8, respectively) (Ho et al., 1992). Smee et al. (2002) also showed that HPMPCpp is a moderate inhibitor of partially purified cowpox virus DNA polymerase, with a $K_{i(HPMPCpp)}/K_{m(dCTP)}$ value of 11.3. Further, HPMPCpp inhibited the activity the molluscum contagiosum, cowpox virus and HSV-1 DNA polymerases expressed in an in vitro transcription and translation system, although kinetic data were not shown (Watanabe and Tamaki, 2008). As with HPMPApp, we did not calculate a K_i value for HPMPCpp and vaccinia virus DNA polymerase, but did present data showing this compound is a less favored substrate for the viral enzyme than is dCTP. The K_m of HPMPCpp was determined to be 23 ± 6 μM, higher than the calculated K_m for dCTP (3.8 ± 0.7 μM) (Magee et al., 2005). Also as seen for HPMPApp and vaccinia virus DNA polymerase, the V_{max} of the two substrates were similar (3.0 ± 0.4 and 2.4 ± 0.2 pmol/min, respectively) (Magee et al., 2005). These results are comparable to those previously obtained using HCMV DNA polymerase, where the K_m values for HPMPCpp and dCTP were 18.7 ± 1.4 and 0.67 ± 0.07 μM, respectively and the V_{max} values for the two substrates were 0.27 ± 0.09 and 0.39 ± 0.08 pmol/min, respectively (Xiong et al., 1996).

HPMPCpp has also inhibits HIV-1 reverse transcriptase (RT), with a $K_{i(HPMPCpp)}/K_{m(dCTP)}$ value of 4.6 for a DNA template and 5.9 for an RNA template (Cherrington et al., 1996). However, subsequent work has indicated that HPMPCpp is not active against HIV-1 RT (Frangeul et al., 2008). In determining the 50% inhibitory concentration of HPMPCpp, these authors showed that HIV-1 RT activity is not inhibited by concentrations of this compound up to 2 mM. This enzyme could however, use HPMPCpp as a substrate and incorporate it into DNA (Frangeul et al., 2008), a result that we have recently confirmed (Magee et al., 2011).

2.3.3. HPMPGpp

The addition of HPMPG to virus-infected cells inhibits HSV-1 DNA synthesis (Terry et al., 1988). This compound's active metabolite, HPMPGpp, is a competitive inhibitor of HSV-1 DNA polymerase with respect to dGTP and selectively inhibits this viral enzyme relative to human DNA polymerase α (Terry et al., 1988). Although $K_{m(dGTP)}$ values were not provided, the $K_{i(HPMPGpp)}$ values were determined to be 0.032 ± 0.015 and 2.3 μM for HSV-1 DNA polymerase and human DNA polymerase α, respectively (Terry et al., 1988). It is important to note that this study used the racemic mixture of (*R,S*)-HPMPGpp, and not (*S*)-HPMPGpp alone (Terry et al., 1988).

2.3.4. HPMPTpp

Few data have been obtained on HPMPT or its active metabolite HPMPTpp, most likely because this compound exhibits little to no antiviral activity (Table 1). The explanation for this lack of activity

is unclear but may be due to an inability of cellular kinases to activate HPMPT to HPMPTpp, an inability of HPMPTpp to inhibit DNA polymerases, or both.

3. Mechanism of action of HPMP nucleosides

Evidence obtained using a number of virus polymerases (DNA polymerases and reverse transcriptase), as well as human DNA polymerases, indicates that HPMP nucleoside diphosphates (HPMPXpp, where X is adenine, cytosine, guanine or thymine) can be used as substrates by these enzymes and incorporated into the 3′-terminus of the growing DNA strand. Unlike many other antiviral nucleoside and nucleotide analogs however, the addition of HPMPX to the 3′ end of the primer does not result in a termination of DNA synthesis. Rather, this incorporation results in a number of different effects on enzyme activity, as described below.

3.1. HSV-1 DNA polymerase

Merta et al. (1990) demonstrated that HPMPApp could replace dATP in reactions catalyzed by purified HSV-1 DNA polymerase using activated calf thymus DNA as the substrate. Significant DNA synthesis occurs in these reactions, although not to the levels observed when dATP is present. In contrast, PMEA diphosphate, an obligate chain terminator, cannot replace dATP to support DNA synthesis (Merta et al., 1990). Although HPMPApp substrate usage was not examined directly, these results suggest HPMPA is incorporated into DNA and where it can promote continued DNA synthesis.

3.2. HCMV DNA polymerase

The first studies to thoroughly examine the mechanism of action of HPMP nucleosides against DNA polymerases looked at the interactions between HPMPCpp and HCMV DNA polymerase (Xiong et al., 1997, 1996). Xiong et al. (1997) showed with primer extension analyses using defined primer template pairs that HPMPCpp can be used as an alternative substrate for this enzyme, and this compound is faithfully incorporated into DNA opposite a template dGMP. These authors suggested that the rate of incorporation of CDV and dCMP into DNA are similar, based on observations of the extension of the original primer strands (Xiong et al., 1997). The addition of a single HPMPC molecule to the primer terminus does not lead to chain termination and HCMV DNA polymerase can use a synthetic primer terminating in HPMPC or dCMP with similar affinities (K_m values of 165 ± 42 and 90 ± 8 nM, respectively) (Xiong et al., 1996). However, the incorporation of a single HPMPC molecule does slow subsequent elongation (Xiong et al., 1997). In contrast, when two consecutive molecules are incorporated into DNA, or when two molecules of HPMPC separated by a natural nucleotide are incorporated, elongation is dramatically reduced or terminated. Further, when present at the 3′-terminus of the primer strand, HPMPC is not excised by the 3′-to-5′ proofreading exonuclease activity of HCMV DNA polymerase (Xiong et al., 1997). In addition, when present in the template strand, pausing is observed at positions immediately prior to, at, and immediately after the HPMPC molecule. However, HPMPC in the template strand is not an absolute block to DNA synthesis as full-length extension past the lesion is also observed (Xiong et al., 1997). Interestingly, the degree of inhibition of HCMV DNA polymerase is much larger when HPMPC is located in the template strand than with a template incorporating HPMPC into the nascent strand. The incorporation of HPMPC into DNA therefore results in several mechanisms of HCMV DNA polymerase inhibition.

3.3. Vaccinia virus DNA polymerase

We have previously studied the effects of HPMPApp and HPMPCpp on primer extension reactions catalyzed by vaccinia virus DNA polymerase (Magee et al., 2008, 2005). As described in Sections 2.3.1 and 2.3.2, both compounds can be used as substrates by this enzyme. HPMPApp was shown to be a more efficient substrate for vaccinia virus DNA polymerase than was HPMPCpp (Magee et al., 2008, 2005). Indeed, HPMPApp is as good as, if not a better substrate than, the natural substrate dATP. In contrast, HPMPCpp is a less efficient substrate for this enzyme than dCTP. Both HPMPA and HPMPC are incorporated into the 3′-terminus of the growing DNA strand and these HPMPA/C-terminated primers can also be extended by each polymerase, indicating that the hydroxymethyl moiety on each compound can act as a 3′-hydroxyl group to initiate nucleophilic attack on the incoming dNTP. The presence of either compound in the primer strand did however, lead to the slowing or pausing of continued DNA synthesis (Magee et al., 2008, 2005). The incorporation of a single HPMPA or HPMPC residue by vaccinia virus DNA polymerase results in pausing at the N + 1 position, where N is the expected site of incorporation of the compound. When two consecutive molecules of HPMPA or HPMPC are incorporated into DNA by this enzyme, a slowing of DNA synthesis was also observed relative to control reactions incubated with the natural nucleotides (dATP and dCTP, respectively); pause sites were located at positions N, N + 1, N + 2, and N + 3. This slowing of DNA synthesis following HPMP nucleoside incorporation is more profound when HPMPC is incorporated in the primer strand than when HPMPA was present, particularly when two molecules are incorporated. In all of these reactions however, the production of full-length DNA strands was observed (Magee et al., 2008, 2005). These results indicate that HPMPA and HPMPC are not simple chain terminators. They can be incorporated into DNA and this permits the continuation of DNA synthesis.

One of the issues these studies highlight is the danger of relying upon simplified biochemical assays as screens for drugs with antiviral properties. HPMPA is a highly effective inhibitor of vaccinia virus growth in vivo, with EC_{50} values ranging from 0.3 to 8.1 μg/ml (Baker et al., 2003; De Clercq et al., 1986, 1987), and yet the HPMPApp metabolite is just as good a substrate for vaccinia virus DNA polymerase as is dATP in primer extension assays. As we note below, chain extension assays do not always accurately reflect the primary mode of action of this compound.

The effect of HPMPA and HPMPC on the 3′-to-5′ exonuclease activity of vaccinia virus DNA polymerase was also investigated. Although HPMPA and HPMPC can be excised from DNA bearing these compounds at the primer terminus, oligonucleotides containing either compound at the penultimate position of this terminus, equivalent to the N + 1 structure described above, are refractory to exonuclease activity (Magee et al., 2008, 2005). These results are different from what is seen with HCMV DNA polymerase (Section 3.2). The HCMV enzyme's 3′-to-5′ exonuclease activity cannot excise a molecule of HPMPC located at the primer terminus (Xiong et al., 1997).

The ability of HPMPA and HPMPC to be incorporated into DNA by vaccinia virus DNA polymerase without causing chain termination prompted an examination of the effects of these compounds when positioned in the template strand. Similar to the results observed using HCMV DNA polymerase (Section 3.2) (Xiong et al., 1997), the presence of HPMPA or HPMPC in the template results in inhibition of DNA synthesis. However, unlike HCMV DNA polymerase, vaccinia virus DNA polymerase is completely inhibited by drug in the template; nucleotide can be incorporated opposite the HPMPA/C residue but further extension does not occur. This inhibition of extension is observed even if oligonucleotides terminating one nucleotide past the HPMPA/C residue are used to prime

DNA synthesis. Interestingly, these latter primers are rapidly degraded by one nucleotide by the vaccinia virus DNA polymerase 3′-to-5′ exonuclease activity, suggesting that these structures are not favored substrates.

Jesus et al. (2009) examined the effects of HPMPC on the vaccinia virus replicative cycle, and found that treatment of virus-infected cells with this compound inhibits genome encapsidation and virion morphogenesis. Further, analysis by atomic force spectroscopy of genomic DNA synthesized in the presence of HPMPC showed aggregated structures that were not seen when this compound is absent. These authors suggested that the incorporation of HPMPC into DNA alters the structure of the nucleic acid and prevents its packaging into virions (Jesus et al., 2009). More recently we have solved the solution structure of a DNA duplex containing a single molecule of HPMPC (Julien et al., 2011). This compound is well accommodated in the DNA structure and comparison with an isosequential control DNA duplex shows a root mean square deviation of only 1.5 Å over all common atoms. The stability of the HPMPC-containing DNA duplex however, is less than the control DNA with a lower melting temperature relative to the latter (Julien et al., 2011). We favor the hypothesis that once an HPMPX moiety has been incorporated into DNA, it creates a subtle disturbance in the structure of the helix that then creates an impediment to the transit of the molecule through the DNA binding domains of a DNA polymerase or packaging helicase. A good analogy would be the difficulty encountered trying to screw a nut over a bolt bearing a stripped thread.

3.4. Adenovirus DNA polymerase

HPMPApp inhibits adenovirus DNA replication at the level of elongation, but not at initiation (Mul et al., 1989). These authors did not demonstrate HPMPA incorporation into DNA catalyzed by adenovirus DNA polymerase, but did present data showing HPMPApp inhibits, but does not stop elongation.

3.5. HIV-1 RT

Frangeul et al. (2008) first demonstrated the ability of HIV-1 RT to incorporate HPMPC into DNA, although HPMPCpp was also determined to be an inefficient substrate. More recently, we showed that HPMPApp and HPMPCpp can replace dATP and dCTP, respectively, to support DNA synthesis catalyzed by HIV-1 RT (Magee et al., 2011). The incorporation of either compound into DNA slows, but does not absolutely terminate continued DNA synthesis. Interestingly, the incorporation of two consecutive molecules of HPMPC, but not HPMPA, results in a severe inhibition of HIV-1 RT activity. The presence of either compound in the template strand also inhibits HIV-1 RT; this inhibition is more profound with HPMPC than with HPMPA (Magee et al., 2011).

3.6. Human DNA polymerases

The ability of HPMP nucleosides to inhibit the growth of a variety of different tumors and transformed cell lines, including those with and without a known viral cause (Andrei et al., 1998a,b; Collette and Zechel, 2011; Johnson and Gangemi, 1999; Liekens et al., 1998, 2001a,b; Redondo et al., 2000; Sherman et al., 2002; Snoeck et al., 2000; Spanos et al., 2005; Yoshizaki et al., 2008), as well as their ability to inhibit the replication of viruses that use cellular DNA polymerases to replicate their genomes (Andrei et al., 1997; Topalis et al., 2011) suggests that cellular DNA polymerases are also inhibited by these compounds. Indeed, rat cellular DNA polymerases α, δ, and ε can use HPMPApp as a substrate and incorporate two to four consecutive HPMPA molecules into a growing DNA strand (Birkus et al., 2004; Kramata et al., 1996). Further, both DNA polymerases δ and ε can excise HPMPA from a primer terminus, with more efficient removal of the drug by polymerase ε (Birkus et al., 2004). In addition, the mitochondrial abnormalities observed in biopsies from patients with BV virus nephropathy treated with HPMPC (Talmon et al., 2010) suggests that DNA polymerase γ may use these compounds as substrates as well.

3.7. Model of the mechanism of action of HPMP nucleosides

The inhibition of polymerases, including DNA polymerases and reverse transcriptase by HPMP nucleosides occurs after their incorporation into DNA and is due to a number of different factors, including the inhibition of DNA synthesis by the presence of compound in the primer strand and inhibition of DNA synthesis by these compounds in the template strand; inhibition of 3′-to-5′ exonuclease activity also plays a role (Fig. 5). The inhibition of DNA synthesis by the presence of these compounds in the template is of particular interest, as this mechanism is not relevant for most other antiviral nucleoside or nucleotide analogs because they act as either obligate or functional chain terminators. One notable exception is the antiviral drug entecavir, a 2′-deoxyguanosine analog that, like the HPMP nucleosides, possesses a 3′-hydroxyl moiety. Entecavir also has a complex mechanism of inhibition against HIV-1 RT, inhibiting DNA synthesis when present in either the primer or the template strands (Domaoal et al., 2008; Tchesnokov et al., 2008).

4. Resistance to HPMP nucleosides

The complex mechanism of action of HPMP nucleosides against DNA polymerases and reverse transcriptases has implications for the development of resistance to these drugs. The in vitro selection of resistance to HPMPA and/or HPMPC has been described for HSV-1, HCMV, HHV6, vaccinia virus, cowpox virus, camelpox virus,

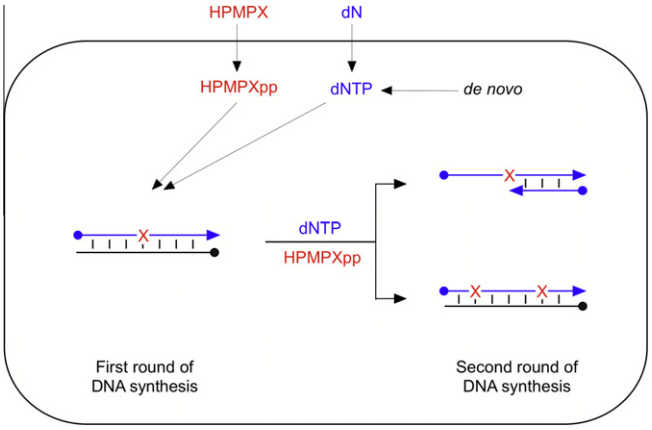

Fig. 5. The mechanism of action of the HPMP nucleosides. HPMP nucleosides (HPMPX) are taken up into cells by endocytosis and then converted to their active intracellular metabolites, HPMP nucleoside diphosphates (HPMPXpp), by cellular enzymes. As analogs of dNTPs, HPMPXpp can be used as substrates by polymerases (DNA polymerases and reverse transcriptases) and incorporated into DNA (X residues [red] embedded in newly synthesized ordinary DNA [blue]). This incorporation of HPMPX into the primer strand results in a slowing of subsequent DNA elongation, but not termination. Since these primer strands are eventually fully elongated, they can serve as template DNA in the second round of DNA synthesis (at right). In this case, the presence of HPMPX in what is now a new template strand (top right) also causes inhibition of DNA synthesis; this inhibition can either cause a slowing or termination of further DNA elongation, depending on the enzyme. The semi-conservative nature of DNA replication also results in further rounds of HPMPX incorporation opposite the old, original, DNA template (black strand, bottom right). HPMPX in the DNA primer strand can also inhibit the DNA polymerase 3′-to-5′ exonuclease activity, potentially creating mutations as well as slowing replication (not shown).

Table 4
Virus resistance to HPMP nucleosides.

Virus[a]	Resistance described				References
	HPMPA	HPMPC	HPMPG	HPMPT	
HSV-1	+	+	Unk[b]	Unk	Andrei et al. (2007), Andrei et al. (2000)
HSV-2	Unk	+	Unk	Unk	Wyles et al. (2005)
HCMV	+	+	Unk	Unk	Cihlar et al. (1998), Scott et al. (2007), Smith et al. (1998), Snoeck et al. (1996)
HHV6	Unk	+	Unk	Unk	Bonnafous et al. (2008)
Vaccinia	+	+	Unk	Unk	Andrei et al. (2006), Becker et al. (2008), Kornbluth et al. (2006), Smee et al. (2002)
Cowpox	+	+	Unk	Unk	Smee et al. (2002)
Camelpox	+	+	Unk	Unk	Smee et al. (2002)
Monkeypox	+	+	Unk	Unk	Smee et al. (2002)
Adenovirus	Unk	+	Unk	Unk	Gordon et al. (1996), Kinchington et al. (2002)

[a] Virus abbreviations are HSV-1 (herpes simplex virus type 1), HSV-2 (herpes simplex virus type 2), HCMV (human cytomegalovirus), HHV6 (human herpes virus 6).
[b] Unknown or not tested.

monkeypox virus and adenovirus (Table 4). In some cases resistance loci create cross-resistance to many different related nucleoside phosphonate compounds (Duraffour et al., 2012; Gammon et al., 2008). Resistance to HPMPC, the only member of the HPMP nucleoside class to be approved for use in humans, has also been found in clinical isolates of HSV-2 and HCMV (Table 4).

In many of these cases, resistance has been mapped to viral DNA polymerase genes and the specific residues conferring resistance have been located in both the polymerase domain and the 3′-to-5′ exonuclease domain of these enzymes. Although more work is needed to examine the interactions of HPMPXpp with these drug-resistant polymerases, it is probable that mutated residues in the polymerase domain allow the enzyme to select against incorporation of the compounds into DNA, or to bypass HPMPX residues in the template strand, while mutations in the 3′-to-5′ exonuclease domain facilitate removal of the compound after incorporation. For example, in vitro selection for vaccinia viruses that are resistant to HPMPC and related compounds repeatedly selects for an A314T substitution in the exonuclease domain and for an A684V mutation in the polymerase domain (Andrei et al., 2006; Gammon et al., 2008). The A314T mutation can be modeled to fall within an extended β-hairpin region in the homologous RB69 DNA polymerase structure (Shamoo and Steitz, 1999) and has been speculated to play some role in transferring DNA from the polymerase to exonuclease domains. This is supported by in vitro enzyme assays where polymerases encoding the A314T substitution excise HPMPC from the penultimate position in a primer strand far faster than wild type vaccinia DNA polymerase (Gammon and Evans, 2009). The A684V mutation falls in a highly conserved part of the polymerase domain active site and likely influences the positioning of another highly conserved nearby tyrosine residue that plays an important role in nucleotide selection (Andrei et al., 2006). This mutation could thus favor either selection against HPMPXpp nucleotides or promote trans-lesion bypass when the enzyme encounters a HPMPX moiety in the template. Viruses encoding the A684V mutation are weak mutators (Andrei et al., 2006), a phenotype compatible with either hypothesis.

The inhibitory effect caused by HPMP nucleosides in the template strand may explain why it is so difficult to select for viruses exhibiting high-level resistance to these compounds. The few mutants that have been identified (e.g. the vaccinia A314T and A684V mutations) are not highly resistant and exhibit reduced virulence in mice (Andrei et al., 2006). Although one can select for higher levels of resistance with continued passage, these mutant viruses exhibit even greater levels of impairment. In many ways these compounds create lesions resembling a form of DNA damage. This damage is not necessarily a problem for cells as it can be accommodated using bypass polymerases and repaired using cellular DNA repair systems. [However, the mitochondria may be less capable of repairing such damage, perhaps accounting for the mitochondrial toxicity reported for some of these compounds (Talmon et al., 2010).] It is much more of a problem for viruses, especially because no mutant polymerase could ever completely avoid incorporating these compounds into DNA and thus avoid creating an impediment to second strand synthesis. It appears to be difficult for viruses to transit a mutational landscape selecting for DNA polymerases that exhibit a combination of three complex traits (efficient drug excision, great selectivity against HPMPXpp incorporation, and a tolerance for damaged templates) without negatively affecting fitness. This is of special relevance in the biodefense realm, where the studies with vaccinia virus shows that high virulence appears to be incompatible with drug resistance in an Orthopoxvirus (Andrei et al., 2006; Becker et al., 2008; Duraffour et al., 2012; Gammon et al., 2008; Kornbluth et al., 2006; Smee et al., 2002, 2005). This suggests that drug-based countermeasures will not be readily defeated through the malicious creation (or natural evolution) of drug-resistant strains and provides some support for the development of compounds like CMX001 as a smallpox therapeutic (Painter and Hostetler, 2004).

Although drug resistance has been more extensively evaluated using vaccinia virus, some of the same themes seem to be applicable to other viruses. The Hostetler laboratory has showed that the alkoxyalkyl ester derivatives of HPMP nucleosides retain activity against HIV-1 strains possessing a number of resistance mutations at clinically relevant concentrations (Hostetler et al., 2006; Magee et al., 2011). It will be of interest to determine the types of mutations that will develop upon the in vitro selection of resistance of HIV-1 to HPMP nucleosides. Since these compounds exert major effects on HIV-1 RT when present in the template strand, we predict that resistance will be more difficult to achieve than that obtained with other antiretroviral compounds that act as obligate chain terminators and that novel mutations will develop.

5. Conclusions

The complex mechanism of action that has been described for HPMP nucleosides, particularly the inhibition of enzyme activity by the presence of these compounds in the template strand, points to a new avenue for targeted antiviral drug design. Nucleoside and nucleotide analogs can be developed that are efficient and selective substrates for viral polymerases and that cause little inhibition of nucleic acid synthesis when being incorporated into DNA or RNA. These analogs would instead exert their effects in subsequent rounds of replication. Modifications to these drugs could then be made, if necessary, to improve oral bioavailability, uptake, and metabolism. Future work will also include an elucidation of the structural basis for the inhibition of DNA polymerases and reverse

transcriptases, and a determination of the mechanism of inhibition of the diphosphoryl derivatives of these compounds on the polymerases of HBV and HCV. These experiments will help to determine if the complex mechanism of action that has been observed against HCMV DNA polymerase, vaccinia virus DNA polymerase, and HIV-1 RT is more broadly applicable to these other virus systems. In addition, it will be of interest to test the efficacy of HPMP nucleosides, and their alkoxyalkyl ester derivatives, against other viruses, like influenza, measles, or respiratory syncytial virus, to determine the full antiviral activity spectrum of these drugs. Both the parent compounds and their derivatives should be tested, in case high levels of drug are required for activity, as is seen in the case of HIV-1 and hepatitis C virus.

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References

Aduma, P., Connelly, M.C., Srinivas, R.V., Fridland, A., 1995. Metabolic diversity and antiviral activities of acyclic nucleoside phosphonates. Mol. Pharmacol. 47, 816–822.

Aldern, K.A., Ciesla, S.L., Winegarden, K.L., Hostetler, K.Y., 2003. Increased antiviral activity of 1-*O*-hexadecyloxypropyl-[2-(14)C]cidofovir in MRC-5 human lung fibroblasts is explained by unique cellular uptake and metabolism. Mol. Pharmacol. 63, 678–681.

Andrei, G., Snoeck, R., Reymen, D., Liesnard, C., Goubau, P., Desmyter, J., De Clercq, E., 1995. Comparative activity of selected antiviral compounds against clinical isolates of varicella-zoster virus. Eur. J. Clin. Microbiol. Infect. Dis. 14, 318–329.

Andrei, G., Snoeck, R., Vandeputte, M., De Clercq, E., 1997. Activities of various compounds against murine and primate polyomaviruses. Antimicrob. Agents Chemother. 41, 587–593.

Andrei, G., Snoeck, R., Piette, J., Delvenne, P., De Clercq, E., 1998a. Antiproliferative effects of acyclic nucleoside phosphonates on human papillomavirus (HPV)-harboring cell lines compared with HPV-negative cell lines. Oncol. Res. 10, 523–531.

Andrei, G., Snoeck, R., Piette, J., Delvenne, P., De Clercq, E., 1998b. Inhibiting effects of cidofovir (HPMPC) on the growth of the human cervical carcinoma (SiHa) xenografts in athymic nude mice. Oncol. Res. 10, 533–539.

Andrei, G., Snoeck, R., De Clercq, E., Esnouf, R., Fiten, P., Opdenakker, G., 2000. Resistance of herpes simplex virus type 1 against different phosphonylmethoxyalkyl derivatives of purines and pyrimidines due to specific mutations in the viral DNA polymerase gene. J. Gen. Virol. 81, 639–648.

Andrei, G., Gammon, D.B., Fiten, P., De Clercq, E., Opdenakker, G., Snoeck, R., Evans, D.H., 2006. Cidofovir resistance in vaccinia virus is linked to diminished virulence in mice. J. Virol. 80, 9391–9401.

Andrei, G., Fiten, P., Froeyen, M., De Clercq, E., Opdenakker, G., Snoeck, R., 2007. DNA polymerase mutations in drug-resistant herpes simplex virus mutants determine in vivo neurovirulence and drug–enzyme interactions. Antivir. Ther. 12, 719–732.

Bachmeyer, C., Moguelet, P., Baud, F., Lescure, F.X., 2009. Efflorescence of facial *molluscum contagiosum* as a manifestation of immune reconstitution inflammatory syndrome in a patient with AIDS. Eur. J. Dermatol. 19, 257–258.

Baker, R.O., Bray, M., Huggins, J.W., 2003. Potential antiviral therapeutics for smallpox, monkeypox and other orthopoxvirus infections. Antiviral Res. 57, 13–23.

Balzarini, J., Holy, A., Jindrich, J., Naesens, L., Snoeck, R., Schols, D., De Clercq, E., 1993. Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine. Antimicrob. Agents Chemother. 37, 332–338.

Baxter, K.F., Highet, A.S., 2004. Topical cidofovir and cryotherapy–combination treatment for recalcitrant molluscum contagiosum in a patient with HIV infection. J. Eur. Acad. Dermatol. Venereol. 18, 230–231.

Beadle, J.R., Wan, W.B., Ciesla, S.L., Keith, K.A., Hartline, C., Kern, E.R., Hostetler, K.Y., 2006. Synthesis and antiviral evaluation of alkoxyalkyl derivatives of 9-(S)-(3-hydroxy-2-phosphonomethoxypropyl)adenine against cytomegalovirus and orthopoxviruses. J. Med. Chem. 49, 2010–2015.

Becker, M.N., Obraztsova, M., Kern, E.R., Quenelle, D.C., Keith, K.A., Prichard, M.N., Luo, M., Moyer, R.W., 2008. Isolation and characterization of cidofovir resistant vaccinia viruses. Virol. J. 5, 58.

Birkus, G., Rejman, D., Otmar, M., Votruba, I., Rosenberg, I., Holy, A., 2004. The substrate activity of (S)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenine diphosphate toward DNA polymerases alpha, delta and epsilon. Antivir. Chem. Chemother. 15, 23–33.

Bonnafous, P., Boutolleau, D., Naesens, L., Deback, C., Gautheret-Dejean, A., Agut, H., 2008. Characterization of a cidofovir-resistant HHV-6 mutant obtained by in vitro selection. Antiviral Res. 77, 237–240.

Briand, S., Milpied, B., Navas, D., Thomare, P., Stalder, J.F., 2008. 1% Topical cidofovir used as last alternative to treat viral infections. J. Eur. Acad. Dermatol. Venereol. 22, 249–250.

Bronson, J.J., Ghazzouli, I., Hitchcock, M.J.M., Webb, R.R., Kern, E.R., Martin, J.C., 1989. Synthesis and antiviral activity of nucleotide analogues bearing the (*S*)-(3-hydroxy-2-phosphonylmethoxy)propyl moiety attached to adenine, guanine and cytosine. In: Martin, J.C. (Ed.), Nucleotide Analogues As Antiviral Agents. American Chemical Society, Washington, DC, pp. 88–102.

Bronson, J.J., Ho, H.-T., De Boeck, H., Woods, K., Ghazzouli, I., Martin, J.C., Hitchcock, M.J.M., 1990. Biochemical pharmacology of acyclic nucleotide analogues. Ann. NY Acad. Sci. 616, 398–407.

Buller, R.M., Owens, G., Schriewer, J., Melman, L., Beadle, J.R., Hostetler, K.Y., 2004. Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. Virology 318, 474–481.

Cherrington, J.M., Allen, S.J., McKee, B.H., Chen, M.S., 1994. Kinetic analysis of the interaction between the diphosphate of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, ddCTP, AZTTP, and FIAUTP with human DNA polymerases beta and gamma. Biochem. Pharmacol. 48, 1986–1988.

Cherrington, J.M., Fuller, M.D., Mulato, A.S., Allen, S.J., Kunder, S.C., Ussery, M.A., Lesnikowski, Z., Schinazi, R.F., Sommadossi, J.P., Chen, M.S., 1996. Comparative kinetic analyses of interaction of inhibitors with Rauscher murine leukemia virus and human immunodeficiency virus reverse transcriptases. Antimicrob. Agents Chemother. 40, 1270–1273.

Cihlar, T., Chen, M.S., 1996. Identification of enzymes catalyzing two-step phosphorylation of cidofovir and the effect of cytomegalovirus infection on their activities in host cells. Mol. Pharmacol. 50, 1502–1510.

Cihlar, T., Fuller, M.D., Mulato, A.S., Cherrington, J.M., 1998. A point mutation in the human cytomegalovirus DNA polymerase gene selected in vitro by cidofovir confers a slow replication phenotype in cell culture. Virology 248, 382–393.

Cihlar, T., Ho, E.S., Lin, D.C., Mulato, A.S., 2001. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids 20, 641–648.

Collette, D.C., Zechel, M.A., 2011. Novel treatment of atypical human papillomavirus-associated epithelial hyperplasia with cidofovir. J. Oral Maxillofac. Surg. 69, 2383–2386.

Connelly, M.C., Robbins, B.L., Fridland, A., 1993. Mechanism of uptake of the phosphonate analog (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) in Vero cells. Biochem. Pharmacol. 46, 1053–1057.

Cundy, K.C., 1999. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. Clin. Pharmacokinet. 36, 127–143.

Dal Pozzo, F., Andrei, G., Lebeau, I., Beadle, J.R., Hostetler, K.Y., De Clercq, E., Snoeck, R., 2007. In vitro evaluation of the anti-orf virus activity of alkoxyalkyl esters of CDV, cCDV and (S)-HPMPA. Antiviral Res. 75, 52–57.

Davies, E.G., Thrasher, A., Lacey, K., Harper, J., 1999. Topical cidofovir for severe molluscum contagiosum. Lancet 353, 2042.

De Clercq, E., 1997. Acyclic nucleoside phosphonates in the chemotherapy of DNA virus and retrovirus infections. Intervirology 40, 295–303.

De Clercq, E., 2007. The acyclic nucleoside phosphonates from inception to clinical use: historical perspective. Antiviral Res. 75, 1–13.

De Clercq, E., 2011. The clinical potential of the acyclic (and cyclic) nucleoside phosphonates. The magic of the phosphonate bond. Biochem. Pharmacol. 82, 99–109.

De Clercq, E., Holy, A., 2005. Acyclic nucleoside phosphonates: a key class of antiviral drugs. Nat. Rev. Drug Discov. 4, 928–940.

De Clercq, E., Neyts, J., 2009. Antiviral agents acting as DNA or RNA chain terminators. Handb. Exp. Pharmacol., 53–84.

De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 1986. A novel selective broad-spectrum anti-DNA virus agent. Nature 323, 464–467.

De Clercq, E., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I., Holy, A., 1987. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines. Antiviral Res. 8, 261–272.

Domaoal, R.A., McMahon, M., Thio, C.L., Bailey, C.M., Tirado-Rives, J., Obikhod, A., Detorio, M., Rapp, K.L., Siliciano, R.F., Schinazi, R.F., Anderson, K.S., 2008. Pre-steady-state kinetic studies establish entecavir 5′-triphosphate as a substrate for HIV-1 reverse transcriptase. J. Biol. Chem. 283, 5452–5459.

Duraffour, S., Snoeck, R., Krecmerova, M., van Den Oord, J., De Vos, R., Holy, A., Crance, J.M., Garin, D., De Clercq, E., Andrei, G., 2007. Activities of several classes of acyclic nucleoside phosphonates against camelpox virus replication in different cell culture models. Antimicrob. Agents Chemother. 51, 4410–4419.

Duraffour, S., Andrei, G., Topalis, D., Krečmerová, M., Crance, J.-M., Garin, D., Snoeck, R., 2012. Mutations conferring resistance to viral DNA polymerase inhibitors in camelpox virus give different drug-susceptibility profiles in vaccinia virus. J. Virol. 86, 7310–7325.

Flores-Aguilar, M., Huang, J.-S., Wiley, C.A., De Clercq, E., Vuong, C., Bergeron-Lynn, G., Chandler, B., Munguia, D., Freeman, W.R., 1994. Long-acting therapy of viral retinitis with (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. J. Infect. Dis. 169, 642–647.

Frangeul, A., Bussetta, C., Deval, J., Barral, K., Alvarez, K., Canard, B., 2008. Gln151 of HIV-1 reverse transcriptase acts as a steric gate towards clinically relevant acyclic phosphonate nucleotide analogues. Antiviral Ther. 13, 115–124.

Gammon, D.B., Evans, D.H., 2009. The 3′-to-5′ exonuclease activity of vaccinia virus DNA polymerase is essential and plays a role in promoting virus genetic recombination. J. Virol. 83, 4236–4250.

Gammon, D.B., Snoeck, R., Fiten, P., Krecmerova, M., Holy, A., De Clercq, E., Opdenakker, G., Evans, D.H., Andrei, G., 2008. Mechanism of antiviral drug resistance of vaccinia virus: identification of residues in the viral DNA polymerase conferring differential resistance to antipoxvirus drugs. J. Virol. 82, 12520–12534.

Gil-Fernandez, C., Garcia-Villalon, D., De Clercq, E., Rosenberg, I., Holy, A., 1987. Phosphonylmethoxyalkylpurines and -pyrimidines as inhibitors of African swine fever virus replication in vitro. Antiviral Res. 8, 273–281.

Gordon, Y.J., Romanowski, E., Araullo-Cruz, T., Seaberg, L., Erzurum, S., Tolman, R., De Clercq, E., 1991. Inhibitory effect of (S)-HPMPC, (S)-HPMPA, and 2′-nor-cyclic GMP on clinical ocular adenoviral isolates is serotype-dependent in vitro. Antiviral Res. 16, 11–16.

Gordon, Y.J., Araullo-Cruz, T.P., Johnson, Y.F., Romanowski, E.G., Kinchington, P.R., 1996. Isolation of human adenovirus type 5 variants resistant to the antiviral cidofovir. Invest. Ophthalmol. Vis. Sci. 37, 2774–2778.

Hartline, C.B., Gustin, K.M., Wan, W.B., Ciesla, S.L., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2005. Ether lipid-ester prodrugs of acyclic nucleoside phosphonates: activity against adenovirus replication in vitro. J. Infect. Dis. 191, 396–399.

Heijtink, R.A., Kruining, J., de Wilde, G.A., Balzarini, J., de Clercq, E., Schalm, S.W., 1994. Inhibitory effects of acyclic nucleoside phosphonates on human hepatitis B virus and duck hepatitis B virus infections in tissue culture. Antimicrob. Agents Chemother. 38, 2180–2182.

Ho, H.T., Woods, K.L., Bronson, J.J., De Boeck, H., Martin, J.C., Hitchcock, M.J., 1992. Intracellular metabolism of the antiherpes agent (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine. Mol. Pharmacol. 41, 197–202.

Hostetler, K.Y., Aldern, K.A., Wan, W.B., Ciesla, S.L., Beadle, J.R., 2006. Alkoxyalkyl esters of (S)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine are potent inhibitors of the replication of wild-type and drug-resistant human immunodeficiency virus type 1 in vitro. Antimicrob. Agents Chemother. 50, 2857–2859.

Ibarra, V., Blanco, J.R., Oteo, J.A., Rosel, L., 2000. Efficacy of cidofovir in the treatment of recalcitrant molluscum contagiosum in an AIDS patient. Acta Derm. Venereol. 80, 315–316.

Jesus, D.M., Costa, L.T., Goncalves, D.L., Achete, C.A., Attias, M., Moussatche, N., Damaso, C.R., 2009. Cidofovir inhibits genome encapsidation and affects morphogenesis during the replication of vaccinia virus. J. Virol. 83, 11477–11490.

Jiang, Z.G., Cohen, J., Marshall, L.J., Major, E.O., 2010. Hexadecyloxypropyl-cidofovir (CMX001) suppresses JC virus replication in human fetal brain SVG cell cultures. Antimicrob. Agents Chemother. 54, 4723–4732.

Johnson, J.A., Gangemi, J.D., 1999. Selective inhibition of human papillomavirus-induced cell proliferation by (S)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl]cytosine. Antimicrob. Agents Chemother. 43, 1198–1205.

Julien, O., Beadle, J.R., Magee, W.C., Chatterjee, S., Hostetler, K.Y., Evans, D.H., Sykes, B.D., 2011. Solution structure of a DNA duplex containing the potent anti-poxvirus agent cidofovir. J. Am. Chem. Soc. 133, 2264–2274.

Keith, K.A., Wan, W.B., Ciesla, S.L., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2004. Inhibitory activity of alkoxyalkyl and alkyl esters of cidofovir and cyclic cidofovir against orthopoxvirus replication in vitro. Antimicrob. Agents Chemother. 48, 1869–1871.

Kern, E.R., Hartline, C., Harden, E., Keith, K., Rodriguez, N., Beadle, J.R., Hostetler, K.Y., 2002. Enhanced inhibition of orthopoxvirus replication in vitro by alkoxyalkyl esters of cidofovir and cyclic cidofovir. Antimicrob. Agents Chemother. 46, 991–995.

Kinchington, P.R., Araullo-Cruz, T., Vergnes, J.P., Yates, K., Gordon, Y.J., 2002. Sequence changes in the human adenovirus type 5 DNA polymerase associated with resistance to the broad spectrum antiviral cidofovir. Antiviral Res. 56, 73–84.

Kornbluth, R.S., Smee, D.F., Sidwell, R.W., Snarsky, V., Evans, D.H., Hostetler, K.Y., 2006. Mutations in the E9L polymerase gene of cidofovir-resistant vaccinia virus strain WR are associated with the drug resistance phenotype. Antimicrob. Agents Chemother. 50, 4038–4043.

Kramata, P., Votruba, I., Otova, B., Holy, A., 1996. Different inhibitory potencies of acyclic phosphonomethoxyalkyl nucleotide analogs toward DNA polymerases alpha, delta and epsilon. Mol. Pharmacol. 49, 1005–1011.

Lebeau, I., Andrei, G., Dal Pozzo, F., Beadle, J.R., Hostetler, K.Y., De Clercq, E., van den Oord, J., Snoeck, R., 2006. Activities of alkoxyalkyl esters of cidofovir (CDV), cyclic CDV, and (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine against orthopoxviruses in cell monolayers and in organotypic cultures. Antimicrob. Agents Chemother. 50, 2525–2529.

Liekens, S., Andrei, G., Vandeputte, M., De Clercq, E., Neyts, J., 1998. Potent inhibition of hemangioma formation in rats by the acyclic nucleoside phosphonate analogue cidofovir. Cancer Res. 58, 2562–2567.

Liekens, S., Neyts, J., De Clercq, E., Verbeken, E., Ribatti, D., Presta, M., 2001a. Inhibition of fibroblast growth factor-2-induced vascular tumor formation by the acyclic nucleoside phosphonate cidofovir. Cancer Res. 61, 5057–5064.

Liekens, S., Verbeken, E., De Clercq, E., Neyts, J., 2001b. Potent inhibition of hemangiosarcoma development in mice by cidofovir. Int. J. Cancer 92, 161–167.

Lin, J.C., De Clercq, E., Pagano, J.S., 1991. Inhibitory effects of acyclic nucleoside phosphonate analogs, including (S)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine, on Epstein-Barr virus replication. Antimicrob. Agents Chemother. 35, 2440–2443.

Magee, W.C., Hostetler, K.Y., Evans, D.H., 2005. Mechanism of inhibition of vaccinia virus DNA polymerase by cidofovir diphosphate. Antimicrob. Agents Chemother. 49, 3153–3162.

Magee, W.C., Aldern, K.A., Hostetler, K.Y., Evans, D.H., 2008. Cidofovir and (S)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenine are highly effective inhibitors of vaccinia virus DNA polymerase when incorporated into the template strand. Antimicrob. Agents Chemother. 52, 586–597.

Magee, W.C., Valiaeva, N., Beadle, J.R., Richman, D.D., Hostetler, K.Y., Evans, D.H., 2011. Inhibition of HIV-1 by octadecyloxyethyl esters of (S)-[3-hydroxy-2-(phosphonomethoxy)propyl] nucleosides and evaluation of their mechanism of action. Antimicrob. Agents Chemother. 55, 5063–5072.

Meadows, K.P., Tyring, S.K., Pavia, A.T., Rallis, T.M., 1997. Resolution of recalcitrant molluscum contagiosum virus lesions in human immunodeficiency virus-infected patients treated with cidofovir. Arch. Dermatol. 133, 987–990.

Merta, A., Votruba, I., Rosenberg, I., Otmar, M., Hrebabecky, H., Bernaerts, R., Holy, A., 1990. Inhibition of herpes simplex virus DNA polymerase by diphosphates of acyclic phosphonylmethoxyalkyl nucleotide analogues. Antiviral Res. 13, 209–218.

Merta, A., Votruba, I., Jindrich, J., Holy, A., Cihlar, T., Rosenberg, I., Otmar, M., Herve, T.Y., 1992. Phosphorylation of 9-(2-phosphonomethoxyethyl)adenine and 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine by AMP(dAMP) kinase from L1210 cells. Biochem. Pharmacol. 44, 2067–2077.

Morrey, J.D., Korba, B.E., Beadle, J.R., Wyles, D.L., Hostetler, K.Y., 2009. Alkoxyalkyl esters of 9-(s)-(3-hydroxy-2-phosphonomethoxypropyl) adenine are potent and selective inhibitors of hepatitis B virus (HBV) replication in vitro and in HBV transgenic mice in vivo. Antimicrob. Agents Chemother. 53, 2865–2870.

Mul, Y.M., van Miltenburg, R.T., De Clercq, E., van der Vliet, P.C., 1989. Mechanism of inhibition of adenovirus DNA replication by the acyclic nucleoside triphosphate analogue (S)-HPMPApp influence of the adenovirus DNA binding protein. Nucleic Acids Res. 17, 8917–8929.

Naesens, L., Lenaerts, L., Andrei, G., Snoeck, R., Van Beers, D., Holy, A., Balzarini, J., De Clercq, E., 2005. Antiadenovirus activities of several classes of nucleoside and nucleotide analogues. Antimicrob. Agents Chemother. 49, 1010–1016.

Naesens, L., Bonnafous, P., Agut, H., De Clercq, E., 2006. Antiviral activity of diverse classes of broad-acting agents and natural compounds in HHV-6-infected lymphoblasts. J. Clin. Virol. 37 (Suppl 1), S69–75.

Neyts, J., De Clercq, E., 1997. Antiviral drug susceptibility of human herpesvirus 8. Antimicrob. Agents Chemother. 41, 2754–2756.

Okleberry, K.M., Warren, R.P., Smee, D.F., 1997. Metabolism of ganciclovir and cidofovir in cells infected with drug-resistant and wild-type strains of murine cytomegalovirus. Antiviral Res. 35, 83–90.

Painter, G.R., Hostetler, K.Y., 2004. Design and development of oral drugs for the prophylaxis and treatment of smallpox infection. Trends Biotechnol. 22, 423–427.

Palú, G., Stefanelli, S., Rassu, M., Parolin, C., Balzarini, J., De Clercq, E., 1991. Cellular uptake of phosphonylmethoxyalkylpurine derivatives. Antiviral Res. 16, 115–119.

Pauwels, R., Balzarini, J., Schols, D., Baba, M., Desmyter, J., Rosenberg, I., Holy, A., De Clercq, E., 1988. Phosphonylmethoxyethyl purine derivatives, a new class of anti-human immunodeficiency virus agents. Antimicrob. Agents Chemother. 32, 1025–1030.

Quenelle, D.C., Collins, D.J., Kern, E.R., 2003. Efficacy of multiple- or single-dose cidofovir against vaccinia and cowpox virus infections in mice. Antimicrob. Agents Chemother. 47, 3275–3280.

Quenelle, D.C., Collins, D.J., Wan, W.B., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2004. Oral treatment of cowpox and vaccinia virus infections in mice with ether lipid esters of cidofovir. Antimicrob. Agents Chemother. 48, 404–412.

Quenelle, D.C., Collins, D.J., Herrod, B.P., Keith, K.A., Trahan, J., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2007. Effect of oral treatment with hexadecyloxypropyl-[(S)-9-(3-hydroxy-2- phosphonylmethoxypropyl)adenine] [(S)-HPMPA] or octadecyloxyethyl-(S)-HPMPA on cowpox or vaccinia virus infections in mice. Antimicrob. Agents Chemother. 51, 3940–3947.

Redondo, P., Idoate, M., Galofre, J.C., Solano, T., 2000. Cidofovir inhibits growth of B16 melanoma cells in vivo. Br. J. Dermatol. 143, 741–748.

Reymen, D., Naesens, L., Balzarini, J., Holy, A., Dvorakova, H., De Clercq, E., 1995. Antiviral activity of selected acyclic nucleoside analogues against human herpesvirus 6. Antiviral Res. 28, 343–357.

Scott, G.M., Weinberg, A., Rawlinson, W.D., Chou, S., 2007. Multidrug resistance conferred by novel DNA polymerase mutations in human cytomegalovirus isolates. Antimicrob. Agents Chemother. 51, 89–94.

Shamoo, Y., Steitz, T.A., 1999. Building a replisome from interacting pieces: sliding clamp complexed to a peptide from DNA polymerase and a polymerase editing complex. Cell 99, 155–166.

Sherman, M.D., Feldman, K.A., Farahmand, S.M., Margolis, T.P., 2002. Treatment of conjunctival squamous cell carcinoma with topical cidofovir. Am. J. Ophthalmol. 134, 432–433.

Shigeta, S., Konno, K., Baba, M., Yokota, T., De Clercq, E., 1991. Comparative inhibitory effects of nucleoside analogues on different clinical isolates of human cytomegalovirus in vitro. J. Infect. Dis. 163, 270–275.

Smee, D.F., Bailey, K.W., Wong, M.-H., Sidwell, R.W., 2000. Intranasal treatment of cowpox virus respiratory infections in mice with cidofovir. Antiviral Res. 47, 171–177.

Smee, D.F., Sidwell, R.W., Kefauver, D., Bray, M., Huggins, J.W., 2002. Characterization of wild-type and cidofovir-resistant strains of camelpox, cowpox, monkeypox, and vaccinia viruses. Antimicrob. Agents Chemother. 46, 1329–1335.

Smee, D.F., Wandersee, M.K., Bailey, K.W., Hostetler, K.Y., Holy, A., Sidwell, R.W., 2005. Characterization and treatment of cidofovir-resistant vaccinia (WR strain)

virus infections in cell culture and in mice. Antivir. Chem. Chemother. 16, 203–211.
Smith, I.L., Taskintuna, I., Rahhal, F.M., Powell, H.C., Ai, E., Mueller, A.J., Spector, S.A., Freeman, W.R., 1998. Clinical failure of CMV retinitis with intravitreal cidofovir is associated with antiviral resistance. Arch. Ophthalmol. 116, 178–185.
Snoeck, R., Sakuma, T., De Clercq, E., Rosenberg, I., Holy, A., 1988. (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, a potent and selective inhibitor of human cytomegalovirus replication. Antimicrob. Agents Chemother. 32, 1839–1844.
Snoeck, R., Andrei, G., De Clercq, E., 1996. Patterns of resistance and sensitivity to antiviral compounds of drug-resistant strains of human cytomegalovirus selected in vitro. Eur. J. Clin. Microbiol. Infect. Dis. 15, 574–579.
Snoeck, R., Noel, J.C., Muller, C., De Clercq, E., Bossens, M., 2000. Cidofovir, a new approach for the treatment of cervix intraepithelial neoplasia grade III (CIN III). J. Med. Virol. 60, 205–209.
Spanos, W.C., El-Deiry, M., Lee, J.H., 2005. Cidofovir incorporation into human keratinocytes with episomal HPV 16 results in nonselective cytotoxicity. Ann. Otol. Rhinol. Laryngol. 114, 840–846.
Srinivas, R.V., Connely, M., Fridland, A., 1997. (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC) inhibits HIV-1 replication in epithelial cells, but not T-lymphocytes. Antiviral Res. 35, 23–27.
Talmon, G., Cornell, L.D., Lager, D.J., 2010. Mitochondrial changes in cidofovir therapy for BK virus nephropathy. Transplant Proc. 42, 1713–1715.
Tchesnokov, E.P., Obikhod, A., Schinazi, R.F., Gotte, M., 2008. Delayed chain termination protects the anti-hepatitis B virus drug entecavir from excision by HIV-1 reverse transcriptase. J. Biol. Chem. 283, 34218–34228.
Terry, B.J., Mazina, K.E., Tuomari, A.V., Haffey, M.L., Hagen, M., Feldman, A., Slusarchyk, W.A., Young, M.G., Zahler, R., Field, A.K., 1988. Broad-spectrum antiviral activity of the acyclic guanosine phosphonate (R, S)-HPMPG. Antiviral Res. 10, 235–251.
Topalis, D., Lebeau, I., Krecmerova, M., Andrei, G., Snoeck, R., 2011. Activities of different classes of acyclic nucleoside phosphonates against BK virus in primary human renal cells. Antimicrob. Agents Chemother. 55, 1961–1967.
Toro, J.R., Wood, L.V., Patel, N.K., Turner, M.L., 2000. Topical cidofovir: a novel treatment for recalcitrant molluscum contagiosum in children infected with human immunodeficiency virus 1. Arch. Dermatol. 136, 983–985.
Valiaeva, N., Prichard, M.N., Buller, R.M., Beadle, J.R., Hartline, C.B., Keith, K.A., Schriewer, J., Trahan, J., Hostetler, K.Y., 2009. Antiviral evaluation of octadecyloxyethyl esters of (S)-3-hydroxy-2-(phosphonomethoxy)propyl nucleosides against herpesviruses and orthopoxviruses. Antiviral Res. 84, 254–259.
Vesely, J., Merta, A., Votruba, I., Rosenberg, I., Holy, A., 1990. The cytostatic effects and mechanism of action of antiviral acyclic adenine nucleotide analogs in L1210 mouse leukemia cells. Neoplasma 37, 105–110.
Votruba, I., Bernaerts, R., Sakuma, T., De Clercq, E., Merta, A., Rosenberg, I., Holy, A., 1987. Intracellular phosphorylation of broad-spectrum anti-DNA virus agent (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine and inhibition of viral DNA synthesis. Mol. Pharmacol. 32, 524–529.
Watanabe, T., Tamaki, K., 2008. Cidofovir diphosphate inhibits molluscum contagiosum virus DNA polymerase activity. J. Invest. Dermatol. 128, 1327–1329.
Wyles, D.L., Patel, A., Madinger, N., Bessesen, M., Krause, P.R., Weinberg, A., 2005. Development of herpes simplex virus disease in patients who are receiving cidofovir. Clin. Infect. Dis. 41, 676–680.
Wyles, D.L., Kaihara, K.A., Korba, B.E., Schooley, R.T., Beadle, J.R., Hostetler, K.Y., 2009. The octadecyloxyethyl ester of (S)-9-[3-hydroxy-2-(phosphonomethoxy) propyl]adenine is a potent and selective inhibitor of hepatitis C virus replication in genotype 1A, 1B, and 2A replicons. Antimicrob. Agents Chemother. 53, 2660–2662.
Xiong, X., Smith, J.L., Kim, C., Huang, E.S., Chen, M.S., 1996. Kinetic analysis of the interaction of cidofovir diphosphate with human cytomegalovirus DNA polymerase. Biochem. Pharmacol. 51, 1563–1567.
Xiong, X., Smith, J.L., Chen, M.S., 1997. Effect of incorporation of cidofovir into DNA by human cytomegalovirus DNA polymerase on DNA elongation. Antimicrob. Agents Chemother. 41, 594–599.
Yokota, T., Konno, K., Chonan, E., Mochizuki, S., Kojima, K., Shigeta, S., de Clercq, E., 1990. Comparative activities of several nucleoside analogs against duck hepatitis B virus in vitro. Antimicrob. Agents Chemother. 34, 1326–1330.
Yokota, T., Mochizuki, S., Konno, K., Mori, S., Shigeta, S., De Clercq, E., 1991. Inhibitory effects of selected antiviral compounds on human hepatitis B virus DNA synthesis. Antimicrob. Agents Chemother. 35, 394–397.
Yoshida, M., Yamada, M., Tsukazaki, T., Chatterjee, S., Lakeman, F.D., Nii, S., Whitley, R.J., 1998. Comparison of antiviral compounds against human herpesvirus 6 and 7. Antiviral Res. 40, 73–84.
Yoshizaki, T., Wakisaka, N., Kondo, S., Murono, S., Shimizu, Y., Nakashima, M., Tsuji, A., Furukawa, M., 2008. Treatment of locally recurrent Epstein-Barr virus-associated nasopharyngeal carcinoma using the anti-viral agent cidofovir. J. Med. Virol. 80, 879–882.
Yu, K.L., Bronson, J.J., Yang, H., Patick, A., Alam, M., Brankovan, V., Datema, R., Hitchcock, M.J., Martin, J.C., 1992. Synthesis and antiviral activity of methyl derivatives of 9-[2-(phosphonomethoxy)ethyl]guanine. J. Med. Chem. 35, 2958–2969.
Zabawski Jr., E.J., Cockerell, C.J., 1999. Topical cidofovir for molluscum contagiosum in children. Pediatr. Dermatol. 16, 414–415.
Zhang, Y., Schols, D., De Clercq, E., 1999. Selective activity of various antiviral compounds against HHV-7 infection. Antiviral Res. 43, 23–35.