Antiviral Research 114 (2015) 21–46

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

# Insights into the mechanism of action of cidofovir and other acyclic nucleoside phosphonates against polyoma- and papillomaviruses and non-viral induced neoplasia

G. Andrei *, D. Topalis, T. De Schutter, R. Snoeck

*Laboratory of Virology and Chemotherapy, Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, Belgium*

ARTICLE INFO

*Article history:*
Received 3 March 2014
Revised 22 September 2014
Accepted 21 October 2014
Available online 30 October 2014

*Keywords:*
Cidofovir
Acyclic nucleoside phosphonates
PMEG
Human papillomavirus
Human polyomaviruses
Non-viral induced neoplasias

ABSTRACT

Acyclic nucleoside phosphonates (ANPs) are well-known for their antiviral properties, three of them being approved for the treatment of human immunodeficiency virus infection (tenofovir), chronic hepatitis B (tenofovir and adefovir) or human cytomegalovirus retinitis (cidofovir). In addition, cidofovir is mostly used off-label for the treatment of infections caused by several DNA viruses other than cytomegalovirus, including papilloma- and polyomaviruses, which do not encode their own DNA polymerases. There is considerable interest in understanding why cidofovir is effective against these small DNA tumor viruses. Considering that papilloma- and polyomaviruses cause diseases associated either with productive infection (characterized by high production of infectious virus) or transformation (where only a limited number of viral proteins are expressed without synthesis of viral particles), it can be envisaged that cidofovir may act as antiviral and/or antiproliferative agent. The aim of this review is to discuss the advances in recent years in understanding the mode of action of ANPs as antiproliferative agents, given the fact that current data suggest that their use can be extended to the treatment of non-viral related malignancies.



**Contents**

1. Introduction . . . 22
2. Similarities and differences between polyomaviruses (PyVs) and papillomaviruses (PVs) . . . 22
3. Clinical impact of human polyomaviruses . . . 29
4. Clinical importance of human papillomaviruses (HPVs) . . . 30
5. PME derivatives . . . 32
   5.1. *In vitro* and *in vivo* antiproliferative activities . . . 32
   5.2. Mechanism of antiproliferative effects of PME derivatives . . . 33
6. Cidofovir (CDV) activity against viruses not encoding for their own DNA polymerases . . . 35
   6.1. *In vitro*, *in vivo* and clinical evidences for the anti-polyomavirus activity of CDV . . . 35
   6.2. Polyomavirus replicative cycle: potential targets for CDV . . . 36
   6.3. *In vitro*, *in vivo* and clinical evidences for the anti-papillomavirus activity of CDV . . . 36
   6.4. Why is CDV selective against HPV-induced hyperproliferation? . . . 37
7. Extension of the clinical use of CDV for the treatment of tumors not induced by oncogenic viruses . . . 38
8. Can the antitumor activity of CDV be synergized? . . . 40
9. Conclusions and perspectives . . . 41
   Acknowledgements . . . 42
   References . . . 42

* Corresponding author at: Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. Tel.: +32 16 33 73 72; fax: +32 16 33 73 40.
*E-mail address:* graciela.andrei@rega.kuleuven.be (G. Andrei).

http://dx.doi.org/10.1016/j.antiviral.2014.10.012
0166-3542/

## 1. Introduction

The acyclic nucleotide analogue cidofovir {(CDV), 1-[(*S*)-3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine, HPMPC}, displays potent activity against a broad spectrum of DNA viruses. The intravenous formulation of CDV has been formerly licensed for the treatment of human cytomegalovirus (HCMV) retinitis in AIDS patients in 1996. However, this compound is mostly used off-label for the treatment of severe infections caused by various DNA viruses other than HCMV (De Clercq, 2007, 2011). Different formulations of CDV have been employed for the management of acyclovir resistant and/or foscavir resistant herpes simplex virus infections, poxvirus-associated diseases including molluscum contagiosum virus and orf virus, life-threatening adenovirus and human polyomavirus (PyV) infections as well as human papillomavirus (HPV)-associated hyperproliferative diseases. A summary of the applications of CDV as an antiviral and antiproliferative agent in the treatment of human diseases is presented in Table 1.

CDV belongs to the class of acyclic nucleoside phosphonates (ANPs), which are well-known for their antiviral properties. In addition to CDV, two other ANPs got approval for the treatment of viral infections (De Clercq and Holy, 2005; De Clercq, 2007, 2006). Tenofovir {PMPA, (*R*)-9-[2-(phosphonylmethoxy)propyl]adenine} and adefovir {PMEA, 9-[(2-phosphonylmethoxy)ethyl]adenine} are active against retro- and hepadnaviruses, their oral prodrugs forms being licensed for the therapy of human immune deficiency virus (HIV) (tenofovir) and of chronic hepatitis B virus (HBV) infections (tenofovir and adefovir).

ANPs have been shown to enter the cell by an endocytosis-like process and they are converted intracellularly to their diphosphate metabolites by cellular enzymes (De Clercq and Holy, 2005). The diphosphate forms of the ANPs (i.e. CDVpp, PMEApp and PMPApp) interact as competitive inhibitors/alternative substrates with respect to the normal substrates (i.e. dCTP and dATP). Incorporation of one molecule of PMEApp or PMPApp into the growing DNA strand results inevitably in DNA chain termination whereas CDVpp requires two consecutive incorporations to efficiently terminate DNA synthesis, as has been shown for HCMV (Xiong et al., 1996, 1997). The selective antiviral activity of ANPs results from the higher affinity of the ANPpp for viral DNA polymerases [that is herpesvirus and poxvirus DNA polymerases and HIV or HBV reverse transcriptases] than for cellular DNA polymerases α, δ, and ε. Fig. 1 illustrates the intracellular activation of CDV and its mode of action against viruses encoding for their own DNA polymerases. The mechanism of action of ANPs as antiviral agents has been extensively summarized in various reviews (De Clercq, 2003, 2007, 2011; Andrei and Snoeck, 2010; De Clercq and Holy, 2005) and will not be further discussed here.

Besides their well-recognized antiviral characteristics, CDV as well as some PME derivatives, such as PMEA, PMEDAP {9-[(2-phosphonylmethoxy)ethyl]-2,6-diaminopurine} and PMEG {9-[(2-phosphonylmethoxy)ethyl]guanine} (Fig. 2), possess antiproliferative properties, although their mechanisms of antitumor efficacy appear to be dissimilar considering that CDV is not an obligate chain terminator, in contrast to the PME derivatives, and that the effects of CDVpp on cellular DNA polymerization are weaker compared to the diphosphate forms of the PME derivatives (Wolfgang et al., 2009).

In this review, we focus on the antiproliferative activities of ANPs and we debate on their mode of action against viruses, such as polyomaviruses (PyVs) and papillomaviruses (PVs) that do not encode for their own DNA polymerases. Also, the potential use of ANPs for the treatment of non-viral induced tumors will be discussed.

## 2. Similarities and differences between polyomaviruses (PyVs) and papillomaviruses (PVs)

Until 2000, PVs and PyVs were grouped together in the family Papovaviridae (“pa–po–va” stands for papilloma–polyoma–vacuoliting agent SV40). Since then, the family Papovaviridae is obsolete and the Papillomaviridae and Polyomaviridae families were recognized by the International Committee on Taxonomy of Viruses (ICTV) (Johne et al., 2011; de Villiers et al., 2004).

Table 2 summarizes the main similarities and differences between PyVs and PVs. These two viral families have a non-enveloped icosahedral capsid (composed of 72 capsomers) surrounding a double-stranded circular DNA genome of ~5 kbp in PyVs and of ~8 kbp in PVs. Both viruses use overlapping genes and differential splicing to pack the maximum amount of genetic material in the minimum space. All open reading frames (ORFs) are located on only one (PVs) or both (PyVs) strands of DNA, as depicted in Figs. 3A and 4A, respectively. Two classes of genes, the early (E) genes (which are required for viral DNA replication) and late (L) genes (coding for the structural proteins) exist in both PyVs and PVs.

**Table 1**
Clinical uses of cidofovir as an antiviral and antiproliferative agent, either approved by the US Food and Drug Administration or supported by clinical data (De Clercq, 2003, 2006, 2011; Snoeck and De Clercq, 2002). Herpes simplex 1 (HSV-1) and 2 (HSV-2), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), human herpesvirus 6 (HHV-6), 7 (HHV-7), 8 (HHV-8). *Foscavir: foscarnet* sodium injection.

| Route of administration | Clinical indication |
|---|---|
| Systemic (intravenous) | • HCMV retinitis in AIDS patients (approved)<br>• HSV-1, HSV-2 and VZV infections (particularly those that are resistant to acyclovir and/or foscavir)<br>• HCMV infections, mainly those resistant to ganciclovir due to mutations in the UL97 gene<br>• EBV, HHV-6, HHV-7 and HHV-8 (Kaposi’s sarcoma associated herpesvirus) infections<br>• Polyomavirus infections due to JCPyV [progressive multifocal leukoencephalopathy (PML)] and polyoma BKPyV [hemorrhagic cystitis]<br>• Systemic adenovirus infections |
| Systemic (intravenous) or topical (gel/cream) | • Molluscum contagiosum, orf and other poxvirus infections such as monkeypox and smallpox<br>• Complications of smallpox vaccine (vaccinia) |
| Topical (gel/cream) | • Mucocutaneous HSV-1 or HSV-2 infections (particularly those resistant to acyclovir and/or foscavir |
| Topical (eyedrops) | • Keratoconjunctivitis due to HSV or adenovirus |
| Topical (intravitreal) | • HCMV retinitis |
| Topical (gel/cream), intralesional injection, infrequently systemic administration) | • Human papillomavirus-associated lesions:<br>– Recurrent laryngeal papillomatosis<br>– Anogenital warts<br>– Common warts<br>– Cervical/vulvar/anal/penile intraepithelial neoplasia |