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Evaluation of Cidofovir (HPMPC, GS-504) against adenovirus type 5 infection in vitro and in a New Zealand rabbit ocular model

Clarissa B. R. de Oliveira, Douglas Stevenson, Laurie LaBree, Peter J. McDonnell, Melvin D. Trousdale*

The Doheny Eye Institute, the Department of Ophthalmology and the Department of Preventive Medicine, University of Southern California School of Medicine, Los Angeles, CA 900033, USA

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Abstract

The antiviral inhibitory activity of Cidofovir [1-[(*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate, HPMPC, GS-504] against adenovirus type 5 (Ad5) in the New Zealand rabbit ocular replication model was evaluated. The 50% inhibitory dose (ID_{50}) of Cidofovir was determined to be 4.7–9.5 μg/ml against four adenoviruses (two Ad5, Ad8 and Ad14) by plaque reduction assay in A549 cells. Twenty-four New Zealand rabbits received intrastromal inoculation and topical application of 2×10^6 plaque-forming units (PFU) per eye of Ad5 McEwen, a clinical isolate. Cidofovir was administered topically at three different concentrations twice per day, beginning 16 h postinoculation and continuing for 20 consecutive days. The inhibitory effects were determined by measuring suppression of virus replication and by observation of the clinical effects. Compared to the placebo group, the 1% and 0.5% Cidofovir-treated groups showed significantly reduced Ad5 ocular titers, fewer days of viral shedding and less severe subepithelial opacities ($P = 0.0001$). The 1% Cidofovir group had the lowest humoral antibody titer against adenovirus antigens, but the difference was not significant ($P = 0.24$). Cidofovir proved to have potent antiviral activity against adenovirus replication and may have great promise for the treatment of adenovirus infection. Further investigation is recommended.

Keywords: Adenovirus; Antiviral; Cidofovir; HPMPC; Nucleotide analogue

1. Introduction

Ocular adenoviral infections occur world-wide and are associated with epidemics (Gordon et al., 1991a). Two important syndromes are caused by adenovirus: epidemic keratoconjunctivitis (EKC) and pharyngoconjunctival fever (PCF). In the most common of these, EKC, the virus is highly

* Corresponding author. Address: Doheny Eye Institute, 1450 San Pablo St., Los Angeles, CA 90033, USA. Fax: + 213 342 6688.

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contagious, causing ocular manifestations of follicular and papillary conjunctivitis, punctate keratitis, anterior uveitis and subepithelial corneal opacities. Although various adenovirus subtypes have been implicated in the etiology of these ocular syndromes, the most commonly associated adenovirus types are Ad8 and Ad19 (Tsai et al., 1992). Since different adenovirus serotypes can cause EKC, subsequent infection with another serotype often occurs, causing long-lasting immunity to each serotype.

At the moment, there is no effective topical antiviral therapy to inhibit viral replication and reduce the symptoms and duration of the disease (Gordon et al., 1992a). The available antivirals neither prevent nor lessen the corneal sequelae induced by virus infection. Nor is there any treatment to prevent transmission of the virus to either the second eye or to other people.

Cidofovir is a new, broad-spectrum, antiviral agent. It is a potent phosphonate nucleotide analogue that displays activity against cytomegalovirus infections and against a range of other herpesviruses including acyclovir-resistant herpes simplex virus (Lalezari et al., 1994; Yang and Datema, 1991). Cidofovir has been shown to work by inhibition of viral DNA polymerase and appears to be potent and selective in blocking DNA synthesis (Gordon et al., 1992b; Chatterjee et al., 1992; Neyts et al., 1991).

In vitro antiviral activity of Cidofovir against adenoviral isolates (Ad1, Ad5, Ad8) from patients with EKC was previously reported by Gordon et al. (1991b). In vivo studies have demonstrated that Cidofovir inhibits Ad5 replication in the New Zealand rabbit ocular model (Gordon et al., 1992b). When Cidofovir was administered for up to 10 days it significantly reduced Ad5 ocular titers and decreased the number of virus-shedding days. The antiviral action of Cidofovir was shown to last for several days or even weeks both in vitro and in vivo, demonstrating the prolonged efficacy of the drug. The purpose of the present study was to evaluate Cidofovir in vitro and to determine the efficacy of three different concentrations of Cidofovir against Ad5 McEwen in a New Zealand rabbit ocular model. We evaluated the amount and duration of viral shedding in ocular cultures and the severity of keratoconjunctivitis during and after Cidofovir therapy.

2. Materials and methods

2.1. Reagent, cells and viruses

The Ad5 McEwen strain was provided by Y.J. Gordon, M.D. (Eye and Ear Institute of Pittsburgh, PA), and Ad5 WT from Thomas E. Shenk, Ph.D. (Purdue University). The Ad8 and Ad14 were purchased from American Type Culture Collection (ATCC, Rockville, MD). A549 cells, an epithelial-like cell derived from human lung carcinoma (CCl-185, ATTC), were used for virus isolation and plaque assays. These cells were grown and mantained in minimum essential medium (MEM), supplemented with 10% heat inactivated fetal bovine serum (FBS), 200 units of penicilin, 100 μg/ml of gentamicin, 2 μg/ml of amphotericin B and 200 μg/ml of streptomycin.

2.2. Animals

Twenty-four New Zealand albino female rabbits, weighing 1.5–2 kg each were obtained from Irish Farms (Los Angeles, CA). All animal investigations conformed to the ARVO Resolution on the Use of Animals in Research, and all animals were housed in facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

2.3. Virus inoculation

The rabbits were anesthetized by intramuscular injection of four parts ketamine (100 mg/ml) plus one part xylazine (100 mg/ml) using 0.2 ml/kg body weight. 0.5% Proparacaine hydrochloride was applied topically to each eye and the eyes were then inoculated with 1×10^6 PFU (50 μl) of Ad5 McEwen intrastromally in five different sites to form five focal blebs (dice pattern, 10 μl per bleb). The cornea was scarified superficially (eight scratches) around the central intrastromal injection. This procedure was followed by topical application of an additional 50 μl of AD5 McEwen.

The lids were then closed and the eyes massaged for 30 s.

2.4. Examination of rabbit eyes

Two uninformed observers examined the rabbit eyes by slit-lamp biomicroscopy, every other day after virus inoculation. Conjunctivitis, cornea edema and iritis were evaluated and scored as follows: none (0), mild (1), moderate (2) and severe (3). Corneal subepithelial opacities were scored similarly: none (0), up to 5 opacities (1), 5–10 opacities (2) and over 10 opacities (3).

2.5. Cultures

Each eye was rinsed with 30 ml Dulbecco's Phosphate Buffered Solution (DPBS) at 3 h postinoculation. The first ocular swab in each eye was performed 4 h after rinsing. Each eye was swabbed in the upper and lower fornices and around the scratched areas with a cotton applicator. The swab was then placed in 1 ml maintenance media and frozen at −70°C. Swabs were taken daily through day 21. Ocular viral titers were determined by plaque assay.

2.6. Antiviral compound

Cidofovir solutions were provided as part of a research grant from Storz Ophthalmics (Pearl River, NY). Cidofovir was prepared for topical use in three different concentrations (1%, 0.5% and 0.1%) in an aqueous formulation of phosphate buffer, sodium chloride, benzalkonium chloride, and edetate disodium (pH 7–7.4). Control eye drops consisted of the vehicle alone. The rabbits were divided into four coded treatment groups of six animals each (placebo, 1% Cidofovir, 0.5% Cidofovir and 0.1% Cidofovir). Each animal was treated topically with a coded eye drop. The topical drug was delivered bilaterally, twice per day beginning approximately 16 h postinoculation, for 20 consecutive days.

2.7. Determination of viral titer

The ocular swabs were thawed in a 37°C water bath and serially diluted 10-fold through 10^{-3}. Each dilution (0.1 ml/well) was inoculated into duplicate wells onto A549 monolayers in a 24-well plate. The inoculated plate was held for 3 h at 37°C in a 5% carbon dioxide–water vapor atmosphere for virus adsorption. After 3 h the wells were rinsed with Hanks Balanced Salt Solution (HBSS), then overlayed with 0.5 ml/well of 0.5% agar in MEM base media. The plate was incubated at 37°C in a 5% carbon dioxide–water vapor atmosphere. The wells were examined daily for 7 days for the presence of cytopathic effects (CPE). Each plate was stained with 0.5% gentian violet on day 7 postinoculation. The number of plaques per well was counted and scored as PFU/ml.

2.8. In vitro determination of 50% inhibitory dose (ID_{50}) of Cidofovir

100 PFU of isolates of Ad5 McEwen, Ad5 WT, Ad14 or Ad8 (0.1 ml/well) were inoculated onto A549 monolayers grown in 24-well plates and incubated at 37°C 3 h for adsorption. The monolayers were rinsed with HBSS and triplicate wells overlayed with 0.5% agar containing one of six different concentrations (100, 10, 1, 0.1, 0.01, 0.001 μg/ml) of Cidofovir. Triplicate wells received an agar overlay containing no drug. Daily examination of the wells was perfomed to assess CPE development. The plate was stained as described above, the number of viral plaques per well was counted, and ID_{50} values were calculated.

2.9. Humoral antibody response

The rabbits were sacrificed on day 21 postinoculation, and peripheral blood was collected and processed for serum. Serum samples were frozen at −20°C, then later tested for humoral antibody response using standard ELISA (Voller et al., 1978).

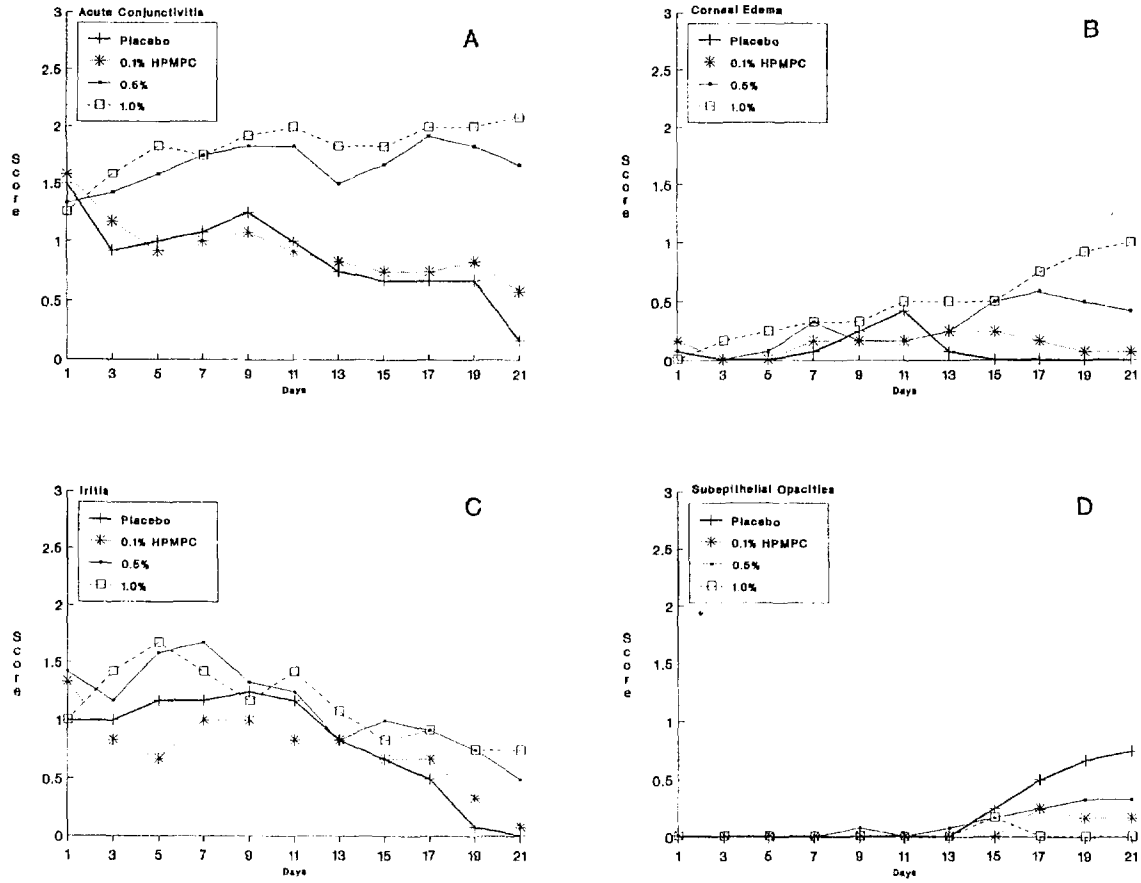

Fig. 1. Comparison of the severity score of different clinical signs (acute conjunctivitis, corneal edema, iritis and subepithelial opacities) in NZ rabbit eyes following the administration of three different concentrations of Cidofovir.

2.10. Statistical methods

The data was analyzed statistically after the experiment was completed. Analysis of variance was used to test for differences in acute conjunctivitis, corneal edema, iritis and subepithelial opacities between the four treatment groups. Where overall significance was observed, multiple comparison t-tests were performed. For non-normally distributed data, Kruskal-Wallis tests were used. The significance level for all analyses was $P \leqslant 0.05$.

3. Results

3.1. In vitro determination of 50% inhibitory dose (ID_{50}) of Cidofovir

A comparison of the antiviral inhibitory activity of Cidofovir between strains and serotypes of adenovirus showed the mean ID_{50} for Ad5 WT, Ad5 McEwen, Ad14 and Ad8 to be 9.5, 7.3, 5.4 and 4.7 μg/ml, respectively. The differences were not statistically significant among the different serotypes.

3.2. Animal studies

Ocular examination of the rabbits revealed acute conjunctivitis was present on day 1 postinoculation (Fig. 1). A moderate grade of acute conjunctivitis was associated with the 1% and 0.5% Cidofovir concentration groups. The 0.1% Cidofovir concentration group and the placebo group demonstrated a graded reduction in the severity of acute conjunctivitis ($P = 0.0001$), with a peak at day 9, and showed a further reduction after day 19 (Table 1).

Mild iritis was found in all groups on day 1 after inoculation. A marked reduction in the severity of iritis was observed in the placebo group following day 11. The 1% Cidofovir concentration group showed peaks on days 5 and 11, followed by a decrease in iritis severity; however the decrease was not significant. Statistically significant differences were documented between the 1% and 0.5% Cidofovir treatment groups and both the placebo and the 0.1% Cidofovir groups ($P = 0.0001$).

The corneal edema evaluation demonstrated some statistically significant differences between groups. There was an increase in the severity of edema with the Cidofovir treated groups.

In all groups, corneal subepithelial opacities began to appear between days 13 and 17. We observed that these opacities had a small nummular or coinlike shape and were generally localized in the center of the cornea. The opacities were most prevalent in the placebo group and a statistically significant difference was found between the placebo group and the 1% and 0.5% Cidofovir groups. For the treated groups, corneal subepithelial opacities were progressively reduced as the concentration of Cidofovir was increased. 1% Cidofovir-treated eyes had no opacities from days 17–21.

Table 1
Analysis of variance among treatment groups*

Parameters measured	P**	Pairwise comparisons***
Conjunctivitis	0.0001	b, c < a, d
Edema	0.0001	b, c < a < d
Iritis	0.0001	c, b < d, a
Opacities	0.0001	d < a, bc < b
Virus in tears****	0.0001	a, d < b, c

*Code: b = placebo, c = 0.1%, a = 0.5%, d = 1%
**Analysis of variance P-value among drug groups, controlling for day.
***Groups are listed in ascending mean order. Groups separated by a comma are not statistically significantly different. Groups separated by < are statistically significantly different using Tukey's multiple comparison adjustment ($P < 0.05$).
****Amount of infectious Ad5 in ocular specimens during the treatment period is presented in Fig. 2.

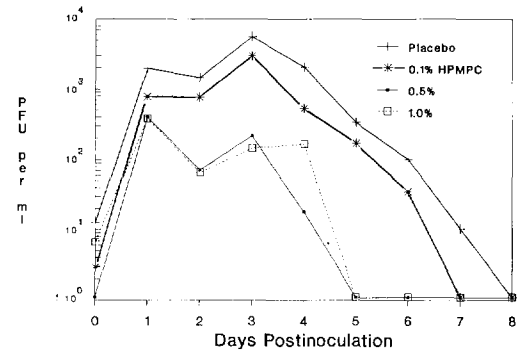

Fig. 2. Amount of infectious Ad5 present in ocular specimens during the treatment period.

Groups treated with higher concentrations of Cidofovir showed stronger grades of acute conjunctivitis, corneal edema and iritis suggesting possible ocular irritation by Cidofovir. In addition, ocular discharge occurred in most rabbits beginning on day 7 postinoculation and severe follicular hypertrophy was present in the upper fornices of some rabbit eyes.

3.3. Determination of Ad5 in tear samples

1% and 0.5% Cidofovir treatment groups demonstrated a significant reduction in Ad5 ocular titers as compared to the placebo group (Fig. 2) ($P = 0.0001$). All groups showed peaks between days 1 and 4 after virus inoculation. Marked reduction in the duration of viral ocular shedding was seen in the Cidofovir treatment groups when compared with the placebo group. The duration of shedding was 7 days for the placebo-treated group, 6 days for the 0.1% Cidofovir-treated group, and 4 days for 0.5% and 1% Cidofovir-treated groups.

3.4. Serological analysis determined by ELISA

The mean ELISA antibody titer for the placebo group was 20 267. The mean antibody titers for the treated groups were 20 933, 18 133 and 6667, respectively, for the 0.1%, 0.5% and 1% Cidofovir-treated groups. No statistically significant differences were found between any of the groups ($P > 0.05$), although the lower mean serum antibody titer of animals treated with 1% Cidofovir is compatible with the expected outcome of an effective treatment which would suppress virus replication, reduce the antigenic load and possibly result in a decreased immune response.

4. Discussion

Despite the self-limited nature of the EKC disease, patients can experience numerous symptoms which may eventually lead to continued morbidity. The chronic phase, marked by corneal involvement, can lead to long-term corneal sequelae. Available therapy is limited to symptomatic treatment (Ford et al., 1987) and an effective and potent antiviral is needed. The antiviral Cidofovir has been used in the past to treat other virus infections and has shown promising results. Cidofovir has potent inhibitory activity against cytomegalovirus (CMV) infection and has been reported to be more effective that the antiviral drugs normally used such as acyclovir, ganciclovir or foscarnet. The antiviral activity of Cidofovir in CMV infections was found to be dependent on drug concentration (Otova et al., 1992). Neyts et al. (1992, 1993) and Snoeck et al. (1988) reported Cidofovir to have great efficacy against murine CMV infections in immunodeficient mice and to be a potent inhibitor of CMV plaque formation in in vitro experiments. A synergistic inhibition of cytomegalovirus replication shown by Cidofovir in combination with these other drugs may be promising for future treatment of CMV infections (Snoeck et al., 1992).

Antiviral agents have an important role in the treatment of herpesvirus infection. Cidofovir showed a strong inhibitory effect against herpes simplex virus types 1 and 2 (HSV1 and HSV2) in vitro and in vivo (De Clercq and Holy, 1991; De Clercq, 1993). It exhibited greater activity than acyclovir against a thymidine kinase-deficient strain of HSV1 and in immunocompromised patients with HSV1-related mucocutaneous infections showed usefulness in treating the viral infections secondary to AIDS (Snoeck et al., 1994). For keratitis caused by the HSV1 thymidine kinase-positive strain, it has been shown that Cidofovir stimulates healing as compared to the placebo group (Maudgal and De Clercq, 1991). In primary genital HSV2 infections of guinea pigs and mice results indicated that topical therapy with 1%, 0.5% or 0.3% Cidofovir was also more effective than 5% acyclovir (Bravo et al., 1993). A protective effect against HSV2 infection was demonstrated in a mouse model after a single administration of Cidofovir (Yang and Datema, 1991). In established retinitis caused by HSV, early treatment with Cidofovir markedly delayed the progression of the infection and had a prolonged antiviral effect, proving Cidofovir to be superior to ganciclovir in the treatment of this condition (Flores-Aguilar et al., 1994). Results indicated that Cidofovir was efficacious in inhibiting CMV retinitis and was not toxic to the rabbit retina at the effective dose required to suppress infection (Dolnak et al., 1992).

When several new antiviral agents were tested in vitro, it was found that Cidofovir, S-HPMPA and 2′-nor-cyclic GMP have significant inhibitory activity against several adenoviral serotypes. It was concluded that the effect of these drugs is serotype-dependent. Of the three drugs tested, Cidofovir was the least toxic (Gordon et al., 1991a). Direct local ocular toxicity was not clinically significant at a total dose of less than 10 mg/eye of Cidofovir over 10 days in a New Zealand rabbit ocular model (Gordon et al., 1994).

Prevention studies involving 1 day of pretreatment before inoculation and continuing for 4 additional days showed a significant reduction in the peak viral eye titer and shortened the duration of ocular shedding (Gordon et al., 1992b). Gordon reported that topical Cidofovir treatment for up to 10 days significantly reduced both Ad5 ocular titers and the number of days of viral

shedding compared with that for control eyes (Gordon et al., 1994). In the present study, Cidofovir demonstrated high antiviral activity by suppression of viral replication and decreased duration of ocular shedding. The observation that significant anti-viral activity was achieved with twice daily dosing indicates that Cidofovir has a long-lasting effect relative to other topical anti-viral compounds. Previous studies have shown that a single topical dose of Cidofovir can prevent or reduce the spread of the virus. This could be an important prophylactic method in the case of epidemics that can occur in communities, hospitals, schools and military institutions, and that can lead to substantial economic repercussions (Ford et al., 1987). A reduction in the corneal subepithelial opacities occurred in the 1% and 5% Cidofovir treatment groups. Subepithelial opacities are an important cause of prolonged morbidity in patients and preventing their formation would avoid the need for chronic corticosteroid therapy, with its related complications. As in the clinical situation, corticosteroid use in our model does decrease the subepithelial opacities (Nóbrega et al., 1993). In this experiment, ocular irritation by Cidofovir was found to be dose-dependent. The clinical signs of iritis, corneal edema and conjunctivitis observed in the rabbit were associated with the higher concentrations of the drug. No clinical signs of systemic toxicity were noticed in any rabbits.

The clinical parameters in Ad-induced ocular disease vary substantially in humans and in animal eye models used to study the disease. The actual value of the use of clinical parameters to evaluate antiviral efficacy is uncertain. Gordon states: "Unfortunately, the use of clinical parameters in the disease ocular model (e.g., acute conjunctivitis, subepithelial infiltrates) to evaluate antiviral efficacy did not prove to be reliable because of the confounding variable of intrastromal injection." (Gordon et al., 1994). We feel that it is important to report clinical parameter data, but like Gordon we place more value on virus replication data for efficacy studies.

Our experiment was performed to confirm the efficacy of Cidofovir against ocular Ad infection and to determine whether it reduced morbidity. Future experiments utilizing this model may be modified to obtain more precise results with respect to the clinical findings. The antiviral Cidofovir effectively reduced Ad5 ocular titers in the New Zealand rabbit ocular model and is a promising candidate for the treatment of adenoviral infections. Further studies of the safety of Cidofovir should be performed before testing in patients.

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References

Bravo, F.J., Stanberry, L.R., Kier, A.B., Vogt, P.E. and Kern, E.R. (1993) Evaluation of Cidofovir therapy for primary and recurrent genital herpes in mice and guinea pigs. Antiviral Res. 21, 59–72.

Chatterjee, S., Burns, P., Whitley, R. J. and Kern, E.R. (1992) Effect of (*S*)-1-[(3-hydroxy-2-phosphonyl methoxy] propyl) cytosine on the replication and the morphogenesis of herpes simplex virus type 1. Antiviral Res. 19, 181–192.

De Clercq, E. (1993) Antivirals for the treatment of herpesvirus infections. J. Antimicrob. Chemother. 32, 121–132.

De Clercq, E. and Holy, A. (1991) Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine in various models of herpes simplex virus infection in mice. Antimicrob. Agents Chemother. 35, 701–706.

Dolnak, D.R., Munguia, D., Wiley, C.A., De Clercq, E., Bergeron-Lynn, L.G.L., Boscher, C., Connor, J.D., Sherwood, C., Capparelli, E. and Armani, R. (1992) Lack of retinal toxicity of the anticytomegalovirus drug (*S*)-1-(3-hydroxy-2 phosphonylmethoxypropyl)cytosine. Invest. Ophthalmol. Vis. Sci. 33, 1557–1563.

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

Ford, E., Nelson, K.E. and Warren, D. (1987) Epidemiology of epidemic keratoconjunctivitis. Epidemiol. Rev. 9, 244–261.

Gordon, Y.J., Araullo-Cruz, T., Romanowski, E., Myers, B., Santora, D., Lin, M. and Kowalski, R. (1991) Replication of ocular isolates of human adenovirus is serotype-dependent in rabbit. Curr. Eye Res. 10, 267–271.

Gordon, Y.J., Romanowski, E., Araullo-Cruz, T., Seaberg, L., Erzurum, S., Tolman, R. and De Clercq, E. (1991) Inhibitory effect of (*S*)-Cidofovir, (*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., Romanowski, E. and Araullo-Cruz, T. (1992a) An ocular model of adenovirus type 5 infection in the NZ rabbit. Invest. Ophthalmol. Vis. Sci. 33, 574–579.

Gordon, Y.J., Romanowski, E., Araullo-Cruz, T. and De Clercq, E. (1992b) Pretreatment with topical 0.1% (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine inhibits adenovirus type 5 replication in the New Zealand rabbit ocular model. Cornea 11, 529–533.

Gordon, Y.J., Romanowski, E.G. and Araullo-Cruz, T. (1994) Topical Cidofovir inhibits adenovirus type 5 in the NZ rabbit ocular replication model. Invest. Ophthalmol Vis. Sci. 35, 4135–4143.

Lalezari, J.P., Drew, W.L., Glutzer, E., Miner, D., Safrin, S., Owen Jr., W.F., Davidson, J.M., Fisher, P.E. and Jaffe, H.S. (1994) Treatment with intravenous (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine of acyclovir-resistant mucocutaneous infection with herpes simplex virus in a patient with AIDS. J. Infect. Dis. 170, 570–572.

Maudgal, P.C. and De Clercq, E. (1991) (*S*)-1-(3-hydroxy-2 phosphonylmethoxypropyl) cytosine in the therapy of thymidine kinase-positive and deficient herpes simplex virus experimental keratitis. Invest. Ophthalmol. Vis. Sci. 32, 1816–1820.

Neyts, J., Snoeck, R., Balzarini, J., and De Clercq, E. (1991) Particular characteristics of the anti-human cytomegalovirus activity of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir) in vitro. Antiviral Res. 16, 41–52.

Neyts, J., Balzarini, J., Naesens, L. and De Clercq, E. (1992) Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice. J. Med. Virol. 37, 67–71.

Neyts, J., Sobis, H., Snoeck, R., Vandeputte, M. and De Clercq, E. (1993) Efficacy of (*s*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine in the treatment of intra cerebral murine cytomegalovirus infection in imunocompetent and imunodeficient mice. Eur. J. Clin. Microb. Infect. Dis. 12, 269–279.

Nóbrega, R., McDonnell, P.J., Nakamura, T and Trousdale, M.D. (1993) Induction of ocular disease in rabbits by human adenovirus type-5 (E3 +) and a E3 − deletion mutant and treatment with prednisolone. Invest. Ophthalmol. Vis. Sci. 34 (suppl.), 850. Abstract.

Otova, B., Votruba, I. and Holy, A. (1992) Pretreatment of the host cell with 1-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir) is sufficient for its antiviral effect. Acta Virol. 36, 313–319.

Snoeck, R., Sakuma, T., De Clercq, E., Rosenberg, I. and 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., Schols, D., Balzarini, J. and De Clercq, E. (1992) Activity of different antiviral drug combinations against human cytomegalovirus replication in vitro. Eur. J. Clin. Microbiol. Infect. Dis. 11, 1144–1155.

Snoeck, R., Andrei, G., Gerard, M., Silverman A., Hedderman, A., Balzarini, J., Sadzot, D.C., Tricot, G., Clumeck, N. and De Clercq, E. (1994) Successful treatment of progressive mucocutaneous infection due to acyclovir- and foscarnet-resistant herpes simplex virus with (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir). Clin. Infect. Dis. 18, 570–578.

Tsai, J.C., Garlinghouse, G., McDonnell, P.J. and Trousdale, M.D. (1992) An experimental animal model of adenovirus-induced ocular disease. The cotton rat. Arch. Ophthalmol. 110, 1167–1170.

Voller A., Bartlett A. and Bidwell D.E. (1978) Enzyme immunoassays with special reference to ELISA techniques. J. Clin. Pathol 31, 507 520.

Yang, H. and Datema, R. (1991) Prolonged and potent therapeutic and prophylactic effects of (*S*)-1-[(3-hydroxy-2-phosphonylmethoxy)propyl]cytosine against herpes simplex virus type 2 infections in mice. Antimicrob. Agents Chemother. 35, 1596–1600.