Lack of Retinal Toxicity of the Anticytomegalovirus Drug (S)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl) Cytosine

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The drug (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) is an antiherpes virus group compound with a higher potency and longer duration of action against human cytomegalovirus (CMV) than ganciclovir or foscarnet. Twenty eyes of ten New Zealand white rabbits received 0.1-ml injections of either normal saline or HPMPC at doses of 10, 50, 100, 300, or 1000 ng. The animals were killed on days 14 and 28. Toxicity was assessed by indirect ophthalmoscopy, electroretinography (ERG), and light and electron microscopy. Both a- and b-wave ERG findings and indirect ophthalmoscopic appearance of retinas in all groups were normal. Light and electron microscopy of perfusion-fixed retinal tissue revealed no morphologic changes at doses of 100 ng or lower. The pharmacokinetics of eight rabbits injected intravitreally with 100 ng of HPMPC showed a 24.4-hr half-life for the drug. These results indicate that HPMPC is not toxic to the rabbit retina at 500-1000-fold the dose that is effective in suppressing CMV infections. Doses of 100 ng also were injected into the vitreous of monkey eyes. Intravitreal injections of HPMPC may be efficacious in inhibiting CMV retinitis for longer dosing intervals than can be used with other anti-CMV compounds. Invest Ophthalmol Vis Sci 33:1557-1563, 1992

Human cytomegalovirus (HCMV) has been recognized as an important pathogen in the immunocompromised patient, especially in those with acquired immune deficiency syndrome (AIDS). In patients with AIDS, HCMV retinitis is a leading cause of blindness. Ganciclovir is currently the drug of choice for the treatment of HCMV. Licensed for use in the United States since 1989, ganciclovir usually is able to control CMV infection, but it is toxic, therapy often must be interrupted, and the CMV disease relapses after therapy is discontinued. Recently, a new class of acyclic nucleoside phosphonate analogues with broad-spectrum anti-DNA virus activity was discovered.1-2 In this class of nucleoside phosphonate derivatives, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) was found to be the most potent and selective inhibitor of CMV in vitro.3 However, HPMPC differs from ganciclovir in several ways. First, HPMPC requires only a short exposure time to produce a marked inhibition of CMV cytopathogenicity and viral DNA synthesis; ganciclovir requires continuous contact with the virus-infected cells to achieve its effect.4-6 Second, HPMPC produces a long-lasting antiviral effect, which explains the efficacy of the compound when dosed infrequently in murine CMV-infected mice.7 The infrequent (ie, weekly) dosing required of this antiviral drug has important clinical advantages in local intravitreal treatment of persistent or recurring retinitis.
Table 1. Intravitreal injections of HPMPC

<table>
<thead>
<tr>
<th>Drug concentration</th>
<th>No. eyes used at 2 wk sacrifice</th>
<th>4 Wk sacrifice</th>
<th>Histology results</th>
<th>Electroretinogram results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td></td>
<td>50</td>
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<td>Normal</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1</td>
<td>Abnormal*</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* Vacuoles in retinal pigment layer and shortening of the photoreceptor outer segments.

Third, HPMPC is polar and highly water soluble; it thus may be suitable for inclusion in liposome delivery systems for intraocular use.

Most antiviral agents (ie, acyclovir) used for the treatment of herpesvirus infections depend on phosphorylation by the virus-encoded thymidine kinase for their antiviral activity. The virus HCMV, which does not encode for a specific thymidine kinase, therefore does not show marked sensitivity to these antiviral drugs. The drug HPMPC is active against thymidine kinase-deficient herpes simplex virus and varicella-zoster virus, including strains that are resistant to the "classic" antiviral drugs such as acyclovir. Ganciclovir-resistant CMV strains have been isolated from immunocompromised patients treated with the drug for CMV infection. This resistance might be based on impaired phosphorylation of ganciclovir. Such strains might be sensitive to acyclic nucleoside phosphonate analogues (ie, HPMPC) because these compounds are phosphorylated differently.

Thus, HPMPC has several properties that make it particularly interesting for intraocular delivery. First, the compound produces a long-lasting antiviral effect that might require less frequent dosing. Because of its polar nature, the compound could be encapsulated into liposomes. A repository preparation (such as liposomes) might be advantageous for local intraocular treatment of CMV retinitis. Also, HPMPC has a high therapeutic index and is selective for CMV (as determined by the ratio of the 50% inhibitory concentration for cell growth to the 50% inhibitory concentration for CMV plaque formation). Higher drug concentrations might be given intravitreally with minimal toxic side effects. Finally, ganciclovir-resistant CMV strains have been isolated from immunocompromised patients treated with the drug for CMV infection.

As suggested, this resistance might be related to impaired intracellular phosphorylation of the compound. Therefore, such ganciclovir-resistant strains may be sensitive to HPMPC because the acyclic nucleoside phosphonates are phosphorylated differently. These qualities make HPMPC particular interesting for further intraocular study and clinical research.

Materials and Methods

A total of 14 New Zealand white rabbits, weighing 2.5–3 kg each, were used. All experiments were done
in accordance with the guidelines of the University of California at San Diego Office of Veterinary Affairs and the ARVO Resolution on the Use of Animals in Research. The rabbits were anesthetized with intramuscular injections of ketamine 75% and xylazine 25%. We administered proparacaine 1% to anesthetize the cornea topically. Under sterile conditions, 0.1 ml of aqueous was removed from the anterior chamber with a 27-gauge needle; this caused a decrease in intraocular pressure before drug delivery. The rabbits received intravitreal injections of 0.1 ml of HPMPC at doses of either 10, 50, 100, 300, or 1000 μg or of normal saline. The drug was injected with a 25-gauge needle 1–2 mm posterior to the limbus into the vitreous. Each rabbit was examined by indirect ophthalmoscopy 7 and 14 days postinjection. After 14 and 28 days, a group of rabbits was killed, and their tissues were perfusion fixed. All rabbits were anesthetized deeply with 80 mg/kg of ketamine combined with 80 mg/kg of xylazine, then given an intravenous injection of sodium pentobarbital. After insertion of an arterial cannula and administration of 1 ml of heparin (5000 units), 1 l of normal saline was perfused at room temperature. Next, 1 l of fixative containing paraformaldehyde 2% and glutaraldehyde 2% in 0.1 M Sorenson’s phosphate (pH 7.1) was perfused. After the eyes were enucleated, a small incision was placed at the limbus, and the eyes were placed for post fixation in a refrigerator for 1 hr. The samples were transferred to 0.1 M Sorenson’s phosphate buffer, washed three times, and refrigerated for gross sectioning.

Gross sectioning of each eye produced three sections for histologic examination. After bisecting each

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**Fig. 2.** One micron-thick methacrylate-embedded section of the retina and underlying choroid from a rabbit receiving 100 μg of HPMPC. All layers of the retina show normal organization and cytoarchitecture. The rod layer (R) overlying the choroid (C) shows a regular linear arrangement of outer rod segments overlying the retinal pigmented epithelium. The inner (IN) and outer nuclear (ON) layers are regularly arranged without evidence of cytology. Original magnification X1600.

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**Fig. 3.** One micron-thick methacrylate-embedded section of the retina and underlying choroid from a rabbit receiving 300 μg of HPMPC. The rod layer (R) overlying the choroid (C) shows irregularities of the outer rod segments overlying a vacuolated retinal pigmented epithelium (arrows). Original magnification X1600.
eye through the optic nerve, one area above and one
area below the medullary ray were taken from one
half of the globe for electron microscopic examina-
tion. A section was removed from the other half of the
globe for thin light microscopic examination (2-μm
sections). Osmium postfixed tissue was prepared after
each sample was embedded in agar 2.25%.10 For thin
light microscopy, the sections were embedded in plastic in glycol methacrylate. For electron microscopy,
the sections were embedded in epon-araldite. We cut
2-μm sections on a microtome (Reichert 2040, Hei-
delberg, Germany) and stained them with toluidine
blue for study under a light microscope.

Electroretinography (ERG) was done on all rabbits
preoperatively and postoperatively before they were
killed at either 14 or 28 days. Before the procedure,
the eyes were dilated with phenylephrine 2.5% and
tropicamide 1% and dark adapted for 30 min. A mix-
ture of ketamine 80% and xylazine 20% was used for
anesthesia. The eyes were positioned 6 inches from
the Grass stimulator light source (Grass Instruments,
Quincy, MA). Silver-impregnated nylon electrodes
were placed on the corneas. Four to eight flash ERGs
were averaged, transferred to an amplifier and digital-
analog convertor designed by us, and then transmit-
ted to a personal computer. The data were collected
on eyes containing HPMPC or normal saline. Other
control data included a data bank of normal ERG
amplitudes and latencies in New Zealand white rab-
bits corrected for the age and weight of the animals (Table 1).

We did a limited pharmacokinetic study to deter-
mine the half-life of this compound. We injected 100
μg of HPMPC in 0.1 ml intravitreally into eight New
Zealand white rabbits. The animals were killed simi-
larly, except for the perfusion fixation procedure, at 1,
4, 8, 16, 24, 48, 72, and 96 hr after the injection. The
eyes were enucleated, and the vitreous and serum sam-
ples were obtained for analysis of HPMPC concentra-
tion using high-performance liquid chromatography.
Vitreal drug concentrations were analyzed by compo-
partmental and noncompartmental methods. The
elimination half-life was determined from a least-
squares fit on the terminal log-linear portion of the
concentration–time curve using program TDMS
(Healthware, San Diego, CA).

One Papio cynocephalus monkey also was tested.
Under general anesthesia and sterile conditions as pre-
Previously described, 100 μg of HPMPC was injected into one eye. The animal was killed, and the tissues were perfusion fixed. After enucleation, the eye was sectioned for light microscopic study to determine the retinal toxicity.

**Results**

The vitreous remained clear throughout the postinjection period in all 14 rabbits and the monkey, except for those rabbits receiving 1000 μg. This dose caused a vitreous haze. The retinas were attached and normal appearing in all animals. The rabbit eyes that received 10, 50, 100, 300, and 1000 μg of the drug had normal ERG findings (amplitude and latency) before and after the intraocular injection (Figs. 1A–B). Light microscopic examination of the animals that received 10, 50, and 100 μg showed normal retinal morphology at all times (Fig. 2). Scattered areas of vacuoles in the retinal pigmented layer and degeneration of the photoreceptor layer were observed at doses of 300 and 1000 μg (Fig. 3). Electron microscopic examination of retinal tissue in an eye injected with 100 μg of HPMPC revealed no retinal abnormalities (Figs. 4A–B). In the monkey, light microscopy showed normal retinal morphology at the 100-μg dose (Fig. 5). A limited pharmacokinetic study found a terminal elimination half-life of 18.6 hr (Fig. 6). Noncompartmental analysis revealed a mean residence time of 24.4 hr. Although there appeared to be a more rapid fall in vitreal drug concentrations initially (suggesting multicompartamental kinetics), using a two-compartment model did not improve the fit (Aike criterion).

**Discussion**

The virus CMV is a major cause of morbidity and mortality in the immunocompromised host. Such infections may present as various pathologic entities, including retinitis, pneumonitis, colitis, or encephalitis. Ganciclovir commonly is used to treat CMV infections in the immunocompromised patient, but it has many side effects, including bone marrow toxicity.1 Another compound, foscarnet also has been approved for treatment of HCMV retinitis. This compound has disadvantages, including renal toxicity, anemia, tremor, and nausea.12 The drug HPMPC is a more potent and selective inhibitor of CMV replication in vitro than is ganciclovir. The main target of its antiviral action appears to be viral DNA polymerase. The 50% inhibitory doses for HCMV of foscarnet, ganciclovir, and HPMPC in cell culture are 15, 0.7, and 0.07 μg/ml, respectively. In addition, HPMPC inhibits cell proliferation and cellular DNA synthesis only at a concentration 1000-fold higher than that.
required to inhibit viral replication and DNA synthesis.

Thus, HPMPC is a potent and selective anti-CMV agent. Its antiviral activity, unlike that of ganciclovir, persists when it is removed from the cells. This may be related to the persistence of active metabolites of the acyclic nucleoside phosphonates in the cell and may have important clinical relevance because it would allow longer dosing intervals in the treatment of CMV retinitis. Additional studies have proved the effectiveness of HPMPC in vivo in suppressing the course of thymidine kinase-deficient herpes simplex virus and CMV infections in mice, rats, and rabbits.13,14 To our knowledge, no studies have shown the effectiveness of HPMPC on CMV retinitis in vivo.

Based on our results, a 100-μg dose of HPMPC (1000-fold the concentration required to inhibit CMV plaque formation by 50%) is not toxic to the rabbit retina as shown by light microscopy, electron microscopy, and electrophysiologic testing for up to 4 weeks. Intravitreal injections of HPMPC at concentrations greater than 100 μg/0.1 ml resulted in moderate adverse effects on the retina, especially the photoreceptor layer. The ERGs were normal for all drug doses, including the 1000-μg dose, 4 weeks postinjection.

Considering the important advantages of HPMPC over ganciclovir, additional studies of the efficacy of HPMPC in vivo are warranted before it could be considered for treating CMV retinitis in humans. Further investigations might entail the following: local intraocular treatment, systemic use of the drug at wider intervals, and use of a liposome-encapsulated repository preparation of the drug for intravitreal therapy. For the treatment of CMV retinitis, local intravitreal therapy should be considered because of the systemic toxicity of all currently used anti-CMV drugs. Advantages of this treatment over systemic administration are the accessibility of the retina to the compound and the potential for higher and prolonged levels of the drug. Local intraocular therapy might avoid the toxicity seen with drugs delivered systemically.15

Currently, intravitreal ganciclovir is given to patients with AIDS and CMV retinitis who are intolerant of or unwilling to receive ganciclovir intravenously. Several investigators have used intravitreal injections of ganciclovir in attempting to control retinitis.16-18 But problems with the short intravitreal half-life of this drug have resulted in frequent dosing. Therefore, a more potent compound like HPMPC, which has long-lasting antiviral activity when administered intraocularly, might be advantageous.

Intraocular repository preparations would be beneficial in the treatment of CMV retinitis. Recognizing that CMV retinitis may be diagnosed in the absence of systemic disease and considering the disadvantages of systemic toxicity and the necessity of daily intravenous therapy with ganciclovir and foscarnet, intravitreal preparations in the repository form might address these concerns. Studies by our group have indicated that a multivesicular liposome system is not toxic to the rabbit retina.16 We found that polar derivatives, which are water soluble, and compounds of high molecular weight are retained well in a multivesicular liposome system. Additional studies of polar drugs, such as HPMPC, encapsulated in the aqueous phase of liposomes might show the importance of this therapy.

Key words: HPMPC, toxicology, cytomegalovirus, retinitis

References


