Characterization of a Novel Intraocular Drug-Delivery System Using Crystalline Lipid Antiviral Prodrugs of Ganciclovir and Cyclic Cidofovir

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PURPOSE. In an earlier study, a novel intraocular drug-delivery system was reported in which hexadecyloxypropyl-phosphoganciclovir (HDP-P-GCV) was used as a prototype. The hypothesis was that many biologically effective compounds could be modified to crystalline lipid prodrugs and could be delivered directly into the vitreous in a long-lasting, slow-release form. This study was undertaken to characterize this new drug-delivery system further, by using small particles of HDP-P-GCV and hexadecyloxypropyl-cyclic cidofovir (HDP-cCDV).

METHODS. HDP-P-GCV was microfluidized into 4.4-μm (median) particles, injected into rabbit vitreous. The vitreous drug concentration was then measured at different time points. Crystalline HDP-cCDV was synthesized, suspended in 5% dextrose, and injected into the rabbit’s vitreous at 10, 55, 100, 550, or 1000 μg in 50 μL vehicle per eye, to determine the highest nontoxic dose.

RESULTS. Microfluidized particles of HDP-P-GCV showed an increased drug release rate compared with the large-particle drug formulation, with area under concentration-time curve (AUC) of 219.8 ± 114.1 (n = 3) versus 108.5 ± 47.2 (n = 3) for unmodified HDP-P-GCV during the 12-week period after a 2.8-micromole intravitreal injection. There was a 103% increase of the drug released from the microfluidized formulation of HDP-P-GCV versus the unmodified formulation. Intravitreal injections of HDP-cCDV at doses of 100 μg/eye or lower were not toxic. After the 100 μg/eye injection, HPLC analysis showed a vitreous HDP-cCDV level of 0.05 μM at week 5, which declined to 0.002 μM at week 8. The concentration at week 8 (0.002 μM) remained above the IC50 for cytomegalovirus (0.0003 μM). The pretreatment study demonstrated an antiviral effect that lasted 100 days after a single intravitreal injection.

CONCLUSIONS. This crystalline lipid prodrug intravitreal delivery system is an effective approach to achieving sustained, therapeutic drug levels in the eye. Small microfluidized particles of HDP-P-GCV provide more rapid dissolution and higher vitreous drug levels. (Invest Ophthalmol Vis Sci. 2004;45:4138–4144) DOI:10.1167/iovs.04-00664

Drug delivery to the vitreous, retina, and choroid is a challenging task, because of the formidable obstacles posed by the blood-retinal barrier and tight junctions of the retinal pigment epithelium. Only small fractions of drug administered orally, intramuscularly, or intravenously reach the target. Therefore, application of large and potentially toxic doses of drug is necessary. Another challenge to retinal drug delivery is that drug levels must be sustained for prolonged periods at the target site. It is difficult to use intravitreal injections if the half-life of the injected drug is short, because frequent injections would be necessary. To facilitate localized delivery to the posterior segment through an injectable, sustained-release system, we have developed and reported an intravitreal drug-delivery system, in which a crystalline lipid prodrg of ganciclovir (GCV), HDP-P-GCV, is used.² In our previous study, we demonstrated that a single intravitreal injection of crystalline HDP-P-GCV provides 20 weeks of protection from herpes simplex virus (HSV) infection in a rabbit retinitis model. Further, we hypothesized that changing the drug particle size might alter the ganciclovir release kinetics and that this delivery system could be applied to many low-molecular-weight compounds that are known to have antiviral or antiproliferative effects.² In the present study, we further characterize this long-lasting intraocular drug-delivery system by studying the relationship between HDP-P-GCV particle size and GCV release profile and by synthesizing and testing a new crystalline compound, hexadecyloxypropyl-cyclic cidofovir (HDP-cCDV), using the same technology we reported earlier.² Although we studied two individual compounds, they belong to the same family of lipid conjugated crystalline compounds and they share the properties of crystalline formulation, water insolubility, and long-lasting slow release after intravitreal delivery.

MATERIALS AND METHODS

Synthesis of Compounds

HDP-P-GCV. HDP-P-GCV was synthesized as previously reported.² To prepare a small-particle formulation and eliminate the population of large particles, HDP-P-GCV was suspended in distilled water, and the slurry was subjected to five passes through a microfluidizer (Microfluidics, Newton, MA). The slurry was then flash frozen in a 1-L round-bottomed flask and lyophilized overnight to remove the water. Unmodified HDP-GCV and microfluidized HDP-GCV were subjected to laser light-scattering particle size analysis at Cirrus Pharmaceuticals, Inc. (Durham, NC). Measurements were performed with

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Animal Studies

All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intravitreal Pharmacokinetics of Small Particle Formulation of HDP-P-GCV. Three rabbits received 2.8 micromoles of the drug in 50 μL of 5% dextrose in their left eyes. The dose had been determined to be nontoxic in an earlier study.2 Vitreous sampling was performed at postinjection weeks 1, 2, 3, 5, 8, and 12. Rabbit eyes were well dilated before anesthesia by topical application of a combination of tropicamide 1% and phenylephrine hydrochloride 2.5%. Anesthesia was performed as previously described.6 Under anesthesia, the rabbit eye was proptosed through a hole made on a piece of latex cover slip. Under the direct view of a surgical microscope, 50 to 100 μL of vitreous fluid was aspirated through the pars plana with a 23-gauge needle. Vitreous sampling was performed according to a previously reported method involving a standardized grading scheme.7 A very experienced observer graded retinitis throughout the experiments in an unmasked manner. For the treatment study, the right eyes of 14 rabbits were intravitreally injected with 50 μL of 5% dextrose alone as the control. Five rabbits received an equivalent dose of free CDV in 50 μL of 5% dextrose, four received an equivalent dose of free cCDV in 50 μL of 5% dextrose, and the other five received 50 μL of 5% dextrose. In the pretreatment study, 58 rabbits were divided into four groups: the 21-, 47-, 68-, and 100-day groups. Fifteen rabbits were used at the 21-day time point, 47 rabbits at the 47-day time point, 68 rabbits at the 68-day time point, and 100 rabbits at the 100-day time point. At each time point, five rabbits were used for the pretreatment study and 58 for the pretreatment study. Only the right eye of each rabbit was used. Ophthalmoscopic retinitis grading was performed according to a previously reported method involving a standardized grading scheme. A very experienced observer graded retinitis throughout the experiments in an unmasked manner. For the treatment study, the right eyes of 14 rabbits were intravitreally injected with 0.06 mL of a 5 × 10−6 dilution of a 10−7 mean tissue culture infective dose (TCID 50)/mL HSV-1. When retinitis developed and reached grade 1 (earliest detected retinitis grades are 1 or 2), five infected eyes received 100 μg HDP-cCDV in 50 μL 5% dextrose, four received an equivalent dose of free CDV in 50 μL 5% dextrose, and the other five received 50 μL of 5% dextrose. In the pretreatment study, 100 μg/eye was tested. Fifty-eight rabbits were divided into four groups: the 21-, 47-, 68-, and 100-day groups. Fifteen rabbits were used at each time point of the pretreatment study (except eyes of 13 rabbits were used at the 100-day time point). At each time point, five rabbits received 100 μg (in 50 μL 5% dextrose) of HDP-cCDV, five received an equivalent dose of CDV in 50 μL 5% dextrose, and five received 50 μL 5% dextrose (for the 100-day pretreatment only three rabbits received HDP-cCDV). HSV-1 virus was inoculated as scheduled at 21, 47, 68, or 100 days after the intraocular drug injections. The HSV-1 virus dose, injection method, and clinical retinitis grading were performed as previously described.2,7 Rabbits were killed 2 weeks after development of retinitis. Retinitis was graded on days 3, 6, 9, 11, and 14. Rabbits without retinitis 3 or 4 weeks after HSV-1 inoculation were killed.

For the pharmacokinetic studies, the highest nontoxic dose from the results of the studies, 100 μg/eye, was intravitreally injected into 24 eyes of 16 rabbits, and the remaining 8 eyes were injected with the same volume (50 μL) of 5% dextrose as the control. Two animals, three eyes with drug and one eye with 5% dextrose, were used at each time point: postinjection days 1 and 3 and weeks 1, 2, 3, 5, 8, and 10. After the animals were killed, the globes were enucleated and kept on ice before they were frozen and dissected. The eyes were then submersed in −40°C 2-methylbutane in a beaker sitting in a dry ice-ethanol bath for 30 seconds. The frozen globe was cut into halves through the optic nerve, and the lens and anterior chamber blocks were removed immediately. The globe halves were then placed under the surgical microscope at room temperature for 40 to 70 seconds to allow the interface between retina and vitreous to thaw before the vitreous block was removed with forceps. The retina was gently scraped off the bed of retinal pigment epithelium (RPE)/chorioid layer with a fine spatula. The remaining RPE/chorioid layer was forcefully scraped off underneath the sclera. The dissection was completed under direct view of a surgical microscope within 2 to 3 minutes to avoid cross-contamination. Different tissues from the same eyes were stored separately in the preweighed and prelabeled glass vials. The vials were kept at −70°C until HPLC analysis.

HSV-1 Rabbit Retinitis Treatment Study of HDP-cCDV. For the retinitis intervention study, 72 rabbits were used, including 14 for the treatment study and 58 for the pretreatment study. Only the right eye of each rabbit was used. Ophthalmoscopic retinits grading was performed according to a previously reported method involving a standardized grading scheme. A very experienced observer graded retinitis throughout the experiments in an unmasked manner. For the treatment study, the right eyes of 14 rabbits were intravitreally injected with 0.06 mL of a 5 × 10−6 dilution of a 10−7 mean tissue culture infective dose (TCID 50)/mL HSV-1. When retinitis developed and reached grade 1 (earliest detected retinitis grades are 1 or 2), five infected eyes received 100 μg HDP-cCDV in 50 μL 5% dextrose, four received an equivalent dose of free CDV in 50 μL 5% dextrose, and the other five received 50 μL of 5% dextrose. In the pretreatment study, 100 μg/eye was tested. Fifty-eight rabbits were divided into four groups: the 21-, 47-, 68-, and 100-day groups. Fifteen rabbits were used at each time point of the pretreatment study (except eyes of 13 rabbits were used at the 100-day time point). At each time point, five rabbits received 100 μg (in 50 μL 5% dextrose) of HDP-cCDV, five received an equivalent dose of CDV in 50 μL 5% dextrose, and five received 50 μL 5% dextrose (for the 100-day pretreatment only three rabbits received HDP-cCDV). HSV-1 virus was inoculated as scheduled at 21, 47, 68, or 100 days after the intraocular drug injections. The HSV-1 virus dose, injection method, and clinical retinitis grading were performed as previously described.2,7 Rabbits were killed 2 weeks after development of retinitis. Retinitis was graded on days 3, 6, 9, 11, and 14. Rabbits without retinitis 3 or 4 weeks after HSV-1 inoculation were killed.

Figure 1. Synthesis of hexadecyl-oxypropyl-cyclic cidofovir (HDP-cCDV).

Reagents: a) N,N-di-cyclohexyl-morpholinocarboxamidine, 1,3-dicyclohexylcarbodiimide, pyridine, 100°C. b) 1-bromo-3-hexadecyloxypropene, N,N-dimethylformamide, 80°C.

A laser diffraction instrument (Helos; Sympatec, Lawrenceville, NJ), equipped with an R3 lens (0.5–175 μm; Sympatec). For each measurement, approximately 100 mg of dry sample was dispersed at a feed rate of 75% by a controlled feeder (Vibri; Sympatec), with dry dispersal (Rodos; Sympatec) attachments set at a main pressure of 4.0 bar and a venturi pressure of 100 mbar. The triggering conditions were set to start measurement when channel 25 reached 1% total signal and to stop when channel 25 dropped below 0.5%. Data were analyzed according to the Fraunhofer method by means of analytical software (Windox, ver. 3.2, release 4; Sympatec).

HDP-cCDV. cCDV was prepared from CDV, as described previously,2 except that the compound was isolated as the dicyclohexyl-morpholinocarboxamidine salt. The scheme of the synthesis is illustrated in Figure 1. The purity (greater than 98%) of the compounds synthesized in this study was confirmed by analytical thin-layer chromatography, nuclear magnetic resonance spectroscopy, and mass spectroscopy, as reported previously.4,5

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{N} \\
\text{CH} & \quad \text{O} \\
\text{O} & \quad \text{C} \\
\text{H} & \quad \text{N} \\
\text{H} & \quad \text{O} \\
\end{align*}
\]
Histologic Evaluation of Retinal Damage and Choroidal Inflammation. After death, globes were enucleated and processed for light microscopy. After they were stained with hematoxylin and eosin (H&E), sections obtained next to the vertical meridian were selected from each eye. The section that was used to measure retinal thickness for each eye included the entire anterior and posterior retina in cross section. The thickness of the retina was measured at five locations in the inferior retina and three locations in the superior retina, as illustrated by the pointers in Figure 2. Thickness was measured with a reticule installed in the eyepiece of the microscope. All measurements and grading were performed under 100× magnification. Each scale unit of the reticule is equivalent to 27.8 µm. The thickness of each retina was expressed as the mean of the measurements at all eight locations.

Statistical Methods
The continuous variables, such as the total area under the concentration-time curve (AUC), IOP, and thickness of the retina, were compared between or among groups by t-test or the Tukey test. The ordinal variables, such as retinitis scores, were analyzed across the groups by using the Kruskal-Wallis test, followed by a nonparametric Tukey-type test to locate the differences if a null hypothesis was rejected by the Kruskal-Wallis test. Differences of P < 0.05 were considered to be significant. All statistical simulations were performed with two-tailed comparison unless otherwise indicated.

RESULTS
Effect of Microfluidization of HDP-P-GCV on Particle Size
We subjected an aqueous slurry of HDP-P-GCV to five consecutive cycles of microfluidization. After lyophilization and recovery of the powder, both the unmodified and the microfluidized HDP-P-GCV formulations were subjected to laser light-scattering particle size analysis (Fig. 3). Unmodified HDP-P-GCV (Fig. 3A) showed a bimodal distribution, with a volume median diameter (x50) centered around 8.0 µm. After microfluidization (Fig. 3B), a more monodispersed population of smaller particles was noted, with an x50 of 4.4-µm, a 99th percentile diameter (x99) of 20 µm, and a 90th percentile diameter (x90) of 10 µm. No large particles remained after microfluidization. Finally, we also analyzed an untreated formulation of HDP-cCDV powder (Fig. 3C). This compound showed a population of particles with an x50 of 8.9 µm.

Intravitreal Pharmacokinetics of the Unmodified and Microfluidized Small Particle Formulations of HDP-P-GCV
Intravitreal injection of the microfluidized HDP-P-GCV resulted in an intravitreal drug depot similar to that of the nonmicrofluidized drug. The total AUC of the microfluidized HDP-P-GCV at week 12 was 219.8 ± 114.1 (n = 3) versus 108.3 ± 47.2 (n = 3) for the unmodified HDP-P-GCV after an intravitreal dose of 2.8 micromoles. After logarithmic transformation of the data, a

FIGURE 2. Schematic showing the eight locations in which thickness was measured.

FIGURE 3. Laser light-scattering particle size analysis of HDP-P-GCV and HDP-cCDV formulations. (A) Unmodified HDP-P-GCV; (B) microfluidized HDP-P-GCV; (C) HDP-cCDV.
-test showed no statistical significance ($P > 0.15$). The mean concentration-time curves are shown in Figure 4. The result indicates that microfluidized HDP-P-GCV may provide a faster release rate and higher free drug concentration in the vitreous fluid. This is probably due to the larger surface area of the microfluidized particles, leading to a more rapid rate of dissolution.

**Intravitreal Toxicity and Pharmacokinetics of HDP-cCDV**

The toxicity study showed that the highest nontoxic dose is 100 μg/eye. Doses of 550 and 1000 μg/eye showed local cataract with mild iritis and/or local retinal toxicity (Fig. 5) in two eyes, one with the 550-μg dose and one with the 1000-μg dose. However, the other eye with the 550-μg dose showed no toxicity, with the drug depot floating in the vitreous cavity without contacting any intraocular tissue until the end of the study. The drug depot was still visible at the end of the study (week 8) in the eyes with 100-μg or higher doses (Fig. 6). IOP was measured at baseline, at postinjection day 3; and at weeks 1, 2, 3, 5, and 8 in each eye. At week 8 after drug injection, the eyes with the 100-μg dose or lower showed an average IOP of 8.7 ± 1.0 mm Hg in the right eyes and 9.8 ± 1.1 mm Hg in the left eyes ($P = 0.175$, paired $t$-test); the eyes with the 550- and the 1000-μg doses showed an average IOP of 8.7 ± 2.1 mm Hg in the right eyes and 11.3 ± 2.3 mm Hg in the left eyes ($P = 0.01$, paired $t$-test). At all other time points, IOP was not significantly different between the treated and the control eyes. All eyes had normal ERGs, including the eye with the 1000-μg injection. Pathology confirmed normal retina, vitreous, and choroids in the eyes with 100-μg intravitreal injections.

The pharmacokinetic study revealed that the whole vitreous samples contained an average concentration of 0.54 μM HDP-cCDV at postinjection day 1 and an average concentration of 0.002 μM at postinjection week 8, with an estimated vitreous half-life of 6.3 days. At the end of the experiment (postin-
jection week 10), HDP-cCDV was still detectable in whole vitreous samples (0.0006 μM; Fig. 7). However, the HDP-cCDV was below the limit of detection in the retina.

**HSV-1 Retinitis Treatment Studies**

**Treatment Study.** Of the 14 eyes of 14 rabbits that received 0.04 mL of $1 \times 10^{-7}$ TCID/50 HSV-1, 12 showed development of retinitis at day 4 after virus inoculation. Rabbits were randomly divided into three groups. Retinitis scores were not significantly different among three groups before the intravitreal drug or dextrose injections ($P = 0.77$, Kruskal-Wallis Test). The four eyes that received the 5% dextrose solution after development of retinitis (median score of 1) progressed to complete retinitis with retinal detachment and severe vitreous cloudiness. The four eyes that received intravitreal HDP-cCDV and the four eyes that received cidofovir after induction of retinitis (median scores of 1.5 and 2), showed clinical scores of retinitis similar to those of the 5% dextrose controls at all time points ($P > 0.05$, Kruskal-Wallis test). However, vitreous cloudiness was noticeably less severe than in the control eyes. The measurement of thickness of retina from histologic evaluation revealed that the thickness of the retina in the eyes with intravitreal injection of CDV and HDP-cCDV was 95 ± 40 and 80 ± 10 μm, whereas the retinal thickness of the 5% dextrose-injected eyes was 46 ± 8 μm. There was no significant difference between the CDV and HDP-cCDV-treated groups or between HDP-cCDV-and 5% dextrose-treated groups; however, a significant difference was detected between the CDV- and 5% dextrose-treated groups ($P < 0.05$, Tukey HSD test).

**Pretreatment Study.** During the 3-week pretreatment study, all five rabbits that received intravitreal injection of 5% dextrose showed development of typical retinitis. In contrast, none of the rabbits that received the intravitreal injection of HDP-cCDV or CDV had retinitis. At each time point, retinitis scores were analyzed across three groups by the Kruskal-Wallis test, followed by a nonparametric Tukey-type test to locate the difference(s) if the null hypothesis was rejected by the Kruskal-Wallis test (Table 1. For the 47-day pretreatment, at day 6 after virus inoculation all control eyes showed retinitis with a median grade of 4. The rabbits that received CDV pretreatment all had retinitis with a median grade of 3. In contrast, of the five rabbits that received HDP-cCDV pretreatment, only two had grade 2 retinitis. There is a significant difference in retinitis

**Chart: HPLC Analysis of Whole Vitreous for HDP-cCDV in Rabbit Eyes**

![Graph showing HPLC Analysis of Whole Vitreous for HDP-cCDV in Rabbit Eyes](image)

- **Vitreous half-life: 6.3 days**

**Figure 7.** A trend plot of HDP-cCDV elimination, determined in a pharmacokinetic study of rabbit vitreous over time, after 100-μg intravitreal injections. The estimated vitreous half-life was approximately 6.3 days. Data is presented as the mean ± SD ($n = 3$).
scores during the 14-day grading period between HDP-cCDV-pretreated eyes and CDV-pretreated eyes or control eyes (Table 1). All 14-day HDP-cCDV pretreatment study, at day 14 after virus inoculation, only two rabbits had grade 3 or grade 4 retinitis. The remaining three rabbits had complete protection from HSV-1 infection. The other two groups (10 eyes) with dextrose or CDV pretreatment all had grade 4 retinitis (Table 1). For the 100-day pretreatment study, one of four rabbits in the HDP-cCDV-pretreated group died before virus inoculation. Among the other three rabbits, one did not have retinitis, and the other two had grade 3 retinitis at day 6. The HDP-cCDV pretreated group had lower retinitis scores than the retinitis scores of the dextrose-pretreatment group (Table 1).

**Discussion**

The goal of this study was to characterize further the novel crystalline lipid prodrug intraocular drug-delivery system that we reported earlier with HDP-P-GCV.2 We found that the unmodified crystalline lipid prodrug of GCV possessed a slow-release property after intravitreal injection. In our previous report, HDP-GCV prevented HSV-1 viral infection of the retina for 20 weeks after a single intravitreal injection, whereas a single intravitreal injection of GCV provided less than 1 week of protection.2 In the present study, we demonstrated that microfluidized small-particle HDP-P-GCV may have released a greater amount of free drug into the vitreous fluid than the unmodified large-particle formulation of HDP-P-GCV. AUCs in animals in the large-particle group ranged from 60.7 to 155, whereas AUCs in the small-particle group ranged from 140 to 351. The drug release was twice as high in the small-particle group. The trend was not statistically significant, probably because of the sample size of the data. We believe that small particles have a larger surface area that increases the contact surface with the dissipation medium, resulting in a higher dissolution rate than with large particles. It is possible that very small amounts of crystalline prodrug diffused to a distant part of the vitreous and were sampled into the vitreous tap, and this diffusion may be the cause of the relatively large variation in the vitreous drug concentrations between individual animal eyes. We used three animals per time point to get a mean value, from which the data curve would show a clear trend and valid information. In this study, vitreous fluid was sampled by multiple vitreous taps at different time points. Although we sampled the vitreous fluid through the pars plana and we did not observe vitreous or anterior chamber fibrin formation, it is still possible that multiple vitreous taps cause intraocular environmental change and influence the subsequent vitreous drug level measurement. Data are not available from the current experiments to resolve this concern.

Injection of lower drug levels could eliminate the local retinal toxicity resulting from contact of drug depot with retina due to gravitational effects and positioning.2,5 The current studies were conducted within nonvitrectomized eyes. The drug aggregation and release profile could be quite different if injected into a vitrectomized eye. Further studies are warranted in vitrectomized eyes. Based on our findings, we hypothesize that controlled release could be achieved by using mixtures of different sizes of crystalline drug in an intravitreal administration. These mixtures could be designed to have release profiles tailored to treat different kinds of vitreoretinal diseases.

In the previous report, we first described this novel intraocular drug-delivery system using HDP-P-GCV as a prototype.2

**Table 1. Time Course and Median Retinitis Scores from Pretreatment**

<table>
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<tr>
<th>Group/Time Point</th>
<th>Animals (n)</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 14</th>
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<tr>
<td>HDP-cCDV: 21 days</td>
<td>5</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>5% Dextrose</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Free CDV</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P (Kruskal-Wallis test)</td>
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<td>0.0062</td>
<td>0.0062</td>
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<tr>
<td>P (nonparametric Tukey test)*</td>
<td>HDP vs. Dex &lt;0.01; HDP vs. CDV NS; CDV vs. Dex &lt;0.01;</td>
<td>HDP vs. Dex &lt;0.01; HDP vs. CDV NS; CDV vs. Dex &lt;0.01;</td>
<td>HDP vs. Dex &lt;0.01; HDP vs. CDV NS; CDV vs. Dex &lt;0.01;</td>
<td></td>
</tr>
<tr>
<td>HDP-cCDV: 47 days</td>
<td>5</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5% Dextrose</td>
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<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Free CDV</td>
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<td>4</td>
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<tr>
<td>P (Kruskal-Wallis test)</td>
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<td>0</td>
<td>0</td>
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<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Free CDV</td>
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<td>4</td>
</tr>
<tr>
<td>P (Kruskal-Wallis test)</td>
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<td></td>
</tr>
<tr>
<td>HDP-cCDV:100 days</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5% Dextrose</td>
<td>5</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Free CDV</td>
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<td>P (nonparametric Tukey test)†</td>
<td>HDP vs. Dex &lt;0.05; HDP vs. CDV NS; CDV vs. Dex NS</td>
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</tr>
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</table>

HDP, HDP-cCDV; CDV, cidofovir; Dex, dextrose.

* Nonparametric Tukey test with equal sample sizes (Nemenyi test).
† Nonparametric Tukey test with unequal sample sizes (Dunn test).
We reasoned that the hexadecyloxypropanol moiety could be coupled to many nucleoside phosphates or phosphonates to form solid hydrophobic crystals that slowly dissolve in water. The dissolved molecules enter the cells and are cleaved intracellularly by phospholipase C into hexadecoxypropanol and the parent drug. In the present study, we used the same concept and technique to synthesize an ether lipid ester of cyclic cidofovir, HDP-cCDV. Intravitreal injection of 100 μg or lower doses of HDP-cCDV crystals provided an ideal drug depot that floated in the inferior vitreous cavity without disturbing the visual axis. The vitreous elsewhere was clear and no toxicity was found in the eyes with 100 μg or lower doses. Local toxicity with higher doses was caused by the contact of drug depot with retina or lens, which was similar to the local retinal toxicity caused by intravitreal high-dose HDP-P-GCV. The higher dose forms a larger drug depot in the vitreous, which tends to contact intraocular tissues and cause toxicity. An IOP decrease associated with intravitreal injection of CDV was not observed in the eyes that received 100-μg or lower doses in the present study. The eyes with higher doses showed a mild decrease in IOP at the last time point (8.7 ± 2.1 vs. 11.3 ± 2.3 mm Hg, P = 0.01). However, no hypotony was found. Hypotony (IOP of 5 mm Hg or lower with associated retinal edema) is a well-known complication after local or systemic cidofovir administration. The absence of hypotony may be because cyclic CDV and HDP-cCDV are not picked up avidly by organic anion transporters in the ciliary body. Intravitreal pharmacokinetics showed that HDP-cCDV was still detectable at week 10 after a single intravitreal injection of 100 μg per eye. The estimated vitreous half-life for HDP-cCDV was 6.5 days, which favorably compares to 20 hours for CDV or 10 hours for cCDV. The detected concentration at week 8 was 0.002 μM, which is above the IC_{50} for CMV. In this study, we did not detect HDP-cCDV in the retina, which could be due to low sensitivity of HPLC and fast conversion of HDP-cCDV into CDV by cellular phosphohydrolases. Using HDP-P-GCV, we have shown that the prodrug may be metabolized by cell-surface transporters of vitreous cells. There was little parent drug detectable when produg was incubated with a heat-inactivated vitreous sample, but conversion was detected readily by native vitreous that contains cells. It has been known that CDV is phosphorylated to cidofovir diphosphate, the active form of cidofovir that has a long intracellular half-life. Indeed, the pretreatment studies indicated at least 100 days of pharmacologic effect against HSV-1 infection of the rabbit retina. In the current studies, retinitis was graded in an unmasked manner. It could lead to bias if retinitis severity were similar to that in the treatment study. Therefore, we performed further objective analysis of retinal thickness from the pathologic examination for the treatment groups to confirm the findings. In the pretreatment studies, the severity of retinitis among the three groups was so obvious that it is unlikely that unmasked grading influenced the results.

In summary, this novel intraocular drug-delivery system has promise for challenging refractory chronic vitreoretinal diseases that require prolonged drug treatment. Small crystals of HDP-P-GCV have been found to release more drug over time than large unmodified particles, and the delivery system has been extended to crystalline HDP-cCDV. The concept and technique described in this study can be applied to many compounds, including antiproliferative drugs such as phosphonothioethylguanine (PMEG), arabinofuranosylguanine ( Ara-G), and 5-fluoro-2'-deoxyuridine (5-FdUr), and other anti-proliferative drugs that we are investigating.

References