Intraocular Pharmacokinetics of a Crystalline Lipid Prodrug, Octadecyloxyethyl-Cyclic-Cidofovir, for Cytomegalovirus Retinitis

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Abstract

Purpose: To evaluate the intraocular pharmacokinetics of octadecyloxyethyl-cyclic-cidofovir (ODE-cCDV) after intravitreal injection into rabbit eyes.

Methods: Twenty-seven New Zealand red rabbits (27 eyes) received intravitreal injections of ¹⁴C-labeled ODE-cCDV (100 µg drug suspended in 5% dextrose), and ocular tissues were collected from 3 rabbits at each pre-determined time point (1 h, 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 5 weeks, and 9 weeks) after the injection. The eye globes were enucleated, and the vitreous, retina, and choroids were separated and harvested into preweighed scintillation vials. Levels of ODE-cCDV were measured by counting in a liquid scintillation counter, and pharmacokinetic (PK) parameters were determined. In addition, 3 eyes of 3 animals were used for autoradiography study at day 1, week 3, and week 6.

Results: ODE-cCDV in vitreous as a whole followed a 2-phase first-order elimination, whereas ODE-cCDV in retina and choroid manifested a nearly steady state during the first 3 weeks and then followed a first-order elimination with the apparent elimination half-life of 10.1 and 7.2 days. For vitreous, apparent elimination half-life was 25 days. However, the drug mean residence time was much longer in retina (17.6 days) and choroid (19.6 days) than that in the vitreous (11.6 days). The drug exposure to the retina [area under the curve (AUC) = 1120837.1 ng · day/mL] was greater than the exposure to the vitreous (AUC = 958645.8 ng · day/mL) and the choroid (AUC = 415407.47).

Conclusion: A crystalline lipid prodrug, ODE-cCDV, has longer vitreous half-life than that in other ocular tissues due to its solid drug depot formation in vitreous. Over time, dissolved free ODE-cCDV from drug depot feeds and accumulates in the retina.

Introduction

DELIVERING THERAPEUTICS directly into the eye to treat chorioretinal diseases has emerged as an important strategy in current eye health care.^{1–5} Intravitreal drug delivery has the advantage of avoiding the surgical procedures needed for intraocular implants that are often associated with more complications.^{5,6} However, intravitreal injectable drugs often have short vitreous half-life and require frequent injections. For example, before the approval of ganciclovir (GCV) implants by U.S. Food and Drug Administration in 1996, intravitreal high-dose GCV needed to be injected weekly as a maintenance therapy.⁷ We previously reported an intravitreal drug delivery system that conjugates a long-chain alkoxyalkyl moiety to a candidate compound to render

it lipophilic with low solubility in vitreous, achieving a long intravitreal half-life.^{8,9} Alkoxyalkyl prodrugs penetrate cell membrane easily and are cleaved inside cells by phosphatases of the phospholipase C type, releasing the parent drug.¹⁰ Our previous pretreatment studies demonstrated a period of 100 to 140 days of herpes simplex virus (HSV) retinitis prevention after a single intravitreal injection of alkoxyalkyl-derivatized cidofovir or GCV.^{9,11} Due to the poor water solubility of lipid-derivatized compounds, their dissolution in vitreous is very slow and dissolved free drugs readily migrate into ocular tissues because of their lipophilicity.¹² Therefore, pharmacokinetic studies from vitreous taps or whole vitreous often underestimate the *in vivo* therapeutic duration. For example, in the previous study using 2.8 µmol of the crystalline ammonium salt of hexadecyloxypropyl

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GCV monophosphate for HSV retinitis prevention in rabbit, hexadecyloxypropyl GCV monophosphate was not detectable at the 18th week from the vitreous tap. However, *in vivo* pretreatment demonstrated a significant drug effect after 20 weeks, which was the last time point studied.¹¹ We also studied intravitreal application of hexadecyloxypropyl cyclic cidofovir (HDP-cCDV), a potent inhibitor of human cytomegalovirus (HCMV) replication *in vitro*. The whole vitreous pharmacokinetics of HDP-cCDV indicated a half-life of 6.3 days, which usually provides a 5 to 6 half-lives of therapeutic duration (38 days if using 6 half-lives). However, pretreatment with HDP-cCDV demonstrated 100% protection of retina from HSV infection 68 days after a single intravitreal injection.⁹ In that study, a detailed pharmacokinetic study of the drug in retina and choroid was not performed because the compound was not radiolabeled. We hypothesized that HDP-cCDV was incorporated into retina and choroid to serve as a second drug depot, which further extended the duration of efficacy. In the current study, we have characterized the ocular pharmacokinetics of ¹⁴C-labeled octadecyloxyethyl-cyclic-cidofovir (ODE-cCDV), a closely related prodrug that differs from HDP-cCDV by only 1 atom in overall length of its alkoxyalkyl moiety (Fig. 1). Based on their potent anti-HCMV activity and favorable pharmacokinetic properties, HDP-cCDV or ODE-cCDV may be further developed into a good intravitreal long lasting and slow release therapy for CMV retinitis while eliminating surgery procedures needed for intraocular implant.

Methods

Preparation of ¹⁴C-ODE-cCDV

Octadecyloxyethyl-cyclic-[2-¹⁴C] cidofovir ([¹⁴C] ODE-cCDV) was prepared by custom synthesis by Moravsek Biochemicals and had specific activity 56 mCi/mmol. Unlabeled ODE-cCDV (12 mg, prepared as previously described)¹³ was combined with 25 μ Ci [¹⁴C]-ODE-cCDV and recrystallized from p-dioxane to obtain crystalline [¹⁴C] ODE-cCDV with activity $\sim 0.21 \mu$ Ci/100 μ g. To 2.4 mL of 5% dextrose was added 4.8 mg crystalline [¹⁴C] ODE-cCDV, and the mixture was placed in an ultrasonic bath for 1 h. This suspension was used for injections (100 μ g drug/50 μ L injection). ODE-cCDV is a white microcrystalline powder and has molecular weight of 557.70 amu, which is a new molecule created by a covalent esterification process (conjugation). We have not fully characterized the particle size; however, the size of the crystals is quite small (microscopic) and they disperse easily in 5% dextrose.

Confirmation toxicity study

ODE-cCDV has 2 more carbon than HDP-cCDV in the alkyl chain; hence, they are expected to have similar physiochemical properties and similar ocular safety profile. In accordance with our published data with HDP-cCDV, 100 μ g per rabbit eye was the highest nontoxic dose.⁹ Therefore, 100 μ g/eye was chosen to do a 4-week confirmation toxicity study on 3 rabbits before the pharmacokinetic study. The right eyes were injected with 100 μ g ODE-cCDV and the left eyes were injected with the equivalent volume (50 μ L) of 5% dextrose. The ocular toxicity was monitored by slit lamp biomicroscopy, Tonopen tonometry for intraocular pressure (IOP), indirect ophthalmoscopy on 3rd day as well as every week, electroretinogram at week 4, and histology with light microscopy.

Pharmacokinetic study

Twenty-four New Zealand red rabbits and 24 eyes were intravitreally injected with 100 μ g of ¹⁴C-labeled ODE-cCDV in 50 μ L of 5% dextrose. Only 1 eye of each animal was used for the drug injection. Three 100 μ g doses of ¹⁴C labeled ODE-cCDV were injected into 3 vials containing 1.4 mL phosphate-buffered saline as the controls for radioactivity counting. Three rabbits at each time point (1 h, 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 5 weeks, and 9 weeks after the injection) were sacrificed. The eye globes were enucleated and the vitreous, retina, and choroids were separated and harvested into the preweighed vials as previously described.⁹ The vials were kept at -80°C until radioactivity counting as we described previously¹⁴ to determine the drug level in each type of ocular tissues. The initial concentration in 1.4 mL phosphate-buffered saline or 1.4 mL rabbit vitreous was calculated as 126 μ M. The drug concentration in different tissue was presented as the percent of initial vitreous concentration.

Autoradiography study

Three eyes of 3 animals were used for the autoradiography study after intravitreal injection of 100 μ g of ¹⁴C labeled ODE-cCDV in 50 μ L of 5% dextrose. On day 1, week 3, and week 6 after the intravitreal injection, one rabbit each was sacrificed and the globes enucleated. Both globes of the same rabbit were snap-frozen as we described before⁹ and sagittal cut through optic nerve into 2 halves. The half globes were embedded in optimal cutting medium (OCT) within a cryo mold then frozen and kept at -80°C for cryo sectioning.

FIG. 1. The chemical structures of hexadecyloxypropyl cyclic cidofovir and ODE-cCDV. ODE-cCDV, octadecyloxyethyl-cyclic-cidofovir.

FIG. 2. Autoradiogram showing radioactivity (black) from the cryo sections of the eye globes. At each time point, 2 sections, 1,000 μm apart, were used. The images on the left are corresponding the slides layout on the right. It is clear that no radioactivity was detected from the all sections from the left eyes. The intensity of the radioactivity in the retina did not show obvious diminish from 1-week images to 6-week images.

Twenty-micrometer sections were cut and mounted on a histology slide for autoradiography study. Two sections, 1,000 μm apart, from each block were used. The slides from the 2 eyes of the same animal were paired and 12 slides from 6 eyes of the 3 animals were arranged on a supporting board for radio activity exposure (Fig. 2). The entire board was placed in a light tight box with a general purpose phosphor plate K-screen 20 $\times$ 25 cm (Kodak) for an exposure of 4 months' duration. At the end of the exposure time, the latent image of ^{14}C activity exposure on the phosphore plate was read with a Bio-Rad Molecular Imager FX Pro Plus system.

The use of animals was adherence to the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research.

Results

Confirmation toxicity study

No toxicity was noted by comparing the ODE-cCDV-injected eyes with the 5% dextrose-injected eyes using slit lamp biomicroscopy and indirect ophthalmoscopy. The drug depots were observed in all 3 ODE-cCDV-injected eyes (Fig. 3) and were still visible upon sacrifice 4 weeks after the intravitreal injection.

IOPs were measured at 2 and 4 weeks after the initial intravitreal injections. Compared with the IOPs of the contra lateral eyes, no significant difference was found (OD-OS 0.33 ± 4.8 , $P=0.8$, paired t -test). Electroretinograms from both eyes of the same animals were comparable and histology did not show abnormality (data not shown).

Pharmacokinetic analysis

After ^{14}C -ODE-cCDV intravitreal injection, vitreous drug showed a 2-phase elimination. The first phase was from the injection to the day 21 and the second phase was from the day 21 to the day 63. The noncompartment analysis revealed a half-life of 5.5 days for the first phase and a terminal half-life of 25 days (Table 1). In contrast, ODE-cCDV in retina and choroid stayed above their initial concentration and reached the peak at 2-week time point (Fig. 4). The terminal half-lives in retina and choroid were 10.1 and 7.2 days, respectively (Table 1). The drug concentration-time profile in retina and choroid did not follow similar kinetics to the vitreous but

was near steady state for 3 weeks followed by a mono exponential decay thereafter. The drug mean residence time (MRT) in retina and choroid was much longer than that in vitreous, 17.6 and 19.6 days versus 11.6 days, respectively (Table 1).

Autoradiography study

As shown in Fig. 2, the sections from the left eyes (controls) did not reveal any radioactivity, whereas all sections from the right eyes demonstrated the radioactivity. From day 1 to week 6 after intravitreal injection, retina/choroid showed consistent radioactivity and the retina/choroid radioactivity seems not diminished from the previous 2 time points although this limited study did not allow us to reliably quantitate the radioactivity. The localized high radioactivity in vitreous reflects the drug depot, whereas radioactivity elsewhere in vitreous, reflecting dissolved drug, was extremely low or undetectable. Due to the large lens of rabbit eye, the drug injected into the vitreous was very close to the lens at the injection site. Some drug might have entered the lens; however, no cataract was observed throughout the study.

FIG. 3. Fundus photograph from a rabbit eye at 2nd week after the intravitreal injection, showing whitish ODE-cCDV at the front of the optic nerve head and above the optic nerve head in the vitreous. The 2 bluish rings at the center of the image were artificial light reflex from the added lens for wider view.

TABLE 1. PHARMACOKINETIC PARAMETERS

Pharmacokinetic (PK) parameters	Ocular tissue		
	Vitreous	Retina	Choroid
Rsq_adjusted	0.88	0.99	0.83
Points used	3	3	3
K (day ⁻¹)	0.028	0.068	0.096
Half-life (day)	25	10.1	7.2
Tmax (day)	0.04	14	14
Cmax (ng/mL)	130431.7 ± 24418.8	45215.7 ± 10981.6	16097.9 ± 1656.4
Tlast (day)	63	63	63
AUC last (day · ng/mL)	958645.8 ± 61608.9	1120837.1 ± 182179.0	415407.5 ± 65767.06
AUMC last (day · day · ng/mL)	11149342	19781300	8136700.9
MRT last (day)	11.6	17.6	19.6

Cmax, maximum concentration; Tmax, the time at which the concentration was maximum; K, first-order rate constant of the drug clearance; MRT, mean residence time; AUC, area under curve; AUMC, area under the first moment curve.

Discussion

The current study showed that the crystalline lipid pro-drug, ODE-cCDV, had a long vitreous terminal half-life (25 days), which is much longer than unmodified CDV (42 h).¹⁵ The clearance of ODE-cCDV from vitreous followed first-order kinetics. However, this vitreous pharmacokinetic data reflected the total ODE-cCDV in vitreous including the depot of insoluble drug. The estimated time for ODE-cCDV to be totally cleared from the vitreous would be 125 to 150 days after the initial intravitreal injection if using 5 or 6 half-lives. This long vitreous drug presence and potent anti HCMV properties (ODE-cCDV EC₅₀ = 24 ng/mL)¹⁶ may provided an extended time of protection from CMV infection. The pharmacokinetic data from retina and choroid reflect only ODE-cCDV and metabolites released from the vitreous drug depot and taken up by these tissues. The retina drug concentration–time curve in Fig. 4 demonstrated that drug level in retina in the first 2 weeks showed a slight upward trend, but drug concentration was below the drug concentration in the vitreous. At the 2-week time point, retina drug concentration surpassed the vitreous drug concentration and stayed above until the near end of the study. The total drug exposure reflected by the area under the curve was greater for retina (1142177.9 ng · day/mL) than that for vitreous (954132.67 ng · day/mL). The drug exposure to the choroid was 37% of that of the retina. The drug kinetics in choroid was similar to that in retina with a consistently lower drug level in the choroid than in retina. The MRT of the drug clearly demonstrated that ODE-cCDV stayed in retina and choroid much longer than in vitreous. Compared to the surgical intravitreal implant, which is designed to have zero-order kinetics and can produce consistent drug levels in retina during the most of its release course,¹⁷ this crystalline drug delivery system produced a less consistent drug level in retina. Nonetheless, this delivery system produced a 2 to 3 weeks of constant drug level in retina and choroid, which is 33% of the total release period. Optimization of the formulation by using different particle size population or altering the drug water solubility by varying the length of the carbon chain of the lipid conjugation may further improve this unique drug release profile and provide longer period of consistent drug level in retina and choroid as well as the other intraocular tissues. An ideal release profile would be that the rate of release can keep the steady state of retina concen-

tration above therapeutic level of the HCMV and with minimal ocular toxicity in a time window of 8 to 12 months. The retina ODE-cCDV concentration in the current study was much higher than its EC₅₀ (24 ng/mL) for HCMV¹⁶ during the whole study course. However, a retina concentration above the EC₉₀ is needed for treatment efficacy and eventually the minimal effective concentration has to be determined through an experimental treatment study.

We envision that treatment of CMV retinitis by intravitreal injection of a cidofovir prodrug would be similar to other widely used intravitreal drug injections, such as Avastin for macular degeneration,¹⁸ which can be performed in a clinic office setting. The difference is that the crystalline drug forms a depot in vitreous and provides an extended period of slow release. This approach combines the benefits of surgical implant (slow release of drug) with the added advantage of a simple needle injection. This technique (needle implant)

FIG. 4. Semi-log plot of the concentration–time profile of ODE-cCDV in the vitreous, retina, and choroid. The reference line in the graph indicates the effective concentration for ODE-cCDV to inhibit 50% of human cytomegalovirus replication.

eliminates the need for surgery and provides shorter recovery time as well as reduced rate of complications such as vitreous hemorrhage and retinal detachment.^{19,20}

Autoradiography at week 6 clearly demonstrated the presence of drug in both retina and choroid, and confirmed the pharmacokinetic data. In addition, drug depot in vitreous was demonstrated on the autoradiography but no radioactivity was seen elsewhere in the vitreous. This observation supports our hypothesis that ODE-cCDV first dissolves from the vitreous depot, and then free ODE-cCDV accumulates in retinal cells where it is metabolized by phosphatases of the phospholipase C type, releasing CDV, which is sequentially phosphorylated to CDV-diphosphate, the active antiviral.^{10,12}

Another possibility is that dissociated free ODE-cCDV was partially hydrolyzed in vitreous into ODE-CDV, which incorporated into the retina cell membrane¹² and then slowly metabolized into CDV-diphosphate.¹² In this study, we counted the total radioactivity from the ocular tissue and could not distinguish the intact prodrug (either ODE-cCDV or ODE-CDV) from their metabolites. In future studies, ocular tissues will be subjected to HPLC with radioactive detector to identify the intact drug and its metabolites. In addition, our autoradiography was limited due to small sample size. The quantitation of the drug from the autoradiograms was not able to be performed. The autoradiograms were derived from the cross sections of the globe and the vitreous drug depot (radioactivity) may not evenly show on these cross sections. However, the radioactivity in retina and choroid is more reliable to reflect the amount of the drug because the drug in retina and choroid is the free drug and metabolites. Compared to the previous nonradiolabeled HDP-cCDV whole vitreous pharmacokinetic study, the drug detecting ability in the current study was much better and was able to reveal the clear 2-phase elimination of the drug from the vitreous. The half-life (6.3 days) of HDP-cCDV from the previous study⁹ was mainly the first phase elimination half-life, which was very close to the half-life of 5.5 days from the first elimination phase of the current study.

In summary, the current study demonstrated that depositary ODE-cCDV in vitreous had a long half-life and was consistently feeding the retina with the dissolved free form of ODE-cCDV. During the study period the drug had greater exposure to retina than to vitreous. The drug MRT was longer in retina than that in vitreous. For this type of intravitreal injectable long-lasting lipid prodrug, retina drug pharmacokinetics is more informative for better understanding the drug characteristics than vitreous pharmacokinetics.

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Author Disclosure Statement

No competing financial interests exist.

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