Intravitreal Toxicology in Rabbits of Two Preparations of 1-O-Octadecyl-sn-glycerol-3-phosphonoformate, a Sustained-Delivery Anti-CMV Drug


PURPOSE. To determine intraocular toxicity and efficacy of the lipid prodrug of foscarnet, 1-O-octadecyl-sn-glycerol-3-phosphonoformate (ODG-PFA), as a long-acting, nontoxic intravitreal injectable drug delivery system for cytomegalovirus (CMV) retinitis.

METHODS. ODG-PFA was synthesized by coupling the phosphonate residue of PFA to the 3 hydroxyl of 1-O-octadecyl-sn-glycerol and formulated as micelles and liposomes at concentrations so that, after injection into the rabbit vitreous, the resultant intravitreal concentrations were 0.2 mM, 0.63 mM, and 2 mM in micellar formulation and 0.02 mM, 0.063 mM, 0.2 mM, and 0.63 mM for liposomal formulation. The compounds were injected, and toxicology evaluations were performed.

RESULTS. Intravitreal injections of micellar ODG-PFA resulted in aggregation of the material in the vitreous and variable local retinal damage. Intravitreal injections of the liposomal ODG-PFA revealed even dispersion of the compounds and a clear vitreous, using final concentration in the vitreous of 0.2 mM. No intraocular toxicity was found with the 0.632 mM final concentration. The 50% inhibitory concentration (IC50) for CMV of ODG-PFA was 0.43 ± 0.27 μM, and the therapeutic index of ODG-PFA after intravitreal injection was estimated to be 1470:1.

CONCLUSIONS. Lipid-derivatized foscarnet liposome formulations may be a useful long-acting delivery system for the therapy of CMV retinitis. (Invest Ophthalmol Vis Sci. 1999;40:1487-1495)

Cytomegalovirus (CMV) retinitis is the most common cause of visual loss among patients with acquired immune deficiency syndrome (AIDS), occurring in 30% to 40% of patients. With the introduction of new drugs and treatment methods (before the advent of highly active antiretroviral therapy [HAART]) the incidence of CMV retinitis and its severe complications had increased because of improved patient survival. Even though with the introduction of HAART, the incidence of CMV may be decreasing, and in some patients immune reconstitution may occur, the treatment of CMV retinitis is still a major concern.

Treatments for CMV retinitis include systemic ganciclovir, foscarnet, and recently approved (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC). All these drugs merely delay retinitis progression and have systemic toxicities. Besides the high rate of reactivation during maintenance treatment, a significant percentage of patients show notable bone marrow suppression with ganciclovir and renal toxicity with foscarnet and HPMPC. Some patients cannot tolerate intravenous antiviral therapy because of systemic toxic side effects, and local intraocular therapy has been used. Both ganciclovir and foscarnet have been administered in intravitreal injections but require frequent injections because of the short half-life of the drugs. Frequent intravitreous injections have resulted in cataract, vitreous hemorrhage, retinal detachment, and endophthalmitis. Cidofovir is efficacious intravitreally with infrequent doses, but significant incidences of iritis and visually significant irreversible hypotony have been reported. The ganciclovir intraocular device is available for local intraocular therapy; however, this device is not biodegradable. Aside from the increased incidence of retinal detachment and vitreous hemorrhage, the difficulty of replacing the device after all the drug has been released has been reported, caused by fibrosis of the implant and scleral thinning and necrosis.

It would be useful to develop local therapies for human CMV retinitis (HCMV) that would be effective, minimally toxic, and long-lasting, to enhance the response of retinitis to treatment. We have synthesized lipid prodrugs of phosphonoformate (PFA; 1-O-octadecylglycerol-3-phosphonoformate [ODG-PFA] and the methyl ester of ODG-PFA [ODG-PFA-OMe]), which are highly active against HCMV- and herpes simplex virus-infected cells in vitro. These compounds form liposomes with natural lipids, which are advantageous for intravitreal use. In an attempt to increase optical clarity, ODG-PFA micelles were also prepared to evaluate this approach to intravitreal drug delivery. These compounds could provide a new method of local therapy that would not require expensive and potentially hazardous surgical intraocular drug delivery devices.
and that could be administered intravitreally as a simple in-office injection under local anesthesia. The purpose of this study was to determine the intravitreal toxicity of ODG-PFA in vivo and its therapeutic index as intravitreal therapy in liposomal and micellar formulations in the rabbit eye.

**MATERIALS AND METHODS**

**Synthesis of ODG-PFA**

ODG-PFA was synthesized and characterized as previously described. Briefly, a solution of \( N, N \)-dicyclohexylcarbodiimide in tetrahydrofuran was added in drops to a mixture of 1-O-octadecylglycerol and methylphosphonoformate in tetrahydrofuran while stirring. After 24 hours the solvent was evaporated and the product (ODG-PFA ethyl ester) was purified by flash chromatography. To a suspension of 1-O-octadecyglycerol-3-phosphonoformate ethyl ester in absolute ethanol was added 1 N aqueous sodium hydroxide. The mixture was stirred at room temperature for 1 hour. The resultant suspension was filtered, and the precipitate was washed with ethanol. The precipitate was dried to yield 1-O-octadecylglycerol-3-phosphonoformate (disodium salt) as an amorphous solid.

**Liposome Preparation for Intravitreal Injection**

Liposomal formulations were prepared as previously described by dissolving the drug (dry powder) in chloroform, dioleoyl phosphatidylycholine (PC), and cholesterol (CH) at a ratio of 65:20:15 (PC:CH:drug). The chloroform was removed by evaporation under nitrogen, and the resultant thin film was lyophilized overnight. Sterile 5% dextrose was added to produce a drug concentration in the range of 8 mM to 28 mM. The mixture was sonicated for 1 hour in a water-jacketed cup horn at 45°C, producing a translucent solution of small unilamellar liposomes (diameter <220 nm) which was diluted further for injection with sterile 5% dextrose.

**TABLE 1. Vitreous Clarity with ODG-PFA in Liposome Formulation**

<table>
<thead>
<tr>
<th>Intravitreal Concentrations</th>
<th>No. of Eyes</th>
<th>2 Weeks after Injection</th>
<th>8 Weeks after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VCS*</td>
<td>AS and Fundus</td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>0,0,0,0,0,2†</td>
<td>NI</td>
</tr>
<tr>
<td>ODG-PFA 0.02 mM</td>
<td>4</td>
<td>0,0,0,0,4†</td>
<td>NI</td>
</tr>
<tr>
<td>ODG-PFA 0.06 mM</td>
<td>4</td>
<td>0,0,0,0</td>
<td>NI</td>
</tr>
<tr>
<td>ODG-PFA 0.2 mM</td>
<td>6</td>
<td>1,0,0,0,1,0</td>
<td>NI</td>
</tr>
<tr>
<td>ODG-PFA 0.632 mM</td>
<td>4</td>
<td>1,2,2,2</td>
<td>NI</td>
</tr>
</tbody>
</table>

VCS, vitreous clarity scale: AS, anterior segment; NI, normal; NS, nonsignificant.

† Vitreous hemorrhage, not unclear because of drug.

†† Comparison of scores across all doses using Kruskal-Wallis.

FIGURE 1. Fundus photograph at the second week of an eye intravitreally injected with the middle dose (0.632 mM final vitreous concentration) of micellar ODG-PFA reveals white drug precipitates with clear surrounding vitreous.
TABLE 2. Vitreous Clarity with ODG-PFA in Micelle Formulation

<table>
<thead>
<tr>
<th>Intravitreal Concentrations</th>
<th>No. of Eyes</th>
<th>2 Weeks after Injection</th>
<th>Optic Disc Edema</th>
<th>8 Weeks after Injection</th>
<th>Optic Disc Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LVOS*</td>
<td>VCS†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>0.0</td>
<td>0,1†</td>
<td>NN</td>
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<tr>
<td>PFA (2mm)</td>
<td>2</td>
<td>0.0</td>
<td>0,0</td>
<td>NN</td>
<td></td>
</tr>
<tr>
<td>ODG-PFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 mM</td>
<td>4</td>
<td>2,2,2,2</td>
<td>0.0,0,0</td>
<td>NNNN</td>
<td></td>
</tr>
<tr>
<td>0.632 mM</td>
<td>4</td>
<td>4,4,4,4</td>
<td>0.3,0,0‡</td>
<td>NNNY</td>
<td></td>
</tr>
<tr>
<td>2 mM</td>
<td>4</td>
<td>4,4,4,4</td>
<td>1,1,2,1‡</td>
<td>YYYY</td>
<td></td>
</tr>
</tbody>
</table>
| * 0, no local opacity; 1+, thin strip or filament of opacity; 2+, patch of opacity; 3+, ball of opacity; 4+, condensed ball of opacity.  
† 0-4+ scale same as table 1 description.  
‡ vitreous hemorrhage, not unclear because of drug.  
§ Comparison of scores across all doses using Kruskal-Wallis* or Monte-Carlo** simulation.  
*** Two high doses versus low dose/control group in Kruskal-Wallis analysis.
| LVOS, localized vitreous opacity scale; VCS, vitreous clarity scale; N, negative; Y, positive. |

Micelle Preparation for Intravitreal Injection

Micelles were prepared by dissolving the drug of interest in 0.9% NaCl and sonicating to a fine suspension. After it was heated to 65°C, the solution cleared and showed molecular rearrangement into micelles. This solution was terminally filtered through a 0.2-μm sterile filter and diluted with sterile normal saline for injection.

Animal Studies

Twenty New Zealand White rabbits were used for toxicology study in accordance with the guidelines of the University of California, San Diego, Office of Veterinary Affairs and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits weighing from 2.2 kg to 5.6 kg each were used to evaluate the liposome and micelle formulations. The rabbits were divided into two groups, according to whether they were killed 2 weeks or 8 weeks after drug injection. A total of 40 eyes were evaluated including controls. A total of 40 eyes were evaluated including controls (Tables 1, 2). After intramuscular injections of 20 mg/kg ketamine and 5 mg/kg xylazine for anesthesia, a topical anesthetic was provided. Rabbits were anesthetized with a mixture of ketamine (21 mg/kg) and xylazine (5 mg/kg). Intravenous injection followed by an intravenous injection of 0.3 ml/kg B-Euthanasia (Scheiring-Plough Animal Health, Kenilworth, NJ). After enucleation, the eyes were immersed in fixative containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M Sörensøn's phosphate buffer. After 10 minutes, a 5 mm incision was made at the limbus, and the globe was returned to the fixative for 2 to 5 days until gross examination.

Electroretinography (ERG) was performed on all rabbits after surgery and before death. For the procedure, the animals were anesthetized with 2.5% phenylephrine-HCl and 1% tropicamide and dark adapted for 30 minutes. The animals were anesthetized with a mixture of ketamine (21 mg/kg) and xylazine (5 mg/kg). The eyes were positioned 6 in from a stimulator light source. Silver-impregnated nylon electrodes were placed on the corneas. Three flash ERGs were averaged after amplification and digitization with a digital analog converter designed by us using a personal computer.
In selected rabbit eyes with higher doses of ODG-PFA, fluorescein angiograms were performed with a fundus camera (Canon, Lake Success, NY) using 0.15 ml 10% fluorescein sodium injected through an ear vein.

RESULTS

Vitreous Clarity

Liposome Formulation. After injection, the compound evenly dispersed in the vitreous with a clear view of the fundus in all eyes except the eyes that received highest doses (0.1 ml of 8.85 mM). Comparison of vitreous clarity scores showed a significant decrease in clarity at higher doses (P = 0.003; Kruskal-Wallis; Table 1). In these eyes with high doses, the drug did not evenly disperse. The area at the injection site was the most cloudy, with a clearer view of the fundus outside this area (Table 1). The local opacities gradually dispersed in the vitreous over time. At the eighth week, most of the localized opacities were absent with a clear view of the entire fundus. Fundus examination showed that fundi of all animals were normal.

Micelle Formulation. After injection, the drug precipitated and formed local opacities with clear surrounding optical media (Fig. 1). The size and density of the local opacity was concentration dependent (P = 0.017 and 0.0302; Kruskal-Wallis). The local opacity remained in a relatively stable position in the vitreous cavity near the injection site and showed little dispersion with the middle dose (0.1 ml of 8.85 mM) and high dose (0.1 ml of 28 mM). The low dose (0.1 ml of 2.8 mM) showed good dispersion at the second week after injection, with all local opacity being resolved by the eighth week. The middle and the high doses showed a persistent local opacification, which had not cleared up by the eighth week (Table 2). Fundus examination revealed the significant prevalence of optic disc edema in eyes with the higher doses (P = 0.016; Monte Carlo). One of four eyes treated with the middle dose experienced optic disc edema with retinal detachment.

Electroretinographic and Fluorescein Angiographic Findings

All eyes at all concentrations of liposomal ODG-PFA exhibited normal ERGs when compared with the saline controls (amplitude and latency; Fig. 2). In the micelle formulation group, one eye showed severe reduction in amplitude at the second week. This eye received the middle dose of micellar ODG-PFA and exhibited retinal detachment. The remainder of the eyes with various doses of ODG-PFA in micelle formulation had normal ERGs compared with the eyes that received saline and PFA. Fluorescein angiographic examination of the selected eyes revealed mild optic disc staining only in eyes that had optic disc edema in the micelle formulation group. The remainder of eyes with both formulations exhibited normal fluorescein angiograms.

Histology Results

Liposome Formulation. Light microscopic examination showed normal retinal morphology in all eyes at the second and eighth weeks (Fig. 3; Table 3). Trace or mild vitritis located at the injection site or lower part of the vitreous cavity was found in some eyes. Vitritis was more pronounced in eyes with high doses, but this was not statistically significant (P = 0.242; Kruskal-Wallis). The inflammatory cells were predominantly chronic (lymphocytes and macrophages, some of which contained lipids) with only rare polymorphonuclear leukocytes and eosinophils found. The electron microscopic examination of retinal tissues from eyes with the highest dose of liposomal ODG-PFA (0.632 mM final calculated intravitreal concentration) revealed normal retinal ultrastructure when compared with the saline control (Fig. 4, 5).

Micelle Formulation. Light microscopic examination revealed mild to moderate vitritis, mainly around the local drug aggregation, which was a predominant infiltration of plasma cells and lymphocytes with many eosinophils also present. The vitritis was not statistically dose dependent (Table 4). Some of the eyes showed optic disc inflammation or preapillary inflammation when the drug precipitated close to or contacted the optic nerve head. ODG-PFA intravitreal injections, 5 eyes showed local retinal destruction accompanied by glial proliferation nodules in areas where the drug depot was in contact with the retina (Fig. 6; Table 4). Five eyes showed optic disc edema with or without inflammation. In these five eyes with optic nerve head edema, four received the high dose and one received middle
FIGURE 3. Light microscopic histology showing normal retinal morphology and structures of an eye 8 weeks after intravitreal injection with the high dose (0.632 mM final intravitreal concentration) liposomal ODG-PFA. Hematoxylin-eosin; magnification, ×25.

dose. In the eye receiving the middle dose, retinal detachment was found.

DISCUSSION

1-O-Octadecyl-sn-glycero-3-phosphonoformiate (ODG-PFA) is a newly synthesized lipid prodrug of phosphonoformiate. This prodrug is 93 times more active than foscarnet in cells infected by the AD169 strain of CMV and 11 to 147 times more active than foscarnet against human clinical isolates of CMV; furthermore, blank liposomes without drug do not have any antiviral activity in HCMV-infected cells up to very high lipid concentrations. The compound is 43 times more active than foscarnet in cells infected with HSV and 44 times more active than foscarnet in cells infected with human immunodeficiency virus (HIV)-1 in vitro. Toxicity in MRC-5 cells was not observed at concentrations of up to 100 μM drug. This agent can self-organize into clear micelles or formulate readily into liposomes. We had hoped that micellar formulations of ODG-PFA would provide greater degrees of optical clarity than the liposomal drug, because they are completely clear. However, the micellar formulation showed persistent precipitates in the vitreous after intravitreal injection and, in some cases, induced localized vitritis with local retinal damage. We prepared an 11 mM micellar stock solution of ODG-PFA and diluted it over a 6-log range with 0.9% NaCl. No precipitation was noted, even with dilutions of 1,000,000-fold. However, when rabbit vitreous in 24-well plates at 37°C was exposed to micellar drug, immediate precipitates were noted at a variety of concentrations (data not shown). Thus, the effect was specific for vitreous and was not caused by dilution of the micellar drug. These precipitates may be owing to the drug itself or to protein denatured by micellar detergent action. We have not specifically analyzed the precipitates. The position of the precipitates in vitreous was a key factor for the appearance of vitritis and local retinal damage. When the localized precipitates con-

### Table 3. Eye Tissue Pathology with ODG-PFA in Liposome Formulation

<table>
<thead>
<tr>
<th>Intravitreal Concentrations</th>
<th>2 Weeks after Injection</th>
<th>4 Weeks after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Eyes</td>
<td>Intraocular Tissue</td>
</tr>
<tr>
<td>Saline</td>
<td>3</td>
<td>NI</td>
</tr>
<tr>
<td>ODG-PFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02 mM</td>
<td>2</td>
<td>NI</td>
</tr>
<tr>
<td>0.06 mM</td>
<td>2</td>
<td>NI</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>3</td>
<td>NI</td>
</tr>
<tr>
<td>0.632 mM</td>
<td>2</td>
<td>NI</td>
</tr>
<tr>
<td>*</td>
<td>NS</td>
<td>0.242*</td>
</tr>
</tbody>
</table>

NI, normal; NS, nonsignificant.

*Vitritis scale: 0, no inflammatory cells seen; 1+, a few inflammatory cells at low magnification; 2+, sparse inflammatory cells at low magnification; 3+, dense inflammatory cells at low magnification; 4+, between mild and severe.

† Comparison of scores across all doses using Kruskal-Wallis.
tacted the retina, the eye was more likely to exhibit localized vitritis associated with local retinal damage. The exact nature of the precipitates was not determined. The precipitates formed only with the micellar formulation. The variable local retinal damage findings were probably caused by random movement of localized precipitates of drug and/or proteins in the vitreous. These pilot study results suggest that micellar formulation was not ideal for intravitreal use.

Intravitreal injections of ODG-PFA in liposomal formulation showed no significant evidence of intraocular inflammation at the 2-week and 8-week time points. Electroretinography showed normal amplitudes and wave forms using our previously described methods. Evaluation of the media showed no local opacification of vitreous in eyes with the highest doses. With the other three doses, the vitreous was clear, and retinal function and structures were normal. The highest drug concentration used for micellar formulation was 2 mM versus 0.632 mM for liposomal formulation. This represents a three-fold (half-log) increase. Rather than be specific to the micellar formulation, we also prepared liposomal formulations at the highest concentration in a preliminary study. However, the resultant opaque solution showed inadequate visual clarity in the vitreous at the highest concentrations. Therefore, the highest liposomal concentration for the principal study was set one-half log lower to allow for acceptable visual clarity. There were no precipitates seen with the liposomal formulation at all concentrations tested. According to our in vitro results, the 50% inhibitory concentration (IC_{90}) of ODG-PFA for laboratory strain AD169 HCMV was 0.43 μM and for human clinical isolates was approximately 0.95 μM, without difference in antiviral activity from dimethyl sulfoxide or liposomal formulations. Therefore, if we take 0.632 mM final concentration as the highest nontoxic dose, the therapeutic index of ODG-PFA was 1470:1 for laboratory strain AD169 HCMV or 665:1 for human clinical isolates. This therapeutic index appears to be the highest among the currently available anti-CMV drugs. According to previous reports, foscarnet can be administered intravitreally with 2400 μg in patients without intraocular drug toxicity. This dose yielded approximately 2 mM final intravitreal concentration in human eyes. With the IC_{90} of foscarnet for most HCMV clinical isolates being approximately 137 μM, the therapeutic index for foscarnet should be 14.6:1. This is 46 (665/14.6) times lower than that of ODG-PFA. For ganciclovir, the mean IC_{90} for clinical HCMV isolates has been shown to be 0.55 μg/ml or 3.3 μg/ml, thus the therapeutic index is approximately 188:1 to 109:1 when 400 μg ganciclovir is injected into human eyes. This therapeutic index is also much lower than that of ODG-PFA. Compared with the therapeutic index of 182 (for laboratory-strain AD169 HCMV) for liposomal acyclovir diphosphate dimyristoylglycerol (ACV-DP-DMG), ODG-PFA is eight (1047/182) times more active against HCMV than is ACV-DP-DMG. We know that ODG-PFA is also much more active than HPMPC, which is more toxic in human eyes than in rabbit eyes. In human eyes, if we take the 20-μg intravitreal injection as the highest nontoxic dose and we use 4 ml as the vitreous volume, HPMPC yields a therapeutic index of only 65.8:1 for clinical HCMV isolates, with 0.076 μg/ml for the IC_{90}.

We have previously reported that complete tissue culture medium containing 20% fetal bovine serum does not metabolize ODG-PFA even after 3 days at 37°C. Cells are required to convert ODG-PFA to PFA. We have also incubated ODG-PFA with vitreous without any evidence by chromatography of conversion to PFA. Therefore, we conclude that the drug is metabolized in the tissues by a phosphodiesterase to 1-O-octadecylglycerol and foscarnet. The vitreous merely acts as a reservoir of the compound. This is advantageous for local intravitreal administration.

ODG-PFA can form liposomes with natural lipids, which is also advantageous for local intravitreal administration. This lipid prodrug forms the liposome wall together with naturally occurring phospholipids and cholesterol, enabling a slow release of drug and allowing for liposomal incorporation of essentially 100% of the drug. Previous studies have shown that the intrinsic activity of PFA in inhibiting viral polymerases is virtually abolished by any chemical substitution (reviewed in Ref. 29); thus, the antiviral effect must come from the PFA released from ODG-PFA. It is also true that the lipid prodrug greatly enhances ODG-PFA transport across the cell membrane as evidenced by our prior work, which accounts for the improved therapeutic potential observed in this study. The liposome wall containing the lipid-derivatized antiviral compound may fuse with the cell membranes of retinal cells allowing incorporation and continuous slow release of the drug into the cells to maintain a high concentration of the ODG-PFA in retinal cells where ODG-PFA was converted into PFA to inhibit
viral polymerases. Alternatively, ODG-PFA may be transferred from liposomes to the target cell by bulk transfer or by protein-mediated transfer. In previous studies, our group has used lipid produgs for intraocular drug delivery and has shown longer vitreal half-life with higher therapeutic index. ODG-PFA, at its highest nontoxic dose (final intravitreal concentration of 0.632 mM in this experiment), showed a clear central vitreous; the local opacity in vitreous provided a depot of drug that could provide for gradual release of drug into the vitreous in a concentration that is not damaging to the retina. This may significantly reduce the frequency of intravitreal injections.

In this study some eyes showed mild variable vitritis which was not detected clinically but only by histologic examination. The vitritis with liposome formulation was a chronic inflammation that was characterized by infiltration of lymphocyte and macrophage cell populations. The histiocytic response seen in eyes with the liposomal drug was not seen in eyes with the micelle formulation and may be a response to lipids in the drug vehicle. In contrast, in eyes with vitritis after injection with micelles, the distinguishing feature of the inflammatory response was more eosinophils and a predominant plasma cell infiltration. This vitritis was more prevalent at the 2-week time interval.

Because of the systemic side effects of systemic administration of antiviral drugs, intraocular repository methods have been investigated. At present, ganciclovir implant and HPMPC intravitreal injection are available for local treatment of CMV retinitis, but the former has resulted in severe complications from surgery for implant placement or implant exchange and the latter has led to severe sight-threatening hypotony. Considering the significant advantages of ODG-PFA over currently used anti-CMV drugs, pharmacokinetic studies and additional efficacy studies in a retinitis animal model are highly indicated. This lipid-derivatized foscarnet could develop into a preferred long-acting delivery system for the therapy of CMV retinitis.

Table 4. Eye Tissue Disease with ODG-PFA in Micelle Formulation

<table>
<thead>
<tr>
<th>Intravitreal Concentrations</th>
<th>2 Weeks after Injection</th>
<th>8 Weeks after Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Eyes</td>
<td>Vitritis*</td>
</tr>
<tr>
<td>Saline</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PFA (2 mM)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ODG-PFA</td>
<td>0.2 mM</td>
<td>2</td>
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<tr>
<td></td>
<td>0.632 mM</td>
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<td></td>
<td>2 mM</td>
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</tbody>
</table>

N, negative; Y, positive; NS, nonsignificant.

* Vitritis scale: 0, no inflammatory cells seen; 1+, a few inflammatory cells at low magnification; 2+, sparse inflammatory cells at low magnification; 4+, dense inflammatory cells at low magnification; 5+, between mild and severe.

† Comparison of scores across all doses using Kruskal-Wallis or Monte Carlo simulation.
References


