Scleral Plug of Biodegradable Polymers Containing Ganciclovir for Experimental Cytomegalovirus Retinitis

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PURPOSE. To evaluate the efficacy of a biodegradable scleral plug containing ganciclovir (GCV) in a rabbit model of human cytomegalovirus (HCMV) retinitis.

METHODS. To develop a rabbit model for HCMV retinitis, HCMV solution was injected once into the vitreous cavity of pigmented rabbits. The treated animals were divided into three groups: group A received no treatment, group B was treated once with GCV solution, and group C was treated with a scleral plug containing GCV. Rabbits in group B received an intravitreal injection of GCV solution 1 week after HCMV inoculation. In group C, the scleral plug containing GCV was implanted in the vitreous of the rabbits 1 week after HCMV inoculation. Ophthalmoscopically, vitreoretinal findings in each group were graded from 0+ to 4+ every week for 4 weeks after HCMV injection.

RESULTS. Eyes of group A rabbits showed whitish retinal exudates and vitreous opacities 3 days after HCMV inoculation. These materials increased gradually until 3 weeks after HCMV inoculation. Scores for vitreoretinal lesions were significantly lower in eyes of group B rabbits compared with those of group A at 1 week after GCV injection (P < 0.05). However, vitreoretinal inflammation in eyes of group B rabbits increased again thereafter, and no significant difference in inflammation between groups A and B was found 2 weeks after GCV injection. In eyes of group C, scores for vitreoretinal lesions were significantly lower compared with those in both group A and group B at 3 weeks after HCMV inoculation (P < 0.01).

CONCLUSIONS. The results demonstrated that sustained release of GCV into the vitreous cavity with biodegradable scleral plugs was effective for the treatment of experimentally induced HCMV retinitis in rabbits. (Invest Ophthalmol Vis Sci. 2001;42:2043–2048)

H uman cytomegalovirus (HCMV) retinitis is the most common ocular infection in patients with AIDS. Retinal infection with HCMV develops in 15% to 25% of patients with AIDS.1–4 The two antiviral compounds that are generally used to treat HCMV, ganciclovir (GCV)5–7 and foscarnet,8,9 have several problems associated with intravenous use. The presence of an indwelling catheter and the time required to infuse these medications may also have a negative impact on quality of life.

Direct intraocular treatment may circumvent these systemic problems.10,11 Multiple injections are associated with an increased risk of cataract, astigmatism, endophthalmitis, retinal detachment, and vitreous hemorrhage.11 A sustained-release device was developed to decrease these risks and to produce a longer therapeutic effect.12

Human immunodeficiency virus (HIV) protease inhibitor has been developed and used in combination with conventional reverse transcriptase inhibitor13,14 (so-called highly active antiretroviral therapy [HAART]). HAART decreases virus quantity and remarkably increases the number of CD4+ lymphocytes. It is reported that HAART decreases the incidence of HCMV retinitis by approximately 50% to 60%, in comparison with a single treatment of a conventional reverse transcriptase inhibitor alone. In many cases, patients with HCMV retinitis who receive HAART partially recover their immunity to HCMV and are able to discontinue anti-HCMV therapy. However, some patients cannot tolerate HAART, and others who receive HAART therapy may develop HCMV retinitis.

Increased survival of patients with HIV underscores the need for a prolonged and effective treatment for HCMV retinitis. The device for sustained release of GCV (Vitrasert; Bausch & Lomb, Inc., Claremont, CA) is a widely used device to the control HCMV retinitis.15–20 However, implant surgery of this device has been associated with many complications such as acute- or delayed-onset endophthalmitis, retinal detachment, vitreous hemorrhage, malpositioning of the implant, and temporally decreased vision caused by astigmatism, hypotony, and cataract.21–25 Also, repeated operations are needed to replace the implant,24–26 and long-term risks of implantation are largely unknown. Removal of the implant may be difficult and may result in surgical complications. Recently,29 to avoid these complications, a biodegradable device of GCV-loaded microspheres as a drug delivery system was reported; however, it has not been clinically applied, because dense vitreous opacity caused by polymeric particles continues long after its injection.

To avoid these complications, we prepared a biodegradable polymer containing GCV. From this polymer we made a scleral plug in a form that avoided direct damage to the lens and the retina, allowed a minimal sclerotomy, and could be set into the eye easily.

The goal of this study was to investigate the efficacy of using a scleral plug containing GCV to treat HCMV retinitis in an experimental rabbit model and to examine the presence of surgical complications.

MATERIALS AND METHODS

Materials

We used poly(ε-lactid; PLGA) with an average molecular weight of 70,000 and poly(ε-lactide-co-glycolide) with an average molecular weight of 5,000 and a copolymer ratio of ε-lactic acid to glycolic acid.
of 80:20 (PLGA-70,000/PLGA-5,000: 80/20). The weight-average molecular weight was determined by gel permeation chromatography by the suppliers. GCV (Denosine) was purchased from Nippon Syntex K K (Tokyo, Japan).

**Preparation of Scleral Plugs Containing GCV**

The scleral implants were prepared by dissolving the polymer and GCV in acetic acid, which is a good solvent for the polymer and the drug. The resultant solution was lyophilized (FDU-830; Tokyo Rikakikai, Tokyo, Japan) to obtain a homogeneous cake. The cake then was compressed into a scleral implant on a hot plate (Model HM-19; Koike Precision Instruments, Osaka, Japan) at temperatures ranging from 80°C to 100°C. It was confirmed that GCV degradation did not occur during the preparation process of the implants. The implants had loadings of 25% (wt/wt). The material used in this research (PLGA-70,000/PLGA-5,000: 80/20) can deliver GCV into the vitreous in a therapeutic range adequate to treat HCMV retinitis for more than 200 days in an in vivo study using rabbits.

The scleral implant weighed 8.5 mg and was 5 mm long and 1 mm in diameter. It was shaped similarly to the scleral plugs that are used on a temporary basis during vitrectomy (Fig. 1).

**Cell Culture and HCMV Propagation**

Human fetal lung fibroblasts (HFL-1; Rikagaku Research Institute, Rikagaku, Japan) were cultured in Ham’s F12 medium (ICN Biomedicals, Costa Mesa, CA) supplemented with 15% fetal bovine serum and were propagated on a 75-cm² culture dish. HCMV AD169 (0.1 ml of 1 × 10⁸ plaque-forming units [pfu]/ml), provided by Yukihiro Nishiyama (Nagoya University School of Medicine), was grown on HFL-1 monolayers maintained for 1 hour in a humid atmosphere containing 5% carbon dioxide at 37°C and added to the medium. HCMV AD169 supernatant stock was injected directly onto fresh, confluent monolayers of Hs68 cells in 80-cm² flasks. Infected cultures were maintained at 37°C in a 5% carbon dioxide atmosphere until less than 75% of the cells exhibited a cytopathogenic effect. The cells begin to exhibit such an effect (that is, HCMV propagation) on day 7. All cells exhibited a cytopathogenic effect by day 14, and the HCMV-infected cells were harvested. The quantity of HCMV was 5 × 10⁶ pfu/ml, measured by using the anti-HCMV antibody.

**HCMV Inoculation into Rabbit Eyes**

Pigmented male rabbits, weighing 1.5 to 2.0 kg each, were used. All animals were handled according to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. The rabbits were anesthetized with an intramuscular injection of pentobarbital sodium (20 mg/kg) before treatment. The pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride eye drops. The ocular surface was then anesthetized with topical instillation of 0.4% oxybuprocaine hydrochloride. Thirty eyes of 30 pigmented rabbits were inoculated with 0.1 ml (5 × 10⁶ pfu/ml) HCMV supernatant. For inoculation, HCMV was drawn into a sterile 1-ml tuberculin syringe fitted with a 30-gauge needle. The propagated HCMV was injected into one eye through the sclera, 2 mm posterior to the limbus. The inoculum was injected into the vitreous chamber near the vitreoretinal interface.

**Implant of Scleral Plugs and Free GCV Injection in HCMV-Infected Rabbit Eyes**

The 30 eyes of 30 pigmented rabbits that were inoculated with HCMV were divided into three groups. One week after HCMV (1 × 10⁶ pfu/ml) inoculation, rabbits were anesthetized again and topical anesthetic was instilled into the eyes. The three groups of eyes (10 eyes in each group from 10 different pigmented rabbits) were as follows: Group A, the control group, received no treatment; group B had 0.1 ml (2 mg/ml) GCV injected into the vitreous cavity through the sclera, 2 mm posterior to the limbus, with a 30-gauge needle; and group C, after the sclera was exposed, had a 1-mm sclerotomy performed with a V-lance 3.5 mm from the limbus. The scleral plug of PLGA-loaded GCV was inserted at the sclerotomy site. The conjunctival wound was sutured with 80 Dexon sutures (Tyco International, Madison, NJ; Fig. 2).

**Chorioretinal Grading of Eyes Inoculated with HCMV**

All groups underwent indirect ophthalmoscopy analysis through dilated pupils on days 1, 2, 3, 4, and 7 and weeks 2, 3, and 4 after HCMV inoculation. All examiners were masked to the treatment received, and the treatments were performed concurrently by two observers. Fundus disease was graded on a 0+ to +4 scale of increasing severity, documented by fundus drawing, and recorded by photograph using a direct fundus camera (Kowa Co., Tokyo, Japan). The retinal and choroidal diseases were scored as follows: 0+, no abnormalities; 1+, focal white retinal infiltrates; 2+, focal-to-geographic retinal infiltrates and vascular engorgement; 3+, severe retinal infiltrates, vascular engorge-
ment, and hemorrhage; and 4+, all the foregoing, plus retinal detachment and necrosis.

**Histologic Examination by Light Microscopy**

After a 4-week observation period, selected eyes of group A rabbits were enucleated and processed for histopathologic analysis. Rabbits were killed at 1 month after injection, and the eyes were immediately fixed in phosphate-buffered 2.5% glutaraldehyde and 5% formaldehyde in 0.15 M phosphate-buffered solution (pH 7.2) at 20°C. The specimens were dehydrated in a series of ascending concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of the eye were cut at a thickness of 4.0 μm and mounted on glass slides. After being dewaxed in xylene, sections were hydrated in a series of descending concentrations of ethanol. The hydrated sections were stained with Meyer’s hematoxylin solution at 20°C for 10 minutes, rinsed in tap water for 15 minutes, immersed in 0.5% cosin solution at 20°C for 10 minutes, dehydrated in a series of ascending concentrations of ethanol, cleared in xylene, and mounted in synthetic resin solution (Harleco; Kokusai Shiyaku, Kobe, Japan). All sections were examined in detail by light microscope (Provis AX 70; Olympus Co., Tokyo, Japan).

**Immunofluorescent Detection of HCMV Antigens in Chorioretinal Tissue**

HCMV antigens in chorioretinal tissue sections were detected by indirect immunofluorescence assay. Routinely fixed paraffin tissue sections of selected enucleated rabbit eyes from all groups after 4 weeks after HCMV inoculation were used for this procedure. Slides were hydrated in phosphate-buffered saline (PBS) for 5 minutes at room temperature. Sections were overlaid with mouse anti-CMV monoclonal antibody provided by Kanji Hirai (Tokyo Dental College) as the first antibody, which reacts in 60 minutes at room temperature.

The slides were washed in two changes of PBS for 5 minutes. The sections then were overlaid with 20 μl of a 1:10 dilution of fluorescein isothiocyanate (FITC)–conjugated anti-mouse immunoglobulin as the second antibody at room temperature. Slides were washed two times in PBS for 5 minutes followed by a final 5-minute wash in distilled water before being air dried. A coverslip was placed over the section after the addition of 15 to 20 μl of a glycerol-PBS solution (4:1). Fluorescence was observed by photomicroscopy (Optiphot; Nikon, Tokyo, Japan).

**FIGURE 3.** Fundus photographs after inoculation with HCMV in the control eyes (group A). (A) One week after inoculation, some whitish retinal exudates were visible (chorioretinal grade, +1). (B) Two weeks after inoculation, the retinal exudates were larger and had increased in number (chorioretinal grade, +2). (C) Three weeks after inoculation, the retinal exudates continued to increase in size and were most numerous (chorioretinal grade, +3). (D) Four weeks after inoculation, the quantity and number of retinal exudates had decreased (chorioretinal grade, +0).
Detection of Surgical Complications after Implantation of Scleral Plugs

To detect surgical complications after the GCV-containing scleral plug was implanted, anterior segment examination by slit lamp biomicroscopy and detailed fundus examination by indirect ophthalmoscopy were conducted.

RESULTS

Clinical Course of Experimental CMV Retinitis

In eyes in the no-treatment group (group A), whitish retinal exudates (Fig. 3) developed 3 days after HCMV inoculation and increased gradually until 3 weeks after inoculation. Thereafter, the chorioretinitis decreased until 4 weeks after injection (Fig. 4A).

Histopathologic Examination

In group A eyes, inflammatory cells were seen on the surface of the retina and vitreous, corresponding to lesions of white exudates seen ophthalmoscopically. Destruction of retinal structures was seen in some areas (Fig. 5).

Immunologic Detection of HCMV Antigens

In group A eyes, immunofluorescent particles corresponding to HCMV antigens were observed inside the retina of enucleated eyes (Fig. 6).

Surgical Complications after Implantation of Scleral Plugs

There were no surgical complications, such as vitreous hemorrhage, hyphema, cataract, retinal detachment, or endophthalmitis, in any eyes with scleral implants.

Effects of Treatment

In group B eyes (Fig. 4B), scores for vitreoretinal lesions were significantly lower than those in group A at 2 weeks after HCMV inoculation (1 week after a single injection of GCV). However, the treatment effect was transient. Vitreoretinal inflammation recurred and no difference was found at 3 weeks after HCMV inoculation (2 weeks after GCV injection, Fig. 7). In group C eyes (Fig. 4C), scores for vitreoretinal lesions were significantly lower than those in both group A and group B at 3 weeks after HCMV inoculation (Fig. 8).

DISCUSSION

In this study, the biodegradable scleral plug containing GCV was effective for treatment of HCMV retinitis in a rabbit model. They released GCV through hydrosis and it then degraded in the vitreous. The degradation of the matrix depended on its molecular weight and polymer composition.

The device for sustained release of GCV (Vitrascert; Bausch & Lomb, Inc.) has been widely used for the control of HCMV retinitis. However, the incidence of severe postoperative complications with this device are not low, with a reported incidence of retinal detachment of 11% to 18%. Other postoperative complications including vitreous hemorrhage, endophthalmitis, cystoid macular edema with epiretinal membrane, and cataract have been reported.

Vitrascert provides sustained release of GCV for 5 to 8 months. If the patient’s immune system remains seriously compromised at 5 to 8 months after the first implantation, it must be decided whether to place an additional implant or replace just the implant. Some patients need additional implants in the same eye. This approach has several limitations: multiple large sclerotomies weaken the eye wall, multiple implants reduce the view of the peripheral retina, and the positioning of sclerotomies for subsequent vitrectomy, if necessary, is restricted. When operations for implant are repeated,
the risks of intraocular bleeding and intraocular dislocation during manipulation of the implant may increase.

In contrast to the previous reports,\textsuperscript{21-28} it was easy to perform a minimal sclerotomy to implant the scleral plug used in the current study. No surgical complications occurred, because we had prepared the scleral plug implant in a form that avoided direct damage to the lens and retina. In addition, it was not necessary to replace the implant in a second surgery, because all the implanted material was biodegradable. In this study, no surgical complications such as hypotony or endophthalmitis were observed. Our previous report demonstrated that the scleral wound was sealed with the swelling around the biodegradable plug and was finally replaced with fibrous tissue.\textsuperscript{31}

A rabbit model for HCMV-induced chorioretinal infection has been reported, but it is difficult to develop a complete model because of the extreme species specificity to members of the CMV family.\textsuperscript{32} No reports show CMV replication in the retina. Some reports of rabbit models for HCMV chorioretinitis show failure to complete the experiment because productive virus infection was transient,\textsuperscript{33,34} sustaining a moderate plateau for 4 days, then dropping precipitously, producing no viral recovery by day 8 after inoculation. These findings were in distinct contrast to the relentless progress of untreated HCMV retinitis. It should be noted that none of the retinal pathologic characteristics of the human disease developed.

Attempts by other investigators to reproduce HCMV replication in the rabbit retina have been unsuccessful. In contrast, an experimental model by Laycock et al.\textsuperscript{32} appeared to be capable of sustaining active HCMV replication for at least 3 to 4 weeks, and virus antigens could be detected at the end of this period. Also, the virus was able to undergo multiple consecutive cycles of replication. These investigators demonstrated that this model was useful for evaluating antiviral therapies against human CMV retinal disease. Similarly, our HCMV retinitis model appeared capable of sustaining active HCMV replication for at least 4 weeks, as observed ophthalmoscopically. Also, we were able to detect CMV antigens in retinal tissue immunohistologically.

\textbf{FIGURE 5.} Histopathologic analysis of untreated HCMV-infected retina (group A) 4 weeks after inoculation showed disorganization of normal retinal architecture in the outer and inner retinal layers. Many mononuclear cells were noted. Hematoxylin-eosin; scale bar, 75 μm.

\textbf{FIGURE 6.} Immunofluorescence-detected HCMV antigens localized in inner and outer retinal and epiretinal layers in HCMV-inoculated rabbit eyes. V, vitreous; R, retina. Scale bar, 50 μm.

\textbf{FIGURE 7.} Fundus photograph of a group B retina after HCMV inoculation and treatment with a single injection into the vitreous cavity of 0.1 ml (2 mg/ml) GCV. The quantity and number of white retinal exudates continued to increase (chorioretinal grade, +3).

\textbf{FIGURE 8.} Fundus photograph of a group C retina inoculated with HCMV, 2 weeks after implantation of a scleral plug containing GCV. The quantity and number of white retinal exudates were significantly decreased (chorioretinal grade, +1), compared with the retinal grading of groups A and B.
Implanted scleral plugs containing GCV significantly reduced the retinal inflammatory changes that were visible ophthalmoscopically. Although the HCMV retinitis model was self-limiting and persisted for only 4 weeks, the difference between the control and treated eye was statistically significant.

In conclusion, our results suggest that this vitreous drug delivery system using an implantable biodegradable polymer device containing GCV may be useful in the treatment of HCMV retinitis.

References