Feasibility of Drug Delivery to the Posterior Pole of the Rabbit Eye with an Episcleral Implant

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PURPOSE. To evaluate the feasibility of a nonbiodegradable polymeric episcleral implant as a new controlled intraocular delivery system of betamethasone (BM) to the posterior pole of the eye.

METHODS. The episcleral implant, which is composed of a drug-releasing component and a suture tag, released BM through an ethylene vinyl acetate membrane. The implants were placed on the sclera in 12 eyes of 12 Japanese white rabbits so that the drug-releasing surface could attach to the sclera at the posterior pole. BM concentrations in the aqueous humor, vitreous, and retina-choroid (posterior half and anterior half) were determined by high-performance liquid chromatography (HPLC) at weeks 1, 2, and 4 after implantation. In addition, the intraocular tissue distribution of the drug was evaluated by fluorescein microscopy after implantation of the implant loaded with 6-carboxy fluorescein diacetate (6-CFDA) as a drug marker. Retinal toxicity was evaluated by electroretinography and histologic examination.

RESULTS. The implant showed zero-order release profiles both in vitro and in vivo for 4 weeks. BM concentrations in the retina-choroid after implantation were maintained above the concentrations effective for suppressing inflammatory reactions for at least 4 weeks. The BM concentration was greater in the posterior half of the retina-choroid than in the vitreous. It was confirmed that 6-CFDA penetrated through the sclera and dispersed into the retina-choroid. Fluorescence from 6-CFDA gradually decreased in intensity with increased distance from the implantation site. Electroretinography and histologic study showed no substantial toxic reactions.

CONCLUSIONS. These findings suggest that the episcleral implant may be a useful drug carrier for intraocular delivery of BM, especially for the posterior part of the eye. (Invest Ophthalmol Vis Sci. 2004;45:238–244) DOI:10.1167/iovs.02-1258

Many macular diseases, such as age-related macular degeneration (AMD) and cystoid macular edema (CME), associated with uveitis, diabetic retinopathy, and central retinal vein occlusion require long-term therapy to supply effective doses of drugs to the posterior pole of the eye. Not all routes of administration, however, are appropriate. Topical delivery is a relatively easy method of drug administration and carries little risk. Penetration of the drug, however, is poor in the retina or choroid due to lacrimation and a long diffusional path. Systemic administration of a drug may cause general side effects while achieving therapeutic levels in the eye. Although intravitreal injection can deliver drugs to the posterior part of the eye without producing systemic side effects, multiple injections, as would be required for chronic choroidal disorders, may cause local complications, such as vitreous hemorrhage, retinal detachment, cataract formation, or endophthalmitis. Peribulbar or subconjunctival injection is often used to deliver the drug to the posterior part of the eye. However, serum levels after injections were reportedly as high as after oral administration, and drug concentration in the aqueous humor was also high due to transcorneal diffusion. Recently, intravitreal injection of lipophilic compounds such as triamcinolone acetonide has been reported to be useful to treat various cases of CME or AMD. Although the drugs can remain long-term in the intraocular tissues after injection, the release rate of the drug may not be controlled. Therefore, a local sustained-release drug delivery system with minimal side effects and invasion may be an ideal method.

Materials and Methods

Ethylene vinyl acetate co-polymer (EVA, vinyl acetate content: 53%) was purchased from Aldrich Chemical Co. (Milwaukee, WI); BM from Wako Pure Chemicals Industries (Osaka, Japan); polyvinyl alcohol 205 (PVA; polymerized degree 500, 86.5% to 89% hydrolyzed) from Kura ray Co., Ltd. (Tokyo, Japan); 6-CFDA from Sigma Chemical Co. (Tokyo, Japan), and dichloromethane from Kanto Chemical Co., Inc. (Tokyo, Japan). Other chemicals were of reagent grade.

Preparation of the Implant

BM and 20% PVA aqueous solution were mixed in a mortar (90% BM loading), and the mixture was shaped into granules through a sieve. The granules (75 mg) were filled in a stainless mold for infrared analysis, compressed into a disc by a press under reduced pressure at...
the weight of 10 tons for 5 minutes and a 4-mm diameter disc was stamped out by a trephine.

A stainless steel wire (diameter 0.28 mm) was inserted into the EVA tube (inner diameter 0.52 mm, outer diameter 1.52 mm) and cut approximately 15 mm long. The EVA discs (diameter, 4 mm) were attached to both ends of the EVA tube by heating at 100°C on the hot plate (model HM-19; Koike Precision Instruments, Osaka, Japan).

An EVA disc was fixed on one side of the BM disc surface by heating at 100°C on the hot plate. The BM disc was immersed into the EVA-dichloromethane solution at various concentrations (2.5% or 5%) for 5 seconds, to coat it with the EVA membrane. Next, it was dried at room temperature for 24 hours and then under reduced pressure for 24 hours. Finally, the BM disc was fixed on the end of the EVA disc of the suture tag by heating at 100°C on the hot plate. Figure 1 shows the episcleral implant (top) and a macular explant (bottom).

### In Vitro Release Study

The implant was incubated in 50 mL of phosphate-buffered solution (0.1 M, pH 7.4) in a shaking water bath (BT-25; Yamato Scientific Co., Ltd., Tokyo, Japan) at 37°C. At predetermined intervals, the entire volume was sampled, and 50 mL of fresh medium was added to the sample vial to approximate perfect sink conditions. The concentration of BM in the medium was below 20% of its saturated solubility at all times. The amount of BM released into the medium was measured by HPLC, using a C-18 reversed-phase column (150 × 6.0 mm inner diameter, YMC-Pack ODS-A312; YMC Co., Ltd., Kyoto, Japan). A pump (PU-980; Japan Spectroscopic Co., Ltd., Tokyo, Japan) was used at a constant flow rate of 1 mL/min. The mobile phase was a mixture of methanol and 50 mM potassium dihydrogenphosphate aqueous solution (55:45). The column oven (860-OC; Japan Spectroscopic Co., Ltd.) was equipped and set at 40°C. A spectrophotometer detector (L-4000; Hitachi, Ltd., Tokyo, Japan) was used at a wavelength of 240 nm. Fluorometholone (Wako Pure Chemical Industries) was used as an internal standard.

### In Vivo Release Study

Twelve right eyes of 12 Japanese white rabbits, weighing 2.0 to 2.5 kg each, were used. All animals were handled according to the ARVO Statement for the Use of Animals in Ophthalamic and Vision Research. We used the BM-loaded implants coated with 5% EVA-dichloromethane solution for in vivo studies. The rabbits were anesthetized with a mixture (1:1) of xylazine hydrochloride (2 mg/kg) and ketamine hydrochloride (5 mg/kg). The ocular surface was then anesthetized with a 0.4% oxybuprocaine hydrochloride. A wide area of the upper temporal quadrant of the sclera was exposed with a perilimbal conjunctival incision. An implant was placed in the episcleral space so that the drug-releasing surface could attach to the sclera at the posterior pole (Fig. 2). The end of the implant was fixed with 60 nylon, and the conjunctival wound was sutured with 7-0 silk. Animals were killed with an overdose of intravenous pentobarbital sodium at weeks 1, 2, and 4 after implantation. The sclera was exposed, the stitches were removed, the tissue around the implant was excised, and the implant was extracted from the episcleral space. The eyes with the implants and the contralateral eye were enucleated and immediately frozen at −85°C and the implant and samples of ocular tissues were retrieved. These were the aqueous humor, vitreous, and posterior and anterior halves of the retina-choroid. The ocular tissues were stored at −85°C until the BM concentrations were determined by HPLC.

### Determination of BM in the Implants and Ocular Tissues

The remaining amount of BM in the implant was determined by the described HPLC procedures. The recovered implant was cut by a razor to expose the remaining BM pellet. The remaining BM was dissolved with 50 mL mobile phase of HPLC. BM was also determined by the described HPLC procedures. BM was dissolved with 0.2 mL mobile phase. One hundred microliters of this solution was injected into HPLC to determine the remaining amount of BM in the implant. The remaining percentage of BM in the implant was shown as the percentage of the remaining amount of BM in the recovered implant to the actual loading amount of BM in the implant before implantation. The BM concentration in recovered ocular tissues (aqueous humor, vitreous humor, and two sections of the retina-choroid) was also determined by the described HPLC procedures. BM was extracted from the tissues by the following procedures: 0.1 mL of internal standard solution (2.5 μg/mL fluorometholone) and 3.0 mL of 0.2 M HCl were added to each tissue sample. The mixture was homogenized and centrifuged at 3000 rpm for 15 minutes (KN-70; Kubota, Tokyo, Japan). The supernatant was collected, and BM was extracted twice with 3.0 mL of ethyl acetate. Ethyl acetate phases were then dried under reduced pressure using a centrifugal concentrator (VC-960; Taiotec Co., Saitama, Japan). The residue was dissolved with 0.2 mL mobile phase. One hundred microliters of this solution was injected into HPLC, as described. Under these conditions, the detection limits for BM were 5 ng/g in the vitreous humor and 50 ng/g in both the aqueous humor and retina-choroid. The extraction rate of BM from all studied ocular tissues was approximately 100%. The BM concentrations in ocular tissues were represented as BM weight per weight of wet tissue.

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932922/)  
**Figure 1.** The BM-loaded biodegradable implant (top) and a macular explant (bottom).
Distribution of Fluorescence after Implantation of 6-CFDA-Loaded Implant

To evaluate the intraocular distribution of the drug after implantation, 6-CFDA was used as a drug marker. 6-CFDA is comparatively similar to BM, in that it is a lipophilic compound with a molecular weight of 460.4. The 6-CFDA-loaded implants were prepared by the same procedure described for BM. The 6-CFDA-loaded implants were implanted in two rabbits. Animals were killed at 4 days after implantation and eyes were enucleated. The eyes were embedded in tissue-freezing medium (Tissue Tek; OCT compound; Sakura Finetec Co., Ltd., Tokyo, Japan), frozen, and sectioned at 10 μm on a cryostat. The sections were observed by fluorescein microscopy (AX-70; Olympus Optical Co., Ltd., Tokyo, Japan). Two eyes without implants were also observed by fluorescein microscopy.

Toxicity Study of the Implant

Electrophysiological Evaluation. Four rabbits received the BM-loaded implants in the right eyes. Scotopic electroretinography (ERG-50; Kowa, Co., Ltd., Nagoya, Japan) was performed on both eyes before treatment and 4 and 8 weeks after implantation of the BM-loaded implant. The a- and b-wave amplitudes in the experimental (right) eyes and the control (left) eyes were evaluated.

Histologic Examination. Four rabbits receiving the BM-loaded implants were killed at 4 and 8 weeks after implantation. The eyes were enucleated and immediately immersed in a mixture of 4% glutaraldehyde and 2.5% neutral buffered formalin for 24 hours. Globes were opened at the pars plana and the cornea, lens, and vitreous were carefully removed. The retina-choroid and sclera were dehydrated, embedded in paraffin, and sectioned with a microtome. Sections were stained with hematoxylin-eosin for light microscopy.

Statistical Analysis

An unpaired Student’s t-test was used to compare the release rate from the implants coated with 2.5% and 5% EVA-dichloromethane solution. A paired Student’s t-test was used to determine the statistical difference between a- and b-wave amplitudes of the eyes with implants and those of the control eyes. P < 0.05 was considered to be statistically significant.

RESULTS

In Vitro Release of BM from the Implant

The cumulative release of BM from the implants coated with 2.5% and 5% EVA-dichloromethane solution was recorded (Fig. 3). Increasing the EVA concentration decreased the release rate. The release rate from the implants coated with 2.5%
EVA-dichloromethane solution (22.43 ± 6.8 μg/d) was significantly higher than that from the implants coated with 5% EVA-dichloromethane solution (10.8 ± 2.6 μg/d; P < 0.05). BM was constantly released from all implants for 3 months.

**In Vivo Release of BM from the Implant**

In vivo release of BM from the implant was estimated by measuring the remaining drug in the recovered implant. Figure 4 shows the profile of in vitro and in vivo release of BM from the implant coated with the 5% EVA-dichloromethane solution. The in vivo release from implants also showed a zero-order release profile. The drug release rate from the implant in vivo was relatively faster than that in vitro, although there was no statistically significant difference between them.

**In Vivo Pharmacokinetics of BM in Ocular Tissues**

The concentrations of BM in the vitreous and retina-choroid after implantation of the BM-loaded implants were plotted in Figure 5. The concentrations of BM in the posterior half of the retina-choroid were significantly higher than in the vitreous at all times. In the vitreous, the maximum concentration was 13.44 ng/g at 2 weeks after implantation. The concentrations of BM in the posterior half of retina-choroid and in vitreous were constantly maintained for 4 weeks. BM in the anterior half of the retina-choroid was detected only at 4 weeks but was not detected at 1 and 2 weeks. No BM was detected in the aqueous humor and in the contralateral eyes at all times throughout the study.

**Distribution of Fluorescence after Implantation of the 6-CFDA-Loaded Implant**

Figure 6 shows fluorescence micrographs and light micrographs after implantation of the 6-CFDA-loaded implant. The fluorescence gradually decreased in intensity with the distance from the implantation site. Fluorescence (B, D, F) and light micrographs (C, E, G) of the sclera and retina-choroid. (B, C) At the implantation site, the sclera and retina-choroid showed intense fluorescence. (D, E) At the equator, moderately bright fluorescence was observed at the sclera. The retina-choroid demonstrated mild fluorescence. (F, G) Near the pars plana, only weak fluorescence was observed at the sclera. (B), (D), and (F) correspond with the fields defined by (A).

Original magnification: (A) × 4; (B-G) × 20.
At the equator, moderately bright fluorescence was observed at the sclera. The retina-choroid showed mild fluorescence (Fig. 6D). Near the pars plana, weak fluorescence was observed only at the sclera (Fig. 6F). In the eyes without implants, very weak fluorescence was observed (Fig. 7A).

**Toxicity Studies**

The implants were encapsulated with thin fibrous tissues, but the sclera under the implants showed a normal appearance. Light microscopic examination revealed no substantial changes in the retina and choroid at the implantation site (Fig. 8).

Figure 9 shows the mean a- and b-wave amplitudes before and after implantation. The eyes receiving the implants showed no significant changes of a- and b-wave amplitudes at 4 and 8 weeks after implantation compared with the control eyes ($P > 0.05$, paired Student’s $t$-test).

**DISCUSSION**

In this study, we demonstrated drug distribution in ocular tissues after implantation of a nonbiodegradable BM-loaded episcleral implant. The drug was released from the implant, and it penetrated through the sclera into the retina-choroid and vitreous without producing any significant toxic effects on the eye. The concentrations of BM were maintained above levels effective for suppressing inflammation (150–4000 ng/g) in the retina-choroid for 4 weeks. The in vitro release profile from the device showed an approximately linear release pattern; the drug release could be controlled by changing the concentration of EVA in the coating solution on the surface of implants. The authors of the concept have reported that, in controlled drug delivery systems using the EVA membrane, the drug was released through the EVA membrane on the surface of the implant, and the release rate was controlled by the thickness of the membrane. The nonbiodegradable implant could release BM constantly for at least 3 months, and the in vivo release correlated to the in vitro release. The in vivo release rate was relatively faster than the in vitro release rate, possibly because of the increased BM solubility in vivo.

Recently, transscleral drug delivery has been hypothesized to be an effective mean of achieving therapeutic concentrations of drugs in the posterior part of the eye. It has been reported that scleral permeability is comparable to that of the corneal stroma. A similar to the corneal stroma route, the primary route for solute transport through the sclera is by passive diffusion through an aqueous pathway. The rabbit sclera is permeable to 150-kDa dextran and IgG of the same molecular weight, whereas human sclera is permeable to 70-kDa dextran, and permeability declines exponentially with increasing molecular mass and radius. Therefore, BM, with a molecular mass of 355 Da, may easily penetrate through the sclera by passive diffusion. Permeability of BM through the RPE is unclear, because we did not evaluate the BM concentrations in the retina and choroid separately. However, BM may penetrate through the RPE, because BM was detected in the vitreous cavity. The RPE, the outer blood–retinal barrier, is between the choroid and the neurosensory retina. The RPE has tight junctions and low permeability to many compounds. In general, lipophilic drugs show high affinity for the cells, which may increase the blood–retinal barrier’s permeability. Because BM is a moderately lipophilic drug, penetration may be through the intracellular route by passive diffusion. However, further study may be needed to clarify the distribution of betamethasone in the retina and choroid.

Sustained intravitreal delivery devices, which release dexamethasone or fluocinolone acetonide, have been successfully used to treat severe uveitis. The structure of these devices is very similar to the ganciclovir implant (Vitrasert; Bausch & Lomb Optical Co., Rochester, NY), in which the drug release rate is controlled by the polyvinyl alcohol membrane. These devices were implanted in the vitreous through the pars plana. The drugs from these devices were gradually released, diffused into the vitreous, and were delivered not only to the retina-choroid but also to the aqueous humor. Therefore, when the drug concentration in the retina-choroid reached effective levels, the gradient of drug concentration in the aqueous humor may be large. In this study, BM was not detected in the aqueous humor. Therefore, to deliver the drug more effectively to the macular region without increasing the drug concentration in the aqueous humor, this system may be more useful than intravitreal drug delivery systems. Furthermore, previous studies have shown that transvitreal permeation of the retina is limited for tissue plasminogen activator (70 kDa),...
because the inner limiting membrane is the barrier to penetration into the retina.\textsuperscript{37} On the contrary, large molecules such as immunoglobulin (150 kDa) have been reported to penetrate the sclera.\textsuperscript{14} Therefore, the episcleral implant may be more useful for site-specific treatment in the retina-choroid and the intraocular delivery of large molecular compounds such as bioactive protein and antibody than intravitreal implants.

One of the most advantageous aspects of this system is to deliver the drug more efficiently to the macular region. Because it is difficult to place an implant directly at the posterior pole, we designed this implant, which is similar to a macular expant. This implant can be inserted easily between the superior and inferior oblique muscles without having to cut the lateral rectus muscle. Also, there is little risk of destroying the short posterior ciliary arteries during surgery, which can occur during the macular buckling procedure.\textsuperscript{15,16} Implantation of this implant may be less invasive than that of other intraocular devices. It has been reported that the implantation of intravitreal devices sometimes involves complications such as cataract, retinal detachment, vitreous hemorrhage, and endophthalmitis.\textsuperscript{39,40} In this study, we used stainless steel for the device. In general, ocular implants should avoid steel because magnetic resonance scanning can dislodge these implants and cause injury if the metal stays with the implant. Therefore, nonmagnetic components should be used, such as titanium, which is used in a macular explant in humans. In addition, although no substantial toxic reactions were observed electrophysiologically and histologically, the observation period in this study is relatively short. Therefore, long-term toxicity studies would be required before the clinical application.

Our studies demonstrated that the episcleral implant appears to be biocompatible. In conclusion, our findings suggest that a transscleral drug delivery system with an episcleral implant may have a potential usefulness in the treatment of vitreoretinal disorders, especially macular diseases. Because advanced safety and effectiveness of the implant must be ensured in clinical application, further investigations are necessary.

References


