Intraocular Tissue Distribution of Betamethasone after Intrascleral Administration Using a Non-biodegradable Sustained Drug Delivery Device

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**Purpose.** To evaluate the tissue distribution of betamethasone (BM) after implantation of a nonbiodegradable intrascleral implant as a new, controlled intraocular delivery system.

**Methods.** Nonbiodegradable intrascleral implants designed to release BM for at least 1 month were placed in the sclera of pigmented rabbits. The BM concentrations in the aqueous humor, vitreous, and retina-choroid were determined by high-performance liquid chromatography (HPLC) at 3, 7, 14, and 28 days after implantation. The BM concentrations in three sections of retina-choroid were also investigated. Retinal toxicity was evaluated by electoretinography and histology.

**Results.** The BM released from the intrascleral implant in vitro and in vivo showed zero-ordered release profiles for 4 weeks. The BM concentrations in the retina-choroid after placement of the intrascleral implants remained higher than effective concentrations for suppressing various inflammatory processes for at least 28 days. The BM concentrations in the retina-choroid around the implant site were more than 10 times higher than in the opposite side throughout the study. No substantial toxic reactions were observed by electoretinography or histology.

**Conclusions.** These findings suggested that the nonbiodegradable intrascleral implant could be a useful drug carrier for intraocular delivery of BM without producing severe retinal toxicity. The intrascleral site may be considered for effective intraocular drug distribution after implantation. (Invest Ophthal Vis Sci. 2003;44:2702–2707) DOI:10.1167/iovs.02-0956

Pharmaceutical approaches may be used to control many intraocular inflammatory and proliferative diseases, such as uveitis, diabetic retinopathy, and proliferative vitreoretinopathy. However, topical delivery is not viable, because of its extensive diffusion path and lacrimation. Also, systemic administration requires large doses of the drug, resulting in general side effects.

Although intravitreal injection of a drug aqueous solution or suspension can deliver drugs to the posterior segment without systemic side effects, the low-molecular-weight compound solution is eliminated from the vitreous within several hours. Also, repeated long-term intravitreal injections are required for posterior segment diseases, which may cause complications, such as vitreous hemorrhage, retinal detachment, or endophthalmitis. Lipophilic compounds can remain active for a long period in intraocular tissues after intravitreal injection of the suspension, but the drug release rate cannot be controlled. Therefore, a sustained-release drug delivery system with minimal side effects and invasion may be an ideal method to develop.

Some particle drug delivery systems for maintenance of long-term intraocular tissue concentration of drug have been investigated. Although intravitreal injection of particle carriers, such as microspheres and liposomes, may be useful for the treatment of vitreoretinal disorders, small particles suspended in the vitreous may impair the clarity of the ocular media. Some intravitreal implants have already been used clinically. Intraocular sustained delivery devices, which release dexamethasone or fluorocinolone acetonide, have been successfully used to treat severe uveitis. The structure of these devices is very similar to that of the ganciclovir implant (Vitrascen; Ciron Vision, Claremont, CA), in which the drug release rate is controlled by the poly (vinyl alcohol) membrane. However, the application of these devices has involved complications such as cataract, retinal detachment, and vitreous hemorrhage, produced by the implantation of the devices.

Recently, it has been hypothesized that transscleral delivery may be an effective method of achieving therapeutic concentrations of drugs in the posterior part of the eye. We have recently demonstrated that steroid-loaded biodegradable intrascleral implants can deliver the drug at therapeutic levels in the vitreous and retina-choroid. However, there has been a disparity between in vitro and in vivo release kinetics in the system of biodegradable intrascleral implants. In terms of the release profile, the system using a nonbiodegradable polymer membrane is more controllable than that using a biodegradable polymer. Furthermore, nonbiodegradable devices can release drugs longer than biodegradable devices. Therefore, a drug delivery system using nonbiodegradable polymer may be suitable for some chronic intraocular diseases, such as diabetic vitreoretinopathy and age-related macular degeneration.

In this study, we designed a new betamethasone (BM)-loaded nonbiodegradable intrascleral implant composed of ethylene vinyl acetate (EVA) copolymer. The device was implanted in a scleral pocket of pigmented rabbits. We investigated in vitro and in vivo release of BM from the implant and intraocular tissue levels of BM. We also determined the distribution of BM in the retina-choroid after implantation by measuring BM concentration in three different parts of the retina-choroid (around the implantation site, opposite the implantation site, and at the posterior pole).

**Materials and Methods.**

Ethylene vinyl acetate copolymer (vinyl acetate content: 33%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). BM was pur-
The intrascleral 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°. At predetermined intervals, the entire volume was sampled, and 50 mL of fresh medium was added to the sample vial to approximate a perfect sink condition. 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-9800; 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 dihydrogen phosphate aqueous solution (55:45). The column oven (8620-CO; Japan Spectroscopic Co.) was equipped and set at 40°. A spectrophotometer detector (model 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**

Sixteen right eyes of 16 pigmented 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 Ophthalmic and Vision Research. 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 topical instillation of 0.4% oxybuprocaine hydrochloride. After the sclera was exposed, a scleral pocket was made with a crescent knife 2 mm from the limbus at a depth that was one half the total scleral thickness. The intrascleral implant was then inserted into the scleral pocket. The scleral and conjunctival wounds were sutured with 7-0 silk. Animals were killed with an overdose of intravenous pentobarbital sodium at 3, 7, 14, and 28 days after implantation, and the implanted eyes and the contralateral eyes were enucleated. The eyes were immediately frozen at −85°, and the intrascleral implants and samples of ocular tissues (aqueous humor, vitreous, and three different sections of retina-choroid) were also retrieved. Ocular tissues were stored at −85° until the BM concentration was determined by HPLC.

**Determination of Betamethasone in Intrascleral Implants and Ocular Tissues**

The recovered implants were cut by razor to expose the remaining BM pellet. The remaining BM was dissolved in a 50-mL mobile phase of HPLC, and 100 μL of this solution was injected into HPLC. The in vivo release of BM from the intrascleral implant was calculated by the following equation: (the actual loading amount of BM in the implant before implantation) − (the remaining amount of BM in the recovered implant)/(the actual loading amount of BM in the implant before implantation) × 100.

The BM concentrations in recovered ocular tissues (aqueous humor, vitreous, three different sections of retina-choroid) were also determined by HPLC. BM was extracted from the tissues by the following procedures: Internal standard solution (0.1 mL of 2.5 μg/mL) 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 in a centrifugal concentrator (VC-960; Taisco Co., Saitama, Japan). The residue was dissolved in 0.2 mL of 50 mM potassium dihydrogen phosphate aqueous solution (55:45). The column oven (8620-CO; Japan Spectroscopic Co.) was equipped and set at 37°. At predetermined intervals, the entire volume was sampled, and 50 mL of fresh medium was added to the sample vial to approximate a perfect sink condition. 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-9800; 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 dihydrogen phosphate aqueous solution (55:45). The column oven (8620-CO; Japan Spectroscopic Co.) was equipped and set at 40°. A spectrophotometer detector (model 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.

**Histologic Analysis**

Two of the rabbits receiving the BM-loaded intrascleral implants were killed at 4 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 divided into three pieces (around the implantation site, opposite the implantation site, and the posterior segment). The divided pieces of retina-choroid and sclera were dehydrated, infiltrated, embedded in paraffin, and sectioned with a microtome. Sections were stained with hematoxylin-eosin for light microscopy.

**Electrophysiological Study**

Retinal function was evaluated by scotopic electroretinography (ERG) before treatment and 4 weeks after implantation of the BM-loaded intrascleral implants. Scotopic ERG was recorded after 60 minutes of dark adaptation (ERG-50; Kowa Co., Ltd., Nagoya, Japan). A silver-plated electrode was placed on each earlobe, one serving as the reference and the other as the ground.

**Statistical Analysis**

An unpaired t-test was used to assess whether the BM concentration in two different sections of retina-choroid (opposite the implantation site and the posterior segment) had decreased compared with the retina-choroid around the implantation site at 3, 7, 14, and 28 days after implantation of the intrascleral implants. A paired t-test was used to assess whether the ratio of the b-wave amplitude of the treated eye to the control eye had decreased after treatment. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**In Vivo Release**

In vivo release was determined by measuring the remaining drug in recovered implants. Figure 3 shows the profile of in vitro and in vivo BM release from the intrascleral implants coated with 2.5% EVA/dichloromethane solution. The in vivo release from devices also showed zero-ordered release profiles, the same as in vitro release profiles, with \( R^2 > 0.99 \). In this study, no deformation or dislocation of a device from the scleral pocket was observed.

The in vitro and in vivo correlations for the BM-loaded nonbiodegradable intrascleral implant were explored by comparing the in vitro drug release data with the in vivo release data. A linear correlation between in vivo and in vitro release data were observed with \( R^2 > 0.99 \). The drug release rate from implants in vivo was approximately 1.8 times faster than in vitro.

**Pharmacokinetics of BM in Ocular Tissues**

Concentrations of BM in the vitreous and in the retina-choroid after implantation of the BM-loaded intrascleral implants coated with 2.5% EVA/dichloromethane solution were plotted in Figure 4. The concentrations in the retina-choroid, which were calculated in three different parts of the tissue, were significantly higher than in the vitreous throughout this study. In the retina-choroid, the maximum concentration was obtained at 1 week after implantation (122.6 \( \mu \)g/g). In the vitreous, it was observed at 2 weeks after implantation (229 ng/g). No BM was detected in aqueous humor or in contralateral eyes at any time throughout this study.

**Distribution of BM in the Retina-Choroid**

The BM concentrations were measured in three different parts of retina-choroid: around the implantation site, opposite the implantation site, and at the posterior pole (Fig. 5). At all times, the BM concentrations in the retina-choroid decreased, depending on the distance from the implantation site. The BM concentrations at the side opposite the implantation site \( (P < 0.01) \) and at the posterior pole \( (P < 0.05) \) were significantly

**FIGURE 2.** In vitro release profiles of BM from the intrascleral implant coated with 1% and 2.5% EVA/dichloromethane solution. The data are the mean ± SD of results in four experiments.

**FIGURE 3.** In vivo and in vitro release profiles of BM from the intrascleral implant. The data are the mean ± SD of results in four experiments.

2704 Okabe et al. IOVS, June 2003, Vol. 44, No. 6
lower than that around the implantation site at 14 days after implantation. The BM concentration around the implantation site showed the highest level (560.9 μg/g) at 7 days after implantation. The gradient of BM concentrations gradually decreased with time. At 28 days after implantation, the BM concentration in the retina-choroid around the implantation site was more than 14 times higher than in the side opposite the implantation site.

**Toxicity Studies**

Figure 6 shows a light micrograph of the retina around the implantation site. Histologic study revealed no substantial changes in the retina at any site after application of the BM-loaded intrascleral implants.

The ratios of scotopic b-wave (implanted eye/fellow eye) before and after implantation were 1.16 ± 0.14 and 0.94 ± 0.13, respectively. The eyes receiving the implant showed no statistically significant electrophysiologic changes, compared with the control eyes.

**DISCUSSION**

In this study, we determined the drug distribution in ocular tissues after implantation of an intrascleral nonbiodegradable BM-loaded device. When released from the implant, the drug penetrated the retina-choroid and vitreous. The concentration of BM was maintained at higher than effective levels for suppressing inflammation (0.15–4.00 μg/mL) in the retina-choroid for 28 days. The profile of in vitro release from the implant showed an approximately linear pattern, and the drug release could be controlled by changing the concentration of EVA in the solution used for coating the surface of the device. It has been reported that the drug is released through the membrane on the surface of devices, and the release rate is controlled by the thickness of the membrane. The nonbiodegradable implant constantly released BM for at least 1 month in the sclera, and the in vivo release correlated closely with the in vitro release. However, the in vivo release rate was approximately 1.8 times faster than the in vitro release rate, possibly due to the increased BM solubility in vivo.

BM was shown to be fairly permeable through the sclera and retina-choroid and to reach the vitreous cavity. Recently, transscleral drug delivery has been hypothesized to be an effective means of achieving a therapeutic concentration of drug in the posterior part of the eye. It has been reported that scleral permeability is comparable to that of the corneal stroma, in that the primary route for solute transport is by passive diffusion through an aqueous pathway. The rabbit sclera is permeable to solutes ranging in molecular weight up to...
to 150 kDa, and scleral permeability declines exponentially with increasing molecular weight and molecular radius.\textsuperscript{8} Therefore, it seems that BM, with a molecular mass of 355 Da, could penetrate easily through the thinned sclera by passive diffusion. In this study, we did not evaluate the permeability of BM through the retinal pigment epithelium (RPE), because we did not measure concentrations in the retina and choroid separately. However, BM penetrated the RPE, because it was detected in the vitreous. The RPE, which is the outer blood-retinal barrier, is between the choroid and the retina. The RPE has tight junctions of the nonleaky type and has low permeability to many compounds.\textsuperscript{24,25} Generally, when drugs penetrate through the cell membrane of various tissues, two potential pathways exist: the transcellular and the intercellular. It is believed that the lipophilic drugs penetrate by the former route. Because BM is a moderately lipophilic drug, it may penetrate mainly through the transcellular route. Further study is needed to clarify the distribution of BM in the retina and choroid separately.

In vivo release studies demonstrated that the implant released the drug at an almost constant rate. However, the BM concentrations in the retina-choroid and vitreous were not maintained at a constant level. The maximum concentrations of BM were observed at 1 and 2 weeks after implantation in the retina-choroid and in the vitreous, respectively. In this system, it may take time to diffuse the drug through the sclera into the eye. After the attainment of steady state flux, theoretically the BM concentrations should have been maintained at a constant level. However, they decreased with time after that point. Probably, diffusion of the drug through the sclera decreases, because encapsulation of the implant with thin connective tissues was observed histologically at the end of the study (data not shown).

We investigated the distribution of BM by measuring the drug concentrations at the three sections of retina-choroid, which were around the implantation site, the side opposite the implantation site, and at the posterior pole. The gradient of BM concentration in the retina-choroid was observed. The BM concentrations in the retina-choroid decreased, depending on the distance from the implantation site, and the BM concentrations opposite the implantation site ($P < 0.01$) and at the posterior pole ($P < 0.05$) were significantly lower than that around the implantation site at 14 days after implantation. At 7 days after implantation, the BM concentration around the implantation site was more than 1000 times higher than in the opposite side. Although the gradient of BM concentration in the retina-choroid gradually decreased with time after implantation, the highest value was approximately 14 times higher than the lowest value at 4 weeks after implantation. In the posterior pole, the BM concentration was maintained over that level needed to suppress inflammation throughout the study. Our findings suggest that the intrascleral implant may be placed as close as possible to the lesion in the eye. If the drugs applied for intrascleral implant were selected according to their effect and toxicity, then they can be delivered to the posterior pole from an anterior implantation site.

A few intraocular steroid delivery systems have already been used clinically. Intravitreal sustained delivery devices that release dexamethasone or flucinolone acetonide have been successfully used to treat severe uveitis.\textsuperscript{3,4} These devices were implanted in the vitreous through the pars plana. The drugs released from these devices gradually diffused into the vitreous, and were delivered to the retina-choroid. When the drug concentration in the retina-choroid reached the steady state, the gradient of drug concentration may not have been as large as that in the system with an intrascleral implant. Because approximately 90% of the vitreous is water, the diffusion of drug could be significantly faster in the vitreous than in the retina-choroid.\textsuperscript{26,27} It has been reported that transvitreal penetration into the retina is limited for relatively large molecules, such as tissue plasminogen activator (70 kDa), because the inner limiting membrane (ILM) is a barrier to penetration.\textsuperscript{26} In contrast, large molecules such as immunoglobulin (150 kDa) have been reported to penetrate the retina through a transscleral route.\textsuperscript{11} We speculate that intrascleral implants may be more useful for site-specific treatment in the retina-choroid and for intraocular delivery of large molecular compounds, such as bioactive protein and antibody, than intravitreal implants.

In safety studies, no substantial toxic reactions were detected histologically in the retina around the implantation site, despite the high BM concentration. However, when we apply the intrascleral implant to some more toxic drugs such as anticancer agents, further investigations may be required.

In conclusion, our findings suggest that a new intrascleral drug delivery system using an implantable nonbiodegradable polymer device may be useful to treat vitreoretinal disorders. Further investigations are needed to assess its safety and effectiveness before clinical application can be recommended.

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\section*{References}


