Biodegradable Intrascleral Implant for Sustained Intraocular Delivery of Betamethasone Phosphate

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PURPOSE. To evaluate the feasibility of using a biodegradable intrascleral implant for intraocular sustained delivery of betamethasone phosphate (BP).

METHODS. The intrascleral implant (0.5 mm thick and 4 mm in diameter) was made of poly(DL-lactide) containing 25% beta-methasone phosphate. The in vitro release of BP from the implant was evaluated by high-performance liquid chromatography (HPLC). The implants were placed into a scleral pocket in the rabbit's eye. The concentrations of BP in the aqueous humor, vitreous, and retina-choroid were measured by HPLC. The toxicity and biocompatibility of the implant were evaluated by slit lamp examination, electoretinography, and light microscopy.

RESULTS. In vitro studies demonstrated that the implants released BP in a biphasic pattern for at least 8 weeks. The BP concentrations in the vitreous and the retina-choroid remained within the concentration range capable of suppressing inflammatory responses for more than 8 weeks. The BP concentration was greater in the retina-choroid than in the vitreous. In the aqueous humor, BP was below the detection limit during the observation period. No significant toxicity to the retina was observed. Also, the implant showed good biocompatibility in the eye.

CONCLUSIONS. These results suggest that the intrascleral implant would be a promising system for delivery of steroid to the posterior segment of the eye. (Invest Ophthalmol Vis Sci. 2003;44:740–744) DOI:10.1167/iovs.02-0375

Cortisosteroids suppress inflammation and cicatrization. Many intraocular inflammatory and proliferative diseases, such as uveitis, diabetic retinopathy, and proliferative vitreoretinopathy, require long-term treatment with the drug. However, it is difficult to deliver effective doses of drugs to the posterior part of the eye. Systemic administration necessitates large doses of the drug, resulting in general side effects. Topical eye drops penetrate poorly into the posterior part of the eye, because of lacrimation and the length of the diffusion path. Intraocular injections must be administered multiple times to maintain drug concentrations within a therapeutic range for a long period of time and sometimes cause complications, such as vitreous hemorrhage, retinal detachment, or endophthalmitis. A sustained-release drug delivery system with minimal side effects and invasion may be the ideal method.

It has been hypothesized that transscleral delivery is an effective method of achieving therapeutic concentrations of drugs in the posterior part of the eye.1–4 With its large surface area and high degree of hydration, the sclera is permeable to solutes of a wide range of molecular weights.2,4,5 Subconjunctival or peribulbar injection is a means of transscleral drug delivery, and it can provide a higher intravitreous or subretinal concentration of the drug than oral administration.5–9 However, extensive systemic absorption occurs with this method.

In this study, we used a biodegradable intrascleral implant containing betamethasone phosphate (BP) to evaluate its feasibility as a sustained intraocular drug delivery system. The device was implanted in the scleral pocket. We investigated the in vitro and in vivo release of BP from the implant and the safety of the implant in the rabbit eye.

MATERIALS AND METHODS

Poly(DL-lactide) (PLA), with an average molecular mass of 20 kDa was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). BP was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of reagent grade.

Preparation of the Intrascleral Implant

The intrascleral implant was prepared by dissolving 250 mg BP and 750 mg PLA in 20 mL of acetic acid. The resultant solution was frozen at ~80°C and lyophilized for 48 hours to obtain a homogeneous cake. Seventy-five milligrams of the cake was placed in a stainless mold for infrared analysis (P/N202-32010; Shimadzu, Kyoto, Japan) and compressed by a press (SSP-10; Shimadzu) under reduced pressure at the weight of 10 tons for 5 minutes. A disc (thickness: 0.5 mm, diameter: 15 mm) was obtained. The disc for an intrascleral implant was then stamped out from this disc. The intrascleral implant weighed approximately 7 mg and was 0.5 mm thick and 4 mm in diameter (Fig. 1). The intrascleral implant had a BP loading of 25% wt/vol.

In Vitro Release Study

The intrascleral implant was placed in 2 mL of 0.1 M phosphate-buffered saline (pH 7.4) in a closed vial. Then it was immersed in a shaking water bath at 37°C. At predetermined intervals, the entire volume was sampled, and 2 mL fresh medium was added to the sample vial to approximate a perfect sinking condition. The amount of BP released into the medium was measured by high-performance liquid chromatography (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 (LC-10AS; Shimadzu) was used at a constant flow rate of 1.2 mL/min with a degasser (KT-16; Showa Denko Co., Ltd., Tokyo, Japan). The mobile phase was a mixture of methanol and 50 mM potassium dihydrogenphosphate aqueous solution (55:45). The column oven (CTO-10A) was equipped and set at 40°C. A spectrophotometric detector (SPD-6A; both from Shimadzu) was used at a wavelength of 240 nm.

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Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Submitted for publication April 15, 2002; revised August 12, 2002; accepted September 23, 2002.

Commercial relationships policy: N.

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In Vitro Release of BP from the Implant

The cumulative release of BP from the intrascleral implants is plotted in Figure 3. The data show biphasic release profiles, with an initial burst and a second stage. An initial burst (35%) was observed during the first day, and then BP was gradually released over 50 days.

In Vivo Release of BP from the Implant

Figure 4 shows the profile of in vivo release of BP from the implant at the sclera. The profile was obtained by estimating the percentage of BP remaining versus the initial content in the implant. In contrast with the in vitro release profile, no initial burst was observed. In addition, more than 80% of BP was released at 28 days.

Determination of BP in the Implants and Ocular Tissues

The amount of BP remaining in the implants and samples of ocular tissue was determined by the described HPLC procedures. In vivo release data were determined by measuring BP in recovered implants that had been placed intrasclerally for various time periods. BP was extracted from the tissues by the following procedures: 0.1 mL of the internal standard solution (fluorometholone in methanol: 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 10 minutes (CF7D2; Hitachi, Tokyo, Japan). The supernatant was collected, and BP was extracted twice with 3 mL of ethyl acetate. Ethyl acetate phases were then dried under reduced pressure with a centrifugal concentrator (VC-960; Taitec Co., Saitama, Japan). The residue was dissolved in mobile phase with 0.5 mL of mobile phase. One hundred microliters of this solution was injected into HPLC, as described. Under these conditions, the detection limit for BP was 30 ng/mL. The BP concentrations in ocular tissues were represented as BP weight per weight or volume of wet tissue.

Toxicity Study of the Implant

Clinical Observations. Slit lamp examination, measurement of intraocular pressure, and indirect ophthalmoscopy were performed to evaluate the possible toxic effect of the implant before implantation and at 1, 2, 4, 8, 12, and 16 weeks after implantation.

Electrophysiological Evaluation. Scotopic electroretinography (ERG) was performed on both eyes of each of four rabbits at baseline and 1, 2, 4, and 8 weeks after placement of the implant. The electroretinographic responses (ERG-50; Kowa Co., Ltd, Nagoya, Japan) were analyzed by dividing the b-wave amplitude recorded from eyes with the implants by those recorded from the contralateral control eyes.

Histopathologic Examination. Possible adverse effects of the intrascleral implant on ocular tissues were evaluated by light microscopy. At 16 weeks after implantation, when the implants appeared to have completely degraded, four eyes were enucleated after rabbits were killed with an overdose of pentobarbital sodium. The eyes were immersed in a mixture of 4% glutaraldehyde and 2.5% neutral buffered formalin for 24 hours. Globes were opened at the equator and divided into anterior and posterior segments. The cut specimens were dehydrated, infiltrated, embedded in paraffin, and sectioned with a microtome. Sections were stained with hematoxylin and eosin.

Statistical Analysis

A paired Student’s t test was used to compare the ERG b-wave amplitude ratio before and after implantation. Results were considered statistically significant at P < 0.05.

RESULTS

In Vitro Release of BP from the Implant

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In Vivo Pharmacokinetics of BP in Ocular Tissues

The BP concentrations in the vitreous and the retina-choroid after implantation are shown in Figure 5. The level of BP in the retina-choroid was significantly higher than in the vitreous at all times. Both in the vitreous and in the retina-choroid, maximum concentrations were observed at 2 weeks after implantation. Thereafter, the levels of BP gradually decreased. In the aqueous humor, BP was below the detection limit during the observation period.

Clinical Observation

Slit lamp examination showed no significant inflammatory reaction at the implantation sites. The cornea and anterior chamber were clear and silent. Indirect ophthalmoscopy demonstrated no abnormal findings during the observation period. One week after implantation, the implantation sites were swollen, and partly exposed in the scleral pocket. The protrusion of the implantation sites decreased with time. At 16 weeks after implantation, the sites were completely flattened. No increase of intraocular pressure was noted during the observation period.

Electrophysiological Evaluation

Right-to-left ratios of the scotopic b-wave showed no statistically significant difference between baseline and each time point (P > 0.09; Fig. 6).

Histologic Examination

Histologic findings indicated complete degradation of the implant at 16 weeks after implantation. The implantation site was replaced with loose connective tissue. A few multinucleated giant cells were observed around the implantation site (Fig. 7A). The retina near the implantation site showed no abnormalities (Fig. 7B).

DISCUSSION

In this study, we demonstrated the feasibility of using a biodegradable intrascleral implant as an intraocular sustained release system for BP. The drug was released from the implant intrasclerally and penetrated the retina-choroid and vitreous. The concentration of BP was maintained at an effective level for suppressing inflammation in the retina-choroid for more than 8 weeks, without showing any toxic effect on the eye.

The profile of in vitro BP released from the implant showed a biphasic release pattern, with an initial burst and a second stage derived from diffusional release. This profile was similar to that of a biodegradable plug-shaped scleral implant containing BP, which we previously reported.10 The initial burst may have been due to the rapid release of the drug deposited on the...
Intrascleral Betamethasone Phosphate Delivery Device

One of the most advantageous aspects of this system is that the device releases the drug continuously in the sclera. It is reported that the lag time for solute diffusion across the sclera is between 0.15–4.00 µg/mL. The drug release into the intrascleral space was greater over this period. In an intravitreal BP release system using a plug-type implant, BP concentration was higher in the retina/choroid than in the vitreous at all times. Presumably, BP may not be distributed in the aqueous humor or vitreous. BP permeated the scleral pocket, which enhances permeation of BP through the thinned sclera. Olsen et al. have reported that the permeation of dexamethasone through the sclera with surgical thinning is significantly higher than through sclera without surgical thinning. The scleral permeability to BP in the rabbit may be higher than that in the human since the rabbit sclera is thinner than the human sclera. However, it has been reported that the scleral permeability to small molecules between human and rabbit is not significantly different. The retinal pigmented epithelium (RPE), which is the outer blood–retina barrier, is between the choroid and the retina. The RPE has tight junctions of the nonleaky type and has low permeability to many compounds. BP may pass through the paracellular spaces by passive diffusion.

The BP levels in the retina/choroid and vitreous showed a slow decline from week 2 to week 8, although the decline in drug release into the intrascleral space was greater over this period. In an intravitreal BP release system using a plug-type implant, BP concentration was higher in the retina/choroid than in the vitreous at all times. Presumably, BP may not distribute by simple diffusion in the eye, and the clearance of the drug in the retina/choroid may be much slower than in the vitreous. Accumulation of the drug in the retina/choroid has to be taken into consideration in intracocular drug delivery systems. Furthermore, in this study, we only measured BP concentrations. Betamethasone phosphate is metabolized into betamethasone by hydrolysis. The disparity between the drug levels in the tissue and drug release from the implant may come from these factors.

McCartney et al. have postulated the existence of a trans-scleral route of corticosteroid penetration into the rabbit eye after subconjunctival injection. Recently, Weijtens et al. reported several series of studies on the concentration of dexamethasone in vitreous and serum by three different administration routes: subconjunctival injection, peribulbar injection, and oral administration. In these studies, dexamethasone disodium phosphate and dexamethasone were used for subconjunctival or peribulbar injection and for oral administration, respectively. Serum levels after subconjunctival and peribulbar injection are comparable to those achieved by a high oral dose. The higher intravitreous concentration after subconjunctival injection or peribulbar injection is caused by diffusion from the serum and additional transscleral diffusion. Betamethasone may show kinetics in the eye similar to those of dexamethasone, because it is a stereoisomer of dexamethasone. Furthermore, its effective concentrations for suppressing various inflammatory processes (0.15–4.00 µg/mL) are thought to be the same as dexamethasone.

One of the most advantageous aspects of this system is that the device releases the drug continuously in the sclera. It is reported that the lag time for solute diffusion across the sclera is similar to or actually longer than the drug–sclera contact time during conventional administration of the drug. Although only part of the drug diffuses across the sclera after subconjunctival or peribulbar injection, most of the drug is absorbed systemically. To achieve therapeutic concentration in the vitreous, multiple injections may be needed, resulting in local and general side effects from the drug. This intra-

In contrast to the in vitro release profile, the in vivo implant released approximately 8% of the drug for 1 week with no initial burst effect. Therapeutically, the implant released the drug faster than it did in vitro. The surrounding environment of the implant in the sclera may not be the same sinking condition as that in vitro. At the early stage, water absorption of the implant may be less than that in vitro, resulting in no rapid release of the drug from the implant. In this condition, the water channels may not be well developed in the matrix. Also, the pH inside the implant may decrease, which enhances autocatalysis at the center of the polymer matrix. Finally, the drug may release rapidly after the establishment of communication between the inside and the outside. This difference between in vitro and in vivo conditions has to be taken into consideration in this system.

Transscleral drug delivery has been hypothesized to be an effective means 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. As in the corneal stroma, the primary route for solute transport through the sclera is passive diffusion through an aqueous pathway. The rabbit sclera is permeable to solutes ranging in molecular weight up to 150 kDa, and the permeability declines exponentially with increasing molecular weight and molecular radius. In this study, BP permeated the sclera fairly well. BP has a small molecular weight of 516 Da, and the device containing BP was inserted into the scleral pocket, which enhances permeation of BP through the thinned sclera. Olsen et al. have reported that the permeation of dexamethasone through the sclera with surgical thinning is significantly higher than through sclera without surgical thinning. The scleral permeability to BP in the rabbit may be higher than that in the human since the rabbit sclera is thinner than the human sclera. However, it has been reported that the scleral permeability to small molecules between human and rabbit is not significantly different. The retinal pigmented epithelium (RPE), which is the outer blood–retina barrier, is between the choroid and the retina. The RPE has tight junctions of the nonleaky type and has low permeability to many compounds. BP may pass through the paracellular spaces by passive diffusion.

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Figure 7. Light micrograph of an eye that received the implant, 16 weeks after implantation. Hematoxylin and eosin stain. (A) The implantation site was replaced with loose connective tissues. A few multinucleated giant cells (arrows) were present. (B) The retina near the implantation site showed no abnormalities in its architecture. Original magnification, ×50.
scleral sustained drug delivery system would permit drug flux and minimize systemic absorption of the drug by the conjunctival vasculature.

In terms of intraocular steroid delivery systems, a few systems have already been used clinically. Intravitreous sustained delivery devices, which release dexamethasone or fluorocinolone acetoniode, have been successfully used to treat severe uveitis. The structure of these devices is very similar to the ganciclovir implant (Vitrascert; Bausch & Lomb Surgical, San Dimas, CA), in which the drug release rate is controlled by the membrane of poly(vinyl alcohol). The 2-mg fluorocinolone acetoniode device can release the drug at a constant rate for more than 2.5 years. In this system, the kinetics of the drug in the vitreous may be predictable because the drug is released from the device directly in the vitreous humor. In contrast, the kinetics of the drug in our system depends mainly on physical and chemical characteristics of the drug, such as molecular weight or water solubility. Probably, the drugs that can be applied to the intrascleral implant are limited.

In our studies, the scleral implant appeared to be biocompatible. No substantial toxic reactions were observed electrophysiologically or histologically. In conclusion, our results suggest that this new intrascleral drug delivery system involving an implantable biodegradable polymer device may have usefulness in treating vitreoretinal disorders. Because advanced safety and effectiveness of the intrascleral implant must be ensured in clinical application, further investigations are needed.

References


