A New Vitreal Drug Delivery System Using an Implantable Biodegradable Polymeric Device

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Purpose. The authors evaluated the feasibility of using an implantable biodegradable polymeric device to deliver drugs into the vitreous humor.

Methods. Two types of devices were prepared by compression-molding polymers of poly(DL-lactic acid) of two different molecular weights. The molecular weights of the poly(DL-lactic acid) used were 5,600 (device-1) and 9,100 (device-2). Sodium fluorescein (NaF) served as a hydrophilic drug marker. The release of the dye from the devices was studied in vitro. The intravitreal kinetics of NaF was evaluated in rabbits in vivo by fluorophotometry. The eyes were evaluated electrophysiologically and histologically to determine if there were toxic effects.

Results. Device-1 and device-2 released NaF for more than 25 and 45 days, respectively, in vitro. Detectable concentrations of NaF were present in the vitreous up to 17 days (device-1) and 28 days (device-2). Both types of devices were well tolerated, with no noted toxic effects.

Conclusions. These results suggested that this device may be a potentially effective system to deliver drugs in the vitreous. Invest Ophthalmol Vis Sci. 1994;35:2815-2819.

It is difficult to supply therapeutic levels to the posterior part of the eye after systemic dosing. For example, the blood–retinal barrier prevents the transport of most systemically administered drugs into the posterior part of the eye. Nevertheless, many vitreoretinal disorders require this type of drug treatment. Injections of drugs into the vitreous have been used to obtain therapeutic effects. Their kinetics in the vitreous and toxicity to the eye have been reviewed. To achieve therapeutic concentration levels, frequent intravitreal injections are needed. These injections may, however, cause various complications, such as vitreous hemorrhage, retinal detachment, or infection. To solve these problems, controlled-release systems of drug delivery to the vitreous have been studied. We have previously demonstrated the effectiveness of using biodegradable polymer microspheres in the vitreous. Biodegradable polymer microspheres were used as injectable vehicles that released the drug through hydrolysis and gradually degraded with time.

In this study, we have developed an implantable biodegradable polymeric device as a new drug delivery system in the vitreous. This study investigated the kinetics and biocompatibility of the device after implantation in rabbit eyes.

MATERIALS AND METHODS

Synthesis of Biodegradable Polymers

Homopolymers were synthesized by polycondensation of DL-lactic acid (Musashino Shoji, Tokyo, Japan). The polymerization products were purified by precipitation with distilled water from a methanol solution. The weight–average molecular weight was determined by gel permeation chromatography using standard polystyrenes (HLC-8020, Toyo Soda, Tokyo, Japan). By varying the time and temperature of polymerization, two molecular weights of poly(DL-lactic acid) (PDLLA) were obtained: 5,600 and 9,100.

Preparation of Devices

A total of 10 mg of sodium fluorescein (NaF) (Fluorescein, Alcon; Fort Worth, TX) and 990 mg of a single...
batch of each molecular weight of the polymer were dissolved in 10 ml of acetonitrile (Wako Pure Chemical Industries, Osaka, Japan) mixed with distilled water by 10 vol%. The resulting solution was rapidly frozen in liquid nitrogen and lyophilized (FDU-839, EYELA, Tokyo, Japan) to make a homogeneous powder. The devices were fabricated from this powder by use of the compression-molding technique. Two types of devices were prepared: device-1 (MW = 5,600) and device-2 (MW = 9,100). The devices were shaped similarly to the temporary metallic scleral plug, which is used during vitrectomy (Fig. 1).

In Vitro Release Study

Three each of device-1 and device-2, 1% by weight of NaF, were placed in individual vials that contained 10 ml of phosphate-buffered saline at pH 7.4 and were immersed in a shaker bath kept at 37°C. The released medium was periodically removed and replaced with the same amount of fresh phosphate-buffered saline. To approximate a perfect sink condition, the frequency of changing the medium was adjusted during the course of the release study to ensure that the concentration of NaF was below 20% of its saturation value at all times. The amount of NaF in the medium was determined by fluorescent spectrophotometry (Shimadzu, Model RF-5000S, Spectrofluorophotometer, Kyoto, Japan) at the excitation wavelength of 480 nm and the emission wavelength of 508 nm. The experiment was performed in triplicate for each device.

In Vivo Release Study

We used 20 eyes of 20 albino rabbits, each weighing between 1.8 and 2.3 kg. All animals were handled according to the ARVO Resolution on the Use of Animals in Research. They were anesthetized with a mixture (1:1) of xylazine hydrochloride (4 mg/kg) and ketamine hydrochloride (10 mg/kg). The pupils were dilated with 1 drop each of 0.5% tropicamide and 2.5% phenylephrine hydrochloride eyedrops. The ocular surface was then anesthetized with a single topical instillation of 0.4% oxybuprocaine hydrochloride. A paralimbal conjunctival incision was made 5 mm from the temporal limbus in the right eye. Sclera was exposed, and a 1-mm sclerotomy was made with a microsurgical vitreal retinal blade 2 mm from the limbus. Devices were implanted at the sclerotomy site. Penetration into the vitreous cavity was confirmed with the vitreous lens. The left eye served as an untreated control. Group 1 (ten eyes) received device-1. Group 2 (ten eyes) received device-2. The conjunctival wound was sutured with 8–0 virgin silk. Topical antibiotic ointment was applied to the eyes of each animal to minimize the risk of postoperative infection.

The concentration of NaF in the vitreous was measured for up to 30 days with an automatic scanning fluorophotometer (Fluorotron Master, Coherent; Palo Alto, CA). The mean concentrations of NaF in the vitreous were determined. Slit-lamp biomicroscopic examinations of the anterior segments and fundus examinations by indirect ophthalmoscopy were performed. Electrophysiologic studies were performed under scotopic conditions before and 30 days after implantation. The electroretinographic responses (ROX-5T, Heiwa Electronic Industrial, Tokyo, Japan) were analyzed by dividing the voltages recorded from eyes with the devices by those recorded from the contralateral control eyes. The ratios obtained were subjected to statistical analysis using Student's t-test.

Histologic studies using light microscopy were performed on five eyes from each group at 1 and 3 months after implantation. The eyes were enucleated immediately after rabbits were killed with an overdose of pentobarbital sodium. The eyes were immersed in the 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.

RESULTS

In Vitro Release Study

The release profiles of NaF from the devices are shown in Figure 2. Device-1 and device-2 released NaF for more than 25 and 45 days, respectively. Significant early burst effects were not observed.

In Vivo Release Study

The concentration of NaF in the vitreous was measurable up to 17 days in group 1 and to 28 days in group 2.
Vitreal Drug Delivery Using a Polymeric Device

Drug Delivery Using a Polymeric Device

Time (days)

FIGURE 2. In vitro release profiles of NaF from polymer devices. Device-1 (open circle) and device-2 (closed circle) released NaF for more than 25 and 45 days, respectively. The percent released is presented as the mean standard deviation (SD), \( n = 3 \).

Peak concentrations were observed at 7 days in group 1 and at 17 days after implantation in group 2. Thereafter, NaF levels gradually decreased with time. Detectable (with a limit of detection of 1.0 ng/ml) concentrations of NaF in the vitreous humor were measurable for up to 17 days and 28 days from device-1 and device-2, respectively.

Both types of the devices swelled in the vitreous and then gradually decreased in size. Contact between the swollen device and the ocular lens was observed in two eyes in group 1, but no opacification resulted. No inflammatory reactions in the vitreous were observed in either group. One eye in group 1 developed a flat retinal detachment around the implantation site. Devices in both groups decreased in density and appeared to be almost degraded 3 months after implantation, as seen by slit-lamp biomicroscopy.

Right-to-left ratios of the scotopic b-wave before implantation were 0.98 ± 0.23 and 1.03 ± 0.17 (mean ± SD) in group 1 and group 2, respectively. The right-to-left ratios 50 days after implantation were 1.07 ± 0.14 and 1.12 ± 0.25 in group 1 and group 2, respectively. No significant differences were observed between the right-to-left ratios of the b-wave between groups.

Histologically, mild foreign body reactions were observed around the devices 1 month after implantation in both groups (Fig. 4). By 3 months after implantation, the devices were almost degraded and replaced by connective tissue (Fig. 5). The retina, lens, and other tissues were normal.

DISCUSSION

We evaluated a new drug delivery system in the vitreous with an implantable biodegradable polymeric de-

![FIGURE 2](image2.png)

FIGURE 2. In vitro release profiles of NaF from polymer devices. Device-1 (open circle) and device-2 (closed circle) released NaF for more than 25 and 45 days, respectively. The percent released is presented as the mean standard deviation (SD), \( n = 3 \).

![FIGURE 3](image3.png)

FIGURE 3. Mean concentration (±SD, \( n = 10 \)) of NaF in rabbit vitreous after implantation of the device. Detectable concentrations of NaF from device-1 (open circle) and device-2 (closed circle) were found in the vitreous up to 17 days and 28 days, respectively.

![FIGURE 4](image4.png)

FIGURE 4. Light micrographs of an eye implanted with device-2 1 month after implantation. Device (arrowheads) has perforated into the sclera. Mild inflammatory reactions are observed around the device. S, sclera; C, conjunctiva. Hematoxylin and eosin stain, original magnification \( \times 45 \).
vice. Polymeric delivery systems have been successfully developed as a controlled drug delivery system.\textsuperscript{21-24} We previously demonstrated the effectiveness of biodegradable polymer microspheres of this use.\textsuperscript{15,16} Biodegradable polymer microspheres have been used as injectable vehicles, which released the drug through hydrolysis and then degraded in the vitreous. Liposomes also have been used as injectable vehicles. Many investigations have been reported on liposomes as a vitreal drug delivery system.\textsuperscript{3-9} Liposomes have already been used clinically;\textsuperscript{10} however, both of these systems may temporarily disturb the transparency in the vitreous as they disperse in the vitreous humor.

Biodegradable polymer rods containing 5-fluorouracil also have been investigated to treat experimental proliferative vitreoretinopathy.\textsuperscript{11,12} The devices could deliver therapeutic levels of 5-fluorouracil for at least 2 weeks. However, because they are not fixed in the vitreous, the devices have to be moved into contact with the retina. Recently, a new sustained-release delivery system with a nonbiodegradable polymer device has been reported.\textsuperscript{13} It releases ganciclovir through an ethylene vinyl acetate and polyvinyl alcohol membrane. Therapeutic concentration levels of ganciclovir were maintained for more than 80 days without toxicity. Clinical application of this device is a potential therapy for cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome.\textsuperscript{14} This device, however, appears to be too large for the eye and is not degradable. Therefore, it may be of limited use for clinical applications. Because our new device is fixed in the sclera and it releases the drug into the vitreous as the device degrades, there is no need to remove it. Repeated implantation also can be performed.

It is difficult for biodegradable polymer microspheres to encapsulate hydrophilic drugs and obtain good release profiles without burst effects.\textsuperscript{17} Microspheres incorporating with NaF have shown significant burst effects.\textsuperscript{25} In the conventional method of preparing the microspheres, microcrystals of NaF may form in the matrix, and the distribution of NaF molecules in the matrix may not be homogeneous. These microcrystals may create many pores on the surface of the microspheres, which in turn increase the surface area of the matrix. This increased surface area is itself associated with more rapid degradation and further release. In the present study, we used NaF as a hydrophilic drug marker. Both devices released NaF without early burst effects in the vitreous. The distribution of NaF molecules in the matrix may be homogeneous by our method. The devices release NaF through the matrix of polymers by diffusion. The degradation of the matrix occurs with time. As expected, the surface area of the devices from which NaF diffuses into aqueous media increases more slowly than does the surface area of the microspheres. These two factors may lead to better release profiles from the devices than from the microspheres. The degradation of the matrix depends on its molecular weight and polymer composition.\textsuperscript{26,27} Device-1, composed of PDLLA (MW = 5,600), released NaF faster than device-2, which was manufactured from PDLMA (MW = 9,100); therefore, it appears possible to control the release rate by varying the molecular weight of the polymer.

NaF, which is released from the device, diffuses in the vitreous and is actively transported across the blood-retinal barrier outwardly from the vitreous cavity.\textsuperscript{28} The concentrations of NaF in the vitreous may be determined by three factors: the release rate of NaF from the device, the diffusion rate of NaF in the vitreous, and the outward transport rate of NaF from the vitreous cavity. To control the drug concentrations in the vitreous, these three factors have to be taken into consideration. Furthermore, the degradation rate of the polymers in the vitreous humor in vivo also may be different from that in vitro.

Biodegradable polymers cause mild foreign body reactions that gradually decrease during or after their degradation.\textsuperscript{29,30} Histologically, mild foreign body reactions were observed around both types of our devices. Both types of the devices, however, produced no significant inflammation in the vitreous. Although one eye in group 1 developed a flat retinal detachment, it may have been produced during the implantation. Contact between swollen devices and the ocular lens was observed in two eyes in group 1. PDLLA tends to swell in aqueous media with time, especially PDLMA of lower molecular weight.\textsuperscript{18} Device-1 swelled more than device-2. Furthermore, the pars plana is poorly developed in rabbits, which may also have caused contact with the lens. Swelling of the devices has to be taken into consideration.

In conclusion, our results suggest that this new
Vitreal Drug Delivery Using a Polymeric Device

vitreous drug delivery system using an implantable biodegradable polymer device may have potential usefulness in vitreoretinal disorders.

Key Words
poly(DL-lactic acid), drug delivery, sodium fluorescein, vitreous, rabbit

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References