Injectable Microspheres with Controlled Drug Release for Glaucoma Filtering Surgery

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The authors evaluated the effects of biodegradable poly (lactic acid) microspheres that provided the controlled release of the antimetabolic agent adriamycin (ADR) to prevent post surgical fibrosis after glaucoma filtering surgery. Fifty six eyes of 28 rabbits underwent posterior lip sclerotomy and received a 0.2 ml subconjunctival injection that contained microspheres 90° from the filtering site immediately after surgery. Microspheres containing ADR (100 or 200 μg) were randomly administered to one eye. The fellow eyes served as controls and received microspheres without the drug. Intraocular pressure in the eyes treated with the microspheres that contained the drug was significantly lower than that in the control eyes from days 7–12 in the 100 μg group and from days 6–16 in the 200 μg group (P < .05). Eyes that received ADR had a significantly longer patent filtering bleb compared with the control eyes (P < .05). No corneal complications were observed in the eyes treated with 100 μg of ADR and the control eyes. Peripheral corneal opacities (25%) and epithelial erosion (17%) were observed in the eyes that received the 200 μg dose, but the cornea returned to normal after 4 wk. These results suggest that controlled-drug-release microspheres with an antimetabolic agent may be promising for preventing fibrosis after surgery.


Glaucoma filtering surgery usually fails because of post surgical scarring, a process in which fibroblasts play a prominent role. To inhibit the proliferation of fibroblasts, many antimetabolic agents have been evaluated in vitro. One potent antimetabolic agent, 5-fluorouracil (5-FU), has improved the success of glaucoma filtering surgery. Clinically, frequent subconjunctival injections are needed to inhibit bleb failure. These injections, however, have produced problems such as patient discomfort and ocular complications. To solve these problems, controlled drug delivery systems of several antimetabolic agents have been evaluated, including sustained drug delivery from liposomes, slow release from bioerodible polymers and membranes, and collagen implants.

We previously demonstrated that microspheres of biodegradable polymers have played a significant role as a drug delivery system in the vitreous. An advantage of the microspheres is that they can be injected through a 27 G needle, whereas other polymer materials must be implanted through surgery. In the present study, we evaluated the effect of injectable microspheres of poly (lactic acid; PLA) with controlled release of the antimetabolic agent adriamycin (ADR) for glaucoma filtering surgery in rabbits.

Materials and Methods
Preparation of Microspheres

The PLA microspheres, containing 10% ADR (Sigma Chemical Co, St. Louis, MO), were prepared by the solvent evaporation method. The molecular weight of PLA was 3400. We dissolved 20 mg of ADR and 180 mg of PLA in 0.5 ml of distilled water and 4.5 ml of acetonitrile at 40°C, and emulsified the solution in 500 ml of liquid paraffin containing 2% (weight/weight) sorbitan monooleate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) by agitation. The oil in oil emulsion was agitated for 24 hr under atmospheric pressure at room temperature until the acetonitrile solvent was evaporated. Thereafter, the microspheres were collected by centrifugation (10,000 rpm at 0°C for 10 min), washed with hexane to remove the liquid paraffin and the emulsifier on the surface of the micro-
spheres, and dried under vacuum until the solvent evaporated. The microspheres were almost homogeneous and spherical with a diameter of about 10 μm.

In Vitro Release Studies

One milligram of the microspheres was suspended in 5 ml of phosphate buffered saline at pH 7.4, and the resulting suspension was stored in a vial and immersed in a shaker bath kept at 37°C. The release medium periodically was removed and replaced with the same amount of fresh medium. During the first day, the medium was changed four times. The amount of ADR released in the medium was measured by fluorescence spectrophotometry. The experiment was performed in triplicate.

Surgical Procedure

After an intramuscular injection of ketamine hydrochloride (50 mg/kg) and a retrobulbar injection of 2% lidocaine hydrochloride (1 ml) were administered, a posterior lip sclerotomy was performed on both eyes of 28 pigmented rabbits, each weighing between 2.1 and 3.5 kg. All operations were performed by the same surgeon (HK). A lid speculum was inserted, and the superotemporal conjunctiva was incised near the fornix with spring scissors. The conjunctiva was carefully dissected anteriorly to the limbus. Tenon’s tissue overlying the sclera was excised, and a groove incision was made with a razor blade and extended anteriorly into the corneal stroma. The area previously was cauterized over the sclera to prevent bleeding. The anterior chamber then was entered through the filtering site, and a 1 × 3 mm block of scleral tissue and trabecular meshwork was excised with the use of Vannas scissors. The edges of the sclerotomy were cauterized for hemostasis, and the iris was cauterized to prevent bleeding before a peripheral iridectomy was performed. The conjunctiva was closed with continuous 8-0 virgin silk suture to produce a watertight closure. The anterior chamber was reformed with sterile saline solution, and the bleb was checked for leakage.

At the end of the surgical procedure, 0.2 ml of the microsphere suspension was injected subconjunctivally through a 27 G needle 90° from the filtering site. The suspension was injected within 30 min after the microspheres had been placed in a suspension. One eye of each rabbit was randomized to determine which would receive microspheres containing ADR (100 or 200 μg) versus the control microspheres without the drug. Topical antibiotic ointment was applied to both eyes of each animal to minimize the risk of post surgical infection.

Measurement of the intraocular pressure (IOP) and slit-lamp bi microscopic examinations of the anterior segment were performed daily for 30 days after surgery. IOP was measured with pneumotonometry (Alcon Applanation Pneumatonograph; Digilab Inc., Cambridge, MA), which had been calibrated for use in the rabbit by relating pneumotonometric IOP readings to actual IOPs obtained manometrically. Before each IOP measurement, one drop of 0.4% oxybuprocaine hydrochloride was applied topically to each eye. No general anesthesia was used while IOP was measured.

Before surgery, six rabbits were designated to be killed for histologic examination. The data from the eyes of these rabbits were not used in the statistical analysis. We evaluated data from 22 rabbits. Of these rabbits, 10 rabbits received 100 μg of ADR and 12 received 200 μg.

Statistical analysis was performed using the paired t-test and paired Wilcoxon’s test to compare the experimental eye of each rabbit with the fellow control eye. A level of P < .05 was considered statistically significant.

The investigations using animals in this study conformed to the ARVO Resolution on the Use of Animals in Research.

Histologic Preparation

Six of the rabbits treated with the microspheres containing 200 μg of ADR were killed with an overdose of pentobarbital sodium 1, 2, 3, 4, 6, and 12 wk after surgery. A subtotal exenteration of the orbits was performed immediately, preserving the conjunctiva. The globes were immersed in the mixture of 4% glutaraldehyde and 2.5% neutral buffered formalin for 24 hr. Globes were opened at the equator and divided into anterior and posterior segments. The cut specimens were dehydrated, infiltrated, and embedded in paraffin. Six-micron step sections of the filtering site and the injection site of microspheres were cut with a microtome. Sections were stained with hematoxylin-eosin and studied by light microscopy.

Results

In Vitro Studies

The release profile of ADR from the microspheres is shown in Figure 1. ADR was released from the microspheres for at least 20 days without a significant “burst” effect.

In Vivo Studies

The postoperative IOP levels in the experimental eyes treated with the microspheres containing ADR were significantly lower than those in the control eyes from days 7–12 in the 100 μg group and from days
6–16 in the 200 μg group ($P < .05$, paired Wilcoxon's test; Fig. 2). Eventually, however, both eyes returned to their preoperative IOP levels. The experimental groups showed a longer duration of filtering blebs—average, 11.8 ± 1.9 days (mean ± standard deviation) in the 100 μg group and 14.0 ± 1.8 days in the 200 μg group, compared with 8.9 ± 1.0 days and 8.8 ± 1.4 days in the control eyes, respectively ($P < .05$, paired $t$-test; Fig. 3).

Wound dehiscence that required repeated suturing occurred 6 days after surgery in one eye treated with the microspheres containing 100 μg of ADR. The conjunctival wound had been closed with continuous 8–0 virgin silk suture.

Corneal epithelial erosion was observed in two eyes (17%) treated with the microspheres containing 200 μg of ADR, but resolved 2 wk after surgery. Peripheral corneal opacities were seen near the filtering site in three eyes (25%) treated with the microspheres containing 200 μg of ADR. These opacities appeared between 5 and 7 days after surgery, and all were accompanied by peripheral new vessel growth. These findings also were observed in 3 eyes (14%) in the control group. These new vessels and opacities, however, gradually decreased. No lens opacities were observed.
Histologic Studies

One week after surgery, eyes treated with the microspheres containing ADR had open filtering ostia and subconjunctival cavities that contained a few inflammatory cells and a little fibrous tissue. The filtering ostia of the control eyes were filled with loose connective tissue, and their subconjunctival cavities contained many inflammatory cells and dense fibrous tissue (Fig. 4). Two weeks postoperatively, the filtering ostia of both eyes were filled with connective tissue.

At the injection sites of the microspheres, microspheres were degraded over time and were phagocytosed by several multinucleated giant cells (Fig. 5). This foreign body reaction gradually was decreased with time. Twelve weeks after surgery, only remnant pieces of microspheres could be recognized in the connective tissue.

The retina and ciliary body were normal in all six eyes examined.

Discussion

In the present study, we evaluated a new sustained delivery system of a potent antimetabolie agent, ADR. The PLA microspheres, which are biodegradable polymers, release the drug by diffusion through the matrix of polymers. The degradation of the matrix occurs over time. This may enhance the rate of release. We previously demonstrated that the microspheres of biodegradable polymers are promising as a drug delivery system in the vitreous.20 Using this type of system, we can control the release rate of the drug by changing the amount of the drug loading or the molecular weight of the matrix oligomer.21 A suspension of the microspheres can be injected through a 27 G needle. Different controlled drug delivery systems of antimetabolic agents have been explored to eliminate frequent subconjunctival injections and decrease side effects. Bioerodible polymers composed of bis (p-carboxyphenoxy) propane (or hexane) and sebacic acid,14-16 and collagen implants18,19 have been used to deliver antimetabolic agents at the filtering site. They are implanted adjacent to the filtering site and directly inhibit post surgical fibrosis. Collagen implants, however, have incited granulomatous inflammatory reactions.19 Liposomes, small bilayer vesicles composed of phospholipids, have been used to provide a sustained delivery system of antimetabolic agents.12,13 Liposomes also can be administered by subconjunctival injections. Compared to liposomes, microspheres are more controllable for release of the drug and are stable for the preservation.

The fungal glycoside anthracycline antibiotic ADR is thought to exert its antimetabolic effect by intercalating between adjacent base pairs on a DNA strand.
and inhibiting its template activity. We chose ADR because in vitro data suggest that it is approximately 100 times more potent than 5-FU in inhibiting the proliferation of fibroblasts.\textsuperscript{1,2} A high potency agent is suitable because we can administer a small amount of microspheres to achieve the desirable effect. In addition, a good releasing profile of ADR from the PLA microspheres was obtained.

The results of the present study showed that the microspheres containing 100 and 200 μg of ADR effectively reduced postoperative IOPs within a certain duration and maintained filtering blebs, compared with those in the control eyes. This effect was more prominent in the eyes treated with the higher dose of ADR. Histologic findings showed that the filtering ostium in the treated eye was patent 1 wk after surgery. However, the filtering ostium already was filled with connective tissue 2 wk after surgery. Although the microspheres released ADR for at least 20 days in vitro, therapeutic effects in vivo lasted for only 16 days in the eyes that received the microspheres containing 200 μg of ADR. This probably was due to the enhancement of the degradation of the microspheres in vivo and to inadequate drug concentration to inhibit the fibrosis at the filtering site after 17 days. Previous studies with the biodegradable polyanhydride discs that contained 5-FU applied to glaucoma filtering surgery in rabbits showed similar results.\textsuperscript{10,11} They were implanted adjacent to the filtering site and directly inhibited the fibrosis at the filtering site. However, they need to be implanted at surgery and can be extruded through the conjunctiva. Administration of microspheres can be done through a fine needle and requires no additional surgery. The other advantage of the microspheres is that an additional administration can be done easily at an office at any time during the follow-up period.

Corneal epithelial defects is a significant complication after subconjunctival injections of 5-FU. Peripheral corneal opacities and new vessel growth were found in 25% of the eyes treated with the microspheres containing 200 μg of ADR. The eyes treated with the microspheres containing 100 μg of ADR showed no corneal complication, although three eyes (14%) in the control group showed corneal lesions similar to those in the 200 μg group. These corneal lesions were observed mainly near the filtering site, not at the injection site of the microspheres, and gradually disappeared with time. Therefore, surgical invasion may have been a possible cause of these corneal lesions.

In conclusion, our results suggest this new drug delivery system, using the PLA microspheres, may be a therapeutic method for glaucoma filtering surgery. Subconjunctival administration of the microspheres with other agents can be applied to the treatment of other ocular disorders.

**Key words:** adriamycin, glaucoma filtering surgery, microspheres, poly (lactic acid), subconjunctival injection

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**References**