Creation of a Drug-Coated Glaucoma Drainage Device Using Polymer Technology

In Vitro and In Vivo Studies

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Objective: To create and test a slow-release anti-fibrotic drug-coated glaucoma drainage device using in vitro and in vivo experiments.

Methods: A slow-release device incorporating mitomycin C in poly(2-hydroxyethyl methacrylate) disks was developed using redox-polymerization techniques. A standardized preparation of this drug delivery device was attached to the Ahmed glaucoma valve (model FP7; New World Medical, Inc, Rancho Cucamonga, California). Semicircular disks (5 mm x 6 mm) of P(HEMA)-mitomycin C containing varying concentrations of mitomycin C per gram dry weight of the gel were attached to the lower half of an Ahmed glaucoma valve plate. Water was pumped through the modified Ahmed glaucoma valve at a rate comparable to that of aqueous humor outflow, and mitomycin C release was measured. Modified and unmodified Ahmed glaucoma valves were implanted in a rabbit model, and drug release and fibrosis were assessed after 3 months.

Results: The P(HEMA)-mitomycin C device released mitomycin C in vitro over 1 to 2 weeks. Studies in rabbits revealed that mitomycin C was released from the disks during the 3-month implantation. Histologic analysis demonstrated a significant reduction in inflammatory reaction and fibrosis in the resulting blebs.

Conclusion: Our slow-release drug-coated glaucoma drainage device decreased fibrosis and inflammation in the resulting bleb in a rabbit model.

Clinical Relevance: This device could reduce the failure rate of glaucoma drainage devices.


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the plate. After in vitro release tests to study the rate of drug release from the matrix, the efficacy of the slow-release device was measured in a cell-culture system using human conjunctival fibroblasts. The results demonstrated that the P(HEMA) polymer enabled release of mitomycin C and inhibited the proliferation of fibroblasts in a dose-dependent manner. The objectives of the present study were (1) to develop techniques for preparation and standardization of P(HEMA) disks loaded with mitomycin C; (2) to demonstrate that the device released mitomycin C both in vitro and in vivo when attached to a commercially available flexible silicone Ahmed glaucoma valve (AGV) (model FP7; New World Medical, Inc, Rancho Cucamonga, California); and (3) to determine the effects of the modified device after implantation in a rabbit model.

METHODS

The P(HEMA) sheets were prepared as described previously by polymerization of 2-hydroxyethyl methacrylate by redox polymerization in the presence of a cross linker, N,N-methylenebis-acrylamide on 2 glass slides with a 0.75-cm spacer. After removing impurities by repetitive swelling in ethanol and water for 12 hours each for 2 days, P(HEMA) sheets were cut into semicircular disks measuring 5 × 6 mm to fit the lower half of the AGV plate. A 1-mm trephine (Katena Products Inc, Den- ville, New Jersey) was used to punch a hole in the middle of each disk. The P(HEMA) disks were loaded with mitomycin C at varying concentrations (0.17, 0.35, and 0.8 mg of mitomycin C per gram of dry gel) using a previously described solvent evaporation technique. In this technique, a good solvent, ethanol, for mitomycin C, and its mixture with water were used for the drug-loading experiments.

Previously sterilized AGVs were placed in a sterile hood. A 1-mm hole was punched in the center of the lower half of the end plate. The mitomycin C–loaded (HEMA) disks were then attached to the lower half of the AGV end plate by passing a silicone rivet through the central holes of the 2 devices. The entire procedure was performed under sterile conditions. The modified GDD was resterilized using UV light under the hood for 5 minutes. The UV light was used to prevent degradation of mitomycin C in the P(HEMA) matrix. Twenty-four modified valves were prepared using this technique.

IN VITRO EXPERIMENTS

The purpose of this experiment was to test whether the modified AGV would release the mitomycin C in a slow and sustained fashion. An in vitro experimental device was constructed using a syringe pump (model 55-1144; Harvard Apparatus Inc, Holliston, Massachusetts) to inject sterile water through the tubing of the modified AGV. The injection rate was kept constant to emulate aqueous humor circulation in the human eye (2.5 µL of aqueous humor per minute or 3.6 mL/d). The experiment was designed to simulate the postoperative human eye as closely as possible. A 30-mL syringe filled with sterile water was attached to the syringe pump. The syringe was attached to one end of a 23-gauge butterfly needle system. The 23-gauge needle was attached to the silicone tube of the modified AGV (Figure 1). A scintillation vial was used as the collecting media. The modified AGV was suspended inside the vial, and the vial was sealed with flexible film (Parafilm; Pechiney Plastic Packaging, Inc, Neenah, Wisconsin) to prevent evaporation. The entire experiment was carried out at room temperature.

Figure 1. Left, Syringe pump used to emulate the flow of aqueous humor through the modified Ahmed glaucoma valve (model FP7; New World Medical, Inc, Rancho Cucamonga, California). Right, Enhancement of vial (oval) in which fluid was collected after flow through the modified Ahmed glaucoma valve. The Ahmed glaucoma valve with the attached poly(2-hydroxyethyl methacrylate)–mitomycin C–coated (purple) disk is shown in the foreground.

After 24 hours, the AGV was removed from the vial and suspended in a new vial. The water that was flushed over the P(HEMA) matrix during 24 hours was tested for mitomycin C. This experiment was repeated daily for 1 week. The amount of mitomycin C in the collected samples was determined by UV-visible light spectroscopy (UV-Vis; Varian, Inc, Palo Alto, California). Mytomycin C has strong absorption at 360 nm in ethanol. The amount of mitomycin C was calculated from a previously formed calibration curve constructed at 360 nm. Release of mitomycin C was monitored for 7 days.

IN VIVO EXPERIMENTS

In vivo experiments were performed on rabbit eyes to test the modified AGVs for (1) testing of the efficacy of the device in terms of release of mitomycin C; (2) validation of the sterilization techniques used; and (3) verification that the concentration of mitomycin C used was sufficient to inhibit fibrosis without collateral damage to the underlying sclera and the surrounding muscles. All investigations conformed to the regulations of the Association for Research in Vision and Ophthalmology, the National Institutes of Health, and the guidelines set forth by the Tulane University Institutional Animal Care and Use Committee.

Twenty-two albino New Zealand rabbits were used in this experiment. The right eye of all rabbits was used for implantation of the AGV. The rabbits were divided into 4 groups. Group 1 (n=4) received AGVs with no mitomycin C–coated device attached; group 2 (n=6) received the modified AGV with 0.17 mg of mitomycin C per gram of dry gel; group 3 (n=6) received the modified AGV with 0.35 mg of mitomycin C per gram of dry gel; and group 4 (n=6) received the modified AGV with 0.8 mg of mitomycin C per gram of dry gel.

The surgery was performed in standard fashion. After adequate anesthesia using ketamine hydrochloride, the right eye of the rabbit was prepared with povidone-iodine (Betadine; Purdue Pharma LP, Stamford, Connecticut). A 7-0 polyglactin 910 (Vicryl; Ethicon, Somerville, NJ) suture was passed through the supratemporal limbus to rotate the eye downward. Conjunctival peritomy was performed at the limbus in the supratemporal quadrant, followed by posterior dissection in the same plane. The dissection was carried further between the superior and lateral rectus muscles posteriorly. The AGV was brought to the operative site and primed with balanced salt solution. The end plate was tucked into the supratemporal quadrant and secured to the underlying episclera with 2 interrupted 10-0 nylon sutures approximately 7 mm from the limbus. The silicone tube was cut 0.5 mm anterior to the limbus using Westcott scissors. A 23-gauge butterfly needle was used to enter the
anterior chamber 0.25 mm posterior to the limbus, and 0.1 mL of viscoelastic (Healon; Advanced Medical Optics, Inc, Santa Ana, California) was injected. The silicone tube was inserted into the anterior chamber through the needle tract and secured to the surrounding sclera with a 10-0 nylon suture. The conjunctiva was secured to the limbus with an interrupted 10-0 polyglactin 910 suture.

The eye was treated with antibiotic and corticosteroid drops along with cyclopentolate hydrochloride, 1% (Cyclogel; Falcon Pharmaceuticals, Ltd, Fort Worth, Texas), 4 times a day for 2 weeks. All animal eyes were examined for signs of infection and tube erosion on days 1, 3, 7, and 14 and monthly thereafter. The animals were killed 3 months after implantation. The eyes were enucleated taking care not to disturb the bleb and the implant. After enucleation, 10% formalin was injected into the vitreous cavity to fix the eyes. After 24 hours, the eye was dissected, with the first incision passing through the middle of the bleb. The implant end plate was isolated during this process and removed from the eye. The mitomycin C–coated device attached to the end plate of the AGV was then examined. Histology slides were prepared involving the bleb and the surrounding tissues and stained with hematoxylin-eosin and trichrome to highlight the fibrous capsule. The histologic sections were examined using light microscopy by a pathologist (S.H. or C.E.M.) who was blinded to the different groups. Basement membrane measurements were made in micrometers at ×20 magnification.

**RESULTS**

**IN VITRO EXPERIMENTS**

Figure 2 shows the cumulative mitomycin C release results from the in vitro experiments. The initial rate of mitomycin C release was faster than the release rate after 5 days. This is reasonable because the drug molecules that are situated at and around the surface are released first on contact with water, and the drug molecules deeper inside the P(HEMA) gels are released later. After 1 week, however, a light purple could still be observed in the P(HEMA)–mitomycin C device (Figure 1), indicating the presence of residual mitomycin C.

**IN VIVO EXPERIMENTS**

There were no instances of infections, unexpected animal deaths, or other complications. All of the operations resulted in formation of a thick-walled vascular bleb (Figure 3). No avascular cystic blebs were observed even in the mitomycin C–treated groups. After the enucleation, the posterior edge of the bleb was sliced open, and the valve plate was removed from the bleb without disturbing the bleb. The AGVs were examined by one of us (R.S.A.) with the naked eye. The modified AGVs retained the attached P(HEMA) device. Before implantation, the disks were purple, indicating the presence of mitomycin C. At the end of the experiments, the disks were colorless, indicating that the mitomycin C had been completely released from the disks (Figure 4). This was further confirmed by extraction of 3 of the sample disks recovered from the animal experiments. No mitomycin C was found at spectroscopic analysis of these ethanol extracts, confirming that the disks had released all of the mitomycin C during the 3 months of the study.

**HISTOLOGIC ANALYSIS**

The fibrous capsule thickness in various locations and the degree of inflammation were assessed. The fibrous capsule thickness varied significantly in the roof and the anterior edge of the bleb (the side toward the limbus)
when compared with blebs in animals implanted with unmodified AGVs (Figure 5, Figure 6, and Figure 7). No statistically significant differences were noted among the 3 concentration groups (0.17, 0.35, and 0.8 mg of mitomycin C per gram of dry weight of P[HEMA]). When these 3 groups were combined in the analysis, the differences between the unmodified and modified AGVs were highly significant. The thickness at the limbal edge of the bleb was reduced from a mean of 16.7 µm to 4.3 µm (P < .001) in animals implanted with the P(HEMA)-mitomycin C modified AGV, whereas the thickness at the roof of the bleb was reduced from a mean of 21.7 µm to 6.6 µm (P < .001). Although there seemed to be a reduction in fibrous capsule thickness at the posterior edge of the bleb (the side facing the optic nerve; 13.7 vs 7 µm), the means were not statistically different (P = .24). There was no difference in the fibrous capsule thickness along the floor of the bleb, adjacent to the sclera between the groups (20.8 vs 17.1 µm; P = .55). The degree of inflammation was significantly higher in group 1 (AGV without mitomycin C) than in the other groups with the mitomycin C–coated device.

Trabeculectomy and other perilimbal surgical procedures result in formation of a bleb that acts as a reservoir at the limbus. These blebs are associated with various adverse effects such as bleb leaks, bleb-related infections, and bleb dysesthesia. Postoperative fibrosis leading to failure of trabeculectomy leads to excessive scar tissue formation in the perilimbal area, making that area less suitable for a second surgical procedure. These outcomes have led to the increasing use of GDDs in the management of glaucoma. Surgery using GDDs results in formation of the bleb 8 to 10 mm posterior to the limbus. The presence of the biomaterial of the GDD and the accumulation of aqueous humor in the subconjunctival space stimulates an inflammatory reaction and fibrosis, which leads to formation of a well-defined bleb surrounding the GDD plate. The combination of ongoing inflammation and fibrosis leads to failure of the operation. From a clinical standpoint, the bleb proceeds through 3 stages: (1) a hypotensive phase, which lasts approximately 1 to 4 weeks during which the IOP is typically low, and the bleb is ill defined and diffuse and exhibits congested blood vessels; (2) a hypertensive phase, which lasts from 1 to 6 months and is associated with increased IOP, and the bleb becomes localized and well defined with formation of a dense fibrous capsule separating the aqueous humor from the conjunctival blood vessels; and (3) a stable phase, which is achieved at the end of 6 months and is characterized by the presence of a bleb with no or little inflammation and well-controlled IOP, usually in the midteens (range, 15-17). The incidence of the hypertensive phase has been reported to be between 10% and 50% and varies with the various GDDs. In general, it is accepted that the incidence is lower with the Baerveldt implant compared...
with the AGV. During this phase, the IOP can potentially increase to 30 to 50 mm Hg. The overall failure rate of GDDs is approximately 10% each year, leading to a 50% failure rate at 5 years. This is the result of continued fibrosis. One-time application of antifibrosis drugs such as mitomycin C or fluorouracil during the operation has not been effective in reducing fibrosis in surgical procedures incorporating GDDs, unlike that after trabeculectomy. In the tube-vs-trabeculectomy study comparing the Baerveldt implant with trabeculectomy in patients with previously failed trabeculectomies, during the first year of follow-up, in the GDD group, IOP was more likely to be controlled, persistent hypotonia or repeat operation because of glaucoma was averted, and there were fewer postoperative complications compared with the trabeculectomy with the mitomycin C–treated group. A recent article suggested increased success with lower incidence of the hypertensive phase in patients receiving repeated injections of fluorouracil into the bleb after application of mitomycin C during surgery in patients undergoing AGV implantation. Thus, surgery using a GDD has several advantages over traditional trabeculectomy. However, the inflammatory reaction that results in the hypertensive phase and the continued fibrosis leading to complete failure are important problems that must be overcome to improve the surgery. The creation of a slow-release mitomycin C drug-coated GDD is an attempt to overcome these problems.

The drug delivery system was verified in both in vitro (laboratory) and in vivo (cell culture and rabbit) models. The effect of mitomycin C treatment on the bleb reflects the pattern of release of mitomycin C on contact with the aqueous humor. In the presence of aqueous humor, the P(HEMA) polymer swells, releasing the mitomycin C into the aqueous humor. Because for the most part, the AGV plate prevents contact of the aqueous humor with the sclera, most of the effect of the drug should be found on the roof and the anterior side wall of the bleb, as was demonstrated at histologic analysis. In the groups treated with mitomycin C, the fibrous capsule was significantly thinner in the roof and the anterior edge of the bleb compared with the group that received no mitomycin C. However, there were no differences among the groups with 3 different concentrations of mitomycin C. It may be that the degree of anti-inflammatory and antifibrotic action of mitomycin C was similar in the range of concentrations used in these experiments.

These experiments were conducted in rabbits, and the results may not translate to human beings. However, if these results can be reproduced in human beings, these modified AGVs with the slow-release P(HEMA)–mitomycin C matrix may result in a lower incidence of the hypertensive phase and an increased overall success rate by virtue of thinning the fibrous capsule and decreasing the inflammatory reaction. We chose not to measure the IOP in the postoperative period in the rabbits because IOP measurements are not accurate in the rabbit model owing to a thin cornea. To our knowledge, the incidence and occurrence of the hypertensive phase in the rabbit model has never been reported. The long-term success rate of these procedures in the rabbit is unknown. For these reasons, we chose to concentrate on the histologic findings of the bleb as the conclusive evidence that the device works.

**CONCLUSIONS**

Our in vivo and in vitro experiments prove that mitomycin C is released in a sustained-release fashion from P(HEMA) disks after their attachment to the AGV. That mitomycin C was recovered after UV sterilization demonstrates that the technique of sterilization used in these experiments was successful in ensuring sterility while not damaging the drug. The concentration of MMC in the P(HEMA) disks was high enough to prevent significant fibrosis in the bleb while averting formation of avascular blebs.

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Virtual Spectacles

Spectacles have enhanced the lives of countless people over the last few hundred years, yet most of us take them for granted. In addition to a few individual collectors and enthusiasts, there are several small societies and museums dedicated to visual aids such as the Ocular Heritage Society in the United States and the British Optical Association Museum in London, England. Now there is an incredible online collection of everything related to visual aids created by David Fleishman, MD, a retired ophthalmologist in Massachusetts, with the help of more than 1100 contributors from around the world.

On his Web site you can learn about the history of visual aids from their invention in the 13th century in Italy to the present day. There is a guide to various design features with the corresponding time period and a spectacle terminology glossary. There are special topics such as articles about optical fans and the spectacles of famous people, from Abraham Lincoln to Sigmund Freud and Audrey Hepburn. Every article is accompanied by beautiful images of spectacles (Figure) and people, both real and mythical, wearing them throughout history. There is also a list of museums around the world with collections of antique spectacles.

You will need some time to fully appreciate the value of this Web site, with its 260 pages and 4700 images representing more than 625 institutions. When you are done, you can quiz yourself with educational games and enjoy visual games and optical illusions. It is still a work in progress, with plans for new pages on monocles and the history of telescopes to be added in the near future.

Ilya Rozenbaum, MD
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Figure. Antique spectacles made by John McAllister, who established the first optical shop in America in Philadelphia, Pennsylvania.