Laser-Cured Fibrinogen Glue to Repair Bleb Leaks in Rabbits

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Objective: To determine whether laser-cured fibrinogen glue can close bleb leaks in rabbits.

Methods: Full-thickness filtration surgery with intraoperative mitomycin and a sutured limbus-based conjunctival flap was performed in 1 eye each of 19 New Zealand albino rabbits. On the second postoperative day, a 2- to 3-mm hole was made in the bleb. In 9 rabbits, the hole was glued using fibrinogen glue with indocyanine green dye added. The glue was “cured” with a diode laser. Eyes that had been glued and developed a subsequent leak had the glue reapplied on the day the leak was detected.

Results: The glue remained on the conjunctiva for an average (mean±SD) of 1.9±1.8 days (range, 0-5 days). The last day of bleb leak for the rabbits with glued eyes was 1.6±2.4 days; for the control rabbits, it was 8.0±4.4 days (P=.001, Mann-Whitney U test).

Conclusion: Laser-cured fibrinogen glue is effective in closing bleb leaks in rabbits.


Bleb leaks after trabeculectomy can occur either in the immediate postoperative period or much later, in established blebs. A leaking bleb may lead to hypotony maculopathy, choroidal effusions, suprachoroidal hemorrhage, shallow or flat anterior chamber, cataract progression, peripheral anterior synechia, endophthalmitis, or bleb failure. Many techniques for managing bleb leaks have been reported. We report a technique to close bleb leaks in a rabbit model using diode laser–cured fibrinogen glue made from single-donor human plasma with indocyanine green dye (ICG) added during the acute postoperative period.

Results: The average (mean±SD) number of days the glue was present on the conjunctiva was 1.9±1.8 days (range, 0-5 days). Immediately after gluing the hole, the glue had a hardened consistency (Figure 1 and Figure 2), but as early as postoperative day 1, the glue appeared softened. The size of the spot of glue became smaller with each day, and eventually it “dissolved” or fell off the conjunctiva.

The last day the bleb was found to leak in each rabbit is shown in Figure 3. Three of the rabbits in the glued group started leaking after initial sealing of the bleb hole and required repeated laser-cured fibrinogen glue application (1 additional application for 2 rabbits; 1 rabbit required 2 additional applications). The glue was reapplied on the day the leak was detected. The last day of bleb leak for the glued group was 1.6±2.4 days (range, 0-5 days). The last day of bleb leak for the control group was 8.0±4.4 days (range, 0-17 days). This difference is shown in Figure 3 and was statistically significant (P=.001, Mann-Whitney U test).

There was no statistically significant difference in intraocular pressure between rabbit eyes that were glued and those that were not for each of the 4 days following puncture of the bleb (P value range, .06-.56; Mann-Whitney U test).

Histologic findings in all eyes studied included fibroblast proliferation and lymphocytic infiltrate, absence of an eosinophilic or foreign body reaction, and...
MATERIALS AND METHODS

Full-thickness filtration surgery was performed in the right eye of 19 New Zealand albino rabbits. Ten rabbits served as controls and 9 rabbits made up the treatment group (sample size calculated on a 2-sided test with a power of 80% and a 5% significance level). The study was conducted according to guidelines established by the University of Minnesota Animal Care Committee, Minneapolis.

Prior to surgery, intraocular pressure was measured with pneumotonometry. Following pneumotonometry, the rabbits were fully anesthetized using an intramuscular injection of ketamine hydrochloride and xylazine hydrochloride. A traction suture was placed through the cornea. A limbus-based conjunctival flap was created and a tenonectomy was performed to prevent the Tenon capsule from occluding the holes that were to be subsequently created.

A cellulose sponge soaked with mitomycin, 0.5 mg/mL, was placed between the sclera and the conjunctival flap for 5 minutes. The sponge was removed, and the site was aggressively irrigated with saline solution. A full-thickness sclerotomy was made at the limbus with a Kelly punch through the Descemet membrane. The conjunctival incision was closed with a running 10-0 nylon suture.

Intraocular pressure was measured daily using topical anesthesia. Two days after the filtering procedure, the rabbits were again fully anesthetized and a 2- to 3-mm hole was punched in the most exuberant part of the bleb using a 15° microsurgical blade.

Nine of the rabbits underwent immediate closure of the bleb hole using laser-cured fibrinogen glue. The glue was prepared by drawing human blood to fill a 30-mL syringe containing 20 IU/mL of heparin sodium and 11.5 mmol/L of trisodium citrate. The blood was centrifuged at 1000g for 20 minutes. The plasma was removed and placed into two 15-mL falcon tubes and frozen at −80°C for a minimum of 24 hours. Freezing the plasma several days resulted in more easily prepared glue. The tubes were thawed and placed into two 50-mL tubes containing a water and crushed ice slurry at 0°C. The “tube inside a tube” was spun at 1000g for 30 minutes. The plasma was decanted leaving the fibrinogen precipitate, which had the consistency of a thick liquid. Indocyanine green dye (Cardio-Green, Becton Dickinson Microbiology Systems, Cockeysville, Md) was added using 1 drop (~20 µL) of 2.5-mg/mL concentrate through a 30-gauge needle.

After the hole was made in the bleb in the 9 study rabbits, the area was dried using a cotton-tipped applicator. The fibrinogen glue was applied to the hole using a 1-mL serological pipet. The glue was “cured” or coagulated using a diode laser (OcuLight SL, Iris Medical Instruments, Mountain View, Calif) with an endolaser probe (700-800 mW; duration, 5 seconds). The endolaser probe was held approximately 5 mm above the glue and passed back and forth over the glued area. This process turned the fibrinogen glue into a dry, firm, golden-brown adhesive and was repeated until all the glue was cured. Subsequently, a moistened fluorescein strip was applied to determine if the bleb was continuing to leak. The procedure was repeated until no leak was found. The remaining glue was frozen at −80°C, and could be reused up to several weeks later.

After creating the bleb leak, all rabbits were monitored daily using topical anesthesia. Data obtained included intraocular pressure, presence or absence of glue, and presence or absence of bleb leak. The eyes were examined with a portable slitlamp and tested for leaks with a moistened fluorescein strip painted over the bleb. The bleb hole was assumed to be permanently closed when there was no leak observed for 4 to 5 days.

During preliminary studies, it was found that if the leak was not immediately resealed, it would continue leaking for several days. Thus, if a leak was found in the study rabbits that had undergone the gluing procedure, the glue was immediately reapplied using the same procedure outlined earlier.

Histopathologic examination was performed on 3 eyes of the study rabbits and 2 eyes of the control rabbits. These eyes were enucleated 12 days after punching a hole in the bleb and fixed in 10% neutral buffered formalin. The globes were sectioned parallel to the meridian of the surgical procedure. Tissue sections were stained with hematoxylin-eosin and periodic acid–Schiff stains.

COMMENT

Various adhesive agents have been tried for repairing leaking blebs, including cyanoacrylate glue.7,8 Despite some promising results in sealing leaking blebs, cyanoacrylate glue is not without problems. The irregular, hard surface of the glue usually requires that it be covered with a bandage lens or a collagen shield for patient comfort, and the glue has a tendency to damage the surrounding conjunctiva if it is dislodged. Fibrin adhesive made from pooled human plasma (Tisseel, Immuno AG Industriestr., Vienna, Austria) has been used to seal bleb leaks in animals; however, neither cyanoacrylate glue nor Tisseel is approved for human use in the United States. Neither Tisseel nor cyanoacrylate glue was used in this study.

While fibrin adhesive made from pooled human plasma may potentially carry blood-borne pathogens, the use of the patient’s own blood as the source of the fibrinogen eliminates this risk. Asrani and Wilensky9 described the use of autologous fibrin tissue glue and thrombin to treat 12 episodes of postfiltration bleb leaks during a 1-year period. Kajiwara10 described the successful repair of a leaking filtration bleb with fibrin glue after unsuccessful treatment of the same bleb with resuturing and cyanoacrylate adhesive.

There was no difference between the appearance of blebs in the glued and the control rabbit eyes after the leak had stopped. The intraocular pressure was not helpful in determining which blebs were leaking; however, the same can be seen in humans after filtration surgery. While a very low pressure may instigate the search for a bleb leak, a normal intraocular pressure does not preclude the existence of a leak.

The laser-cured fibrinogen glue appeared to be well tolerated. Grossly, the area around the glue was not vas-
cularized. On histologic examination, the reaction at the surgical site in eyes that underwent trabeculectomy alone was indistinguishable from those with subsequent application of fibrinogen glue. We attribute the lack of thermal damage to the ICG dye component of the fibrinogen glue. The ICG dye has its maximum absorption (805 nm) very close to the wavelength of the diode laser (810 nm), allowing maximum energy absorption in the fibrinogen and ICG dye and minimal energy absorption by the surrounding undyed tissue.

The fibrinogen glue alone is easily wiped from the surface of the conjunctiva after it is applied. This is helpful if the initial placement of the glue is not ideal and needs to be reapplied, but points out the shortcoming of using the fibrinogen without the laser: the glue alone does not adhere well to the conjunctiva, particularly with a constant seepage of aqueous. The fibrinogen and ICG dye, on the other hand, adheres firmly to the conjunctiva after the application of the diode laser.
ter it is cured with the diode laser. Oz et al.\textsuperscript{11} found that the use of the diode laser alone was not as effective in sealing aortotomies as the use of a diode laser combined with fibrinogen glue. Their finding supports the idea that a stronger bond results from the use of the fibrinogen glue and application of the diode laser together than from the use of either the fibrinogen glue or the diode laser alone.

We believe that the use of laser-cured fibrinogen glue may offer another useful approach to the treatment of bleb leaks after filtration surgery. Although it has yet to be tested in humans, it has the theoretical advantages of being an office procedure that uses a commercially available laser, is able to be repeated, and does not expose the patient to exogenous blood-borne pathogens.

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REFERENCES


News

American Board of Ophthalmology Time-Limited Certificate Renewal

To enter the American Board of Ophthalmology’s certificate renewal process, you must hold a time-limited certificate issued by the board in 1992 and thereafter. Requests for recertification from ophthalmologists who hold lifetime certificates issued before 1992 will be considered on a case-by-case basis. The application filing period will run from January to June each year. All candidates are required to participate in the knowledge and practice assessment components or certificate June each year, which can be completed within a 2-year period. Candidates will be asked to select 1 of 3 pathways for knowledge assessment: a take-home written examination, a recertification oral examination, or the current certifying oral examination. All are required to participate in the office record scan review process for practice assessment. During the renewal process, all individuals must hold a current, valid, and unrestricted license to practice medicine in the United States, its territories, or Canada and must attain 500 continuing medical education credits from an Accreditation Council for Continuing Medical Education–approved institution. A total of 50 credits a year is recommended, with 60% in category 1; 80% must be related to the practice of ophthalmology. For further information concerning this process, you are invited to contact the board office: Denis M. O’Day, MD, Executive Director, 111 Presidential Blvd, Suite 241, Bala Cynwyd, PA 19004; or phone: 610-664-1173; or fax: 610-664-6503.