Enzymatic Sclerostomy
Pilot Human Study

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Objective: To evaluate the feasibility and safety of enzymatic sclerostomy as a new modality to lower intraocular pressure in patients with open-angle glaucoma.

Methods: This single-center, prospective, noncomparative, interventional case series included 15 blind symptomatic eyes of 15 patients with primary open-angle glaucoma. Enzymatic sclerostomy was performed with the patient under topical or peribulbar anesthesia. A specially designed polymethylmethacrylate enzyme applicator filled with a mean±SD of 123±13 µg of collagenase was introduced through a 5-mm peritomy, and affixed to the limbus by means of cyanoacrylate tissue glue. After 22 to 24 hours, the applicators were removed and the patients were followed up for 1 year. Intraocular pressure changes from baseline and complications related to the procedure were the main outcome measures.

Results: Controlled thinning of the treated sclera associated with aqueous percolation and shallow filtration bleb was seen in all eyes in the immediate postoperative period. The mean±SD intraocular pressure decreased from 43.5±9.8 mm Hg (while the patients were receiving a mean±SD of 1.75±0.75 antiglaucoma medications) preoperatively to 24.8±10.6 mm Hg (a 43.0% decrease from baseline with no antiglaucoma medication) on the first postoperative day and to 34.8±10.5 mm Hg (a 20.0% decrease from baseline with no antiglaucoma medication) at the end of 1 year. Ophthalmic adverse effects were limited to the treated area and included immediate postoperative transient conjunctival reaction ranging from mild chemosis to conjunctival maceration. Immediate full-thickness perforation developed in 1 eye; the patient was treated and excluded from data analysis. Two eyes developed symptoms related to increase in intraocular pressure after 9 months; the patients were treated and excluded from further data analysis. No systemic complications were noted.

Conclusions: Enzymatic sclerostomy demonstrated immediate and sustained intraocular pressure reduction and provided symptomatic relief in blind eyes with primary open-angle glaucoma. The procedure, however, needs further technical refinement.

Arch Ophthalmol. 2002;120:548-553

TRABECULECTOMY is an established surgical modality in the treatment of glaucoma. However, it has a long learning curve and is associated with several sight-threatening complications. Imaginative procedures such as microtrabeculectomy,1 holmium and erbium laser sclerostomy,2,4 ultrasonic insonification,5 excimer laser filtration surgery,6 deep sclerectomy,7 and viscocanalostomy8 have been proposed as minimally invasive alternatives to conventional trabeculectomy.

Clostridial collagenase has been used in humans for discolysis in cases of prolapsed intervertebral disk,9 for removal of the cheloid plaques in Peyronie disease,10 and as an adjunct in vitrectomy.11 The potential of collagenase to act as a “biological knife” to selectively digest collagen inspired us to develop a treatment modality for glaucoma that we named enzymatic sclerostomy, wherein highly purified collagenase is used to create deep focal scleral digestion, possibly enabling micropercolation of aqueous humor. Our group has studied the directional selectivity of propagation of collagenase through the scleral collagen lamellae and has demonstrated the efficacy of this method in reducing the intraocular pressure in a rabbit model.12 We have established the optimal duration of exposure to collagenase in humans by evaluating variable periods of application with constant enzyme con-
PATIENTS AND METHODS

The study was performed at the VST Center for Glaucoma Care, LV Prasad Eye Institute, Hyderabad, India, with approval from the institutional review board. Fifteen consecutive patients (15 eyes) with primary open-angle glaucoma who met the inclusion criteria and agreed to participate in the study were enrolled. All the eyes were legally blind and symptomatic because of elevated intraocular pressure, and had had no previous ocular surgery. Each patient had functional vision in the fellow eye. To minimize variations due to differences in the collagen structure, patients selected were uniformly Asian-Indian in race, and the age range was restricted (45-75 years). The patients signed an informed consent and agreed to adhere to the follow-up protocol.

Preoperative evaluation (by J.A.D. and S.G.H.) included slitlamp biomicroscopy of the ocular surface and the anterior segment, intraocular pressure measurement by Goldmann applanation tonometry, evaluation of the anterior chamber angle by Goldmann 2-mirror gonioscope, and optic disc examination with a +60 diopter lens. In patients who were taking topical antiglaucoma medications, the drugs were discontinued at least 1 day before the enzymatic sclerostomy was scheduled. When required, however, the use of topical antiglaucoma medications in the fellow eye continued. An internist performed systemic evaluation to rule out conditions associated with abnormal collagen structure.

 Highly purified collagenase (nuclease, approved as investigational drug 149149), lot 60901, containing 5150 U/vial,14 was supplied as lyophilized powder (BioSpecifics Technologies Corp; Lynbrook, NY). Cysnaocrylate tissue glue was acquired commercially (Braun; Melsungen, Germany). The forceps used for grasping the enzyme applicator were designed and manufactured at the Tools Laboratory of the Weizmann Institute of Science (Rehovot, Israel). Polyacrylamide-glutaraldehyde enzyme applicators were manufactured (ASCON; Madras, India) in conformance with the design previously used.3 The applicators were manually filled with lyophilized collagenase powder in the range of 100 to 150 µg (mean±SD, 123±13 µg), stored at −4°C, and used within 72 hours.

The surgery was performed in the operating room under an operating microscope by 1 of 3 surgeons (J.A.D., S.G.H., and A.K.M.). Topical anesthesia (4% lidocaine drops) was used in 8 eyes and peribulbar anesthesia (1:1 mixture of 2% lidocaine and 0.5% bupivacaine, 5 mL) in 7 eyes. A lid speculum was introduced and a 5-mm peritomy was performed at the superior limbus. Wet-field cautery was applied to achieve hemostasis. The exposed sclera at the intended site of application was thoroughly dried with a cellulose sponge. A drop of tissue adhesive was placed on the ventral surface of the applicator in the trough encircling the well containing the enzyme (Figure 1). The applicator was grasped during the procedure by means of specially designed forceps. It was then firmly applied to the sclera with its anterior edge corresponding to the anterior edge of the anatomic limbus (Figure 2). Fixation of the applicator to the sclera was verified by attempting to mechanically displace it over the scleral surface. Fixation was deemed good when there was no movement of the applicator over the sclera. Fixation was graded fair when there was minimal movement, and poor when there was edge lift or significant movement. Where the applicator fixation was poor, a new applicator with fresh glue was applied to

centration and placement location and have demonstrated ocular and systemic safety.13 Preliminary human studies performed at the Belinson Hospital, Petah-Tikva, Israel; Aravind Eye Hospital, Madurai, India; and California Pacific Medical Center, San Francisco, included 16 blind eyes of 16 patients. Placement of the enzyme delivery system at the anatomic limbus and a mean±SD enzyme exposure time of 22±2 hours were found most effective in reducing intraocular pressure without causing full-thickness scleral digestion or ocular and systemic toxicity. On the basis of the results of our earlier experimental and preliminary human studies, we planned a controlled interventional noncomparative case series to evaluate the efficacy and safety of enzymatic sclerostomy in human subjects.

RESULTS

Of the 15 patients, 11 were men and 4 were women, ranging in age from 45 to 75 years. Three patients were newly diagnosed with primary open-angle glaucoma and were previously untreated. Twelve patients were taking 1.75±0.75 (range, 1-3) types of antiglaucoma medication for symptomatic relief at enrollment. Use of topical antiglaucoma medication was discontinued at least 1 day before surgery. The baseline intraocular pressure was 43.5±9.8 mm Hg. Three patients were excluded from further data analysis, 1 because of full-thickness scleral perforation requiring scleral grafting and 2 because of an elevation of intraocular pressure after 9 months requiring additional treatment (transscleral cyclophotocoagulation in 1 eye and topical timolol maleate 2 times a day in 1 eye). The final follow-up was at 12 months after enzymatic sclerostomy.

The collagenase-specific activity as determined by the manufacturer showed no decay from the day of enzyme charging in the applicators until the actual day of application.

Patients reported no pain or discomfort during application. Applicator position and fixation were inadequate in 5 eyes, and new applicators were applied (twice in 4 cases and 9 times in 1 case) until satisfactory position and fixation were obtained. However, when evaluated after 22±2 hours, during the removal of the applicators, the position of the enzyme applicator was good only in 12 eyes; in 2 eyes it was placed too far anterior and in 1 eye too far posterior. The fixation of the applicator to the sclera was deemed good in 10 cases, fair in 3 cases, and poor in 2 cases. The glue was adequately distributed in the applicator trough in 9 cases. In 2 cases, the glue was excessive and partially obstructed the well that contained collagenase, thereby possibly impeding the contact of the enzyme with sclera. In 4 cases, the glue

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(Reprinted) Arch Ophthalmol. 2002;120:548-554. doi:10.1001/archopht.120.5.548

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ensure satisfactory fixation. The conjunctiva was repositioned to completely cover the applicator (Figure 3), and it was held in position with an 8-0 polyglactin suture or a drop of cyanoacrylate tissue glue. Ciprofloxacin, 0.3% ointment, was applied and the eye was patched. The treated eye was evaluated 18 to 20 hours after application (while the enzyme applicator was still in place) by slitlamp biomicroscopy and Goldmann applanation tonometry. Special note was made of the position of the conjunctival flap, conjunctival reaction, position of the enzyme applicator, presence of aqueous leak, anterior chamber depth, anterior chamber inflammation, and position of the lens. The conjunctival reaction was graded on a scale of 1 to 5 as follows: grade 1, localized chemosis over the applicator; grade 2, as in grade 1 combined with conjunctival hemorrhage over or around the applicator; grade 3, diffuse hemorrhage surrounding the applicator and/or thinning of the conjunctiva over the applicator; grade 4, thinning of the conjunctiva around the applicator and/or localized melt over the applicator; and grade 5, conjunctival maceration over and around the applicator. Enzyme applicators were removed with the patient under topical anesthesia (4% lidocaine drops) a mean ± SD of 22±2 hours after application. The applicator was removed along with the glue in 1 piece. It was examined under an operating microscope to evaluate the adequacy of the glue in the trough and to check if there was accidental spread of the glue to the well containing the enzyme. The area of scleral application was wiped with a cellulose sponge and then thoroughly irrigated with Ringer lactate solution to remove enzyme residue. The position and depth of scleral digestion and the presence of aqueous micropercolation were carefully noted. The depth of scleral digestion was graded as follows: none, no perceptible effect on sclera; fair, concave crater with brownish or bluish hue at the base and minimal aqueous percolation; good, deep concave crater with brownish or bluish hue at the base with continuous and diffuse aqueous percolation; excessive, marked scleral thinning with uveal tissue shining through but with no frank uveal prolapse; and perforation, full-thickness scleral melt and uveal prolapse. The conjunctiva was repositioned to fully cover the treated area, and it was fixed in place with an 8-0 polyglactin suture or with a drop of cyanoacrylate tissue glue. The eye was not patched after removal of the enzyme applicator.

Each surgical procedure of enzyme application was analyzed for the position, fixation, and conjunctival coverage of the applicator. Factors evaluated at the time of removal of the applicator included conjunctival reaction, position and fixation of the applicator, adequacy and distribution of glue, shape and depth of scleral digestion, and the presence of aqueous percolation from the treated site (Figure 4).

All patients received topical 0.3% gentamicin sulfate eyedrops 4 times a day for 1 week and topical 0.1% betamethasone phosphate eyedrops 4 to 6 times a day, tapered over a period of 4 weeks. Patients were examined on postoperative days 1, 2, and 3; at weeks 1, 2, and 4; and every 3 months thereafter. Specific inquiries were made regarding patient comfort during the procedure and at each follow-up visit. Change in intraocular pressure compared with the baseline and the occurrence of complications were the main outcome measures. The statistical significance of the change in intraocular pressure was determined with the 2-tailed paired t test. All data are presented as mean ± SD unless otherwise specified.

did not fully fill the trough, possibly inadequately limiting the area of enzymatic effect. The surgical procedure lasted less than 5 minutes in each of the 10 eyes where the first attempt at application was successful.

An examination 18 to 20 hours after enzyme application (before applicator removal) disclosed in all the eyes a uniformly deep and quiet anterior chamber, round and regular pupil, and undamaged lens. The intraocular pressure was 28.5±12 mm Hg (range, 10-54 mm Hg), representing a decrease of 34.5% from the baseline. All the applicators were in the original position and were well covered by the conjunctiva. The conjunctival reaction was grade 1 in 3 cases, grade 2 in 3 cases, grade 3 in 2 cases, grade 4 in 5 cases, and grade 5 in 2 cases. This seemed to correlate with the degree of applicator fixation.

Applicators were removed 22±2 hours after placement. The patients reported no pain or discomfort. Applicators were removed in less than 3 minutes in all cases. The applicator and the glue that bound the applicator to the sclera could be dislodged in one piece by using the application forceps with gentle force. No difficulties were encountered in the removal of the applicator or repositioning of the conjunctiva to the limbus.

Morphologically, the scleral digestion appeared in the shape of a cup with sharp borders, deepest at the center and sloping steeply at the periphery. The depth of enzymatic scleral digestion was good in 7 eyes, fair in 3 eyes, and poor in 3 eyes. In one case (patient 14), enzymatic digestion of the sclera was excessively deep, progressing to the Descemet membrane. In this case, the amount of collagenase used was 125 µg, the applicator was positioned anterior to the limbus, application was successful in the second attempt, and fixation was good with an adequate amount of glue. In another case (patient 15), a full-thickness sclerocorneal perforation (measuring 1 mm in diameter) with uveal prolapse resulted. The amount of collagenase used in this case was 116 µg, application was successful in the first attempt, fixation was good, and amount of glue was adequate, but the position of the applicator was anterior to the limbus. Slitlamp examination 3 hours before the applicator was removed showed grade 1 conjunctival reaction, deep and quiet anterior chamber, round and central pupil, and an intraocular pressure of 21 mm Hg. The applicator was removed 24 hours after enzyme application, showing a full-thickness focal perforation. The perforation was managed with a small scleral patch graft and a peripheral iridectomy, and the patient was excluded from further data analysis. The patient experienced pain in the operated-on eye, which subsided in 2 weeks. Follow-up was continued, with careful attention given to signs of continuous collagenase digestion of the sclera or other intraocular collagenous tissues. One year after the initial surgery, the scleral patch graft remained well positioned and no signs of collagen tissue digestion
were observed. Intraocular pressure ranged from 17 to 20 mm Hg with treatment with 0.5% timolol maleate twice a day for the duration of follow-up.

In all cases, the conjunctival reaction completely resolved in 1 to 2 weeks and the conjunctiva over the treated site resembled a shallow filtering bleb in the early postoperative period. Nevertheless, the actual presence of filtration blebs could not be ascertained because of the presence of conjunctival chemosis and hemorrhage. A shallow filtering bleb was seen in 5 eyes at 1 week after the sclerostomy (Figure 5), persisting in 4 eyes for 2 weeks and in 3 eyes for 1 month. No bleb was recognized in any of the eyes 3 months after treatment.

Intraocular pressure dynamics after enzymatic sclerostomy are presented in Figure 6. The baseline intraocular pressure was 43.5±9.8 mm Hg (range, 24-56 mm Hg). The mean intraocular pressure on the day after removal of the enzyme applicator was 24.8±10.6 mm Hg (range, 11-42 mm Hg), representing a 43.0% decrease from the baseline. The mean intraocular pressure was 31.0±11.4 mm Hg (range, 15-45 mm Hg), a 28.7% decrease from the baseline; at 1 week, 32.4±9.3 mm Hg (range, 21-42 mm Hg), a 25.5% decrease from baseline, at 1 month; and 34.8±10.5 mm Hg (range, 21-50 mm Hg), a 20.0% decrease from baseline, at 1 year. The reduction in intraocular pressure due to enzymatic sclerostomy was statistically significant (P<.001 at 1 day, at 1 week, at 1 month, and at 1 year). All patients were comfortable with the treatment, required no antiglaucoma medication, and remained so until 9 months after enzymatic sclerostomy. Between the 9th and 12th months of follow-up, 2 patients (patients 4 and 12) reported symptoms related to elevated intraocular pressure. Symptoms were controlled by topical 0.5% timolol maleate twice daily in patient 12, and by semiconductor diode laser transscleral cyclophotocoagulation in patient 4. These 2 patients were excluded from further data analysis. In all, 12 (86%) of 14 patients had symptomatic relief after enzymatic sclerostomy. Ten (91%) of 11 patients (excluding 1 patient who underwent transscleral cyclophotocoagulation) who were taking antiglaucoma medication for symptomatic relief before enzymatic sclerostomy did not need medication after the procedure.

**COMMENT**

By causing an overall decrease of 43.0% in the intraocular pressure immediately after treatment and a sustained...
lowering effect of 20.0% at 1 year, as well as relieving symptoms in 86% of patients without antiglaucoma medication, enzymatic sclerostomy, although still in its technical infancy, has demonstrated its potential as a treatment for open-angle glaucoma.

Encouraged by the results of enzymatic sclerostomy in laboratory animals and on the basis of preliminary experience in humans in several institutions, we decided to evaluate 15 blind, previously unoperated-on eyes with primary open-angle glaucoma and elevated intraocular pressure treated enzymatically according to a uniform protocol and followed up for a year. The LV Prasad Eye Institute in India was chosen as the study site because of the availability of a patient population corresponding to the enrollment criteria, its reputation for providing high-standard medical care, and the availability of infrastructure for conducting standard clinical trials.

None of the patients reported pain or discomfort during and after the procedure (hence, no attempt was made to quantify patient discomfort), and patients were equally comfortable under topical anesthesia or with peribulbar block, suggesting that this treatment could be performed as an office procedure.

Conjunctival reaction varied from none to local maceration and seemed proportionate to the adequacy of the applicator’s scleral fixation; this may explain the absence of detectable blebs beyond 3 months after application in cases where the reaction was excessive.

The extent of the scleral digestion varied from none to full-thickness perforation. Reasons for this variability could be patient related (difference in the scleral structure), applicator related (variation in collagenase content), or procedure related (adequacy of application). However, the enzyme concentration, the contact area, and the period of application seemed adequate, since most eyes displayed observable scleral digestion and had a meaningful intraocular pressure–lowering effect.

Full-thickness scleral perforation, a potentially sight-threatening complication that demonstrates the importance of proper positioning of the applicator, occurred in 1 patient. In this patient, the enzyme applicator was misplaced on the peripheral cornea, anterior to the anatomical limbus. There are differences in the collagen architecture and the relative composition of glycosaminoglycan between the cornea and the sclera; these differences may explain the increased susceptibility of the cornea to collagenase, resulting in its perforation. It is possible that collagenase enzyme gained access to the anterior chamber in this eye after perforation. However, the patient continued follow-up and there was no evidence of continued collagenolytic action on the sclera or the cornea. Data from this patient and from the 2 patients who developed symptoms related to increase in intraocular pressure at 9 months and needed additional treatment for the relief of symptoms were excluded from further analysis.

By the conventional definition of intraocular pressure control after glaucoma filtering surgery (intraocular pressure, ≤21 mm Hg), only 2 patients (13%) demonstrated success in our study. However, most patients (80% [12/15]) were taking antiglaucoma medication when enrolled for the study, and their baseline intraocular pressure was measured without an adequate washout period. Therefore, the achievable intraocular pressure–lowering effect of enzymatic sclerostomy may actually be higher. Moreover, the enzymatic treatment was performed entirely on an Asian-Indian population, and the bleb survival as well as the intraocular pressure–lowering effect may be different in other patient populations.15 Twelve (86%) of 14 patients had symptomatic relief after enzymatic sclerostomy, demonstrating the efficacy of the procedure in symptomatic eyes and its potential as an alternative to cyclodestructive procedures. Ten (91%) of 11 patients who were taking antiglaucoma medication for symptomatic relief before enzymatic sclerostomy did not need medication after the procedure.

Sustained lowering of the intraocular pressure was achieved despite the absence of a detectable filtering bleb in most eyes beyond 1 month and in all eyes beyond 3 months. The possibility of alteration in the trabecular cell biological characteristics or augmentation of episcleral drainage in response to collagenase application as a mechanism of intraocular pressure lowering cannot be ruled out except by further studies. It is possible that enzymatically induced alterations in the walls of Schlemm ca-
This study was presented in part as a poster at the annual meeting of the Association for Research in Vision and Ophthalmology, Ft Lauderdale, Fla, March 15, 1998, and in part as a poster at the annual meeting of the American Academy of Ophthalmology, Orlando, Fla, October 26, 1999.

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