Methylcellulose, a Healing Inhibitor Factor in an Animal Model of Trabeculectomy

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PURPOSE. To use an experimental animal model to study the effect on intraocular pressure (IOP) of methylcellulose 2% application in trabeculectomy, followed by histologic assessment.

METHODS. Trabeculectomy was performed on albino rabbits’ eyes. The study comprised two groups, each consisting of five rabbits. The right eyes were subjected to trabeculectomy, and the left eyes served as the control. In both groups 1 and 2, trabeculectomy was performed in the right eyes, but in group 2, trabeculectomy was followed by injection of methylcellulose 2% into the anterior chamber. The methylcellulose was allowed to pass through the trabeculectomy site into the subconjunctival space, and an extra amount was injected by the same cannula under the conjunctiva in the area of the trabeculectomy from the edge of the peritomy. Measurement of IOP was performed with a Slitontometer once weekly for 4 weeks. Specimens were obtained from the operative site, and semithin sections were prepared, stained with toluidine blue, and examined by light microscopy (LM).

RESULTS. A significant difference in the decrease of IOP (P < 0.0001) was observed between the two groups after 4 weeks of surgery. During the 4 weeks after trabeculectomy, the mean IOP was 18.2 ± 0.45 mm Hg without methylcellulose injection and 18.5 ± 0.77 mm Hg in the control eyes, and 9.8 ± 0.84 mm Hg with methylcellulose injection, 18.25 ± 0.7 mm Hg in the control eyes. The histopathological findings in group 1 (without methylcellulose injection) showed the subocular spaces to be less fenestrated, with deposition of dense collagen bundles intermingled with the fibrocytes, some of which were active with prominent mitosis. In contrast, in group 2 (injected with methylcellulose), the subocular space appeared fenestrated with irregularly and widely spaced segmented collagen bundles.

CONCLUSIONS. Methylcellulose may have antihealing properties that serve to decrease IOP in trabeculectomy. Although more work is needed in humans, because human tissue may be different in its response to the same procedure, the use of methylcellulose could be very promising. (Invest Ophthalmol Vis Sci. 2006;47:2515–2519) DOI:10.1167/iovs.05-1147

Trabeculectomy remains the standard procedure for adult glaucoma surgery, but it is associated with common complications including hypotony, acceleration of cataract formation, and, most notably, filtration failure. Jampel et al.1 reported the rate of complications in primary trabeculectomies, including anterior chamber bleeding during surgery (8%) and conjunctival buttonhole (1%). Complications with a frequency of 10% or more included a shallow or flat anterior chamber (13%), encapsulated bleb (12%), ptosis (12%), serous choroidal detachment (11%), and anterior chamber blebbing (hyphema; 10%). There were suprachoroidal hemorrhages (0.7%) and no cases of endophthalmitis. Older patients were more likely to experience serous choroidal detachment, new anterior or posterior synechiae, and wound leak.1 For treatment of filtration failure, 5-fluorouracil (5-FU) and mitomycin C (MMC) were initially reported as adjuncts to improve surgical results by decreasing the healing rate of the filtering site in the 1980s.2,3 Yet, undesirable side effects such as overfiltration problems, scleral ischemia, and thinning of the overlying conjunctiva must be considered when using these adjuncts.4 The purpose of the present study was to find an alternative adjunct for trabeculectomy with the fewest and least-severe side effects, where in cases of flat anterior chamber re-filling after trabeculectomy with methylcellulose 2% to treat excessive filtration. Instead of the increasing probability of bleb failure from the adherence of the conjunctiva-Tenon’s capsule to the sclera at the site of filtration and the elevation of IOP, the bleb was uniformly diffuse, with satisfactory IOP readings after relief of the flat anterior chamber with methylcellulose. This article describes our study of this effect in a clinical animal model and its histopathological assessment.

MATERIALS AND METHODS

The present study consisted of two groups of albino rabbits weighing 2.5 to 3.0 kg. Each group consisted of five rabbits. Animals were kept in separate cages at 21°C in a normal 12-hour light-dark cycle. For the experiments (surgery and enucleation), all animals were anesthetized by intramuscular injection of 1 to 4 mL/kg of a solution of 5 mL ketamine (100 mg/mL) and 5 mL/kg xylazine (20 mg/mL). The right eyes were subjected to trabeculectomy, and the left eyes served as the control. Statistical analysis of the IOP differences between the groups of rabbits was performed with Student’s t-test.

SURGICAL PROCEDURE

The same surgeon (AS) performed all the trabeculectomies. We started with surgery in group 1, followed by group 2. The surgeon was not masked to the treatment groups, but the pathologist was masked. A fornix-based conjunctival peritomy was performed in the upper part of the eye, and the sclera was exposed. A limbus-based half-scleral-thickness flap 4 mm2 was dissected with a 45° super-sharp blade, until clear cornea was reached. A stab paracentesis was performed with the tip of the 15° super-sharp blade in the temporal part of the cornea, followed by a 2 × 2 trabeculectomy, where excision of a 2 mm2 sclera and cornea was performed over the limbus under the scleral flap. The anterior chamber with a 15° super-sharp blade. A peripheral iridectomy was performed. In group 1, the anterior chamber was re-formed with physiologic saline solution and in group 2, with 1 mL methylcellulose 2% injected via the trabeculectomy (the opening of the anterior chamber with the excised part of the sclera and cornea under the scleral flap). The flaps were closed with two 10-0 nylon sutures, which marked the site of surgery. The conjunctiva was

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Submitted for publication August 28, 2005; revised September 26, November 15, and December 31, 2005; accepted March 31, 2006.

Disclosure: A.A.M. Shouman, None; A. Helal, None; M.A. Marzouk, None; E.M.A. Zaki, None

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closed in all cases with 8-0 virgin silk sutures. In group 2, another 1 mL of methylcellulose was injected into the subconjunctival space and through the paracentesis, to deepen the anterior chamber and allow it to pass through the trabeculectomy and into the subconjunctival space.

IOP Measurement

Before surgery, and weekly for 4 weeks afterward, IOP was performed with a Schiotz tonometer, with rabbits under local anesthesia (benoxinate hydrochloride). The weight used on the tonometer was 5.50 g.

Histologic Specimen Preparation

The experiment lasted for 4 weeks, after which the animals were killed with an overdose of phenobarbitone and the eyes immediately enucleated. The labeled areas of the trabeculectomies were dissected (the area of the scleral flap with the surrounding 5 mm), marked with the 10-0 nylon stitches of the flap. The specimens were immediately double fixed in 4% glutaraldehyde and then in 1.3% osmium tetroxide in phosphate buffer (pH 7.3), processed, and embedded in araldite. Semithin sections were stained with toluidine blue and examined by light microscopy (LM). To assess the modulatory effect of the intraoperative use of methylcellulose 2% on the healing process after trabeculectomy, LM examination of the subscleral space was performed to determine the nature of the cellular infiltrate and the density of the collagen bundles. Experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the cantonal veterinary service in Egypt.

RESULTS

During the procedures and during the follow-up period, no mortalities occurred among the rabbits. There was no intraoperative complication, apart from the difficult scleral dissection. In one rabbit in the methylcellulose-injection group, because of the sclera’s thinness, there was mild bleeding on peripheral iridectomy that was tamponaded by the methylcellulose injection. IOP was measured in both eyes under topical anesthesia, a maneuver that was easy and well tolerated by animals throughout the study. No eyes exhibited postoperative inflammation in the anterior chamber (postoperative inflammation was assessed roughly by the level of eye congestion, redness, and the absence of hypopyon). All corneas stayed clear, and no endophthalmitis was observed.

A significant difference in the amount of IOP decrease \( (P < 0.0001) \) was observed between the two groups at 4 weeks after the surgery (mean IOP was 18.2 ± 0.45 mm Hg without methylcellulose injection, 18.3 ± 0.77 mm Hg in control eyes and 9.8 ± 0.84 mm Hg with methylcellulose injection, 18.25 ± 0.7 mm Hg in control eyes). The overall results of the two groups are shown in Tables 1 and 2.

The decrease in IOP in the eyes with methylcellulose injection was maintained throughout the 4 weeks of follow-up, unlike the other group without methylcellulose injection in which the decrease in IOP was not maintained.

Histopathological Assessment

Light microscopic examination of subscleral tissue from the left control eyes of both groups showed unremarkable pathologic changes, suggesting the stability of the surrounding circumstances and the efficacy of the postoperative medications throughout the period of the experiment. LM examination of the excised tissue from right eyes of group 1 (without methylcellulose injection) showed the subscleral space to be less...
fenestrated, with deposition of dense collagen bundles intermingled with fibrocytes, some of which were active with prominent mitosis. Fibrocytes appeared focally irregular and wavy. The cellular infiltrate was minimal and mostly formed of lymphocytes found in the narrow spaces in between the fibrocytes (Figs. 1, 2). In contrast, LM examination of the excised specimens from right eyes of group 2 (injected with methylcellulose) showed the subscleral space to be fenestrated, with irregular wide spaces. The spaces were surrounded by a network of fine fibrous connective tissue with irregularly and widely spaced segmented collagen bundles. In addition, considerable cellular populations were present, mainly macrophages and lymphocytes. The cellular infiltrate was scattered in the fenestrated spaces in between the fibrous connective tissue and the segmented collagen bundles (Figs. 3, 4).

**DISCUSSION**

The use of 5-FU and MMC as a healing inhibitor in glaucoma surgery became routine in the 1990s. Both of these agents were introduced as adjuncts to trabeculectomy in the early 1980s.2,3 The benefit of postoperative subconjunctival 5-FU injections in eyes at high risk of filtration failure undergoing trabeculectomies was demonstrated in the Fluorouracil Filtering Surgery Study.5,6 Its use is not widespread because of the cumbersome nature of postoperative 5-FU injections and the associated corneal side effects.7,8 The randomized clinical trial comparing intraoperative use of topical MMC and 5-FU showed minimal differences in final IOP, visual acuity, and short- and mid-term complications between the two groups.9 Use of the antifibrotic agents 5-FU and MMC as intraoperative adjuncts to

**Figure 1.** Light micrograph of a semithin section of a rabbit's eye after trabeculectomy without methylcellulose, showing sclera (SC) and compact irregular subscleral tissue (f). Toluidine blue stain; magnification, ×500.

**Figure 2.** Light micrograph of semithin section of rabbit eye after trabeculectomy without methylcellulose showing sclera (SC) and wavy compact subscleral tissue (f), some showing active nuclei (arrowhead). Toluidine blue stain; magnification, ×500.
trabeculectomy, which was initially popular in eyes at high risk for trabeculectomy failure, has now become routine in low-risk eyes undergoing primary trabeculectomy. Although an American Glaucoma Society survey showed that approximately three of four glaucoma specialists use one of these two agents during primary trabeculectomy surgery, there remains no consensus on which agent should be used in this setting.10

An increase in external resistance at the conjunctiva–Tenon’s capsule–episcleral interface is the major cause of failure of filtration surgery.7,11 Scarring of a glaucoma filtering bleb is caused mainly by proliferation of subconjunctival fibroblasts and by their biosynthesis of collagen and other extracellular material.12 Inhibition of wound healing is a crucial factor in successful glaucoma filtration surgery.7 The present study demonstrated the efficacy of methylcellulose as an adjunct in decreasing the IOP in conjunction with trabeculectomy during 4 weeks of follow-up, highlighting its possible role as an inhibitory factor in the healing process in the subconjunctival and episcleral region. This technique is novel: No references were found on PubMed regarding the antihealing properties of methylcellulose of any concentration. No side effects were observed during surgery or the 4 weeks of follow-up.

From a histopathological point of view, the present study highlighted the efficacy of injection of methylcellulose in conjunction with trabeculectomy. We suggest two mechanisms of action during our analysis of the results: Either, methylcellulose 2% acts as a space-occupying agent keeping tissues apart (hydraulic effect) and thus decreases the probability of their touching and adhering by fibrosis, or, it has a healing-inhibiting mechanism. We are in favor of the antihealing theory, as the amount of methylcellulose injected would have diffused subconjunctivally and would have been absorbed rapidly in much less than the 4 weeks of follow-up, if the action were hydraulic, with consequent touching of the conjunctiva-Tenon’s layer to the sclera and ensuing fibrosis with almost the same outcome as the group that were not injected with methylcellulose. This conclusion is supported by the widely fenestrated subscleral space that ensured drainage.

The absence of inflammatory cellular infiltrate during the entire study indicates the tolerability of methylcellulose by the rabbit tissues. However, the presence of a population of macrophages was explained by the necessity of its presence in engulfing the injected material with the possibility of a role in cellular secretion of collagenase enzymes to dissolve the deposited collagen bundles. The presence of dense collagen bundles in the subscleral space of the rabbits in group 1 (without methylcellulose), together with the presence of scattered active fibrocytes, indicates the probable efficacy of methylcellulose 2% injection with trabeculectomy to ensure significant aqueous drainage. As evidenced by the IOP measurements and the histopathological examination, methylcellulose may have an effective role in decreasing the IOP in trabeculectomy.

**Figure 3.** Light micrograph of semithin sections of a rabbit eyes after trabeculectomy with methylcellulose, showing sclera (SC) with sub-scleral tissue having multiple irregular spaces (S) surrounded by fibrous connective tissue (f) with cellular infiltrate formed mainly of macrophages (arrowhead) and lymphocytes (arrow). Toluidine blue stain; magnification, ×500.

**Figure 4.** A different rabbit eye showing the same features as described in Figure 3.
CONCLUSION

Methylcellulose may cause a decreasing rate of healing that serves to maintain decreased IOP after trabeculectomy. Although more work is needed in humans because human tissue may be different in its response to the same procedure, the use of methylcellulose could be very promising.

References