Efficacy of Hyaluronidase in Reducing Increases in Intraocular Pressure Related to the Use of Viscoelastic Substances

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Objective: To evaluate the efficacy of hyaluronidase in preventing increases in intraocular pressure related to injections of hyaluronan-containing viscoelastic substances.

Methods: Twenty-five white rabbits were divided into 5 groups. In groups 1 through 4, 0.15 mL of aqueous humor was removed and replaced with 0.10 mL of a viscoelastic substance in both eyes. Additionally, 10 units of hyaluronidase (0.05 mL) was injected in the anterior chamber of the right eye, whereas the left eye was injected with a volumetrically equivalent dose of balanced saline solution. Viscoelastic substances tested were Healon and Healon GV (Pharmacia & Upjohn, Kalamazoo, Mich), Viscoat (Alcon Laboratories, Fort Worth, Tex), and Ocucoat (Storz Ophthalmics, Clearwater, Fla). In group 5, right eyes were injected with 10 units of hyaluronidase and the left eyes were treated with balanced saline solution.

Results: After injections of viscoelastic substance, intraocular pressure rose rapidly, reaching a peak at approximately 46 hours after injection and returning to pre-injection levels within 24 hours. Hyaluronidase significantly decreased intraocular pressure when used with Healon, Healon GV, and Viscoat, but not with Ocucoat. When injected in the absence of viscoelastic, hyaluronidase appeared to decrease intraocular pressure, but this result was not statistically significant.

Conclusions: Injections of hyaluronidase into the anterior chamber of rabbits effectively prevent increases in intraocular pressure induced by hyaluronan-containing viscoelastic substances. This effect may be related to the ability of hyaluronidase to cleave hyaluronan moieties.

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SODIUM HYALURONATE (NaHa), also known as hyaluronan, is a naturally occurring macromolecule found in many tissues, including the synovial fluid and vitreous body. Aqueous solutions of NaHa have viscoelastic properties. In addition to maintaining a deep anterior chamber during surgery and preventing sudden fluctuations in intraocular pressure, injections of NaHa into the anterior chamber have been shown to protect corneal endothelial cells.1,2 Injections of NaHa do not interfere with intraoperative visibility. For these reasons, NaHa has been used extensively in anterior segment surgery.

Because of the significant contribution of NaHa to eye surgery, several compounds have been developed that serve equivalent purposes. Some compounds are merely preparations of NaHa with different concentrations and molecular weights, whereas others combine NaHa with chondroitin sulfate. Others still use hydroxypropyl methylcellulose as a clear viscous substance.

Despite their advantages, the use of viscoelastic substances has been correlated with significant increases in intraocular pressure postoperatively.1-6 It is thought that retained NaHa blocks the outflow facility of the anterior chamber and prevents the egress of aqueous humor, thus leading to increases in intraocular pressure.7 Though transient, these increases can lead to significant ocular damage. Although strategies have been devised to minimize intraocular pressure elevations, including thorough washout of the anterior chamber at the end of surgery, routine use of antiglaucoma medicines postoperatively, and postoperative paracentesis, none is ideal.

There is evidence that decreases in intraocular pressure can be achieved enzymatically by means of hyaluronidase (Wydase; Wyeth Laboratories, Philadelphia, Pa).6 Hyaluronidase is currently used for subcutaneous and retrobulbar injections of local anesthetics to promote the diffusion of anesthetics. Hyaluronidase is a highly specific, naturally occurring enzyme that cleaves NaHa into disaccha-
MATERIALS AND METHODS

The viscoelastic substances and hyaluronidase used in this study are listed in the Table. Hyaluronidase for intraocular injection was prepared by adding balanced saline solution to 150 U of lyophilized bovine testicular hyaluronidase (Wydase) to obtain a concentration of 10 U of hyaluronidase in 0.05 mL of fluid.

Twenty-five white New Zealand rabbits (weight, 2.0-3.4 kg) were included in this study. Throughout the study, the procedures in the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research were strictly adhered to. At the onset of the investigation, each eye of all rabbits was examined by slit-lamp biomicroscopy to exclude any preexisting abnormality.

Rabbits were anesthetized with intramuscular injection of a combination of ketamine hydrochloride (50 mg/kg) and 0.5 mL of chlorpromazine hydrochloride (100 mg/mL). Proparacaine hydrochloride (100 mg/mL). Proparacaine hydrochloride 0.5% eye drops were instilled into the conjunctival cul-de-sac 1 minute before injection. Preoperative intraocular pressures were measured by tonometry\textsuperscript{14,15} (Tono-pen XL; Mentor, Norwell, Mass) in each eye. The lids were held open with a wire speculum, and after the eyes were immobilized, a syringe fitted with a 30-gauge needle was inserted into the limbus into the anterior chamber and 0.15 mL of aqueous fluid was removed. Next, with a separate 30-gauge needle, 0.10 mL of viscoelastic substance was injected into the anterior chamber of the right eye. With the same needle, 0.05 mL of hyaluronidase (10 U) was injected in the anterior chamber of the same eye. The anterior chamber of the left eye, serving as control, was injected with an equivalent volumetric dose of the same viscoelastic substance (0.10 mL of viscoelastic and 0.05 mL of balanced saline solution). Rabbits were divided into 5 groups of 5 each. In group 5, the right eye was injected with 10 U of hyaluronidase (0.15 mL), and the left eye, serving as control, was injected with 0.15 mL of balanced saline solution.

Immediately after injections, rabbits were examined by biomicroscopy to evaluate possible ocular trauma caused by the injections. Intraocular pressures were measured immediately after injection. Tonometry was repeated at 1, 2, 4, 6, 8, 12, 24, and 48 hours after injection. Ocular examination was performed by means of slit-lamp biomicroscopy and indirect ophthalmoscopy to evaluate the anterior chamber, lens, vitreous, and retina. The data were analyzed statistically by 1- and 2-tailed 2-sample unequal variance Student \( t \) test. At the completion of the study period, all rabbits were killed with a lethal dose of pentobarbital sodium (100 mg/kg given in the marginal ear vein).

Ride components, thus reducing both viscosity and molecular weight of NaHa.\textsuperscript{9} One study using baboon eyes showed an increase in outflow facility after instillation of hyaluronidase.\textsuperscript{10} Anterior- and posterior-chamber in-

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RESULTS

Figure 1 summarizes the average changes of intraocular pressure in control (left) eyes treated with various viscoelastic substances (groups 1-4). Intraocular pressure increased within 1 hour after anterior chamber injection of viscoelastic and reached a peak at approximately 46 hours after injection. Intraocular pressure gradually decreased to approximate preinjection levels at about 24 hours after injection. An increase in intraocular pressure occurred with all of the viscoelastic substances evaluated. Healon GV appeared to have the most profound increase in intraocular pressure (\( P < .05 \) at 2, 4, and 6 hours after injection) compared with other viscoelastics.

Figure 2 represents the intraocular pressures obtained after 10 U of hyaluronidase was injected in the anterior chamber of the right eye (group 5). Compared with control (left eye), intraocular pressures appeared to decrease modestly. These results were statistically significant only at 4 (\( P = .02 \)) and 48 (\( P = .01 \)) hours after injection.

Figure 3 summarizes the results of intraocular pressure changes when Healon, Healon GV, Viscoat, and Ocucoat were used in the presence (hyaluronidase-treated right eye) and absence (control left eye) of hyaluronidase. Differences in intraocular pressure between the treated and control eyes were assessed by the unequal paired Student \( t \) test. In all except the Ocucoat group (Figure 3, D), intraocular pressure was significantly lower in the hyaluronidase-treated right eye. In the Ocucoat group, intraocular pressure was lower in the hyaluronidase-treated eyes, but this result was not statistically significant.
Hyaluronidase Products Used in the Study Group*

<table>
<thead>
<tr>
<th>Group</th>
<th>Product</th>
<th>Brand Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium hyaluronate 1%</td>
<td>Healon</td>
<td>Pharmacia &amp; Upjohn, Kalamazoo, Mich</td>
</tr>
<tr>
<td>2</td>
<td>Sodium hyaluronate 1.4%</td>
<td>Healon GV</td>
<td>Pharmacia &amp; Upjohn, Kalamazoo, Mich</td>
</tr>
<tr>
<td>3</td>
<td>Sodium hyaluronate 3% and chondroitin sulfate 4%</td>
<td>Viscoat</td>
<td>Alcon Laboratories, Fort Worth, Tex</td>
</tr>
<tr>
<td>4</td>
<td>Hydroxypropyl methylcellulose</td>
<td>Ocucoat</td>
<td>Storz Ophthalmics, Clearwater, Fla</td>
</tr>
<tr>
<td>5</td>
<td>Hyaluronidase</td>
<td>Wydase</td>
<td>Wyeth Laboratories, Philadelphia, Pa</td>
</tr>
</tbody>
</table>

*Data from Hutz et al.12 In groups 1 through 4, 0.10 mL of viscoelastic substance was injected into the right eye with 0.5 mL of hyaluronidase, whereas in the left eye, 0.10 mL of viscoelastic substance was injected with 0.5 mL of balanced saline solution. In group 5, 0.15 mL of hyaluronidase was injected into the right eye, whereas 0.15 mL of balanced saline solution was injected into the left eye.

Figure 1. Average intraocular pressure (IOP) after injection of viscoelastic substances into the anterior chamber. Error bars represent SDs.

Figure 2. Average intraocular pressure (IOP; mean ± SD) after injection of 10 U of hyaluronidase in the anterior chamber of right eyes, compared with injection of balanced saline solution in left eyes as control.

COMMENT

Increases in intraocular pressure related to viscoelastic substances used as surgical aids have been extensively studied.1-6 Shortly after injection of a viscoelastic substance into the anterior chamber, intraocular pressure increases rapidly. In rabbits, approximately 6 to 12 hours after injection, intraocular pressure approaches a zenith and begins to decline. Within 12 to 24 hours after injection, intraocular pressure stabilizes to preinjection levels. Our study is in general agreement with previously published results, indicating that intraocular pressure reaches maximum values approximately 6 to 8 hours after injection and returns to preinjection pressures within 24 hours after injection.6 This time-dependent increase in pressure appears to be generally independent of the type of viscoelastic used.

The mechanism of viscoelastic-related intraocular pressure rise is not clearly understood. It has been shown that outflow facility is significantly decreased with anterior chamber injections of NaHa, correlating with increases in intraocular pressure.12 Increased resistance to flow of aqueous humor is thought to occur in the endothelial meshwork, a region with the highest concentrations of NaHa.3 It is thought that viscoelastic substances bind in this region, where the resistance to aqueous flow is highest, and “clog” the trabecular canals. Thus, aqueous humor meets increased resistance across the endothelium of trabecular meshwork, causing an increase in intraocular pressure.9 If this hypothesis is correct, then hyaluronidase, which cleaves NaHa into its disaccharide components (thus reducing both the molecular weight and viscosity), should prevent such a block in outflow facility. Moreover, hyaluronidase should have no effect on blockage of outflow facility caused by viscoelastic substances unrelated to NaHa, such as hydroxypropyl methylcellulose. We have demonstrated that some viscoelastic-related increases in intraocular pressure can be prevented with concomitant use of hyaluronidase. This occurs with all NaHa-containing viscoelastics. We have also shown that hyaluronidase appears to be ineffective in reducing intraocular pressures when hydroxypropyl methylcellulose is used. These results agree well with previous studies, demonstrating the ability of hyaluronidase to prevent the blocking of outflow facility by NaHa.7,8 These results support the notion that pressure-reducing effects of hyaluronidase are related to its ability to cleave NaHa moieties.

The trabecular meshwork’s extracellular matrix contains glycosaminoglycans including NaHa, which are believed to line the trabecular canals and contribute to overall resistance to flow.12 Studies have demonstrated that perfusion of the trabecular meshwork with hyaluronidase results in decreases in intraocular pressure.9 Our study demonstrates that anterior chamber injections of hyaluronidase alone have only a modest effect in decreasing intraocular pressure. Therefore, it appears that the effects of hyaluronidase in preventing viscoelastic-related pressure spikes are at least in part related to the degradation of injected NaHa rather than native trabecular NaHa molecules. However, that hyaluronidase has a pressure-lowering effect indepen-
dent of its ability to cleave the injected NaHa cannot be ruled out.

Despite earlier disappointment with intravitreal injections of hyaluronidase, there is considerable evidence that intraocular injections of testicular hyaluronidase appear to be safe. Calder and Smith used up to 750 U of hyaluronidase in human patients after cataract surgery and reported no adverse effects. In this study, there was no evidence of ocular toxic effects from use of 10 U of hyaluronidase. Nevertheless, more extensive studies may be necessary to establish the intraocular safety of hyaluronidase.

The ability of hyaluronidase to prevent increases in intraocular pressure provides a method of preventing viscoelastic-related postoperative pressure spikes. This enzyme can conceivably be instilled in the anterior chamber at some point after NaHa-containing viscoelastic has been used during anterior segment surgery. This enzyme can possibly also obviate the need to completely evacuate the viscoelastic after surgery. While further study is necessary to establish the safety and efficacy of hyaluronidase in anterior segment surgery, this enzyme appears to hold promise in decreasing viscoelastic-related complications.

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REFERENCES