Effect of Hyaluronic Acid on Intraocular Pressure in Rats

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PURPOSE. To study the effect of acute or chronic intracameral injection of hyaluronic acid on intraocular pressure (IOP) in rats.

METHODS. Acute or chronic injections of hyaluronic acid were performed unilaterally in the rat eye’s anterior chamber, whereas the contralateral eye was injected with saline solution. IOP was assessed daily or weekly by a tonometer in conscious rats. IOP was also assessed in both experimental groups at several intervals during the light–dark cycle.

RESULTS. A single injection of hyaluronic acid induced an increase of IOP that lasted for 8 days ($P < 0.01$), whereas its chronic administration during 9 weeks induced a significant and sustained increase in IOP, compared with the eye injected with vehicle ($P < 0.01$). This hyaluronic acid–induced hypertension was significantly decreased by the application of 1 drop of brimonidine (0.2%), latanoprost (0.005%), or timolol (0.5%). Significant daily variations of IOP were observed in both control and hyaluronic acid–injected eyes, peaking during the dark phase ($P < 0.001$, ANOVA).

CONCLUSIONS. These results suggest that the intracameral administration of hyaluronic acid could be a model of ocular hypertension in rats. (Invest Ophthalmol Vis Sci. 2002;43:2196–2200)

Although the pathogenesis of optic neuropathy in glaucoma is still an open question, increased intraocular pressure (IOP) is probably the most important risk factor in primary open-angle glaucoma. In fact, nearly all glaucoma therapy relies on lowering IOP. The trabecular meshwork in the chamber angle is thought to play a key role in the regulation of aqueous outflow and IOP. It is composed of sheets of trabecular beams that contain lamellae made of extracellular matrix materials, which comprise a significant portion of this tissue and probably of the outflow barrier. Among the materials of the trabecular extracellular matrix, the glycosaminoglycan (GAG) profile (i.e., hyaluronic acid [HA], keratan sulfate, heparan sulfate, and hybrid dermatan sulfate-chondroitin sulfate) has been identified in rabbit, monkey, and human eyes. Trabecular GAGs, particularly, have been implicated in the modulation of outflow resistance and in the development of glaucoma. In the trabecular meshwork obtained from patients with primary open-angle glaucoma, several electron microscopic, histologic, and immunologic studies have noted excessive accumulation of extracellular matrix materials. An abnormal accumulation of acid mucopolysaccharides in the anterior chamber angle was also reported in steroid-induced ocular hypertension. Intense histochemical staining observed in the various layers of human trabecular meshwork suggests that a substantial amount of HA is present in the outflow pathway, and a quantitative analysis indicated that it is the most abundant GAG of the human trabecular meshwork.

We have recently demonstrated that brimonidine, a highly selective $\alpha_2$-adrenoreceptor agonist that decreases IOP, significantly increases hyaluronidase activity in rabbit trabecular meshwork. Based on these results, we suggested that the effect of brimonidine in increasing outflow could be mediated, at least in part, by its stimulation of hyaluronidase activity—that is, by increasing clearance of GAGs. If a decrease of GAG content reduces IOP, it seems likely that an increase of intracameral HA levels would have the opposite effect. In fact, because HA-containing viscoelastic agents have become almost essential tools in human anterior segment surgery to reduce tissue trauma and endothelial cell loss, as well as to serve as a space maintainer, a considerable amount of evidence has been obtained of their effect on IOP. It is well known that these agents are responsible for causing or exacerbating a transient, but occasionally significant, postoperative IOP elevation. An increase in IOP after the injection of hyaluronan-containing viscoelastic substances has also been demonstrated in rabbits.

At present, no information is available about the effect of HA on IOP in rodents. The purpose of the present study was to examine the effect of intracameral injection of HA on IOP to develop a model of ocular hypertension in rats. The effect of intracameral HA on daily variations in IOP was also examined.

MATERIALS AND METHODS

Male Wistar rats (average weight, 200±40 g), were housed in a standard animal room with food and water ad libitum under controlled conditions of humidity and temperature (21 ± 2°C). The room was lighted by fluorescent lights that were turned on and off automatically every 12 hours (on from 6 AM to 6 PM). Rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (0.5 mg/kg) administered intraperitoneally. With a syringe (Hamilton, Reno, NV) and a 30-gauge needle, 25 $\mu$L of HA (10 mg/mL in saline solution; catalog no. H1751; Sigma Chemical Co., St. Louis, MO) was injected into one eye of anesthetized rats, and an equal volume of vehicle (saline solution) was injected in the fellow (control) eye. The eyes were focused under a surgical microscope (Omni MDU XY; Carl Zeiss, Oberkochen, Germany) with coaxial light. The needle moved through the corneoscleral limbus to the anterior chamber with the bevel down. When the tip of the bevel reached the anterior chamber, the liquid progressively increased the chamber’s depth, separating the needle from the iris and avoiding needle-lens contact. Applications were made slowly but using a force sufficient to just empty the syringe content (adjusted to 25 $\mu$L). In the chronic protocol, injections were applied at the corneoscleral limbus beginning at hour 12 and changing the site of the next injection hourly, by rotating the head to achieve better access to the limbus. The injections and IOP assessments were performed after applying 1 drop of 0.5% proparacaine hydrochloride to each eye.
The effect of a single intracameral injection of 1% (wt/vol) HA or saline solution on rats IOP is depicted in Figure 1. Twelve rats were injected with HA in one eye, and the contralateral eye was injected with vehicle. IOP was assessed in both eyes of these animals before the injection (day 0) and was assessed daily, beginning 24 hours after the injection. HA induced an almost twofold increase in IOP compared with the contralateral eye that lasted for 5 days (at the fifth day, control was 11.9 ± 0.9 mm Hg; HA, 21.3 ± 1 mm Hg, P < 0.01). Afterward, although a slight decrease was observed, the IOP remained significantly higher than that in the saline-injected eye until the eighth day after injection (control, 11.9 ± 0.7 mm Hg; HA, 15.5 ± 1; P < 0.01; Fig. 1). To study the IOP in response to the chronic administration of HA, 20 rats were injected once a week (up to 9 weeks) with HA (in one eye) or saline solution (in the contralateral eye). IOP was assessed at 7-day intervals in both eyes of each animal, before the new injection. The mean values of the IOP from these 20 animals assessed weekly are shown in Figure 2. The IOP of the eyes treated with HA reached a steady state level that lasted throughout the duration of the study (10 weeks) and was significantly higher than that of saline-injected eyes (e.g., at the fourth week, control, 11.7 ± 0.6 mm Hg; HA, 21.6 ± 1 mm Hg, P < 0.01). This steady state IOP was similar to that observed in response to a single injection of HA (20.8 ± 0.4 and 20.6 ± 0.5 mm Hg, with the acute and the chronic treatments, respectively). In all cases, saline solution injection did not significantly change IOP compared with that in intact eyes (intact eyes, 12.2 ± 0.4 mm Hg; saline injected eyes, 11.8 ± 0.6 mm Hg). Both in the acute and chronic studies a high degree of group

Statistical analysis of results was made by a Student’s t-test or a two-way analysis of variance (ANOVA), followed by a Dunnett or Tukey test, as stated. All animal use procedures were in strict accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RESULTS

Of 80 rats, 10 showing cataract and 4 with phthisis bulbi were excluded from the experiments. In addition, almost all the animals had localized corneal edema for less than 24 hours, at the site of the injection. No differences in the incidence of this ocular complication were detected between HA- and saline-injected eyes. IOP assessments were performed at least 24 hours after the injection. A tonometer (TonoPen XL; Mentor, Norwell, MA) was used to assess IOP in conscious, unsedated rats, as described by Moore et al. All IOP determinations were assessed by operators who were blind to the treatment applied to each eye. Animals were wrapped in a small towel and held gently, with one operator holding the animal and another making the readings. Five IOP readings were obtained from each eye by using firm contact with the cornea and omitting readings obtained as the instrument was removed from the eye. Differences among readings were less than 10% (SE). The mean of these readings was recorded as the IOP for that eye on that day. Mean IOPs from each rat were averaged, and the resultant mean IOP was used to compute the group mean IOP ± SE. No significant differences were found between the right and left eyes. The measurements during the dark phase were made under dim red light. Twelve animals were used in the study of the effect of a single injection of HA. In this case, after the measurement of IOP, HA was injected and the IOP was registered daily in both eyes of all animals, beginning 24 hours after the injection.

To study its long-term effects, HA was injected once a week into one eye of 20 animals, and saline solution was injected into the other eye. IOP was assessed weekly, before the new injection. IOP measurements were performed at the same time each day or week (between 11 AM and 12 PM) to correct for diurnal variations in IOP. In another set of experiments, IOP was assessed at 4-hour intervals during the light-dark cycle in both eyes of 10 animals. One drop of brimonidine (0.2%; Poen Laboratories, Buenos Aires, Argentina), timolol (0.5%; Alcon Laboratories, Buenos Aires, Argentina), or latanoprost (0.005%; Pharmacia Laboratories, Buenos Aires, Argentina) was applied in each eye, and IOP was assessed 2 hours later. The effect of each drug was examined in 10 eyes, whereas 10 control eyes received 1 drop of artificial tears (Alcon Laboratories).
consistency in IOP was found. All animals, without exception, responded with an increase of this parameter after the injection of HA.

To gain insight in the nature of the hypertension induced by HA, the effect of several hypotensive drugs was examined. One drop of each drug or of artificial tears was applied in HA-treated eyes. As shown in Figure 3, latanoprost (0.005%), brimonidine (0.2%), and timolol (0.5%), applied acutely (2 hours before the assessment of IOP in 10 eyes/group) significantly reduced the HA-induced elevation of this parameter (control, 21.2 ± 1.5 mm Hg; latanoprost, 15.1 ± 1.2 mm Hg; timolol, 15.7 ± 1.0 mm Hg; brimonidine, 14.7 ± 1.2 mm Hg; *P < 0.01). Artificial tears did not affect IOP (data not shown).

To examine the effect of HA administration on daily variations of IOP, this parameter was assessed at 4-hour intervals throughout the light–dark cycle in HA-treated and control eyes of 10 rats (Fig. 4). Although HA-injected eyes showed an IOP significantly higher than that of control eyes at every interval examined (*P < 0.01), this parameter showed significant daily variations, with a similar pattern in both groups (*P < 0.001, ANOVA). The IOP of both HA- and saline-treated eyes peaked during the dark phase, with IOP at 24 and 4 hours significantly higher than at the other intervals (e.g., at 24 hours IOP was 21.3 ± 1 mm Hg and 31 ± 1 mm Hg in saline- and HA-treated eyes, respectively).

**DISCUSSION**

The foregoing results indicate that the acute or chronic injection of HA in the rat anterior chamber significantly increased IOP compared with the vehicle injection in the contralateral eye. A single injection of HA maintained elevated levels of IOP during 8 days, whereas an injection performed once a week induced sustained and significant hypertension that lasted all along the duration of the study (10 weeks).

The concentration of HA used herein (1%) is similar to that currently used in human ocular surgery. In this case, as mentioned, a significant elevation in IOP was observed. Viscoelastic agents are rapidly removed after surgery, and the hypertensive effect lasts for a relatively short period (e.g., no longer than 24 hours). In rabbits, after the injection of hyaluronan-containing viscoelastic substances, IOP rose rapidly, reaching a peak at approximately 46 hours after injection and returning to preinjection levels during the following 24 hours (i.e., 70 hours after the injection). The longer-lasting effect of a single injection of HA on IOP could be attributable to the fact that because the anterior chamber in the rat eye is very shallow, the actual intracameral concentration of HA is higher than that achieved in other species. In addition, less-complete HA washout in rats cannot be ruled out.

In our conditions, basal IOP (in intact eyes) was 12.2 ± 0.4 (mean ± SE, n = 10 eyes). This level, although considerably lower than that described in Brown Norway rats, is in close agreement with that obtained for the same strain (Wistar) by other investigators.

The highest hypertension achieved with the acute injections was similar to that observed after an injection repeated once a week. It has been shown that that mean IOP in the rabbit is proportional to the concentration of HA. Therefore, it seems likely that a similar steady state concentration of HA is achieved after both the acute and chronic injections. At present, the intracameral fate of the injected HA is unknown. However, the slow decrease of the IOP observed after the acute injection suggests that it may leave the anterior chamber by the bulk flow and/or be degraded by local hyaluronidase activity. In this sense, it has been shown that various tissues...
lining the anterior chamber are able to digest the intracameral HA through intralysosomal hyaluronidase.\textsuperscript{8,16} 

In agreement with our results, it has been shown that the intracameral administration of chondroitin sulfate increases IOP in cats\textsuperscript{17} and rabbits.\textsuperscript{18} Both kinds of GAG coexist in the extracellular matrix of the trabecular meshwork of several species and they probably act by a similar mechanism in the elevation of IOP. It has been suggested that GAGs may reduce the functional diameter of the flow channels through the deep corneoscleral intertrabecular spaces and/or regulate flow through the juxtacanalicular basement membrane. Therefore, it seems likely that HA injected intracamerally could act in a way similar to mucopolysaccharide of endogenous origin (e.g., by impeding normal outflow of aqueous humor).

Although acting by different mechanisms, all the topical glaucoma therapies tested in this study, applied acutely and at the currently used doses, significantly reduced the hypertension induced by HA. Brimonidine is a potent \( \alpha_2 \)-adrenoreceptor agonist that reduces IOP by decreasing aqueous humor production and increasing aqueous humor outflow through the uveoscleral pathway. Each of these mechanisms may account by itself for the decrease in the HA-induced hypertension, because timolol (presumably acting through a decrease in aqueous humor production) and latanoprost (presumably acting through an increase in uveoscleral outflow) were as effective as brimonidine in IOP reduction. Although brimonidine has been shown to increase clearance of HA,\textsuperscript{20} no similar effect has been associated with the action of timolol or latanoprost. The results obtained with glaucoma therapies support the hypothesis that the hypertension induced by HA could be associated with a partial obstruction of trabecular outflow, because it may be relieved by decreasing aqueous humor production or by increasing the alternative outflow pathway. Accordingly, it has been demonstrated that timolol is effective in reducing the increase in human IOP secondary to the use of sodium hyaluronate in cataract surgery.\textsuperscript{19}

Rhythmic oscillations of IOP are a common feature both in humans and laboratory animals. In humans, there are studies reporting that peak IOP occurs either during the morning\textsuperscript{20} or during the dark phase.\textsuperscript{21,22} In contrast, animal studies in both rabbit and rats have consistently demonstrated that IOP increases during the night, presumably due to an increase in the rate of aqueous humor production.\textsuperscript{23–25} As shown herein, a significant rhythm of IOP was observed in both control and HA-injected eyes. The pattern of IOP rhythm in HA-injected eyes was similar to that observed in control eyes. If HA causes an obstruction in aqueous humor outflow, it seems likely that an increase in IOP, higher than that observed in control eyes, could be provoked by an increase in aqueous humor production during the night. Although it would be expected that the nighttime elevation in IOP would be greater in HA-injected than that in normal eyes, the curves paralleled each other. It is possible that the nocturnal increase in aqueous humor production, in the presence of a partial obstruction of the outflow pathway, triggered some compensatory mechanism in HA-injected eyes that did not operate in control conditions and that attenuated the increase in IOP.

Although considered the major risk factor for glaucoma neuropathy, the cellular mechanisms by which elevated IOP damages the optic nerve remain unknown. Acquiring this knowledge relies heavily on the use of animal models of chronically elevated IOP. The use of rodents allows experimentation with a sufficient number of animals and could provide a complete picture of both ultrastructural consequences and cell biology of pressure-induced optic nerve damage.

Several groups have developed various ways to increase IOP in the rat eye, generally by impeding the flow of aqueous humor. The model of Moore et al.\textsuperscript{20} involves the injection of a hypertonic saline solution into limbal aqueous humor–collecting veins, whereas the one developed by Shareef et al.\textsuperscript{27} involves the cautery of two or three of the episcleral–extraocular bital veins to block outflow of aqueous humor. The other glaucoma model in rats was performed by laser photocoagulation after injection of India ink into the anterior chamber.\textsuperscript{28} Each of these models has advantages and disadvantages.\textsuperscript{14} Although multiple injections of HA may be needed to obtain sustained hypertension, the injection itself does not seem to affect IOP. Besides, the hypertonic saline solution and the laser photocoagulation models are similarly inconvenient, in that both necessitate multiple injections or applications. On the contrary, several advantages support the usefulness of our model: it is inexpensive and easy to perform; highly consistent hypertension was achieved; it may have a reasonably long course; daily variations in IOP persisted in HA-injected eyes; and in contrast to the model of vein cautery, in all likelihood, HA did not impede the blood flow out of the eye. Furthermore, as shown herein, this model may be used for pharmacologic studies with drugs that affect aqueous outflow, formation, or a combination of both. However, whether the hypertension induced by HA mimics the optic nerve and retinal neuropathy that characterize glaucoma is still an open question that is currently under study.

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**References**


