Obstruction of aqueous outflow by lens particles and by heavy-molecular-weight soluble lens proteins

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Enucleated human eyes were perfused via the anterior chamber at 25 mm Hg pressure with lens particles (whole lens homogenates) in one series of experiments and with soluble lens proteins from human cataractous lenses in another series. Adding 1% of a homogenate of a single cataractous lens to the anterior chamber induced a 68% decrease in outflow. Perfusion with HMW soluble lens proteins (1 mg/ml; MW more than 150 million) caused a 60% decrease in outflow in 1 hr. In neither series was the obstruction to outflow relieved by subsequent irrigation of the anterior chamber with balanced salt solution or alpha-chymotrypsin. The results show that both lens particles and soluble lens proteins can directly obstruct the aqueous outflow pathways of human eyes. Such obstruction may be a significant factor in certain lens-induced glaucomas.

Key words: lens-induced glaucoma, phacolytic, phacotoxic, heavy-molecular-weight lens protein, cataract, hypermature cataract, macrophages, trabecular meshwork, lens

The pathogenetic mechanisms involved in the various lens-induced glaucomas have not been adequately explained. Glaucomas following extracapsular cataract extractions and lens injury may not fit criteria for either phacolytic or phacoanaphylactic phenomena and have tended to confuse the classification of the various lens-induced reactions. The significance of possible "phacotoxic" reactions is also unclear. Because of this uncertainty, we have investigated the direct obstructive properties of particulate lens material and soluble lens proteins under circumstances in which the possibilities of tissue reactions are limited by lack of blood circulation to the eye, in an attempt to identify the simplest factors that may be responsible for interference with aqueous outflow in open-angle lens-induced glaucomas.

Methods

Human eyes enucleated post-mortem were stored at 4°C in a moist chamber and were submitted to quantitative aqueous perfusion within 48 hr after death, according to a standard technique.4,6 Briefly, a reservoir of perfusion fluid was suspended from a lever arm of an electric force transducer which was connected to a strip-chart recorder. Calibration measurements established that from the change in weight of the reservoir the rate of flow could be determined reliably to the nearest 0.1 μl/min with this apparatus.

In preparation for perfusion, the enucleated eye was allowed to come to room temperature and was gently wrapped in gauze, placed in a plastic cup, and submerged to the limbus in the same physiologic salt solution utilized for perfusion. A 5...
mm central comeal trephine button was removed, a radial iridotomy was performed to prevent artificial deepening of the anterior chamber, and the anterior chamber was gently irrigated to remove any pigment which might have come loose into the anterior chamber at the time of iridotomy. A special stainless steel fitting was placed in the cornea and was connected to the fluid reservoir of the monitoring apparatus by means of polyethylene tubing. The cup containing the enucleated eye was placed in a water bath with adjustable temperature. The distance of the eye below the level of the fluid reservoir was varied to establish whatever intraocular pressure was desired. In this study perfusions were performed at 22°C and 25 mm Hg unless otherwise noted.

The eyes were first perfused in this manner for an hour in order to stabilize and to provide an initial flow value. After this, the corneal fitting was removed, and the lens particles or proteins were either instilled into the anterior chamber or infused through the tubing for a variable period from a separate reservoir at the same pressure as for the flow measurements. Following this, the flow was again measured by perfusion in the same manner as in the initial step. The medium for perfusion was Dulbecco's phosphate-buffered salt solution (PBS) (Grand Island Biological Co., Grand Island, N. Y.).

For experiments with particulate lens substance, homogenates of single human senile cataracts were prepared in a Tenbroeck homogenizer in 3 ml of 0.9% sodium chloride, from which the desired dilutions were made. Calcium-containing solutions for homogenization were avoided because of the known effect of calcium in causing aggregation of heavy-molecular-weight (HMW) lens protein. Five percent of a cataract homogenate suspension contained 4.0 to 10.0 mg of total protein (0.2 to 0.5 mg of HMW soluble lens protein—MW more than 150 million); other dilutions were proportional. The cataract homogenate was placed into the anterior chamber unfiltered.

For experiments with unclassified soluble lens proteins separated from particulate material, the homogenates of single cataracts were centrifuged at 14,000 x g for 20 min, and the supernatant was passed through a 1.2 μ Millipore filter before it was infused into the enucleated human eyes. In such experiments from 2.0 to 5.0 mg of soluble lens protein (0.2 to 0.5 mg of HMW protein) were introduced into the anterior chamber.

For experiments involving more specifically the HMW and low-molecular-weight (LMW) soluble lens proteins, these proteins (1 mg/ml) were prepared from human cataractous lenses according to previously published techniques and were passed through 1.2 μ Millipore filters prior to perfusion. In these experiments 0.5 mg of HMW or LMW lens protein (pH 7.4, 295 mOsm) was introduced into the anterior chamber.

Results

Particulate lens material (whole lens homogenate). Placing a small amount (5%) of the homogenate from a single cataractous lens into the anterior chamber of a perfused, enucleated human eye resulted in a severe obstruction of the outflow of the perfusion fluid (Fig. 1). Adding 1% of a single-cataract homogenate to the anterior chamber caused a 68% decrease in outflow (Fig. 2). The obstruction was not relieved by irrigation of the anterior chamber with balanced salt solution or alpha-chymotrypsin (Fig. 1).

Soluble lens proteins. Perfusion of enucleated human eyes with the heterogeneous soluble lens proteins present in the superna-
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0.5% of a Single Cataract Homogenate
Added to Anterior Chamber

Fig. 2. Effect produced on facility of aqueous outflow by varying the proportion (%) of a cataractous whole-lens homogenate added to the anterior chamber of perfused eyes. The number of eyes in each group is shown in parentheses.

Tants of different, individual-cataract homogenates induced a variable decrease in outflow of perfusion fluid that was not reversed by irrigation of the anterior chamber with balanced salt solution or alpha-chymotrypsin. The average results of six perfusions are demonstrated in Fig. 3 (range 20% to 50% decrease in outflow). The magnitude of the obstruction was less with the supernatant than with the corresponding whole lens homogenate (Figs. 1 and 3).

In an attempt to determine whether a selected class of soluble lens proteins could cause such an obstruction, enucleated human eyes were perfused with special preparations of HMW soluble lens proteins from human cataracts. Perfusion with these HMW lens proteins produced a severe obstruction of fluid outflow (Fig. 4). This obstruction was not relieved by irrigation of the anterior chamber with balanced salt solution or alpha-chymotrypsin (Fig. 4). The amount of obstruction increased when perfusion with the HMW lens proteins was prolonged (Fig. 5). When the same experiments with HMW lens proteins were performed in four enucleated human eyes at 37°C instead of 22°C, the same results were obtained, and again obstruction to outflow was not relieved by irrigation with balanced salt solution or alpha-chymotrypsin.

Perfusion of similar amounts of LMW soluble lens proteins from human cataracts failed to cause any obstruction of fluid outflow.

Discussion

In 1955 Flocks et al. first designated as “phacolytic glaucoma” the open-angle glaucoma associated with a leaking hypermature cataract. The macrophagic response to this leaking lens material had previously been described by Zeeman and Irvine. The Irvinet postulated that blocking of the trabecular meshwork by macrophages was the basis for the glaucoma. Flocks et al. believed that the associated glaucoma was due to obstruction of the intertrabecular spaces by macrophages distended with engulfed lens material and by Morgagnian fluid that had escaped from the lens. Goldberg popularized the Millipore filtration identification of the diagnostic macrophages in this condition, but he, too, suspected that the glaucoma was caused by blockage of the angle by both macrophages and protein-
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Fig. 3. Effect of soluble lens protein from human cataracts on aqueous outflow. Six enucleated human eyes were perfused for 1 hr with the supernatant of human cataract homogenates utilized in Fig. 1. Then attempts were made to reverse the effect by washing out the anterior chamber.

acous debris. Nevertheless, in recent years the role of macrophages in producing the glaucoma has been highlighted, and the possible obstruction of the trabecular meshwork by the liberated lens material itself has been mentioned infrequently.13-16

Our studies have demonstrated that, at least in enucleated human eyes, the aqueous outflow pathways are easily obstructed by particulate lens material (Figs. 1 and 2). This suggests that glaucoma following planned or unplanned extracapsular cataract extraction or lens injury may be attributable to the direct obstructive properties of the lens particles themselves. Particles from hypermature cataracts may also contribute to the obstruction of outflow in certain cases of phacomelic glaucoma. Whether such lens particle obstruction is due more to insoluble lens proteins or to cell membrane fragments is not clear from our study. Obstruction due to soluble lens proteins can explain only part of this cataract homogenate-induced decrease in facility (Figs. 1 and 3).

In phacomelic glaucoma a heavy protein flare in the anterior chamber has been observed clinically and has been thought to be due to soluble lens protein which has passed through a spontaneous rent or an area of dissolution of the lens capsule.11-13, 15, 17 It is known that within human cataractous lenses there is an increase in the amount of HMW soluble lens proteins.9, 18 Also, in a separate study we have analytically identified HMW soluble proteins in human aqueous humor in six cases of phacomelic glaucoma.19

Our experiments have demonstrated that perfusion of HMW soluble lens proteins from human cataracts in enucleated human eyes produces a severe obstruction of fluid outflow that increases with the length of the HMW protein perfusion time (Figs. 4 and 5). The amount of HMW soluble lens protein introduced into our experimental eyes is similar to that detected in the aqueous humor in cases of human phacomelic glaucoma.19 LMW soluble lens proteins introduced in similar amounts fail to cause any obstruction of outflow. Our results suggest that direct obstruction of the aqueous outflow pathways by liberated HMW soluble lens protein may be an...
important part of the mechanism of phacolytic glaucoma. Such protein obstruction possibly may be involved also in certain glaucomas following extracapsular cataract extraction.

Although phacolytic glaucoma is well known clinically to be cured by cataract extraction, we found in our experiments in enucleated eyes that obstruction by HMW soluble lens proteins was not relieved by irrigation of the anterior chamber for 3 hr. This has left unexplained what the natural mechanism is by which obstruction to aqueous outflow is alleviated after removal of an inciting cataractous lens in phacolytic glaucoma but suggests that it would be instructive to determine whether normal outflow could be restored by more effective means for deaggregating or enzymatically digesting the HMW proteins, which we assume to be deposited in the aqueous outflow system.

It seems worth considering whether macrophages may be the normal scavenger response to lens material in the anterior chamber and may contribute to removal of lens material from the outflow system. They have generally been thought of as contributing to the obstruction in phacolytic glaucoma, but engorged macrophages, perhaps fewer in number than in phacolytic glaucoma, have been observed in anterior chamber aspirates of children following routine needling and aspiration of cataracts, and these eyes did not appear to have had glaucoma. Possibly the seemingly low incidence of phacolytic glaucoma in children and juveniles with injured and cataractous lenses may be due to a difference in the protein composition of their lenses compared to the lenses of adults. Differences between the juvenile and adult outflow channels possibly may also be significant. It would be instructive to make a comparison of the obstructive properties of juvenile lens proteins in the outflow system in the same manner as we have described for material from adult cataracts.
Since our experiments with adult cataractous lens particles and proteins have shown that they can directly obstruct the aqueous outflow of human eyes but do not seem to wash out readily, future experiments might well be directed at investigating the possible role of macrophages in clearing obstruction, as well as the presumed opposite role of these cells in obstructing the aqueous outflow system.

REFERENCES