Identification of heavy-molecular-weight soluble protein in aqueous humor in human phacolytic glaucoma

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Aqueous humor was obtained by paracentesis at the time of cataract surgery from six patients with phacolytic glaucoma, diagnosed on the basis of acute unilateral open-angle glaucoma associated with an apparently leaking hypermature or mature cataract, and from six control patients with immature cataracts. Three of the latter had primary open-angle glaucoma. Quantities of heavy-molecular-weight (HMW) protein (MW greater than 150 × 10^6) sufficient to obstruct aqueous outflow were identified in all six phacolytic aqueous humor specimens but in none of the controls. Three of the hypermature cataractous lenses from the cases of phacolytic glaucoma were also examined and were found to have 14-fold greater quantities of HMW protein in their liquefying cortex than were present in the cortex of immature cataractous lenses. These findings, correlated with experimental HMW protein perfusion studies in excised human eyes that we have already reported, strongly suggest that direct obstruction of the aqueous outflow channels by liberated HMW soluble lens protein may be a significant and previously unappreciated factor in the pathogenesis of phacolytic glaucoma.

Key words: phacolytic glaucoma, heavy-molecular-weight proteins, aqueous humor, hypermature cataract, lens-induced glaucoma

Previous descriptions of the pathogenesis of phacolytic glaucoma have focused on the role of macrophages distended with engulfed lens material in producing glaucoma.1-9 Quite recently, in vitro experiments10 have shown that heavy-molecular-weight (HMW) protein aggregates11, 12 having a molecular weight greater than 150 × 10^6 daltons, from water-soluble and water-insoluble lens proteins themselves can directly obstruct the aqueous outflow channels and hence, experimentally, cause a phacolytic-like glaucoma. Conceivably, in the clinical situation leaking HMW aggregates from hypermature cataracts could, if present in sufficient concentration, cause the in vivo obstruction of aqueous outflow and the onset of phacolytic glaucoma.

To test this hypothesis, the objectives of this investigation were threefold:

1. To establish whether HMW aggregates are present in the aqueous humor of phacolytic subjects.
2. To measure the HMW protein concentration of the cortical region of hypermature "leaky lenses."
3. To correlate the concentration of HMW protein found in both the phacolytic aqueous samples and the cortical region of hyperma-
ture cataracts to the concentration of HMW protein established as needed to produce experimental phacolytic glaucoma.

Methods

In six patients with clinical phacolytic glaucoma (acute unilateral open-angle glaucoma associated with hypermature or mature cataract and variable biomicroscopic signs of foreign material in the aqueous humor), 0.1 to 0.2 ml of aqueous humor was obtained at the time of cataract surgery. Similar samples were obtained from six control patients with immature cataracts but clear aqueous humor. Three of these control patients had primary open-angle glaucoma, and three had normal intraocular pressure. A small portion of each sample of aqueous humor was examined diagnostically by phase-contrast microscopy for cellular content. The remainder was brought up to a final volume of 2 ml with Tris buffer (0.01M, pH 7.4) and centrifuged at 15,000 × g for 20 min.

Agarose, 150M, from Bio-Rad Laboratories (Richmond, Calif.) was packed in columns measuring 2 by 27 cm. The protein concentration of the initial sample of aqueous humor and of each fraction collected by chromatography was determined by measuring its absorbance at 280 nm. The hypermature (phacolytic) and immature (nuclear sclerotic) cataractous lenses were decapsulated, and the cortex and nucleus were separated. The cortices were homogenized in 2 ml of Tris buffer (0.01M, pH 7.4) and then were centrifuged at 15,000 × g for 20 min. The protein concentrations of the initial lens homogenate, supernatant, and of each fraction collected by chromatography were determined by measurement of its absorbance at 280 nm.

Description of phacolytic glaucoma cases

In each of the six cases diagnosed as typical examples of phacolytic glaucoma in this study, there was rapid onset of ocular pain and redness in one eye. The intraocular pressures ranged from 50 to greater than 90 mm Hg but were normal in the contralateral uninvolved eyes. None of the affected eyes had a history of previous glaucoma or ocular surgery. In each case there was corneal epithelial edema, but the angles were established by gonioscopy definitely to be open. One patient had 180° of angle recession, but in the other five, the angles appeared normal. The cataractous lenses in the glaucomatous eyes were hypermature (with a liquid cortex) in five patients and mature (opacified totally white) in one. The lens was dislocated into the vitreous in one patient and subluxated in one other patient.

On slit-lamp biomicroscopy of the anterior chambers, significant flare was noted in the glaucomatous eyes, but none in the contralateral normal eyes. Cellular reaction in the anterior chamber was judged minimal in four patients and moderate in two. In four eyes circulating white particles in the anterior chamber were observed and interpreted variously as very large cells, cellular aggregates, or small particles of lens material. Several of the cataracts were noted to have patchy white deposits on the anterior lens capsule.

In general, the eyes showed little response to topical antiglaucoma or anti-inflammatory therapy. There was some lowering of intraocular pressure with use of carbonic anhydrase inhibitors and osmotic agents, but in all eyes the intraocular pressure remained greater than 40 mm Hg to the time of cataract extraction. In four of the eyes, cataract extraction was accomplished intracapsularly without difficulty, but in one case extraction was complicated by vitreous loss, and in one it was an unplanned extracapsular extraction.

In the four cases that were not complicated by vitreous loss or extracapsular extraction, intraocular pressure was less than 24 mm Hg during the immediate postoperative period, despite use of alpha-chymotrypsin at the time of cataract extraction. Phase microscopy examination of fresh anterior chamber fluid revealed typical engorged mac-
**Results**

**Presence of HMW aggregates in phacolytic aqueous humor.** To establish the presence of HMW protein in aqueous humor samples from phacolytic subjects, the soluble proteins of these samples were separated by large-pore gel molecular-sieve chromatography (Agarose 150). The typical elution profile of samples from six cases of phacolytic glaucoma is shown in Fig. 1 (open circles). The same type of protein separation was performed for aqueous humor samples from three open-angle glaucoma patients and three normal subjects. The closed circles depict the typical elution pattern found for these samples. The entire population of soluble proteins from the anterior chamber tap of phacolytic specimens eluted early in the chromatogram, indicating a shift toward larger-molecular-weight proteins relative to the retarded, hence smaller-size, protein population of the nonphacolytic samples. On average, the HMW content of the phacolytic aqueous proteins, i.e., void volume fraction, represented roughly 35% the total initial protein concentration. No HMW protein was found in the normal or open-angle aqueous samples.

Those of our cases of phacolytic glaucoma in which phase-contrast microscopy of the aqueous humor revealed few or no macrophages did not differ from the other cases (with plentiful macrophages) in regard to either anterior chamber HMW protein content or in regard to response of intraocular pressure to cataract surgery.

**HMW protein concentration of the cortical region of hypermature cataracts.** The technique of molecular-sieve chromatography was further used to estimate quantitatively the concentration of HMW protein from the cortex of three hypermature (phacolytic) cataracts. Fig. 2 illustrates the elution pattern of the soluble proteins, HMW as well as lower-molecular-weight (LMW) components from hypermature lens cortices (open circles). The three hypermature cataracts showed the same distribution. The distribution of the HMW and LMW protein fractions of the cortical region of immature (nuclear sclerotic) cataracts is depicted by closed circles. The first and most obvious feature to observe from the data is that the concentration of HMW protein in hypermature cortical samples is roughly 14 times greater than that found for cortical samples from immature cataractous lenses. Second, the decrease in LMW protein concentration in hypermature cortical fractions relative to the concentration of LMW protein in nuclear sclerotic cortex in all likelihood suggests the aggregation or shift in protein size to large-molecular-weight moieties as a mechanism for hypermature cataract formation. Interestingly enough, previous investigations have shown that HMW protein is present primarily in the nuclear region of normal and cataractous lenses. Increased levels of HMW protein from nuclei occur with age and cataract disease. Generally, the cortical region of human lenses, normal as well as immature and mature cataractous lenses, contain low levels of HMW protein. The presence of significant quantities of HMW protein in
hypermature cataract cortex apparently distinguishes these lenses from other human lenses, normal and cataractous.

**Correlation of aqueous HMW protein concentration with in vitro obstruction.** Past experiments show that 0.5 mg of HMW protein isolated from cataractous lens nuclei cause, upon perfusion, a decrease of 60% in facility of aqueous outflow.10 In the present studies, the average concentration of protein (280 nm absorption) typically measured for phacolytic aqueous humor samples was approximately 9 mg/ml. The average volume of anterior chamber fluid in the human eye is on the order of 0.25 ml. Hence the total protein contained in the aqueous humor of phacolytic subjects is 2.25 mg. Fig. 1 demonstrated that roughly 35% of the total protein of the phacolytic aqueous elutes as HMW protein. Thus 35% of the total protein (2.25 mg) or roughly 0.8 mg of HMW protein is potentially available to mechanically obstruct the aqueous outflow from the anterior chamber of subjects with phacolytic glaucoma. This concentration of HMW protein is well in excess of the experimental value previously established to cause obstruction.

The intermediate molecular size species of proteins found in the phacolytic aqueous (test tubes 6 to 12, Fig. 1) are probably derived from LMW proteins of either the lens or aqueous and are conceivably of a molecular size sufficient to also contribute to decreased aqueous outflow.

**Discussion**

Our results show that HMW proteins of a molecular size greater than $150 \times 10^6$ daltons occur in all clinical examples of phacolytic aqueous humor samples studied. Roughly 35% of the total initial sample elutes as HMW proteins. No HMW protein was present in the aqueous from normal and open-angle glaucoma subjects. The cortices of hypermature cataractous lenses contain significant quantities of HMW protein. The concentration of HMW protein present in the aqueous of phacolytic patients, i.e., the in vivo situation, is in excess of the concentration of HMW protein that causes in vitro obstruction. These findings strongly suggest that hypermature cataractous lenses may leak sufficient quantities of HMW protein into the anterior chamber for these aggregates to induce mechanical obstruction of aqueous outflow, resulting in phacolytic glaucoma.

The role of macrophages as key factors in inducing phacolytic glaucoma is, as a consequence of this investigation, de-emphasized. It is interesting to remark in this respect that the presence of macrophages in the anterior chamber does not invariably result in phacolytic glaucoma. Engorged macrophages occur after cataract needleling in the anterior chamber aspirates of children.17 Yet no case of phacolytic glaucoma in these children was observed. In a recent series of 20 cases of phacolytic glaucoma, the youngest patient was 35 years old.18 Jedziniak et al.12, 19 and Spector et al.16, 20 have found that HMW protein is virtually absent in infantile and juvenile lenses. The onset of HMW aggregates occurs at 20 years of age. The concentration of HMW protein increases monotonically with age between 20 and 60. The concurrent development of phacolytic glaucoma and HMW aggregate formation strengthens the evidence that HMW protein may be a contributory factor to the disease of phacolytic glaucoma.

**References**