# Aqueous Humor Ascorbate Concentration and Open-Angle Glaucoma

Pei-fei Lee, MD; Kwok-Wai Lam, PhD; Mei-mei Lai, MD

**• The mean value for aqueous concentration in 35 patients with open-angle glaucoma was 22.4 ± 12.9 mg/100 ml. The mean value for aqueous ascorbate in four patients with uncomplicated senile cataract was 11.55 ± 3.01 mg/100 ml. The results indicate that the majority of open-angle glaucomatous eyes do not involve a deficiency of ascorbate, and suggest that ascorbate has no therapeutic value in the management of primary open-angle glaucoma. The magnitude of aqueous ascorbate variation among glaucoma es is probably related to the factors that influence the patency of trabecular meshwork, not the metabolic activity of the ciliary processes.**

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The high concentration of ascorbate in normal aqueous humor has been an interesting observation for decades, and the application of ascorbate as an adjunct in the management of primary open-angle glaucoma has been studied in several laboratories.[1-4] However, its precise role in ocular tissues and the clinical implications remain unclear. Furthermore, the unavailability of literature on the ascorbate concentration in the aqueous humor among glaucoma eyes makes it more difficult to evaluate the therapeutic value of ascorbate. Although reduction of ascorbate in the anterior chamber, associated with increasing intraocular pressure, has been shown in animal models,[5] and buphthalmic rabbits,[6] it is not known if it occurs in human glaucoma eyes. The present study was undertaken as the beginning of our evaluation on the aqueous humor composition in the eyes of patients with primary open-angle glaucoma. The level of aqueous humor ascorbate concentration among 35 patients with primary open-angle glaucoma and its importance with regard to the pathogenesis of glaucoma are discussed in this report.

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From the Departments of Ophthalmology (Drs Lee, Lam, and Lai) and Biochemistry (Dr Lam) Albany Medical College of Union University; and the Section of Ophthalmology, Surgical Service, Veterans Administration Hospital (Dr Lee) Albany, NY. Dr Lai is now with the Department of Ophthalmology, Howard University, Washington, DC.

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Reprint requests to Department of Ophthalmology, Albany Medical College, Albany, NY 12208 (Dr Lee).

## METHODS

**Aqueous Humor Collection.**—Aqueous humor samples were obtained under an operating microscope at the time of glaucoma surgery in all eyes. No glaucoma medication was given for at least 14 hours before the operation. After a routine preparation, a sliding peripheral corneal incision was made with a Wheeler knife. A No. 30G or 27G needle-cannula attached to a disposable tuberculine syringe was used to aspirate aqueous humor via the corneal incision without totally collapsing the anterior chamber. Usually, about two thirds the amount of total aqueous humor was withdrawn without injury to the iris or corneal endothelium with the tip of the needle-cannula. Repeated withdrawal of aqueous humor was not attempted in any case, in order to avoid the contamination of the secondary aqueous humor. If the anterior chamber was lost following the corneal incision, or during the cannula insertion into the anterior chamber, the aqueous sample collection was abandoned. The anterior chamber was then refilled with normal saline and the surgery proceeded as planned.

**Blood Serum Collection.**—Venous blood samples were obtained at the time of the surgery, usually prior to or immediately after the operation. If intravenous infusion was used, then venous blood was taken from the opposite arm in order to avoid a diluted serum sample.

**Biochemical Analyses.**—The colorimetric method of Roe and Kuether[7] was used for analysis of serum ascorbate concentration. High pressure liquid chromatography[8] was used to analyze ascorbate concentration in the aqueous humor because of the limited amount of aqueous available for analysis. Previous reports have shown that the major ultraviolet absorption peak observed in the chromatogram of aqueous humor was ascorbate. Other compounds such as aromatic amines and nicotinamide adenosine dinucleotide eluted near the ascorbate peak were not present in sufficient quantity to interfere with the estimation of ascorbate concentration. The previous report has shown that the results obtained by the chromatographic method were identical to those obtained by the colorimetric method.[8] Ascorbate concentration in serum is much lower than that of aqueous and the interference by neighboring peaks is serious. Therefore, the colorimetric method of Roe and Kuether was used in this study for analyses of serum ascorbate concentration. Protein concentrations were estimated by the method of Lowry et al.[9]

## RESULTS

The frequency distribution of aqueous humor ascorbate concentration among 46 eyes of 35 patients with primary open-angle glaucoma is described in Fig 1. The mean value and SD for ascorbate was 22.4 ± 12.9 mg/100 ml, with a broad range varying from 1.5 to 62.0 mg/100 ml. Aqueous analyses were also done in four eyes (four patients) with senile cataract as a control. The mean value of aqueous ascorbate concentration was 11.55 ± 3.01 mg/100 ml with a range varying from 9.1 to 15.4 mg/100 ml. Venous blood samples were taken at the time of surgery from 35 patients for ascorbate analyses. The correlation between the ascorbate concentrations of serum and aqueous humor is shown in Fig 2. The correlation coefficient, $r$, for the ascorbate concentration of aqueous humor and serum has a value of 0.4 calculated from the equation[10]: $r = \Sigma xy/[(\Sigma x^2)(\Sigma y^2)]^{1/2}$.

Protein analyses of 35 eyes of 29 patients disclosed a mean value and SD of 70.0 ± 62.7 mg/100 ml and a range varying from 17 to 296 mg/100 ml. These values are similar to the data reported by Bessiére et al,[11] yet much lower than serum protein concentration, indicating that the blood aqueous barrier in these eyes is not severely disturbed. There was a slight trend for eyes with high protein concentrations to have low ascorbate concentration (Fig 3). The $r$ value is −0.27. The small increase of protein concentration in some samples could be the result of glaucoma medication. It might not be related to the pathologic condition of glaucoma.

Analyses of aqueous humor and blood were repeated in six patients at the time of the first and second eye operation. The level of ascorbate concentration showed no significant difference between the right or left eye of the same patient. The mean value and SD for aqueous ascorbate between OD and OS were 29.3 ± 10.1 mg/100 ml and 22.6 ± 7.3 mg/100 ml ($P = .07$). The level of aqueous protein concentration between the OD and OS of the same patient was also not significant, 38.9 ± 28.1 mg/100 ml and 38.5 ± 17.4 mg/100 ml, respectively ($P > .5$).

Three patients who underwent reoperation on the same eye had aqueous and blood analyses again carried out at the time of reoperation. There was no significant difference between the first and second samples collected at the time of reoperation. The mean value for aqueous ascorbate was 17.7 ± 9.9 mg/100 ml at the first operation and 21.9 ± 7.2 mg/100 ml at reoperation. The mean value for aqueous protein was 57.0 ± 8.2 mg/100 ml at the first operation and 86.7 ± 84.0 mg/100 ml at reoperation ($P = .5$ for ascorbate and for protein analyses).

## COMMENT

If one assumes that the normal ascorbate concentration in aqueous humor is between 15 and 18 mg/100 ml as reported by Purcell et al,[12] and Becker,[13] the present data observed among the 46 eyes with primary open-angle glaucoma indicate that a majority of the patients have normal or above normal ascorbate concentration in the aqueous humor. This study indicates that the majority of human open-angle glaucoma eyes are not accompanied by a reduction of ascorbate in the anterior chamber as seen in animal models.[5,6] On the contrary, as high as 60 mg/100 ml was observed in far advanced cases. The outflow facility was poor in some patients, in spite of an elevation of ascorbate concentration up to 60 mg/100 ml. Therefore, the possible beneficial effect of ascorbate concentration on outflow facility was not observed in human eyes, as demonstrated in animal models by Lieb and Stark,[1] who suggested that ascorbate increased outflow facility by acting as a spreading factor. It is interesting to note that in this series, three patients who showed a low aqueous ascorbate concentration (< 5 mg/100 ml) demonstrated no other systemic diseases nor was their adrenal cortex stimulated by the adrenocorticotropic hormone, and none were treated with topical steroids. The reason for low ascorbate concentration in these eyes is not clear.

The high ascorbate concentration among some glaucomatous eyes in this series does not encourage the use of ascorbate in the management of open-angle glaucoma. Since ascorbate concentration is normal, or above normal value, further increase of ascorbate will aggrevate the situation due to the osmolarity pressure in the aqueous humor, resulting from elevated ascorbate concentration. This conclusion is in agreement with that of Fishbein and Goodstein[3] and a recent report by Daniel et al.[4] They stated that topical or oral administration of ascorbate had no noticeable effect on the IOP among open-angle glaucoma patients. Their statements are also in agreement with our limited clinical trials on three patients (unpublished data). However, the possibilities of oral ascorbate therapy and its clinical value to eyes with low ascorbate concentration has not been investigated. In view of this uncertainty, the level of ascorbate aqueous concentration should be considered if one wishes to evaluate the therapeutic value of ascorbate in the management

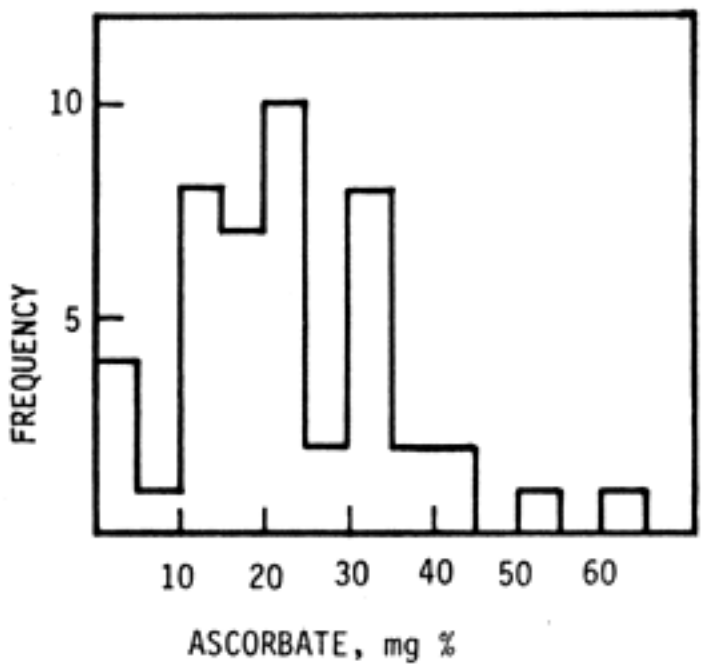


Fig 1.—Frequency distribution of aqueous humor ascorbate concentration in 46 eyes of 35 patients with primary open-angle glaucoma.

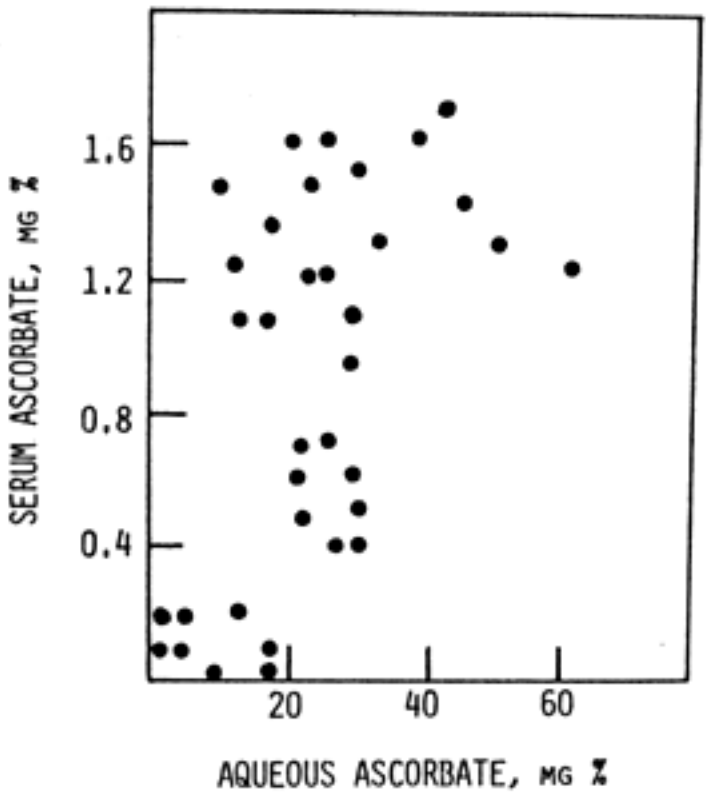


Fig 2.—Correlation between ascorbate concentrations in serum and aqueous humor of 35 patients with primary open-angle glaucoma.

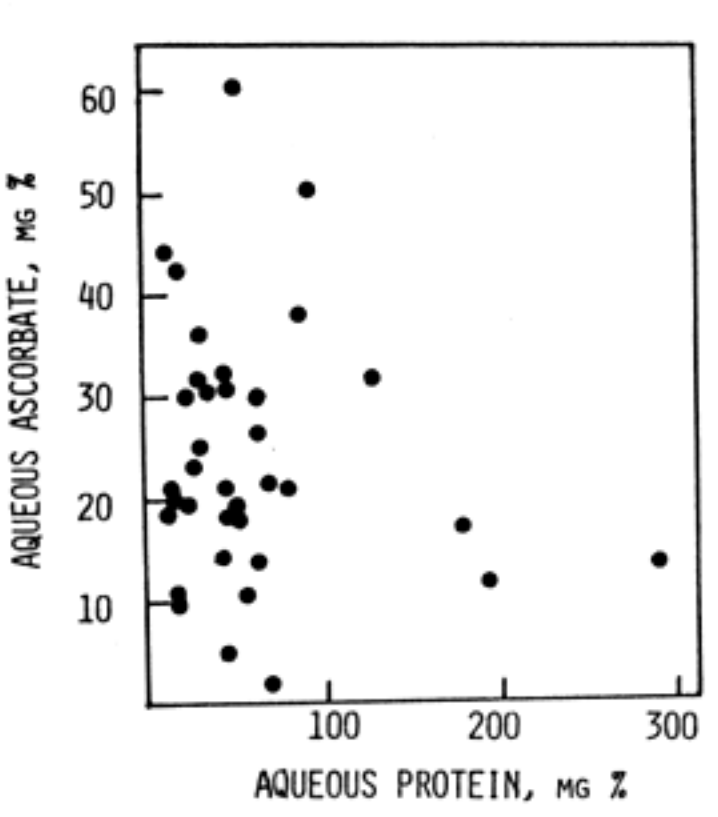


Fig 3.—Correlation between aqueous humor ascorbate and protein concentrations in 35 eyes of 29 patients with primary open-angle glaucoma.

of glaucoma.

The ascorbate concentration in the aqueous humor of normal animal eyes has been shown to be dependent on the ascorbate concentration in the blood, the rate of blood flow, or active-transport activity of the ciliary processes.[14,15] Based on the present analyses of the blood among 35 patients, the variations of aqueous humor ascorbate concentration observed among these glaucomatous eyes show some correlation to that of blood. However, the few cases having extremely high aqueous humor ascorbate concentration did not show unusually high ascorbate in the serum.

The aqueous ascorbate concentrations in the two eyes of two patients were 51.0 and 62.0 mg/100 ml and the serum ascorbate concentration 0.7 and 0.9 mg/100 ml, respectively. The level of 50 mg/100 ml aqueous ascorbate concentration in animal eyes is considered a saturated state, which was observed only when blood ascorbate was drastically increased by systemic administration of excess ascorbate.[14-16] Both of these patients demonstrated far-advanced glaucoma and their aqueous protein concentrations were within the range of normal values (92.0 mg/100 ml and 52.0 mg/100 ml, respectively). The reason for this unusually high level of aqueous ascorbate concentration is not clear. Perhaps further experiments and clinical investigation on the aqueous samples collected for untreated as well as early stages of open-angle glaucoma may disclose certain explanations.

The biochemical function of ascorbate is still unclear. The high ascorbate concentration (beyond 30 mg/100 ml) in some glaucomatous eyes could be due to reduction of use of trabecular meshwork and eye tissues of primary open-angle glaucoma. Alternatively, if the rate of water flowing through the anterior chamber is reduced while ascorbate transport is unchanged, the ascorbate concentration in the anterior chamber would be increased. This hypothesis may explain the clinical condition of the gradual decrease in aqueous outflow facility followed by gradual elevation of IOP in eyes with primary open-angle glaucoma. High ascorbate concentration in the eyes with treated primary open-angle glaucoma, therefore, may represent a sequela of impaired outflow facility rather than increased activity of the active-transport system within the ciliary processes. This hypothesis predicts that (*a*) aqueous ascorbate concentration is within normal range in the eyes with early stage glaucoma in which the trabecular patency is not appreciably affected clinically and (*b*) the magnitude of aqueous ascorbate concentration increases with the degree or the severity of the impairment on trabecular patency. Unfortunately, the data concerning the clinical condition of trabecular patency of these eyes are insufficient for statistical analysis.

The mean value of ascorbate concentration in four eyes with senile cataract was 11.55 ± 3.01 mg/100 ml, ranging from 9.1 to 15.4 mg/100 ml. This value is in agreement with the data reported by Purcell et al.[12] External filtering procedures have apparently no appreciable influence upon the consistency of the level of the aqueous ascorbate and protein concentrations. Our observation that the level of aqueous ascorbate concentration is not altered by the filtering procedure is similar to the observation of Kronfeld[17] on the aqueous ascorbate concentration of surgically aphakic human eyes. Furthermore, oral administration of acetazolamide apparently has no significant influence on the level of aqueous ascorbate concentration. This series includes 16 patients (23 eyes) who had been taking acetazolamide prior to glaucoma surgery. The mean value of ascorbate in the patients with and without acetazolamide treatment were 26.05 ± 10.97 mg/100 ml and 22.60 ± 17.38 mg/100 ml, respectively ($P > .5$). The results indicate that active transport of ascorbate by the ciliary processes among primary open-angle glaucoma eyes is relatively constant.

Comparative evaluation in aqueous ascorbate concentration between different types of glaucoma is not within the scope of this article and will be reported in a separate communication.

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