The inverse correlation of ascorbic and uric acid concentrations in the aqueous humor of human eyes with cataract

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Abstract

Aim: To investigate the free-radical stress in human eyes with cataract by assessing the loss of a physiological reducing agent (ascorbic acid) with the concurrent accumulation of a catabolic product (uric acid) associated with free-radical formation.

Materials and methods: Ascorbic and uric acid concentrations were analyzed by high-pressure liquid chromatography using a mBondapak-NH_2 column eluted by 10 mM ammonium phosphate. This method was used to analyze ascorbic acid and uric acid concentrations in the aqueous humor of 42 cataractous human eyes.

Results: The mean and standard deviation of ascorbic acid and uric acid concentrations in the aqueous humor were 23.4 ± 10.2 and 1.2 ± 0.8 mg/dL respectively. There was a significant ($P=10^{-6}$) inverse correlation ($r=-0.69$) between ascorbic acid concentration and uric acid concentration.

Conclusion: Ascorbic acid concentration in the aqueous humor is affected by free radicals produced from xanthine oxidase. Since the increase in uric acid is less than the loss of ascorbic acid, additional unknown factors may be responsible for the loss of ascorbic acid in the anterior chamber.

Key words: Ascorbic acid, Aqueous humor, Cataract, Free radical, Uric acid

Introduction

Some investigators have postulated that free radicals play a role in cataractogenesis.[1,2] It is difficult to evaluate this hypothesis by a direct analysis of the concentrations of the highly labile free radicals in the anterior chamber. One may, however, estimate the magnitude of free-radical stress indirectly, on the basis of the loss of physiological reducing agents and the accumulation of catabolic products associated with free-radical formation.

Ascorbic acid is one of the major reducing agents in normal aqueous humor. It is readily oxidized to dehydroascorbate by free radicals. Dehydroascorbate does not accumulate inside the cells. It is rapidly reduced by the intracellular enzymes within living cells[3] or it is metabolized further and excreted. There is no mechanism to reverse ascorbic acid oxidation in the anterior chamber or vitreous humor. Therefore, the loss of ascorbic acid in the anterior chamber may be a good indicator of the magnitude of free-radical stress.

Uric acid production catalyzed by xanthine oxidase is accompanied by the release of superoxide as an intermediate product.[4] The elevation of uric acid concentration in the aqueous humor therefore accounts for one of the known sources of free radicals in the anterior chamber.

We investigated the possible inverse correlation of ascorbic and uric acid concentrations in the aqueous humor of 42 cataractous human eyes as an indicator of free-radical stress.

Materials and methods

Informed consent was obtained from patients undergoing cataract extraction. Aqueous humor samples were obtained at the time of cataract extraction from 42 consecutive cases. After the eye was fully anesthetized but before the start of the cataract operation, about 0.05 ml of aqueous humor was aspirated from the anterior chamber through a small paracentesis site at the periphery of the cornea with a 30-gauge needle attached to a tuberculin syringe. The specimens were stored at -5°C until the time of analysis. The patients had no evidence of glaucoma or any known eye diseases other than cataract. None of these patients had gout. There were 26 female and 16 male patients aged from 19 to 83 years. The mean age was 56 years and five patients had a history of hypertension.

Ascorbic acid and uric acid concentrations were analyzed by high-pressure liquid chromatography (HPLC) using a m-Bondapak-NH_2 column (Waters Associates, Medford, MA, USA) equilibrated in 10 mM ammonium phosphate (HPLC grade, Fisher Scientific, Houston, TX, USA). An aqueous humor specimen (1 mL) was injected into the column and eluted with the same solvent at 1 mL per min. The eluant was monitored with a photodiode array detector, which allows one to examine the light absorption spectrum at any given retention time in the chromatogram. The locations of ascorbic acid and uric acid in the chromatogram were determined by the retention time and confirmed by the UV absorption spectra of the compounds eluted from the column. Ascorbic acid concentrations were calculated from the peak height detected at 265 nm.[5] Uric acid concentrations were calculated from the peak height detected at 290 nm.[6]

Results

Ascorbic acid and uric acid in the chromatogram were identified first by their retention time, then confirmed by their UV light absorption spectra. When a mixture of pure uric acid and pure ascorbic acid was injected into the column, their retention times were 4.6 and 7.9 min, respectively (**Figure 1a**). The identity of the peaks at the retention times of 4.6 and 7.9 min were confirmed to be uric acid and ascorbic acid, respectively, by their absorption spectra (**Figure 1b**).

A typical chromatogram of aqueous humor is shown (**Figure 2a**). Ascorbic acid (peak 8) is the dominant peak in the chromatogram recorded at 265 nm, as shown in the upper tracing of **Figure 2a**. The magnitude of peak 8 is reduced and the uric acid peak (peak 7) is increased in the chromatogram recorded at 290 nm, as shown in the middle tracing of **Figure 2a**. The chromatogram recorded at 210 nm showed multiple peaks (1-6 and 9-10) separated from uric acid and ascorbic acid, as shown in the bottom tracing of **Figure 2a**. The identities of peak 7 and peak 8 were confirmed to be uric acid and ascorbic acid, respectively, by their absorption spectra (**Figure 2b**). All the other peaks, 1-6 and 9-10, as shown in the bottom tracing of **Figure 2a**, had high absorption at wavelengths shorter than 240 nm (**Figures 2b** and **2c**).

The mean value of ascorbic acid concentration in the aqueous humor of the cataractous eyes was 23.4 mg/dL, within the range previously reported in the literature.[7-10] The standard deviation was large ($\pm$ 10.2 mg/dL). The mean uric acid concentration was 1.2 $\pm$ 0.8 mg/dL, similar to the value reported by Walker.[10] There was a significant ($P=10^{-6}$) inverse correlation between the ascorbic acid concentrations and the uric acid concentrations (r=-0.69) (**Figure 3**). The specimens with low ascorbic acid concentrations tended to have high uric acid concentrations.

Discussion

If free-radical stress is an important factor in cataractogenesis, a loss of ascorbic acid concentration in the aqueous humor of cataractous eyes would be expected. Aqueous humor from normal human eyes is not available for investigation. Therefore, one cannot compare the ascorbic acid concentrations of cataractous eyes with those of normal eyes. Furthermore, as free-radical stress is not a continuous phenomenon, a broad variation in the magnitude of free-radical stress in cataractous eyes at the time of cataract extraction is expected. In this study, although we could not compare the specimens from cataractous eyes with specimens from normal eyes, we noticed that the ascorbic acid concentration in the aqueous humor correlated inversely with the concentration of uric acid, which is a catabolic product associated with free-radical production. Such a variation in concentration could represent a varying degree of free-radical stress in cataractous eyes at the time of cataract surgery.

The rapid oxidation of uric acid *in vitro* by g-ray irradiation, hydrogen peroxide etc. has led some investigators to consider uric acid as an antioxidant.[11] However, uric acid oxidation is irreversible. Uric acid cannot serve as a reversible antioxidant, as do ascorbic acid, glutathione and vitamin E. In reality, the *in vivo* formation of uric acid by xanthine oxidase is coupled with free-radical formation.[4] Increased uric acid formation is accompanied by increased production of free radicals that potentially may oxidize ascorbic acid. The data indicate a significant ($P=10^{-6}$) inverse correlation between ascorbic acid concentration and uric acid concentration in cataractous eyes. The magnitude of ascorbic acid loss was higher than the elevation of uric acid, suggesting the presence of other factors that also oxidize ascorbic acid. Xanthine oxidase is not the only source of free radicals. Photochemical reactions and other oxidative metabolic reactions also produce free radicals, and the multiple sources of free radicals could affect the correlation between ascorbic acid concentration and uric acid concentration. The significant inverse correlation indicates that xanthine oxidase could be one of the major factors in formation of free radicals in cataractous eyes.

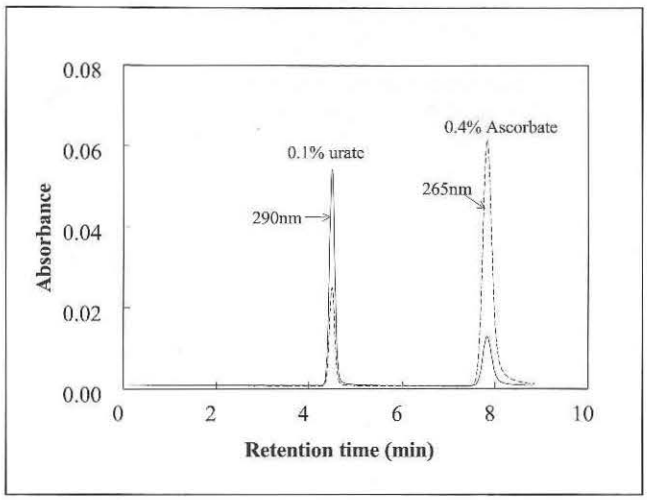

Figure 1a. Retention time of ascorbic acid and uric acid.

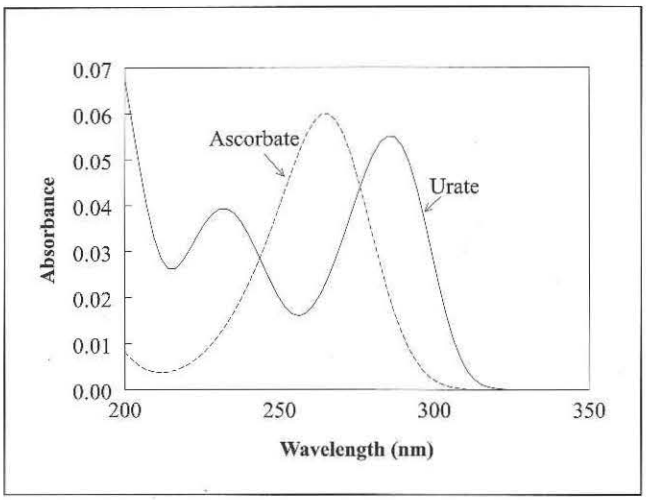

Figure 1b. Absorption spectra of ascorbic acid and uric acid confirming the corresponding absorption wavelength.

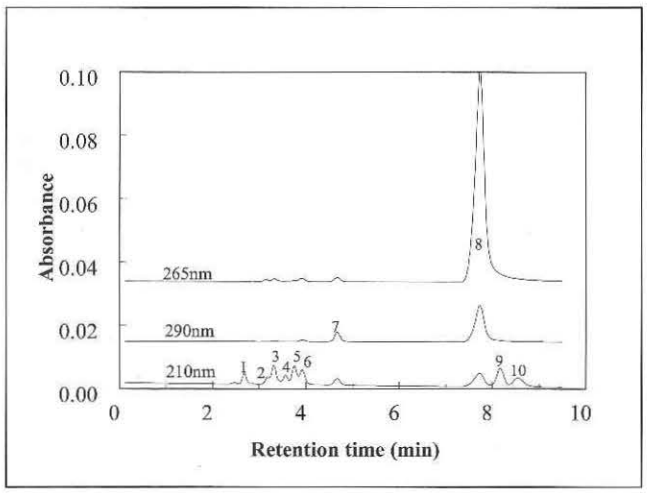

Figure 2a. Aqueous humor chromatogram showing relative dominance of ascorbic acid and uric acid peaks at various wavelengths.

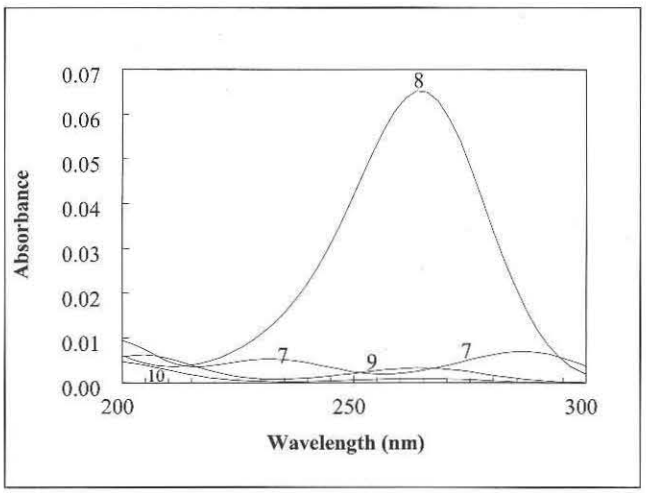

Figure 2b. Absorption spectra confirming the identity of peak 7 being uric acid, and peak 8 being ascorbic acid.

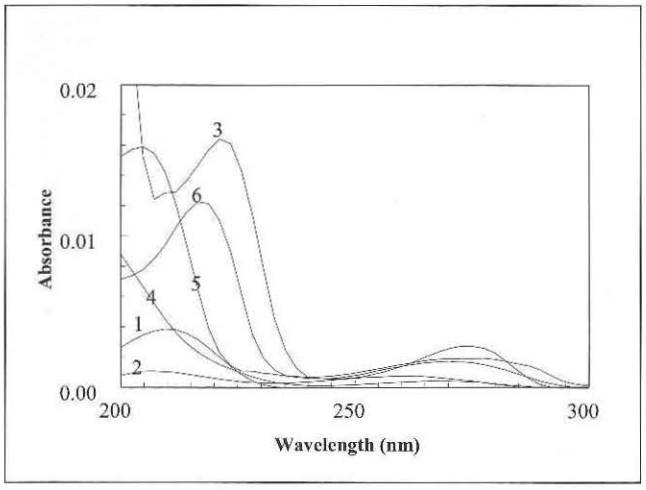

Figure 2c. Absorption spectra showing peaks 1 to 6.

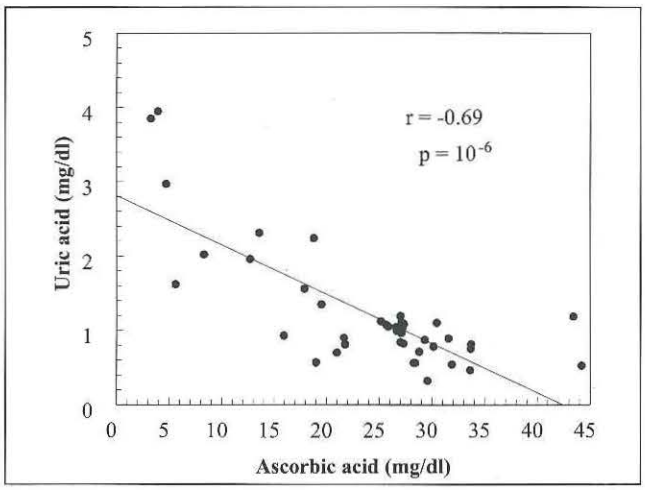

Figure 3. Relative peak absorption of other chemicals in aqueous humor at shorter wavelengths.

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HKJO Quiz

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Question

A 49-year-old gentleman had right tractional retinal detachment secondary to proliferative diabetic retinopathy. Posterior vitrectomy, membrane removal, encircling, endolaser and injection of 5700-centistoke silicone oil were done. He had uneventful recovery after the operation.

Fundal examination two months after operation showed retinal traction with shallow subretinal fluid inferior to the macula. Postoperative examination at three months revealed the same fundal findings, but with an open break inferior to the macula, which was tamponaded by silicone oil.

What is the diagnosis ?

(Answer and discussion on page 13)

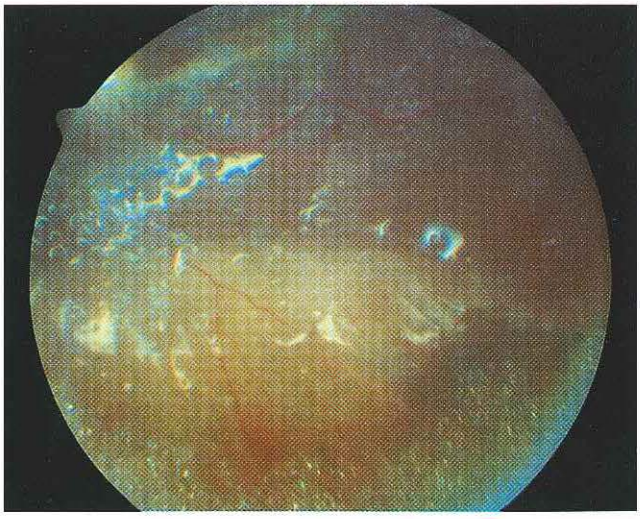

Figure 1. The fundal picture of the right eye eight months after the operation.