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Pentosidine and Autofluorescence in Lenses of Diabetic Patients

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Abstract: Pentosidine, an advanced glycation endproduct, may cause the increased fluorescence found in the lenses of diabetic patients. We measured the autofluorescence in human lenses, in vitro, and quantified the pentosidine to examine its relationship to the autofluorescence. Lens autofluorescence was higher in diabetic than non-diabetic subjects; pentosidine quantities were significantly higher in the diabetic than the non-diabetic group indicating that pentosidine was involved in the greater intensity of autofluorescence in the lenses of patients with diabetes mellitus. **Jpn J Ophthalmol 1997; 41:274–277** © 1997 Japanese Ophthalmological Society

Key Words: Autofluorescence, diabetes mellitus, glycation, pentosidine.

Introduction

Human lenses possess characteristic autofluorescence that intensifies with senescence.[1,2] The major fluorescent substances are oxidized metabolites of tryptophan[3,4] that emit fluorescence at 440 nm, with excitation at 340 nm. Yoshida[5] reported that the intensity of in vitro autofluorescence in lenses of diabetes mellitus (DM) patients is greater than in non-diabetic subjects. Pentosidine, an advanced glycation endproduct produced as the result of a continuous high glucose level, emits fluorescence at 385 nm after excitation at 335 nm and is believed to be responsible for the increased fluorescence in DM patients. In this study, we measured the autofluorescence of human lenses, in vitro, and quantified pentosidine to evaluate the effect of pentosidine on lens autofluorescence and to examine the relationship between the enhanced Maillard reaction (glycation) and diabetic cataracts (Figure 1).

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Subjects and Methods

Ten lenses from patients with diabetic cataracts (DM group; average age, 65.1 ± 11.9) and nine lenses from patients with senile cataracts (non-DM group; average age, 67.1 ± 10.1) with similar degrees of opacity, were studied. Patients who were receiving hemodialysis or treatment for other diseases were excluded. Study procedures were in accord with the principles of the Declaration of Helsinki, 1975, revised in 1983. Whole cataractous lenses, obtained by ultrasonic emulsification and absorption, were treated with additional ultrasound to ensure complete emulsification for measurement samples.

Autofluorescence was measured by fluorophotometer (RF-540; Shimizu, Kyoto) with pentosidine excitation and emission wavelength settings of 335 and 385 nm (ordinate scale: ×1). Pentosidine was then quantified using the technique described by Takahashi et al.[6]

Lens samples (1 mL) were mixed with an equal volume of 12 mol/L HCl and hydrolyzed under nitrogen at 110°C for 20 hours in a sealed glass tube. Hydrolysates were filtered with 0.45 μm pore size membrane filters (DIS-MIC-25cs; Toyo Roshi, Tokyo). The hydrolysate (200 μL) was mixed with 20 mL water and added to an 0.8 × 1.0 cm SP-Sephadex

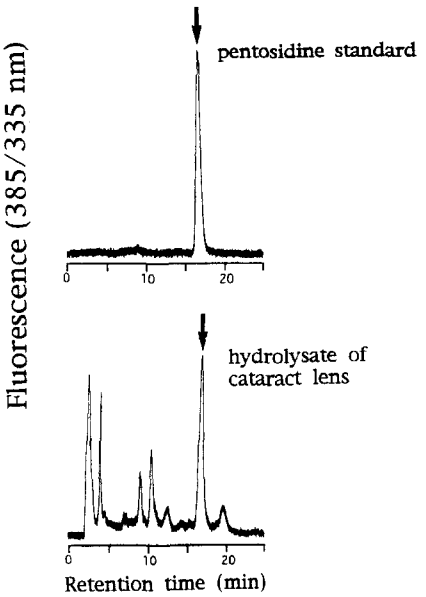

Figure 1. Chromatograms: pentosidine standard and hydrolysate of cataract lens.

C-25 column (Pharmacia LKB, Uppsala, Sweden) equilibrated with water. The column was washed with 20 mL of 0.1 mol/L HCl and eluted with 5 mL 1.0 mol/L HCl. The eluate was evaporated under reduced pressure in a TC-8 concentrator (Taitec, Tokyo) and the residue dissolved in 200 μL 1% n-heptafluorobutyric acid (HFBA). Water used was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Pentosidine was quantified by HPLC (high performance liquid chromatography); the volume of each sample used was 100 μL. The HPLC system included an LC-6A pump, RF-535 fluorescence HPLC monitor, and an SCL-6A system controller (all: Shimizu, Kyoto). A prepacked column with Radial-Pack C_{18}, 10 μm particles (8C1810μ; Waters, Milford, MA, USA) was used. The mobile phase used acetonitrile and 40 mmol/L HFBA (27:73, vol: vol); flow rate was 1.0 mL/min at room temperature. Pentosidine was detected by measurement of fluorescence at 385 nm with excitation at 335 nm. Mann-Whitney U Tests were used for statistical analysis.

Results

In our study, a fluorophotometer with pentosidine-specific wavelength settings was used for lens autofluorescence measurement. We found that the tendency toward autofluorescence was greater in the DM group (3533 ± 12) than the non-DM group (2993 ± 1188) (Figure 2). The amount of pentosidine was significantly higher in the DM group (168.5 ± 101.0) than the non-DM group (81.3 ± 58.6) (pmol/lens; $P < 0.05$; Figure 3). There was a significant correlation between fluorescence intensity and the amount of pentosidine ($r = 0.59$; $P < 0.01$).

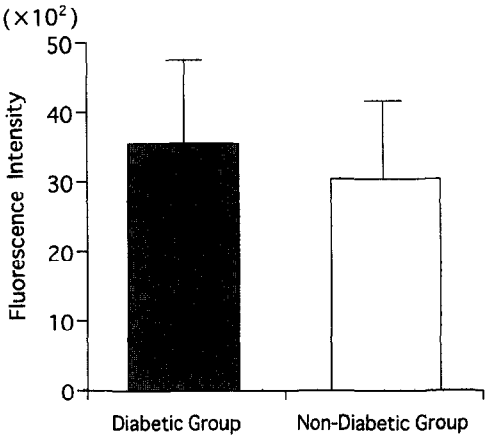

Figure 2. In vitro autofluorescence in human cataractous lenses (Ex 335 nm, Em 385 nm).

Discussion

Glycation (the Maillard reaction in vivo) begins with a reduced sugar such as glucose reacting with the lysine residues of proteins.[7,8] Ketoamine, a slightly stable Amadori compound, is formed in the early stage of the reaction; pentosidine is formed as one of the end-stage metabolites.

Pentosidine was first extracted from cerebral dura mater by Sell and Monnier[9] in 1989. They described it as a fluorescent crosslink consisting of lysine and arginine residues linked by pentose; pentosidine is believed to increase with aging.[9] It is a very stable

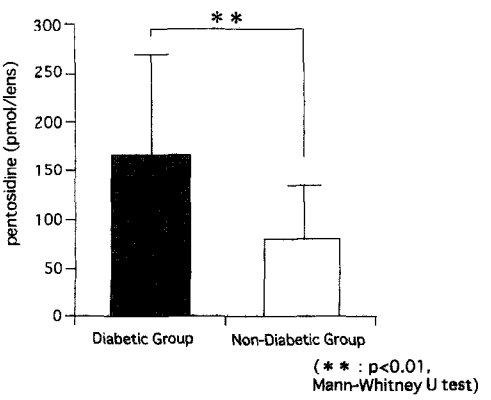

Figure 3. Amount of pentosidine in human cataractous lenses.

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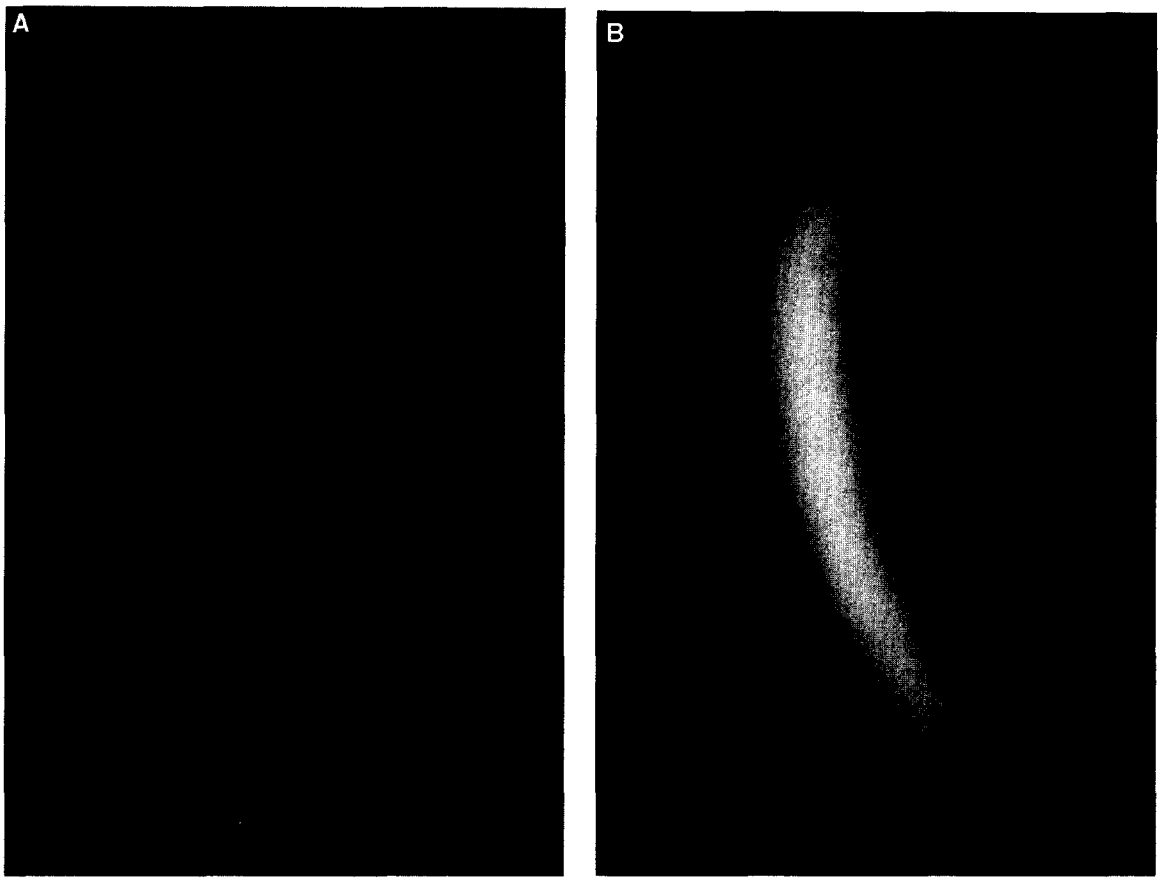

Figure 4. Autofluorescence in human lenses (Ex 340 nm, Em 440 nm). Both lenses obtained from 65-year-old women. (**A**) Lens from a normal subject. (**B**) Lens from a diabetic patient.

substance that can resist up to 20 hours of high temperature, as well as strong acids. It emits fluorescence at 385 nm wavelength when excited at 335 nm. It is also reported to increase in the skin collagen with aging, and to be higher in serum from patients with nephropathy.[10] Because it is a crosslink substance, a relationship between the occurrence and progression of diabetic cataracts is suspected.[8] While

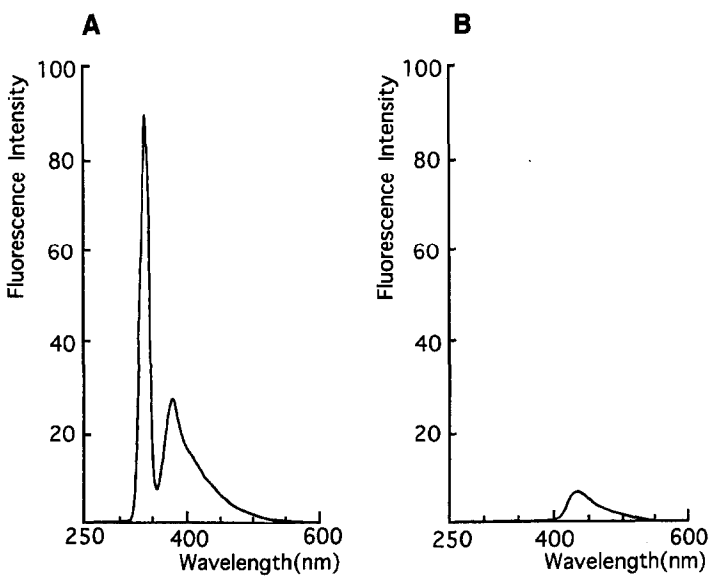

Figure 5. (**A**, **B**) Fluorescence intensity of pentosidine standard (Ex 335 nm). (**B**) Measured through WRATTEN gelatin filter number 2A.

autofluorescence is known to increase with aging, Yoshida[5] found it to be higher in the lenses of diabetic than non-diabetic patients, using a modified anterior ocular camera (Figure 4). This is believed to result from a fluorescent substance formed in the late stage of glycation. Similar advanced glycation endproducts currently identified include pentosidine, pyraline, and crossline (Ex: 379 nm; Em: 463 nm), however, pyraline is reportedly nonfluorescent.

We, therefore, concentrated on examining pentosidine, measuring its fluorescent intensity in a preliminary study using a gelatin filter #2A (Figure 5). This filter blocks wavelengths below 405 nm and was used in the anterior ocular camera by Yoshida[5] to eliminate the effect of false fluorescence when autofluorescence is measured in lenses in vivo. With this filter, we were able to identify the fluorescence of pentosidine: the fluorescent image we obtained, blue with a wavelength of about 440 nm and excitation of intravital ultraviolet at 340 nm, incudes the pentosidine fluorescence as well as that of recently reported oxidative metabolites of tryptophan.[3]

The present study has demonstrated that the intensity of fluorescence attributable to pentosidine is higher and that a greater amount of pentosidine is found in the cataractous lenses of patients with DM, confirming its relationship to the higher autofluorescence observed in such lenses.

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References

1. Satoh K, Bando M. Fluorescence in human lens. Exp Eye Res 1993;16:167–72.
2. Kojima M, Sasaki K. In vivo measurement of human crystalline lens fluorescence. J Kanazawa Med Univ 1985;10:229–85.
3. Iwata S. Metabolism of tryptophan and fluorescent compounds: Suisyoutai (crystalline lens). Tokyo: Medical Aoi Publishers; 1986.
4. Inoue A, Satoh K. Identification of a new fluorescent compound isolated from human lens insoluble protein fraction. Bioorg Med Chem Lett 1993;3:345–6.
5. Yoshida S. Quantification of autofluorescence in human lens. J Dokkyo Med Univ 1994;10:31–42.
6. Takahashi M, Kushida K, et al. Quantification of cross-link pentosidine in serum from normal and uremic subjects. Clin Chem 1993;39:2162–5.
7. Fumitaka H. The Maillard reaction in aging. Saishin Igaku (latest medicine) 1994;49:1385–91.
8. Kato H. In vivo Maillard reaction and disease. Pharmacia 1992;28:466–70.
9. Sell DR, Monnier VM. Structure elucidation of a senescence cross-link from human extracellular matrix. J Biol Chem 1989;264:21597–602.
10. Koji N. The role of glycation in the development of diabetic nephropathy. Kidney and Dialysis 1994;37:699–703.