学 位 論 文 の 要 旨

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学 位 論 文 名　Advanced Glycation End Product Accumulation in Subjects With Open-Angle Glaucoma With and Without Exfoliation

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論 文 内 容 の 要 旨

INTRODUCTION

Progressive optic neuropathy and visual field loss characterize glaucoma, a leading cause of irreversible blindness worldwide including Japan. In open-angle glaucoma (OAG) such as primary OAG (PG) and glaucoma secondary to pseudoexfoliation syndrome (EX), reduction of aqueous humor outflow at the trabecular meshwork (TM) is the main reason for the Intraocular pressure (IOP) elevation. This can be the result of TM cells dysfunction and changes in the amount and quality of the extracellular matrix in the TM, which has been suggested that oxidative stress is involved. We previously identified a significantly lower antioxidant capacity level and higher hydroxylinoleates, oxidation products of the linoleates, in blood samples from subjects with PG and EX as compared with those in control samples. Interestingly, the lower level of blood antioxidant capacity was associated with higher IOP values in patients with glaucoma and control subjects and with worse visual field defects in OAG. More recently, we reported that supplementation with French maritime pine bark/bilberry fruit extracts, which are natural antioxidants compounds, could further reduce the IOP in Japanese patients with controlled PG.

Advanced glycation end products (AGEs), which are the products of a non-enzymatic reaction between reducing sugars and other macromolecules, are critical in aging, as well as metabolic and degenerative diseases. AGEs have been measured in the human body noninvasively on the skin by detecting the skin autofluorescence (sAF) which is highly correlated with AGEs. AGEs formation and accumulation are associated with metabolic diseases such as diabetes mellitus and physiologic changes such as aging. AGEs have been presumed to be associated with the pathophysiologic processes of ocular diseases, such as cataract, diabetic retinopathy, and age-

related macular degeneration. In addition, glaucoma is an age-related chronic neurodegenerative disease affected by oxidative stress, and AGEs accumulate in the optic nerve head and RGC layer. Therefore, we considered that AGEs are associated with the pathogenesis of glaucoma and measured the sAF levels to assess the possible involvement of AGEs in glaucoma.

MATERIALS AND METHODS

The study protocol was approved by the Research Ethics Committee of the Matsue Red Cross Hospital and the Iinan Hospital.

Subjects were recruited consecutively at the Division of Ophthalmology, Matsue Red Cross Hospital, and the outpatient clinic at the Iinan Hospital. A total of 576 eyes of 576 Japanese subjects (254 men and 322 women, mean age ± standard deviation (SD) 71.6 ± 12.8 years) comprised the study population, i.e., subjects with PG (n = 316), exfoliation glaucoma (EG) (n = 127), and non-glaucomatous controls (n = 133). Since the association between diabetes and AGEs has been well documented, patients with severe diabetes that required insulin and was associated with the presence of diabetic retinopathy were excluded. To estimate AGEs, the participants underwent measurements of the sAF levels using the AGEs Sensor (Air Water Biodesign Inc., Kobe, Japan). The sAF values obtained with the excitation and emission wavelengths of 365 nm and 440 nm, respectively, were used to estimate the levels of AGE accumulation. The obtained sAF levels are correlated positively with the level of the hyperglycemia-associated AGE, N δ -(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) which reflect the AGEs accumulation in tissues. The finger clip feature of the device enables measurement of the intensity of the fluorescence from the middle finger of the non-dominant hand where the least skin melanin is present. Although the intensity of autofluorescence from veins is approximately 1.5-fold higher than that of skin, the influence of the veins is negligible in the measurement of the sAF using the AGEs Sensor, since the fingertips have only capillaries and no veins. Therefore, the fingertip is suitable to avoid non-specific skin fluorescence. Trained examiners performed all measurements.

RESULTS AND DISCUSSION

In demographic subject data, there were significant differences among three groups (control, PG, and EG) in almost all parameters (age, sex, the best-corrected visual acuity (BCVA), highest IOP (in the history of medical records to date), the number of glaucoma medications, Mean deviation (MD), lens status (phakia or pseudophakia), diabetes, and hypertension), but not in current smoking habits and IOP (on the day of the AGE measurement).

In the comparisons of AGEs among the three groups, the AGEs levels of the EG group (0.61 ±0.11) were significantly higher than those of the control group (0.56±0.15, $p = 0.0007$) and those of the PG (0.56±0.11, $p < 0.0001$) group, whereas the levels were equivalent in the control and PG groups ($p = 0.5120$).

In possible associations among AGEs and various parameters, age was correlated positively

with the AGEs ($r = 0.11$, $p = 0.0063$), while the BCVA, IOP on the day of the AGE measurement, highest IOP recorded, and number of glaucoma medications used were not correlated with the AGEs. Male sex, pseudophakia, the presence of pseudoexfoliation, diabetes, and systemic hypertension, and no current smoking were associated with higher AGE levels than their corresponding groups.

In the correlations between AGEs and various parameters analyzed by multiple regression analysis to adjust for possible confounding effects among parameters, male sex (standard β = 0.23), EG (0.19), and diabetes (0.09) were correlated with higher AGE levels, while PG (-0.18) and smoking habits (-0.19) were correlated with lower AGE levels. Age, BCVA, IOP, highest IOP, glaucoma medications, lens status, and systemic hypertension were not associated with AGEs.

Our results clearly indicated that subjects with EG had higher AGE levels than those with PG and controls. The specific AGEs such as N ε -(carboxymethyl)-lysine (CML) and pentosidine formed not only as a result of glycation, that is, the Maillard reaction, but also as a result of oxidation. CML was detected in human lens capsules with EX. Although patients with both PG and EG had reduced systemic antioxidant capacity as compared with controls, our previous studies have indicated that the oxidative pathways of PG and EG differ; comprehensive measurement of the serum levels of hydroxylinoleate isomers have found that the enzymatic and singlet oxygen-mediated fatty acid oxidation could be the major oxidation pathways in patients with PG, whereas free radical-mediated oxidation could be a major oxidation pathway in EG. During the Amadori rearrangement, reactive, intermediate products known as α -dicarbonyls or oxoaldehydes including 3-deoxyglucosone and methylglyoxal (MGO) were formed. MGO has also been formed from non-oxidative mechanisms in anaerobic glycolysis and from oxidative decomposition of polyunsaturated fatty acids. Evidence for this came from in vitro experiments in which antioxidants resulted in a reduction in CML formation; metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of proteins led to CML formation. This result suggested that non-enzymatic lipid oxidation had a role in the formation of AGEs. Accordingly, the difference in the underlying oxidation mechanisms between PG and EG could be associated with the significant elevation of AGEs in EG.

CONCLUSION

In conclusion, the levels of AGEs in subjects with EG were higher than those with PG and non-glaucomatous control subjects. This result suggested that specific oxidation and glycation mechanisms underlie the pathogenesis of glaucoma associated with EX.