Effect of Diabetic Retinopathy on Redox State of Aqueous Humor and Serum Albumin in Patients with Senile Cataract

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Purpose:
To investigate the oxidative status of albumin in the aqueous humor and serum of senile cataract patients with diabetes in order to clarify the pathogenesis of this condition.

Methods:
High-performance liquid chromatography (HPLC) was employed to measure the reduced form of albumin (mercaptalbumin) and the oxidized form of albumin (nonmercaptalbumin) in serum and aqueous humor. The mercaptalbumin, nonmercaptalbumin-1, and nonmercaptalbumin-2 fractions in aqueous humor obtained at the start of cataract surgery and in serum obtained intraoperatively were analyzed by HPLC in 7 senile cataract patients with diabetic retinopathy (2 men and 5 women aged 70 ± 9.8 years).

Results:
The mean content (%) of mercaptalbumin, nonmercaptalbumin-1, and nonmercaptalbumin-2 in serum albumin from the diabetic patients was 60.3 ± 7.8, 36.9 ± 6.6, and 2.7 ± 1.7%, respectively, while the corresponding values for aqueous humor albumin were 40.0 ± 14.1, 52.4 ± 12.6, and 7.5 ± 3.6%.
When the mercaptalbumin content (%) of aqueous humor albumin was compared between patients with active and inactive diabetic retinopathy, the respective values were 47.0 ± 9.1% and 22.8 ± 5.7%. A significant correlation mercaptalbumin content (%) of aqueous humor albumin did not show with the HbA1c level, but there was still a relationship ($Y = 5.0 \times - 2.7$, $r = 0.80$, and $p < 0.052$).

Conclusion:
The increase of mercaptalbumin (the reduced form of albumin) in the aqueous humor of patients with diabetic retinopathy probably resulted from an increase of retinal vascular permeability.

Key words : Blood-aqueous barrier, Diabetic retinopathy, Aqueous humor albumin, Serum albumin, Oxidative status

INTRODUCTION

Diabetes is known to induce oxidative stress in the crystalline lens, which is one of the factors causing the onset and progression of cataract [3], and glycosylation and oxidation of the sulfhydryl residues (−SH) of lens proteins have been reported [4, 6, 12, 17]. However, whether diabetes also influences the oxidative status (redox state) of aqueous humor has not been investigated, even though the elevation of serum glucose is associated with an increased glucose level in the aqueous humor [18, 19]. When we examined albumin from the aqueous humor of senile cataract patients in our previous study [9], a decrease in the content of reduced albumin (mercaptalbumin) and an increase in the content of oxidized albumin (nonmercaptalbumin) were found relative to these components of serum albumin [7, 8]. Albumin is a protein that can act as a potent

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sacrificial antioxidant by being oxidized to nonmercaptalbumin. Therefore, it is important to clarify the influence of diabetes on the oxidative status of aqueous humor albumin in senile cataract patients.

Accordingly, the present study examined the oxidative status of albumin in the aqueous humor and serum of senile cataract patients with diabetes.

MATERIALS AND METHODS

Seven cataract patients with diabetic retinopathy (2 men and 5 women ranging in age from 58 to 87 years; mean ± S.D.: 70 ± 9.8 years) were admitted to Ohgaki Municipal Hospital for routine cataract surgery. Informed consent to this study was obtained in all cases. Each patient underwent phacoemulsification and intraocular lens implantation. Approximately 150 μl of aqueous humor was removed through a small corneal limbus incision at the beginning of the operation, with special care being taken to avoid contamination of the specimen with blood. In addition, approximately 10 ml of blood was collected during surgery. The specimens were stored at − 80 ℃ until analysis [8].

HPLC-FD was performed with a system that consisted of a Model AS-8010 autosampler, a Model CCPM double-plunger pump, and a Model FS-8000 fluorescence detector (excitation at 280 nm and emission at 340 nm) combined with a Model SC-8020 system controller (all from Tosoh Co., Tokyo, Japan). A Shodex-Asahipak ES-502N column (10 × 0.76 cm I.D., DEAE column for ion-exchange HPLC, Showa Denko, Co., Tokyo, Japan; column temperature, 35 ± 0.5 ℃ was used. Linear gradient elution was carried out by increasing the ethanol concentration from 0 to 5% for analysis of serum or from 0 to 10% for analysis of aqueous humor. The ethanol gradient was added to 0.05 M sodium acetate-0.04 M sodium sulfate (pH 4.85) (acetate-sulfate buffer) and the flow rate was set at 1.0 ml/min. Details of this method have been published elsewhere [9]. Samples (2 μl of serum or 50 μl of aqueous humor) were injected using an autoinjector.

The HPLC data were subjected to numerical curve fitting and each peak was approximated by a Gaussian function so that the area underneath the peak could be calculated. The mercaptalbumin and nonmercaptalbumin fractions were expressed as a percentage of the area underneath each peak to the total peak area (i.e., each peak area was divided by the total peak area). Results are shown as the mean ± S.D.

RESULTS

Albumin was separated into three components by HPLC. Figure 1 shows representative HPLC profiles of serum albumin (a) and aqueous humor albumin (b) from one of the patients (Case No. 2; 59 years old). It is clear that aqueous humor albumin shows considerable quantitative and qualitative differences from serum albumin. Peak 1 was mercaptalbumin, while peaks 2 and 3 represented nonmercaptalbumin 1 and 2, respectively.

The mean percentages of mercaptalbumin and nonmercaptalbumin in serum albumin and aqueous humor albumin from the 7 senile cataract patients with diabetic retinopathy are summarized in Table 1. The mean content of peak 1 (mercaptalbumin), peak 2 (nonmercaptalbumin-1), and peak 3 (nonmercaptalbumin-2) in serum albumin was respectively 60.3 ± 7.8, 36.9 ± 6.6, and 2.7 ± 1.7%, while these values were 40.0 ± 14.1, 52.4 ± 12.6, and 7.5 ± 3.6% for aqueous humor albumin. These results indicate that serum albumin was mostly mercaptalbumin, while aqueous humor albumin was predominantly nonmercaptalbumin. Table 2 summarizes the clinical data on the 7 patients.

Active diabetic retinopathy was present in 5 of the 7 patients, while inactive retinopathy was seen in 2 patients [11]. HbA1c, an indicator of medium term glycemic control, was high (≧9.0) in 4 out of 7 patients. When these parameters were compared with the mercaptalbumin (%) content of aqueous humor albumin, it was found to be lower in the patients with inactive diabetic retinopathy (Fig. 2). When the aqueous humor mercaptalbumin content (%) was compared with HbA1c, no significant correlation was obtained (Fig. 3) (Pearson's correlation coefficient $r = 0.80$, $p < 0.052$: $Y = 5.0 \times - 2.7$, where Y is mercaptalbumin (%) and X is HbA1c).

Although correlations were also examined between retinal laser photocoagulation or insulin treatment before cataract surgery

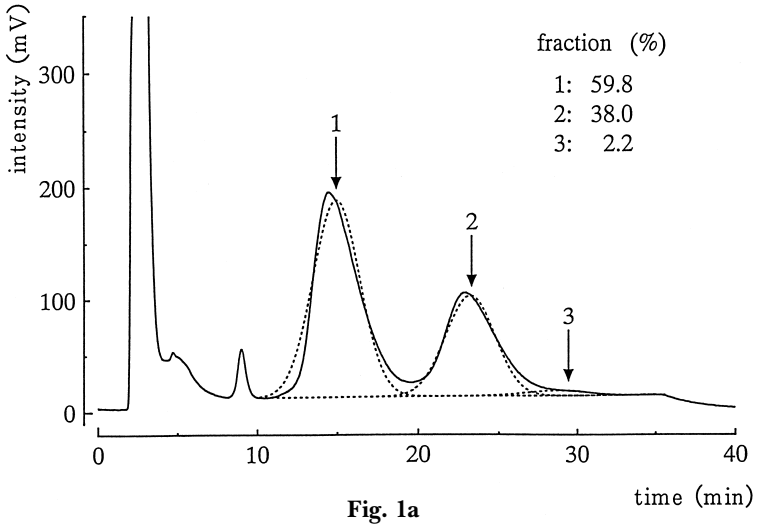

Fig. 1a

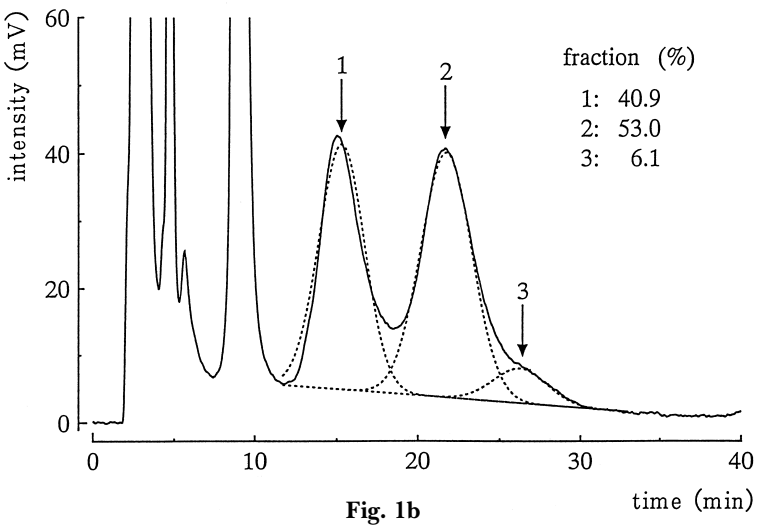

Fig. 1b

Fig. 1 HPLC profiles of (a) serum and (b) aqueous humor from a senile cataract patient with diabetic retinopathy

The data was abianise from patient MT, 59 years old.

An ES-502N column was eluted with an ethanol gradient (0 to 5% for serum and 0 to 10% for aqueous humor) in acetate-sulfate buffer (pH 4.85). Peaks 1, 2, and 3 correspond to mercaptalbumin, nonmercaptalbumin-1, and nonmercaptalbumin-2 (NA-2), respectively. The HPLC profile was subjected to numerical curve fitting (broken line) and the values obtained for each fraction (%) are shown at the top right corner of the Figure. The scale for serum albumin is about 5 times larger than that for aqueous humor because the concentration of total albumin is markedly higher in the serum than in the aqueous humor.

Peak 1 depicts mercaptalbumin, peak 2 depicts nonmercaptalbumin-1, and peak 3 depicts nonmercaptalbumin-2. Numbers shown in the top right corners of Figs 1a and b are included in liste in Table 1.

Table 1 Percentages of mercaptalbumin, nonmercaptalbumin-1, and nonmercaptalbumin-2 in serum and aqueous humor from 7 senile cataract patients with diabetic retinopathy

			Serum			Aqueous humor		
Case No.	Sex	Age	peak 1	peak 2	peak 3	peak 1	peak 2	peak 3
1. (T. K.)	M	87	49.5	44.0	6.5	50.0	38.2	11.8
2. (M. T.)	M	59	59.8	38.0	2.2	40.9	53.0	6.1
3. (K. W.)	F	58	70.2	28.1	1.7	36.6	53.6	9.8
4. (S. O.)	F	69	66.8	31.3	1.9	60.2	37.2	2.6
5. (T. G.)	F	74	51.3	45.7	3.0	47.2	49.7	3.1
6. (T. T.)	F	69	59.7	38.7	1.6	18.8	72.7	8.5
7. (Y. T.)	F	73	65.1	32.7	2.2	26.8	62.6	10.6
Mean		70	60.3	36.9	2.7	40.0	52.4	7.5
S.D.		9.8	7.8	6.6	1.7	14.1	12.6	3.6

peak 1: mercaptalbumin, peak 2: nonmercaptalbumin-1, peak 3: nonmercaptalbumin-2

Table 2 Clinical data of patients with diabetic retinopathy

Patient No.	Aqueous humor fraction of mercaptalbumin (%)	Grade of Diabetic retinopathy	Retinal PC	HgA1c	Insulin Treatment
1	50.0	Active	⊖	10.0	⊖
2	40.9	Active	⊖	9.4	⊕
3	36.6	Active	⊖	6.4	⊕
4	60.2	Active	⊕	9.9	⊕
5	47.2	Active	⊖	11.7	⊖
6	18.8	Non active	⊕	6.4	⊕
7	26.8	Non active	⊕	6.0	⊕

HgA1c = hemoglobin A1c PC = photo coagulation

and the mercaptalbumin content of aqueous humor, no significant relationship was detected (Table 2) [13].

DISCUSSION

This is the first study reporting the oxidative status of albumin in the aqueous humor of patients with diabetic retinopathy. In these diabetic patients, the oxidized forms of serum albumin accounted for about 40% of the total albumin. We previously examined the oxidative status of albumin in non-diabetic patients [9] and found that oxidized albumin was only 34%. Thus, it is conceivable that oxidized form of albumin in serum is uniquely increased in patients with diabetic retinopathy. As already reported, diabetic patients have increased levels of active oxygen species, such as H_2O_2, superoxide or hydroxyl radical, due to oxidative stress associated with hyperglycemia [12, 18], and the albumin acted as a radical scavenger (sacrificial oxidant). Therefore, out results may reflect the oxidative stress associated with diabetic hyperglycemia. However, in the patients with diabetic retinopathy, the level of oxidized albumin was decreased, and that of reduced albumin was increased. The previous study showing that, in the aqueous humor of non-diabetic patients, oxidized albumin accounted for the majority (97% [9]) is in apparent disagreement with the present study. A plausible explanation for this disagreement is the effect of vascular

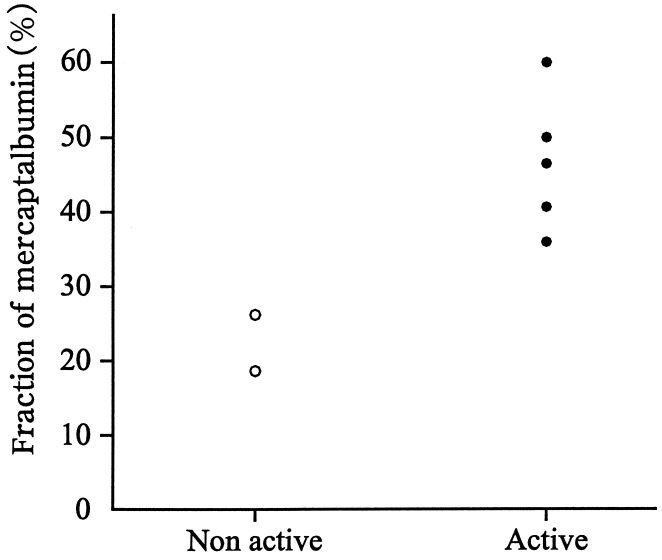

Fig. 2 The grades of diabetic retinopathy and fraction of mercaptalbumin in aqueous humor

The fraction of mercaptalbumin content of aqueous humor tended to be higher in patients with active diabetic retinopathy than in those with non active diabetic retinopathy, although number of patient is still limited. ○: inactive diabetic retinopathy, or ●: active diabetic retinopathy.

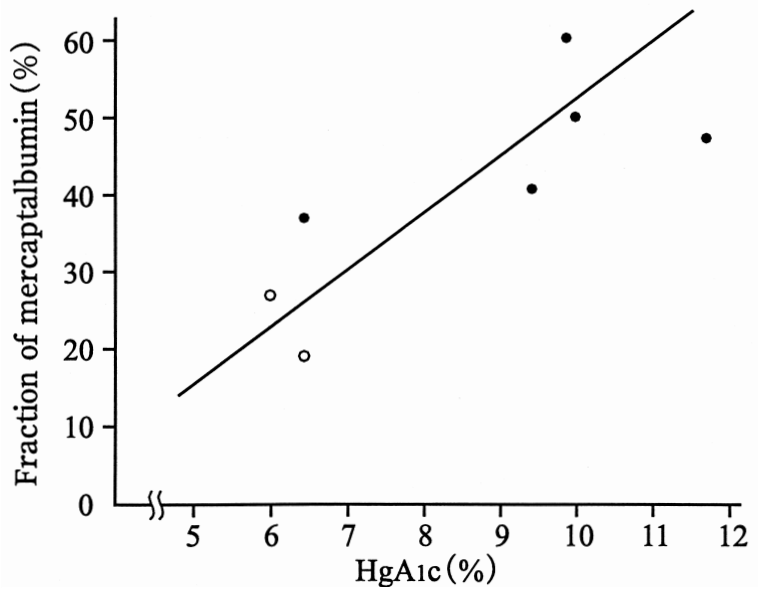

Fig. 3 Comparison between fraction of mercaptalbumin in aqueous humor and HgA1c

The aqueous humor mercaptalbumin (%) was compared with HbA1c, a parameter indicating glycemic control, a significant correlation was not cbtained (Pearson's correlation coefficient $r = 0.80$, $p < 0.052$: $Y = 5.0 \times - 2.7$, where Y is mercaptalbumin (%) and X is HbA1c).
○: inactive diabetic retinopathy, or ●: active diabetic retinopathy.

endothelial growth factor (VEGF) since neovascularization occurs during the progression of diabetic retinopathy, and an increase in VEGF in relation to neovascularization has been demonstrated. Aiello *et al.* reported that an increase of VEGF was observed in the vitreous and the aqueous humor of patients with retinopathy [1, 2]. VEGF is also referred as vascular permeability factor (VPE), since it increases vascular permeability. Therefore, VEGF may increase vascular permeability in diabetic patients, thus leading to an increase of mercaptalbumin in the aqueous humor.

Tanaka *et al.* reported that the VEGF level of patients with active proliferative diabetic retinopathy was significantly higher than that of patients with non-proliferative diabetic retinopathy [20, 21]. Therefore, as was done by Tanaka *et al.*, we divided our patients into groups with and without active diabetic retinopathy for comparison of mercaptalbumin content in the aqueous humor (Fig. 2). Retinopathy associated with neovascularization of the retina and vitreous hemorrhage is defined as active diabetic retinopathy, and while the absence of proliferative changes is termed inactive diabetic retinopathy [11].

We found that mercaptalbumin accounted for 47.0 ± 9.1% of aqueous humor albumin in patients with active diabetic retinopathy versus 22.8 ± 5.7% in patients with inactive retinopathy. Although no significant difference was observed between the 2 groups due to the small number of the patients, the active diabetic retinopathy group tended to have a higher mercaptalbumin content in their aqueous humor.

It therefore seems likely that the increased vascular permeability in active retinopathy results in an increase of mercaptalbumin in aqueous humor [10, 14–16].

In the present study, we compared HbA1c (a parameter indicating glycemic control) with the mercaptalbumin content of aqueous humor albumin in cataract patients with diabetic retinopathy to study the influence of control of DM on the blood-aqueous barrier in the eyeball [5, 19]. As shown in Fig. 3, it was found that the mercaptalbumin content of aqueous humor albumin was 40% or more in patients with high HbA1c values of 9 or more, probably because poorly controlled diabetes led to breakdown of the blood-aqueous barrier.

According to the reports of Moriarty and Inoue who made an indirect assessment of aqueous humor changes using a laser flare cell meter, diabetic patients with more severe retinopathy showed more damage to the blood-aqueous barrier [10, 15]. In this study, we directly collected the aqueous humor and measured aqueous humor albumin by HPLC, finding that the relationship with HbA1c ($r = 0.80$ and $p < 0.052$) was not statistically significant. However, breakdown of the blood-aqueous humor was suggested by the fact that the HbA1c increased as the mercaptalbumin value became higher.

Determination of the oxidative status of albumin in aqueous humor revealed an increase of the reduced form of albumin in patients with diabetic retinopathy, which may result from increased vascular permeability. Progression of retinopathy and a high HbA1c level may result in a further increase of vascular permeability.

However, investigation of a larger numbers of patients is needed to confirm the validity of our observations.

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