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Higher Concentration of Transforming Growth Factor-β in Aqueous Humor of Glaucomatous Eyes and Diabetic Eyes

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Purpose: Diabetic retinopathy and glaucoma are the primary causes of acquired blindness. Cytokines including transforming growth factor (TGF)-β may be involved in these diseases. We therefore collected aqueous humor samples from patients with glaucoma and/or diabetes who were undergoing surgery, and determined the concentration of TGF-β.

Methods: Aqueous humor samples were collected from 80 patients (84 eyes), including 19 eyes with primary open-angle glaucoma (POAG), 22 eyes with diabetes, and 18 eyes with diabetes complicated with POAG. Twenty-five eyes with cataract served as controls. The concentration of TGF-β1 or TGF-β2 was measured by enzyme-linked immunosorbent assay.

Results: The concentration of TGF-β1 was less than 0.1 pg/mL in all of the groups. In contrast to controls who had 1001.4 ± 444.1 pg/mL, the concentration of total TGF-β2 in the diabetes group was 1715.6 ± 882.1 pg/mL, and that in the diabetes complicated with POAG group was 1692.9 ± 361.9 pg/mL. These were significantly higher than that in controls. In contrast to the controls who had 321.2 ± 197.9 pg/mL, the concentration of mature TGF-β2 with POAG was 822.5 ± 484.4 pg/mL, and that of diabetes complicated with POAG was 1058.9 ± 648.4 pg/mL. These were significantly higher than that in the controls. The eyes with diabetes complicated with POAG also had a significantly higher concentration than the eyes with diabetes alone.

Conclusion: Total TGF-β2 and mature TGF-β2 in high concentration may correlate with progression of POAG, diabetes, and diabetes complicated with POAG. **Jpn J Ophthalmol 2002;46:249–253** © 2002 Japanese Ophthalmological Society

Key Words: Aqueous humor, diabetes mellitus, enzyme-linked immunosorbent assay, primary open-angle glaucoma, transforming growth factor-β.

Introduction

Transforming growth factor-β (TGF-β) is a cytokine that acts upon proliferation, migration, differentiation, and apoptosis of cells, and accumulation of extracellular matrix components. TGF-β is synthesized as a precursor formed by about 400 amino acids, consisting of signal peptides, latency-associated peptide (LAP), and mature TGF-β. The precursor forms a dimer by disulfide bonds and then is secreted as latent TGF-β. Latent TGF-β is activated when 112 peptides are removed from its carboxyl terminus by acid, heat or enzymes, and becomes mature TGF-β.[1] In mammals, three isoforms of TGF-β have been identified: TGF-β1, TGF-β2, and TGF-β3. Their biological functions seem to be different according to a study using knockout mice.[1]

In clinical ophthalmology, diabetic retinopathy and glaucoma are primary causes of acquired blindness. There are many patients who have diabetes complicated with primary open-angle glaucoma (POAG). It seems highly likely that cytokines including TGF-β are involved in this association, although the mechanism is unknown.

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Significant amounts of TGF-β have been detected in aqueous humor. It has been reported that while TGF-β1 is negligible in aqueous humor, TGF-β2 is present in sufficient concentration to allow determination.[2,3] In our study, we collected aqueous humor samples from patients with POAG and/or diabetes who were undergoing cataract surgery or combined surgery for cataract and glaucoma. We then determined the concentrations of total (mature plus latent) TGF-β1, total TGF-β2, and mature TGF-β2 in the samples, and compared the results between the groups.

Materials and Methods

Materials

In total, 84 eyes of 80 patients undergoing cataract surgery or combined surgery for cataract and glaucoma were included in this study. We obtained consent from all patients for the study protocol, which was approved by the institutional review board of Shin-Nagata Eye Institute and which conformed to the tenets of the Declaration of Helsinki.

Patients were classified into four groups: those with POAG (group G), those with diabetes (group D), those with diabetes complicated with POAG (group DG), and those with only cataract (group N, which served as control).

Aqueous humor was collected from all eyes undergoing surgery at our institution between January 9 and August 14, 1998. Total TGF-β1 and total TGF-β2 were determined in aqueous humor samples from 8 eyes of 7 patients (age range, 55–79 years, mean ± SD = 66.6 ± 6.9 years; 2 men and 6 women) in group G; 10 eyes of 10 patients (65–88 years, mean = 72.9 ± 7.2 years; 3 men and 7 women) in group D; 10 eyes of 7 patients (52–67 years, mean = 57.4 ± 5.8 years; 8 men and 2 women) in group DG; and 10 eyes of 10 patients (65–84 years, mean = 76.7 ± 6.6 years; 4 men and 6 women) in group N.

In group G, the average preoperative intraocular pressure (IOP) was 21.8 ± 2.7 mm Hg (mean ± SD). According to Kosaki's criteria, 1 eye had Ia visual field loss, 2 eyes had IIb visual field loss, 2 eyes had IIIa visual field loss, and 1 eye had IIIb visual field loss.

In group D, the average hemoglobin A1c (HbA1c) level was 8.2 ± 2.0% (mean ± SD). Six eyes in group D did not have diabetic retinopathy (DR); 4 eyes had simple diabetic retinopathy (SDR).

In group DG, the average preoperative IOP was 24.3 ± 6.2 mm Hg (mean ± SD). Four eyes had Ia visual field loss, 1 eye had IIb visual field loss, 4 eyes had IIIa visual field loss, and 1 eye had IIIb visual field loss. The average HbA1c level was 7.5 ± 2.9% (mean ± SD). Six eyes in group DG did not have DR; 1 eye had preproliferative diabetic retinopathy (PPDR), and 3 eyes had proliferative diabetic retinopathy (PDR).

Mature TGF-β2 was determined in aqueous humor samples from 11 eyes of 11 patients (61–87 years, mean = 74.5 ± 7.1 years; 5 men and 5 women) of group G, 12 eyes of 12 patients (49–83 years, mean = 65.5 ± 10.6 years; 5 men and 7 women) of group D, 8 eyes of 8 patients (58–87 years, mean = 71.6 ± 9.5 years; 5 men and 3 women) of group DG; and 15 eyes of 15 patients (51–85 years, mean = 71.6 ± 8.1 years; 8 men and 7 women) of group N.

In group G, the average preoperative IOP was 20.6 ± 4.9 mm Hg (mean ± SD). Two eyes had Ia visual field loss, 2 eyes had Ib visual field loss, 2 eyes had IIb visual field loss, 1 eye had IIIa visual field loss, 2 eyes had IIIb visual field loss, 1 eye had IV visual field loss, and 1 eye had Va visual field loss.

In group D, the average HbA1c level was 8.8 ± 2.6% (mean ± SD). Nine eyes did not have DR, 1 eye had SDR, 1 eye had PPDR, and 1 eye had PDR.

In group DG, the average preoperative IOP was 21.1 ± 5.2 mm Hg (mean ± SD). Three eyes had Ia visual field loss, 3 eyes had Ib visual field loss, 1 eye had IIIa visual field loss, and 1 eye had IV visual field loss. The average HbA1c level was 8.8 ± 1.9% (mean ± SD). Five eyes did not have DR, and 3 eyes had SDR.

The eyes used for measurement of total TGF-β1 and total TGF-β2 were different than those used for measurement of mature TGF-β2. No patient had undergone cataract surgery previously, and no glaucomatous eye had received laser trabeculoplasty before the surgery.

Collection of Aqueous Humor

At the time of cataract surgery or combined surgery for cataract and glaucoma, preceding the procedure, a wound for limbal paracentesis was made with a 20-gauge V-lance Knife® (Alcon Laboratories, Hemel Hempstead Herts, UK). Aqueous humor was aspirated by using a 27-gauge Top Needle® (Top, Tokyo) attached to a Terumo Syringe® (Terumo, Tokyo), taking care not to contaminate it with blood. Then the aqueous humor samples were quickly frozen, and stored at −20°C until analyzed.

Determination of TGF-β1 and TGF- β2

The concentrations of TGF-β1 and TGF-β2 in the aqueous humor samples were measured by enzyme-

linked immunosorbent assay (ELISA) with the Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA). To prepare samples for determination of total TGF-β1 and total TGF-β2, 0.125 mL of aqueous humor was mixed with 0.025 mL of 1N HCl, and left for 10 minutes at room temperature to allow activation of latent TGF-β. Then the mixture was supplemented with 0.025 mL of 1.2 N NaOH/0.5 M HEPES for neutralization, and diluted with 0.8 mL of Calibrator Diluent. To prepare samples for the determination of mature TGF-β2, 0.125 mL of aqueous humor was diluted with Calibrator Diluent without being treated with acid. Determination was performed immediately after dilution according to the ELISA kit protocol.

Statistical Analysis

Differences in TGF-β2 concentration between the groups were analyzed using the Bonferoni test program.[4]

Results

The concentrations of TGF-β1 were less than 0.1 pg/mL in the samples from all of the groups, and there was no difference between the groups.

The result of determination of TGF-β2 concentration in each group is shown in Figures 1 and 2. Concentrations of total TGF-β2 (mean ± SD) are shown in Figure 1. In contrast to group N, which had 1001.4 ± 444.1 pg/mL, group G had 1699.4 ± 346.3 pg/mL; group D, 1715.6 ± 882.1 pg/mL; and group DG, 1692.9 ± 361.9 pg/mL. Group D and group DG had a significantly higher concentration than group N, respectively ($P < .05$). Group G had a higher concentration than group N, but the difference was not significant. Figure 2 shows concentrations of mature TGF-β2 (mean ± SD). While the concentration was 321.2 ± 197.9 pg/mL in group N, it was 822.5 ± 484.4 pg/mL in group G; 564.2 ± 324.5 pg/mL in group D; and 1058.9 ± 648.4 pg/mL in group DG. Group G had a concentration about 2.5 times higher than group N, and the difference was significant ($P < .05$). The concentration in group D was higher than that in group N, but the difference was not significant. The concentration in group DG was as high as more than three times of that of group N, and the difference was significant ($P < .01$). Group DG also showed a significantly higher concentration than group D ($P < .05$).

The concentrations of total or mature TGF-β2 were not correlated with the age, the sex, the preoperative IOP, the extent of the visual field defect, or the severity of DR. Patients with high HbA1c level tended to have higher levels of TGF-β2, but the difference was not significant.

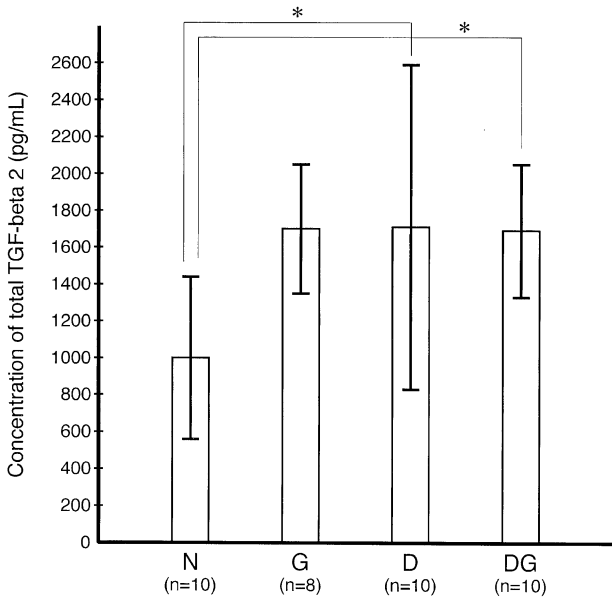

Figure 1. Concentration of total transforming growth factor (TGF)-β2 in aqueous humor. N: total TGF-β2 in normal aqueous humor: 1001.4 ± 444.1 pg/mL (mean ± SD). G: total TGF-β2 in primary open-angle glaucoma (POAG) samples: 1699.4 ± 346.31 pg/mL. D: total TGF-β2 in diabetic samples: 1715.6 ± 882.11 pg/mL. DG: total TGF-β2 in POAG and diabetic samples: 1692.9 ± 361.91 pg/mL. n: number of samples. *$P < .05$.

Discussion

It has been reported that the presence of TGF-β1 in aqueous humor is negligible and that the concentration of TGF-β2 can be determined.[2] Similar results were found in our study: we found the concentration of TGF-β1 to be less than 0.1 pg/mL, but we were able to determine TGF-β2. TGF-β1 is present in the blood at higher concentrations than in the aqueous humor.[5] It has been reported that mRNA for TGF-β2 is present and latent TGF-β2 is secreted in the trabecular cells of porcine eyes.[6] Therefore, TGF-β appears to be not only entering the eye from the blood, but also it is produced locally in the eye. The biological functions of TGF-β, including accumulation of extracellular matrix components and having a stimulatory effect in neovascularization, may be involved in the pathogenesis of diabetic retinopathy or glaucoma.

Tripathi et al reported that the concentrations of total TGF-β2 and mature TGF-β2 in aqueous humor were significantly higher in POAG eyes compared

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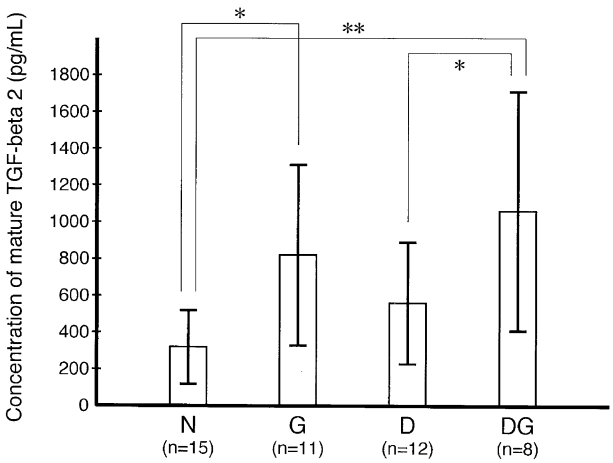

Figure 2. Concentration of mature transforming growth factor (TGF)-β2 in aqueous humor. N: mature TGF-β2 in normal aqueous humor: 321.2 ± 197.91 pg/mL (mean ± SD). G: mature TGF-β2 in POAG samples: 822.5 ± 484.41 pg/mL. D: mature TGF-β2 in diabetic samples: 564.2 ± 324.51 pg/mL. DG: mature TGF-β2 in POAG and diabetic samples: 1058.9 ± 648.41 pg/mL. n: number of samples. $*P < .05$, $**P < .01$.

with control eyes.[7] We showed that the concentration of total TGF-β2 tended to be higher, though not significantly, in POAG eyes than in control eyes, while the concentration of mature TGF-β2 was significantly higher in POAG eyes than in control eyes, being about 2.5 times higher. TGF-β promotes extracellular matrix production by acting upon fibroblasts, glial cells, and other cells, inhibits protease production, and promotes accumulation of extracellular matrix components by promoting production of protease inhibitors.[1,8] TGF-β also inhibits proliferation and migration of trabecular cells in vitro.[9] Trabecular cells express the mRNA for TGF-β2[6] and express receptors that bind to TGF-β2.[10,11] It was reported that TGF-β positively regulates the expression of mRNA for TGF-β1 in trabecular cells.[12] In POAG eyes, the production of latent TGF-β2 in trabecular cells may be enhanced for some reason. In addition, latent TGF-β2 may be readily converted to mature TGF-β2 by some mechanism different from any in normal eyes, resulting in a higher concentration of mature TGF-β2. A high concentration of TGF-β2 may increase the extracellular matrix of the aqueous outflow pathway, thus enhancing resistance to outflow. A high concentration of TGF-β2 may also enhance production of TGF-β2 in trabecular cells, which may increase resistance to outflow all the more. A series of such events may cause the progression of glaucoma.

Diabetic retinopathy, in its development and progression, appears to involve the biological action of TGF-β to promote neovascularization as well as to increase extracellular matrix. In our study, the mean concentration of total TGF-β2 was significantly higher in the eyes of diabetic patients compared with the control eyes, and the mean concentration of mature TGF-β2 was also higher (but not significantly). Hirase et al studied the concentration of TGF-β2 in the vitreous and reported that concentrations of both total and mature TGF-β2 in the vitreous collected during vitrectomy from patients with proliferative diabetic retinopathy were higher than those collected from the vitreous of patients with macular hole.[13] Another report showed that the vitreous of eyes with intraocular fibrosis associated with proliferative vitreoretinopathy contained higher levels of TGF-β2 than the vitreous from eyes with uncomplicated retinal detachment without intraocular fibrosis.[14] Preretinal proliferative membranes in the early stage of formation contain a large number of cells expressing TGF-β type 1 receptors.[15] These findings suggest that TGF-β plays a role in the formation of preretinal proliferative membranes. A higher concentration of TGF-β2 in the eyes of diabetic patients may result in the formation of neovascularization or preretinal proliferative membranes in the eye, which may play a role in the progression of diabetic retinopathy. In the eyes of diabetic patients, secretion of TGF-β2 not only seems to be enhanced in trabecular cells, but also in retina, retinal epithelium, and preretinal proliferative membranes formed due to diabetic retinopathy.[1] In the eyes of diabetic patients, secretion of TGF-β2 may be enhanced simultaneously in several areas by some mechanism different from that in POAG eyes.

In the POAG eyes of diabetic patients, not only was the concentration of total TGF-β2 significantly higher than in control eyes, but also the concentration of mature TGF-β2 was more than three times higher than in control eyes, showing a significant difference. These TGF-β2 levels were also higher than those in the eyes of diabetic patients without POAG. When production and activation of TGF-β2 are enhanced in the eyes of diabetic patients, production of TGF-β2 also seems to be enhanced in trabecular cells. Thus, extracellular matrix components may be overproduced and the resistance to aqueous outflow through the trabecular meshwork may increase, leading to the onset of POAG. There may be several reasons why TGF-β2 is increased in the eyes of diabetic patients, including increased accumulation of blood glucose, high HbA1c levels, and complications with PDR, but in our study we were unable to identify the reasons. Patients with poorly controlled high

levels of blood glucose tended to have higher levels of TGF-β2, but the differences were not significant. The high level of blood glucose may be one reason for the increase in production and activation of TGF-β2, although it could not be proven because we did not measure fasting blood glucose levels.

It is suggested that TGF-β2 plays a major role in the pathology and treatment of diabetes and POAG. If we can identify factors for the increase in secretion of TGF-β2 or for the activation of latent TGF-β2, it would be of great assistance in elucidating the pathology and treatment of POAG and diabetes as well as their complications.

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