Transforming Growth Factor-β Levels in Human Aqueous Humor of Glaucomatous, Diabetic and Uveitic Eyes

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Purpose: Transforming growth factor-β2 is known to be present at elevated levels in the aqueous humor of patients with primary open angle glaucoma (POAG) and diabetes but not in uveitis-related secondary glaucoma. We investigated total TGF-β2 levels and levels of the active form of TGF-β2 in the aqueous humor of eyes with different types of glaucoma.

Methods: The concentration of the total and active form of TGF-β2 was measured in 63 patients with primary open angle glaucoma, neovascular glaucoma complicated with diabetes (NVG), and secondary open angle glaucoma complicated with uveitis (SOAG) using a double antibody ‘sandwich-indirect’ ELISA method.

Results: The levels of total TGF-β2 in the aqueous samples of POAG, NVG, and SOAG were elevated. The levels of active TGF-β2 in the aqueous samples of POAG, and NVG were also elevated, whereas the level of active TGF-β2 was within the normal range in the aqueous samples of SOAG.

Conclusions: These results suggest that the level of TGF-β2 may play a role in the pathology of various types of glaucoma.

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Transforming growth factor-βs (TGF-βs) constitute a family of multifunctional polypeptides of approximately 25 kDa, and exhibit pleiotropic regulatory actions upon most vertebral cell types.1,2 Depending on the cell type, they regulate proliferation, migration, differentiation, cytokine production, synthesis of extracellular matrix (ECM), wound healing, immunosuppression, and in vivo angiogenesis.3 TGF-β exists in at least five genetically distinct isoforms, β1-β5.4 Among these, only three isoforms, namely β1, β2, and β3, are expressed in human ocular tissues.4

TGF-β2 is regarded as the major isoforms in the eye.2-4 Elevated levels of TGF-β2 have been detected in the aqueous humor of glaucomatous eyes,4,7 and reduced levels of active TGF-β2 have been detected in the aqueous humor of uveitic eyes.8

In this study, we evaluated levels of total TGF-β2 and the active form of TGF-β2 in the aqueous humor of patients with different types of glaucoma (POAG, NVG, and SOAG), using a sensitive and specific enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

1. Materials

Aqueous humor was collected in the operating room from 63 eyes of 63 human subjects (age range, 19 to 83 years; mean±standard deviation, 49.88±17.58) undergoing cataract or glaucoma surgery.

Patients were classified into four groups: those with POAG (group P; 14 patients), those with NVG complicated with diabetes (group N; 14 patients), those with SOAG complicated with uveitis (group S; 15 patients), and those with only cataract (group C, control group; 20 patients).

In group P, the average preoperative intraocular pressure (IOP) was 21.8±5.7 mmHg. The patients had established diagnosis of advanced POAG that correlated with advanced visual field loss. The POAG patients received 2.51±0.76 anti-glaucoma medications, and none of the patients had previous ocular surgery of any kind. In group N, the average
preoperative IOP was 33.7±6.2 mmHg. All patients had proliferative diabetic retinopathy with rubeosis iridis. The patients were treated with 2.23±1.18 anti-glaucoma medications and panretinal photocoagulation (PRP). The anterior segment neovascularization was reduced in nine patients, but peripheral anterior synecia formation progressed. Five patients had peripheral anterior synecia closure at the time of PRP. In group S, the average preoperative IOP was 27.3±4.7 mmHg. The patients had uveitis with anterior, intermediate, posterior, and panuveitis of different origins. In group C, patients underwent elective cataract extraction with intraocular lens implantation. All patients had a normal ocular examination. Patients were excluded if they were taking topical medication or had an ocular condition that was being treated with topical or systemic medications.

2. Interventions Preceding Aqueous Humor Sampling

All surgery was performed under local anesthesia, consist of 2% lidocaine hydrochloride, administered by retrobulbar and nasobath injection.

3. Collection of Aqueous Humor

All samplings were performed by the same surgeon. At the time of cataract or glaucoma surgery, a limbal paracentesis was made with a 20 G MVR knife (BD Ophthalmic systems, Bidford on Avon, Warks, B50 4JH, UK). Aqueous humor (100~200 μL) was aspirated into a tuberculin microsyringe prior to any incisional surgical procedures. All samples were rapidly frozen and stored in a -70°C freezer until analysis.

4. Detection and quantification of TGF-β2 by ELISA

The concentration of TGF-β2 in aqueous humor samples was quantified by a double antibody 'sandwich-indirect' enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, U.S.A.), with a sensitivity of 2 pg/ml. Intra- and inter-assay coefficients of variation for ELISA were within 7%. The concentration of TGF-β2 in the aqueous humor samples was determined by comparison with a standard curve of activity of standard TGF-β2.

Briefly, 100 μL standard TGF-β2 or aqueous humor sample was added to a 96-well ELISA plates coated with murine monoclonal anti-TGF-β2-antibodies. After 2 hours of incubation at room temperature, the wells were washed three times with 400 μL wash buffer. Each well was coated with 200 μL of polyclonal anti-TGF-β2 antibodies conjugated to horseradish peroxidase. The mixture was incubated and washed, as previously described. 200 μL substrate solution was added to each well, and the plate was incubated for 20 minutes at room temperature to allow the reaction to proceed. 50 μL stop solution was then added to each well. Absorbance at 450 nm was measured with an immunoreader system (BIO-TEK, Winooski, VT, USA).

5. Acid Activation of the Aqueous Humor Samples

For the determination of total TGF-β2, all of the aqueous humor samples were treated with 10 μL 1N HCl, and left for 10 minutes at room temperature to allow activation. Then, 10 μL 1.2 N NaOH/0.5 M HEPES was added for neutralization, and diluted with 340 μL Calibrator Diluent. Determination of total TGF-β2 was performed immediately.

6. Statistical Analysis

Statistical analysis was performed, using the Kruskall-Wallis
test program. A multiple comparison was conducted, using the Mann-Whitney test. Significant differences were defined by P values below 0.05.

**Results**

The concentration of total and active TGF-β2 in each group are shown in Fig. 1 and 2. The concentrations of total TGF-β2 (mean±standard deviation) in the aqueous humor are shown in Fig. 1. The mean concentration of total TGF-β2 in groups P, N, and S was 3824±890 pg/ml, 3264±221 pg/ml, and 1984±539 pg/ml, respectively. These values were significantly higher than that of group C (1392±177 pg/ml, p<0.05).

The concentrations of active TGF-β2 (mean±standard deviation) in the aqueous humor are shown in Fig. 2. The concentration of active TGF-β2 in the aqueous humor of groups P, N, and S was 2700 pg/ml, 450 pg/ml, and 277±136 pg/ml, respectively, compared with 233±66 pg/ml for group C. Active TGF-β2 levels in groups P and N were statistically significantly higher than that of the control group C (p<0.05).

**Discussion**

The physiologic function of TGF-β2 in the tissue of the eye is not yet fully understood. TGF-β2 has previously been shown to increase accumulation of ECM components and stimulate neovascularization.1-10 TGF-β2 is thought to play an important role in the immunosuppressive function of aqueous humor for the maintenance of anterior chamber-associated immune deviation (ACAID).11

Tripathi et al.10 reported that the mean concentrations of total and active TGF-β2 in the aqueous humor of POAG were 2700 pg/ml and 450 pg/ml, respectively, compared with 1480 pg/ml and 200 pg/ml in the control group. Our study confirmed that the levels of both total and active TGF-β2 were significantly higher in group P than in group C. TGF-β2 plays a regulatory role in the accumulation of ECM by stimulating the synthesis and secretion of matrix proteins and protease inhibitors, and inhibits the synthesis of proteolytic enzymes.12 It also inhibits proliferation and migration of trabecular cells in vitro.13 Thus, high levels of total and active TGF-β2 may contribute to the development of outflow resistance in the pathogenesis of POAG.

Ochiai et al.9 reported total TGF-β2 levels of 1716 pg/ml, and 1001 pg/ml, respectively, in the aqueous of diabetics and control eyes. Another study reported that the concentrations of total and active TGF-β2 in the vitreous of patients with proliferative diabetic retinopathy (PDR) were 2634 pg/ml and 244 pg/ml, respectively.14 The levels of total and active TGF-β2 in the vitreous of nondiabetic subjects were 1305 pg/ml and, 79 pg/ml, respectively.14 We showed elevated levels of both total and active TGF-β2 in NVG related to diabetes. An elevated concentration of TGF-β2 in the aqueous humor of patients with NVG complicated with diabetes may result in neovascularization. Therefore, the production of TGF-β2 in the eyes of NVG patients may have a different outcome from that in the eyes of POAG patients.

TGF-β2 in aqueous humor contributes to the fluid’s immunosuppressive function and inhibits T-cell proliferation. Decreased levels of TGF-β2 in the aqueous humor of uveitis have been reported in a previous study.15 It has been reported that macroglobulin present in the serum binds TGF-β, inhibiting activation. The presence of such proteins in the aqueous humor of uveitic patients may lower the level of active TGF-β2.8 In this study, the level of active TGF-β2 was not elevated when compared with the control; in fact, since the total level of TGF-β2 in SOAG was higher than that of the control, the relative concentration of active TGF-β2 is lower in the SOAG patients. Further studies will be required to improve our understanding of the roles of TGF-β2 in the aqueous humor of various types of glaucomatous eyes.

This study is the first report of increased levels of TGF-β2 in the aqueous humor of NVG complicated with diabetes in Korea. We demonstrate elevated concentrations of total TGF-β2 in the aqueous humor of patients with POAG, NVG, and SOAG. The concentrations of active TGF-β2 in the aqueous humor are also elevated in POAG, and NVG, while the relative concentration of active TGF-β2 in SOAG is decreased. TGF-β2 may play a role in the pathology of various types of glaucoma. Further studies will be needed to elucidate the therapeutic implications for TGF-β2 as an anti-inflammatory or immunosuppressant agent in the treatment of glaucoma.

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