Vascular Endothelial Growth Factor in Aqueous Humor Before and After Intravitreal Injection of Bevacizumab in Eyes With Diabetic Retinopathy

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Objective: To study the concentration of vascular endothelial growth factor in the aqueous humor before and after intravitreal injection of bevacizumab in eyes with proliferative diabetic retinopathy.

Methods: In this prospective, interventional case series, 1.25 mg of bevacizumab was injected into the vitreous cavity as preoperative adjunctive therapy 1 week before pars plana vitrectomy to treat proliferative diabetic retinopathy in 18 eyes in 18 patients. Aqueous humor samples were obtained just before intravitreal injection of bevacizumab and just before vitrectomy 1 week after the injection. Aqueous humor samples also were obtained in patients with cataract without diabetes mellitus (control group). The vascular endothelial growth factor concentration in the aqueous humor was measured using an enzyme-linked immunosorbent assay.

Results: Vascular endothelial growth factor concentration in the aqueous humor ranged from 146 to 676 pg/mL (mean±SD, 326±125 pg/mL) before intravitreal injection of bevacizumab and decreased to less than 31 pg/mL (P<.001) in all eyes 1 week after injection. Intravitreal bevacizumab therapy caused no adverse events. The concentrations in the control group ranged from 80 to 218 pg/mL (mean±SD, 146±40 pg/mL).

Conclusion: Intravitreal injections of bevacizumab resulted in a substantial decrease in vascular endothelial growth factor in the aqueous humor.

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Proliferative diabetic retinopathy (PDR) is a serious complication secondary to diabetes mellitus and a leading cause of blindness.1 Diabetes causes microvascular occlusion resulting in retinal ischemia. The ischemic retina secretes vascular endothelial growth factor (VEGF), an endothelial cell mitogen and an angiogenic inducer, into the vitreous cavity.2-4 Vascular endothelial growth factor increases vascular permeability and causes the formation of new vessels arising from the plane of the retina, resulting in PDR.5 Inhibiting VEGF concentration can decrease vascular permeability and prevent iris and retinal neovascularization in PDR. Photocoagulation applied in eyes with PDR reduces the VEGF concentration in the eyes and results in regression of the iris and retinal neovascularization, reduced leakage, and better visual outcomes.5,7

Bevacizumab (Avastin; Genentech Inc, South San Francisco, California) is a full-length humanized monoclonal antibody to all isoforms of VEGF8 and has been approved by the Food and Drug Administration for intravenous treatment of metastatic colorectal cancer.9 Recently, intravenous injection of bevacizumab was reported effective in treating age-related macular degeneration.10,11 Systemically administered bevacizumab carries a substantial risk of thromboembolic events such as stroke or myocardial infarction in patients with cancer.9,12 The intravitreal dose of 1.25 mg of bevacizumab is about 300- to 400-fold lower than the systemic dose,13 and a lower risk of systemic adverse effects would be expected. Intravitreal injections of bevacizumab then began to be used, and its efficacy without serious adverse events has been reported in neovascular age-related macular degeneration,14-17 central retinal vein occlusion,18,19 and PDR with retinal and iris neovascularization.13,20-23

Intravitreally injected bevacizumab is expected to decrease the intraocular VEGF level, cause regression of the retinal and iris neovascularization, and reduce leakage. However, to our knowledge no report has been published on measurement of intraocular VEGF levels before and after intravitreal injection of bevacizumab in human beings. We measured VEGF concentrations in the aqueous humor before and after intravitreal injection of bevacizumab, which was used as a preoperative adjunctive therapy before vitrectomy to treat PDR.
Aqueous humor samples were obtained from 18 eyes in 18 patients (11 men and 7 women) with PDR. Of the 18 study eyes, 7 had a vitreous hemorrhage, 8 had a tractional retinal detachment, and 3 had diabetic macular edema. Mean patient age was 55.6 years (age range, 31-73 years). The mean ± SD hemoglobin A1C value was 8.0 ± 2.7% (range, 4.9%-17.4%). Laser photocoagulation had been performed in 16 of 18 patients; no patients underwent surgery before the study. Aqueous humor also was sampled from 7 eyes in 7 patients (3 men and 4 women) with diabetic macular edema or ocular disease other than cataract. Mean patient age in this control group was 77.6 years (age range, 70-86 years).

**METHODS**

This study of the off-label use of bevacizumab and collection of aqueous humor before and after intravitreal injection was approved by the Institutional Review Board of Shiga University of Medical Science Hospital, Shiga, Japan. Informed consent was obtained from all participants, including patients with PDR and a control group of patients with cataract.

**SUBJECTS**

Aqueous humor samples were obtained from 18 eyes in 18 patients (11 men and 7 women) with PDR. Of the 18 study eyes, 7 had a vitreous hemorrhage, 8 had a tractional retinal detachment, and 3 had diabetic macular edema. Mean patient age was 55.6 years (age range, 31-73 years). The mean ± SD hemoglobin A1C value was 8.0 ± 2.7% (range, 4.9%-17.4%). Laser photocoagulation had been performed in 16 of 18 patients; no patients underwent surgery before the study. Aqueous humor also was sampled from 7 eyes in 7 patients (3 men and 4 women) with diabetic macular edema or ocular disease other than cataract. Mean patient age in this control group was 77.6 years (age range, 70-86 years).

**OPHTHALMOLOGIC EXAMINATION**

Patients underwent a comprehensive ophthalmologic examination that included measurement of the best-corrected visual acuity and biomicroscopic examination. Fluorescein angiography was performed, and color photographs were obtained using a fundus camera (Topcon TRC-50IA; Tokyo Optical Co Ltd, Tokyo, Japan). The eyes were classified as having diabetic retinopathy, vitreous hemorrhage, tractional retinal detachment, or diabetic macular edema.

**SAMPLE COLLECTION**

Bevacizumab, 1.25 mg/0.05 mL, was injected into the vitreous cavity in the 18 study eyes with PDR 1 week before vitrectomy. A 0.2-mL sample of undiluted aqueous humor was obtained using a 29-gauge needle immediately before injection of bevacizumab and immediately before vitrectomy 1 week after the intravitreal bevacizumab injection. Undiluted aqueous humor also was obtained as a control sample from 7 eyes in 7 patients without other ocular disorders immediately before cataract surgery. All injections and sample collections were performed using a standard sterilization procedure that included the use of topical povidone-iodine and levofloxacin drops. The samples were stored in a deep freezer at −80°C until analysis.

**MEASUREMENT OF VEGF**

The VEGF concentration in the aqueous humor was measured using an enzyme-linked immunosorbent assay for human VEGF (R&D Systems Inc, Minneapolis, Minnesota). The primary antibody against VEGF detected 2 isoforms (VEGF121 and VEGF165) of the 4 VEGF isoforms.24 The limit of the detectable VEGF concentration was 31 pg/mL.

**RESULTS**

The VEGF concentrations in the aqueous humor in eyes with PDR ranged from 146 to 676 pg/mL (326 ± 125 pg/mL) before intravitreal injection of bevacizumab (Figure 1). The VEGF concentrations in the aqueous humor in the control eyes with cataract ranged from 80 to 218 pg/mL (146 ± 40 pg/mL). The mean concentration in eyes with PDR was higher than that in control eyes (P = .002, t test). The VEGF concentration in eyes with PDR dramatically decreased to less than 31 pg/mL, the limit of detection, in all cases 1 week after injection of bevacizumab (P < .001, Wilcoxon signed rank test) (Figure 2).

Of the 18 study eyes, 7 eyes had a vitreous hemorrhage, 8 had tractional retinal detachment, and 3 had diabetic macular edema. The VEGF concentration in the aqueous humor before intravitreal injection of bevacizumab ranged from 146 to 393 pg/mL (339 ± 106) in the group with vitreous hemorrhage, from 159 to 676 pg/mL (335 ± 149) in the group with tractional retinal detachment, and from 196 to 368 pg/mL (271 ± 72) in the group with diabetic macular edema (Figure 1). There were no substantial differences among the groups.

After injection of bevacizumab, no complications developed, such as uveitis, endophthalmitis, effects of ocular toxic effects, or any obvious systemic adverse events. High-quality fundus photographs and fluorescein angiographs were obtained before and after intravitreal bevacizumab injection in 10 eyes. One week after the injection of bevacizumab, substantial regression of neovascularization or fluorescein leakage was seen in 6 of the 10 eyes (60%) (Figure 3).
The mean VEGF concentration in the aqueous humor in eyes with PDR in the present study was similar to that found by Funatsu et al., who reported that the concentrations in the aqueous humor and vitreous fluid in eyes with PDR were substantially higher than in eyes with quiescent PDR. Our results showed that the VEGF concentration in the aqueous humor was also higher than in control eyes. We did not measure the protein level in the aqueous humor, and the concentration of proteins other than VEGF also may increase as a result of increased vascular permeability or disruption of the blood-aqueous barrier. However, the VEGF concentration has a key role in the pathogenesis of diabetic retinopathy.

As expected, intravitreal injection of bevacizumab substantially decreased the VEGF concentration in the aqueous humor in eyes with PDR by at least 10-fold, although the exact concentration 1 week after intravitreal injection of bevacizumab was not determined because the limit of measurement of the enzyme-linked immunosorbent assay was 31 pg/mL. The VEGF concentration in the vitreous seems to be more important than that in the aqueous humor. However, it was reported that the VEGF level in the aqueous humor was substantially correlated with the VEGF level in the vitreous and the aqueous level of VEGF was correlated with the severity of diabetic retinopathy and the activity of PDR. The purpose of this study was to evaluate the changes in the intraocular VEGF concentration before and after intravitreal injection of bevacizumab. However, because it is difficult to obtain vitreous samples before and after intravitreal injection of bevacizumab, we measured the VEGF concentration in the aqueous humor before and after intravitreal injection of bevacizumab. Because we clearly showed that VEGF in the aqueous humor decreased substantially after intravitreal injection of bevacizumab, the VEGF concentration in the vitreous also might decrease substantially after intravitreal injection of bevacizumab. In addition, fluorescein leakage from the retinal neovascularization decreased substantially after intravitreal injection of bevacizumab, as reported previously. Furthermore, bleeding from retinal vessels or new vessels during vitrectomy was reported to be much less than during standard vitrectomy in which bevacizumab therapy was not used, resulting in a higher level of safety during surgery. It is reasonable to think that decreased VEGF concentrations in the vitreous cavity caused decreased fluorescein leakage and minimal bleeding during vitrectomy.

Funatsu et al. reported that the VEGF level in the aqueous was correlated with the severity of diabetic retinopathy according to the modified Early Treatment Diabetic Retinopathy Study retinopathy severity scale. However, there was no substantial difference in the VEGF concentrations in the aqueous humor among the subgroups with vitreous hemorrhage, tractional retinal detachment, or diabetic macular edema. There may be no differences among the types of PDR or we may not have been able to reach a definitive conclusion in the present study because of the small number of eyes in each group.

**Figure 2.** Vascular endothelial growth factor (VEGF) concentration in the aqueous humor in eyes with proliferative diabetic retinopathy before and 1 week after intravitreal injection of bevacizumab. The VEGF levels are shown for eyes with diabetic macular edema (DME), tractional retinal detachment (TRD), and vitreous hemorrhage (VH).

**Figure 3.** Tractional retinal detachment from proliferative diabetic retinopathy in a 43-year-old woman. Fluorescein angiograph before intravitreal injection of bevacizumab (A) and 1 week after the injection (B). Fluorescein leakage decreased substantially after intravitreal bevacizumab injection.
We showed that intravitreal injection of bevacizumab decreased the VEGF level in the aqueous humor and probably the vitreous cavity. We could not measure the exact VEGF concentration 1 week after the intravitreal injection of bevacizumab because of the limits of the enzyme-linked immunosorbent assay used. We found a decrease in the VEGF concentration only 1 week after intravitreal injection of bevacizumab. We do not know how long the reduced level of VEGF is maintained. Further study is warranted to elucidate these issues.

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