Outcome of Vitreous Surgery and the Balance between Vascular Endothelial Growth Factor and Endostatin

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Purpose. To predict the results of vitreous surgery in patients with proliferative diabetic retinopathy (PDR), the correlation between vitreous fluid levels of vascular endothelial growth factor (VEGF) or endostatin and the postoperative outcome was investigated.

Methods. VEGF and endostatin levels in vitreous fluid specimens obtained during vitreous surgery were measured by enzyme-linked immunosorbent assay. Expression of VEGF and endostatin in epiretinal membranes was assessed immunohistochemically. Patients were prospectively followed up for 6 months.

Results. No improvement and/or progression of PDR was seen in 11 (25%) of 44 eyes (progression group). The vitreous fluid level of VEGF was significantly higher in the progression group than in the regression group ($P = 0.0023$). Conversely, the vitreous fluid level of endostatin was significantly higher in the regression group than in the progression group ($P = 0.0299$). Eyes with a high vitreous fluid level of VEGF and a low endostatin level had a significantly greater risk of progression of PDR after vitreous surgery than did eyes with low VEGF and high endostatin levels (odds ratio = 10.00, $P = 0.047$). VEGF and endostatin were detected immunohistochemically in the fibrovascular epiretinal membranes resected from the subjects.

Conclusions. In this study both VEGF and endostatin were expressed in eyes with PDR. VEGF and endostatin levels in the vitreous fluid correlated with the outcome of vitreous surgery for PDR. Therefore, the outcome of PDR surgery can probably be predicted by measuring cytokines and/or growth factors in the vitreous fluid, with VEGF and endostatin being good candidates. (*Invest Ophthalmol Vis Sci.* 2003;44:1042–1047) DOI:10.1167/iovs.02-0374

Predicting the outcome of vitreous surgery for proliferative diabetic retinopathy (PDR) is clinically important. Surgical procedures for PDR have been established during the past 10 years, but many patients with diabetes have not achieved a good outcome.[1] The causes of a poor outcome include rubeotic glaucoma and tractional retinal detachment with fibrous proliferation, and it is thought that the result of surgery may depend on the extent of neovascularization in the eye.[1] To improve the prognosis of vitreous surgery for PDR, it would be useful to have a reliable method of predicting the outcome.

Because PDR causes visual impairment, it is the main target of ocular treatment in patients with diabetes.[1] Current evidence suggests that changes in the balance between stimulators and inhibitors of angiogenesis may activate the angiogenic switch mechanism in PDR.[2] Accordingly, we investigated whether vascular endothelial growth factor (VEGF), an angiogenesis stimulator, and endostatin, an angiogenesis inhibitor, can influence the outcome of vitreous surgery for PDR. Our previous study showed that the vitreous fluid levels of VEGF and endostatin correlate with the severity of diabetic retinopathy and with proliferative fundus changes such as new vessel formation.[3,4] In contrast, the plasma levels of VEGF and endostatin did not correlate with these parameters. Accordingly, we hypothesized that the local balance between VEGF and endostatin in the eye may determine whether retinal neovascularization occurs in PDR. In an attempt to predict the prognosis of PDR, we investigated whether VEGF and endostatin levels in the vitreous fluid have an influence on the outcome of vitreous surgery for PDR—that is, whether it is possible to predict the result of surgery by analyzing samples of vitreous fluid obtained during surgery. We found that the vitreous fluid levels of VEGF and endostatin correlated significantly with the result of vitrectomy and that eyes with high VEGF and low endostatin levels had a significantly greater risk of postoperative progression of PDR.

Patients and Methods

Patients

Samples of undiluted vitreous fluid were harvested at the start of vitrectomy after informed consent was obtained from each subject after an explanation of the purpose and potential adverse effects of the procedure. This study was performed in accordance with the 1975 Declaration of Helsinki, as revised in 1983. Samples of vitreous fluid were obtained from 44 patients with PDR. Vitrectomy was performed for vitreous fluid and/or preretinal hemorrhage in 21 patients and for tractional retinal detachment with fibrous proliferation in 23 patients.

Pars plana vitrectomy was performed by a standardized technique involving three pars plana sclerotomy incisions. Samples of undiluted vitreous fluid (0.3–0.7 mL) were aspirated under standardized conditions from directly above the retina at the beginning of surgery and were immediately transferred to sterile tubes. After collection, the vitreous fluid was removed as far as the vitreous base, followed by segmentation and delamination of proliferative membranes, removal of the posterior vitreous surface, and panretinal endolaser photocoagulation of the retina up to the ora serrata. Retinal detachment was treated with gas tamponade (25% SF6) at the end of vitreous surgery. All operations were performed at Tokyo Women's Medical University Hospital.

Exclusion criteria were previous ocular surgery, a history of ocular inflammation, and retinal detachment associated with a retinal tear.

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Fundus Findings

Preoperative, operative, and postoperative fundus findings were recorded in each subject. The severity of diabetic retinopathy was assessed by standardized fundus color photography and fluorescein angiography (FA), which were performed with a fundus camera that is part of an image-net system (TRC-50IA; Topcon; Tokyo Optical Co., Ltd., Tokyo, Japan) and a preset lens with a slit lamp[3,4] 1 day before vitreous surgery and 6 months afterward. Diabetic retinopathy was graded according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity scale.[5,6] In particular, the severity of new vessels elsewhere (NVE), new vessels on or within 1 disc diameter of the disc (NVD), fibrous proliferation elsewhere (FPE), vitreous hemorrhage, and retinal detachment were graded according to the ETDRS system.[3–5] Retinal photocoagulation was divided into three categories: grade 0, no photocoagulation; grade 1, focal photocoagulation; and grade 2, panretinal photocoagulation (defined as 1200 laser applications or more to the whole retina). The severity of fundus findings in each photograph was graded to calculate the average severity.

Sample Collection

Samples of vitreous fluid were collected into sterile tubes at the time of vitreoretinal surgery and were rapidly frozen at −80°C.[3,4]

Blood samples were also collected from the 44 patients. The blood was immediately placed on ice and subjected to centrifugation at 3000*g* for 5 minutes at 4°C, after which the separated plasma was rapidly frozen at −80°C until assay.[3,4]

Measurement of VEGF and Endostatin

Both VEGF and endostatin were measured in vitreous fluid from the same eye, as well as in plasma samples, using an enzyme-linked immunosorbent assay (ELISA) for human VEGF (R&D Systems, Minneapolis, MN) or an ELISA for endostatin (Cytimmune Sciences, College Park, MD).[3,4] Each assay was performed according to the manufacturer's instructions and our previous reports.[3,4] The VEGF and endostatin levels in vitreous fluid and plasma were within the detection range of the respective assays, because the minimum detectable concentration was 15.6 pg/mL for VEGF and 0.95 ng/mL for endostatin (intra-assay coefficient of variation [CV] was 3.5% and interassay CV was 5.8% for VEGF versus 4.0% and 6.2% for endostatin).

TABLE 1. Clinical and Laboratory Data

Characteristic	No.*	Value
Age (y)	44	55.8 ± 8.4
Sex		
Male	32	72.7%
Female	12	27.3%
Duration of diabetes (y)	44	14.4 ± 6.9
Treatment		
Diet	4	9.1%
Oral hypoglycemic agents	24	54.5%
Insulin	16	36.4%
HbA_{1c} (%)	44	7.5 ± 2.2
Hypertension		
Absent	25	56.8%
Present	19	43.2%
Severity of nephropathy		
Normal	18	40.9%
Mild (albuminuria)	11	25.0%
Moderate (proteinuria)	15	34.1%
Blood VEGF concentration (pg/mL)	44	64.2 ± 30.7
Blood endostatin concentration (ng/mL)	44	6.9 ± 3.6
Vitreous VEGF concentration (pg/mL)	44	1761.5 ± 1594.4
Vitreous endostatin concentration (ng/mL)	44	8.2 ± 3.5

Data are percentage or the mean ± SD.
* Number of patients with available data ($N = 44$).

TABLE 2. Severity of Diabetic Retinopathy at Baseline and after 6 Months

Baseline Severity	Severity after 6 Months						
	47	53	61	71	81	85	Total
71	2	3	2	5	1	0	13
75	3	3	0	0	1	1	8
81	0	4	4	2	2	0	12
85	1	3	3	2	1	1	11
Total	6	13	9	9	5	2	44

Data are the ETDRS retinopathy severity score.

Immunohistochemistry

Immunohistochemistry was performed to confirm the intraocular expression of VEGF and endostatin at the protein level. Resected membranes were fixed in 4% paraformaldehyde, dehydrated, embedded in OCT compound, and frozen in liquid nitrogen. Eight-micrometer sections were cut and stained to detect VEGF or endostatin by an indirect immunofluorescence method. In brief, the sections were incubated in 5% skim milk in phosphate-buffered saline (PBS) for 30 minutes, followed by two washes with PBS. Anti-human VEGF mouse antibody (1:200 in PBS containing 1% skim milk and 1% normal goat serum; Immuno-Biological Laboratories, Fujioka, Japan) or anti-human endostatin rabbit antibody (1:200 in PBS containing 1% skim milk and 1% normal goat serum; Chemicon, Temecula, CA) was added, and sections were incubated for 1 hour at 37°C. After they were washed in PBS, the sections were incubated for 1 hour at 37°C with either fluorescein-labeled goat anti-mouse or anti-rabbit IgG (Alexa Fluor 488; Molecular Probes, Eugene, OR; diluted 1:200 in PBS). After a further wash with PBS, the sections were examined under a photomicroscope (Optiphot-2; Nikon, Tokyo, Japan). Control sections were stained without the primary antibody and did not show any positive reaction.

As a negative control, the samples were treated with nonimmunized IgG instead of the primary antibody. Frozen sections were stained with hematoxylin and eosin to observe the histologic features.

Measurement of Clinical Variables

The following variables were measured at baseline. Hemoglobin A_{1c} (HbA_{1c}) was measured by affinity chromatography (HPLC, normal range: 4.3%–5.8%; Kyoto Chemical, Kyoto, Japan). Systolic and diastolic blood pressures were measured with a mercury sphygmomanometer with the patient in the sitting position, after the patient had rested for 10 minutes. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or treatment with antihypertensive medication. The urinary albumin concentration was measured by an immunoturbidometric method, with a reference of less than 12.0 mg/g creatinine. Microalbuminuria was defined as a urinary albumin concentration of

TABLE 3. Visual Acuity at Baseline and after 6 Months

Baseline Visual Acuity	Visual Acuity after 6 Months				
	<20/200	20/200≦, <20/40	20/40≦, <20/20	20/20≦	Total
<20/200	9	3	3	2	17
20/200≦, <20/40	1	12	9	1	23
20/40≦, <20/20	1	1	0	1	3
20/20≦	0	0	1	0	1
Total	11	16	13	4	44

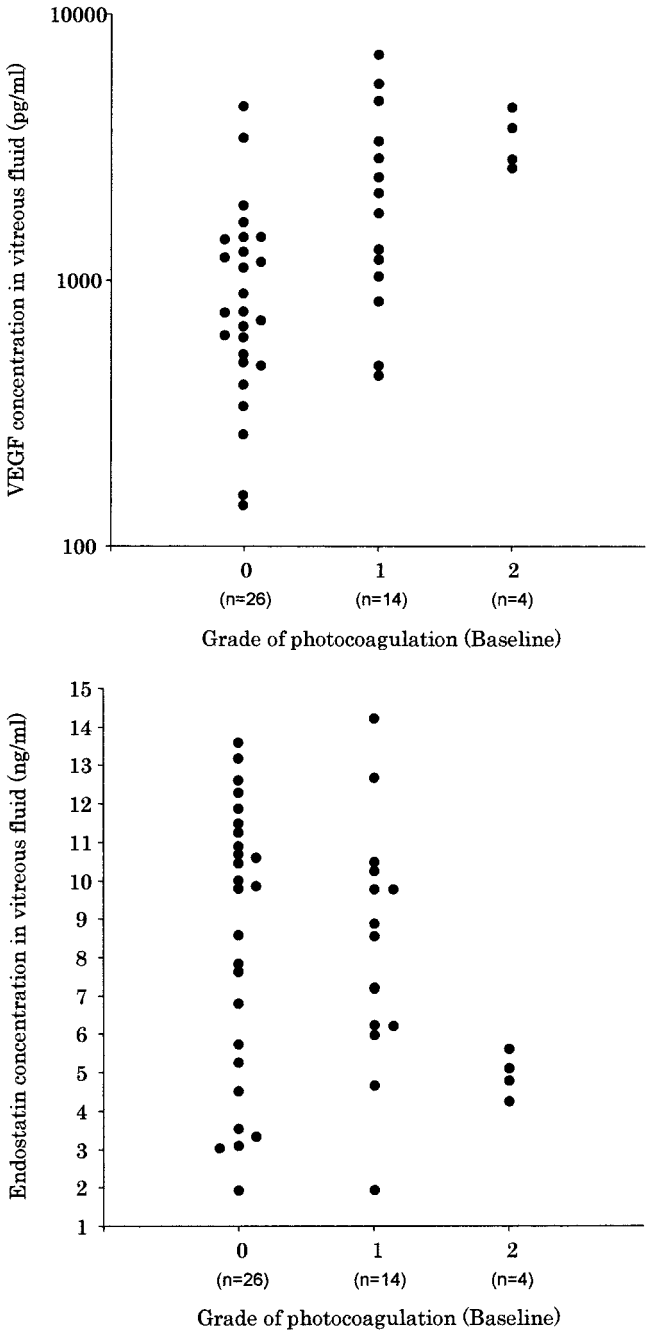

FIGURE 1. (**A**) Correlation between the baseline grade of retinal photocoagulation and the vitreous fluid concentration of VEGF ($\rho = 0.537$, $P = 0.0002$). (**B**) Correlation between the baseline grade of retinal photocoagulation and the vitreous fluid concentration of endostatin ($\rho = -0.258$, $P = 0.0106$). Grade 0, no photocoagulation; grade 1, focal photocoagulation; grade 2, panretinal photocoagulation.

12 mg/g creatinine or more, and proteinuria was defined as a concentration of 300 mg/dL or more.

Statistical Analysis

Analyses were performed on computer (SAS System 8e software; SAS Institute Inc., Cary, NC).[7] Results are presented as the mean ± SD or as the geometric mean ± SD for logarithmic data. To assess the relationship between each angiogenic factor and the ETDRS grade for severity of retinopathy, Spearman's rank-order correlation coefficients were calculated. Odds ratios were calculated with a logistic regression model with three dummy variables. Two-tailed probabilities of less than 0.05 were considered to indicate statistical significance.

TABLE 4. Vitreous Concentrations of VEGF and Endostatin in Eyes According to Progression or Regression of PDR

	No Improvement/ Progression	Regression	*P*
VEGF (pg/mL)	2433.6 ± 986.8	946.1 ± 432.8	0.0023
Endostatin (ng/mL)	6.39 ± 3.29	8.78 ± 3.43	0.0299

RESULTS

Baseline Patient Profile

Complete data were available for 44 of 48 patients; 4 patients were lost to follow-up because of transfer to another hospital or nonattendance at follow-up appointments. Of the 44 patients, 32 were men and 12 were women (Table 1). Their mean age was 55.8 years (range, 39–72) and the mean duration of diabetes was 14.4 years (range, 3–30 years). The mean HbA_{1c} was 7.5% (range, 5.0%–10.2%). All patients were followed up for 6 months. The baseline PDR grade was level 71 in 13 eyes, level 75 in 8 eyes, level 81 in 12 eyes, and level 85 in 11 eyes (Table 2). Previous laser therapy for PDR included panretinal photocoagulation in 26 patients and focal retinal photocoagulation in 14 patients. Four patients had not undergone retinal photocoagulation before vitreous surgery.

Outcome of Vitreous Surgery

At the end of follow-up, PDR showed no improvement and/or progression by 1 level or more in 11 (25%) eyes, whereas regression by 1 level or more was detected in 33 (75%) eyes (Table 2). Twenty-eight patients noted a significant improvement in visual acuity after surgery, and 34 (77%) patients achieved visual acuity of 20/200 or better (Table 3). The remaining 10 (23%) patients had worse visual acuity. Four had diabetic macular edema, four had vitreous hemorrhage, and two had macular atrophy. All patients without panretinal photocoagulation before surgery received additional laser photocoagulation during surgery and within 1 month afterward. In two eyes with very active neovascularization after vitreoretinal surgery, additional retinal photocoagulation was performed among the laser scars to prevent the progression of neovascularization, based on the FA findings.

Vitreous Fluid Levels of VEGF and Endostatin

Vitreous fluid concentrations of VEGF and endostatin correlated significantly with the baseline amount of retinal photo-

TABLE 5. Correlation between the Changes in Fundus Findings and Vitreous Fluid Levels of VEGF or Endostatin

	ρ	*P*
VEGF		
NVE	0.39428	0.0081
NVD	−0.16636	0.2805
Vitreous hemorrhage	0.63469	0.0001
FPE	0.15284	0.3220
Retinal detachment	0.22041	0.1505
Endostatin		
NVE	−0.32238	0.0328
NVD	0.11637	0.4519
Vitreous hemorrhage	−0.20900	0.1734
FPE	−0.12706	0.4111
Retinal detachment	−0.15227	0.3238

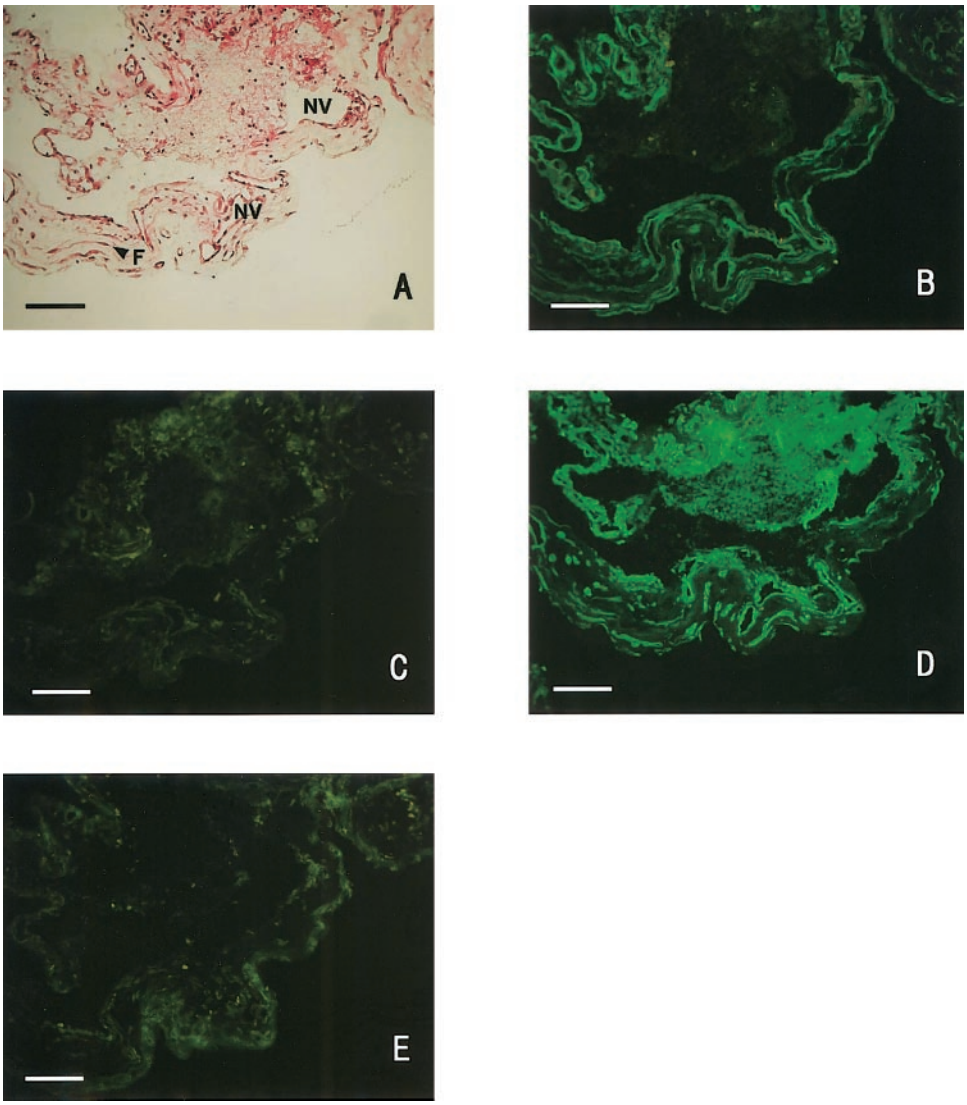

FIGURE 2. Expression of VEGF and endostatin in preretinal membranes. Preretinal membranes from a PDR patient were stained with antisera for endostatin and VEGF. *Green* reaction products for VEGF and endostatin are primarily associated with cellular elements. (**A**) Hematoxylin-eosin stain. The preretinal membrane contained many new vessels (NV) and abundant extracellular matrix (ECM). In the ECM, fibroblast-like (F) cells were present. (**B**, **C**) Immunohistochemistry for endostatin with counterstaining. (**D**, **E**) Immunohistochemistry for VEGF with counterstaining.

coagulation ($\rho = 0.537$, $P = 0.0002$ and $\rho = -0.258$, $P = 0.0106$, respectively; Fig. 1).

Vitreous fluid levels of VEGF were significantly elevated in the patients who showed no improvement and/or progression of PDR (2433.6 pg/mL [994.7–5953.9]) when compared with the patients showing regression of PDR (946.1 pg/mL [417.4–2144.4]; $P = 0.0023$; Table 4). In contrast, the vitreous fluid level of endostatin was significantly higher in the patients with regression of PDR (8.78 pg/mL [1.95–14.22]) when compared with those who showed no improvement and/or progression of PDR (6.39 ng/mL [1.95–12.70]; $P = 0.0299$; Table 4). There was no significant relationship between the vitreous fluid concentrations of VEGF and endostatin ($\rho = -0.255$, $P = 0.0944$).

Vitreous fluid levels of VEGF correlated significantly with the progression of NVE and vitreous hemorrhage ($\rho = 0.39428$, $P = 0.0081$ and $\rho = 0.63469$, $P < 0.0001$, respectively), but did not correlate significantly with NVD, FPE, or retinal detachment ($\rho = -0.16636$, $P = 0.2805$, $\rho = 0.15284$, $P = 0.3220$, and $\rho = 0.22041$, $P = 0.1505$, respectively; Table 5). The vitreous fluid level of endostatin was significantly correlated with regression of NVE ($\rho = -0.32238$, $P = 0.0328$), but not with regression of vitreous hemorrhage, NVD, FPE, or retinal detachment ($\rho = -0.20900$, $P = 0.1734$, $\rho = 0.11637$, $P = 0.4519$, $\rho = -0.12706$, $P = 0.4111$, and $\rho = -0.15227$, $P = 0.3238$, respectively; Table 5).

Expression of VEGF and endostatin in the proliferative membranes resected during surgery was confirmed immunohistochemically. Figure 2 shows a representative case. All the preretinal membranes contained many new vessels (Fig. 2A, NV) and an abundance of extracellular matrix. The matrix also contained fibroblast-like cells (Fig. 2A). Endostain (Fig. 2B) and VEGF (Fig. 2D) were expressed by vascular cells and by the fibroblast-like cells. VEGF was also detected in the extracellular matrix, suggesting that it became trapped there after release from cells.

Balance between Angiogenic Factors

Figure 3 shows patients stratified into four groups according to the changes in PDR and the median vitreous fluid levels of VEGF and endostatin (1213 pg/mL and 8.2 ng/mL, respectively). In group 1 (a high vitreous fluid level of endostatin [≥8.2 ng/mL] and a low level of VEGF [<1213 pg/mL]), 15 (94%) of 16 patients showed improvement in PDR. Five (71%) of seven patients in group 2 showed improvement (low levels of both endostatin [<8 ng/mL] and VEGF [<1213 pg/mL]). Two (33%) of 6 patients in group 3 showed progression of PDR (high levels of both VEGF [≥1213 pg/mL] and endostatin [≥8.2 ng/mL]), whereas 6 (40%) of 15 patients in group 4 showed PDR progression (high VEGF level [≥1213 pg/mL])

and low endostatin level [<8 ng/mL]). The relative risk of PDR progression after vitreous surgery is shown in Table 6 for each group. The odds ratios for groups 2 and 3 were not significantly different from that for group 1, but group 4 had a significantly higher risk of PDR progression after surgery (odds ratio: 10.00, $P = 0.0470$).

Vitreous and Plasma Levels of VEGF and Endostatin Versus Clinical Factors

The vitreous fluid concentration of VEGF was significantly higher than the plasma VEGF level (68.8 pg/mL [15.6–115.0]; $P < 0.0001$). The vitreous fluid concentration of endostatin was also significantly higher than the plasma endostatin level (6.55 ng/mL [1.92–24.70]; $P = 0.0119$). There was no significant relationship between the vitreous fluid level of VEGF and age, duration of diabetes, HbA_{1c}, hypertension, or nephropathy ($P = 0.3550$, $P = 0.6117$, $P = 0.1352$, $P = 0.8699$, and $P = 0.9442$, respectively). There was also no significant relationship between the vitreous fluid level of endostatin and these factors ($P = 0.8406$, $P = 0.2392$, $P = 0.4136$, $P = 0.2825$, and $P = 0.5956$, respectively).

Discussion

In this prospective study, we investigated whether the outcome of vitreous surgery correlates with the balance between levels of VEGF (a stimulator of angiogenesis) and endostatin (an inhibitor of angiogenesis). Vitreous fluid levels of VEGF were significantly elevated in patients with no improvement and/or progression of PDR after surgery, when compared with the levels in patients who showed regression of PDR. In contrast, vitreous fluid levels of endostatin were significantly higher in patients with postoperative regression of PDR than in patients showing no improvement and/or progression of PDR. Expression of both VEGF and endostatin in the resected proliferative membranes was confirmed immunohistochemically. Furthermore, the vitreous fluid concentrations of VEGF and endostatin correlated significantly with the fundus findings and the severity of retinopathy. These results suggest that VEGF is associated with the progression of PDR after vitreous surgery, whereas endostatin correlated with regression of PDR. In this study, we harvested samples of undiluted vitreous fluid at the beginning of surgery and immediately transferred the specimens to sterile tubes. Therefore, the vitreous fluid levels of VEGF and endostatin were not affected by the operation or by endolaser photocoagulation and are likely to reflect the activity of neovascularization and the extent of retinal ischemia. We investigated whether the vitreous fluid levels of VEGF and endostatin are related to the outcome of vitreous surgery and the postoperative course of PDR. It has been hypothesized that the balance between stimulators and inhibitors of angiogenesis correlates with the extent of neovascularization in tumors and in retinal vascular diseases, such as diabetic retinopathy, retinal vein occlusion, and retinopathy of prematurity.

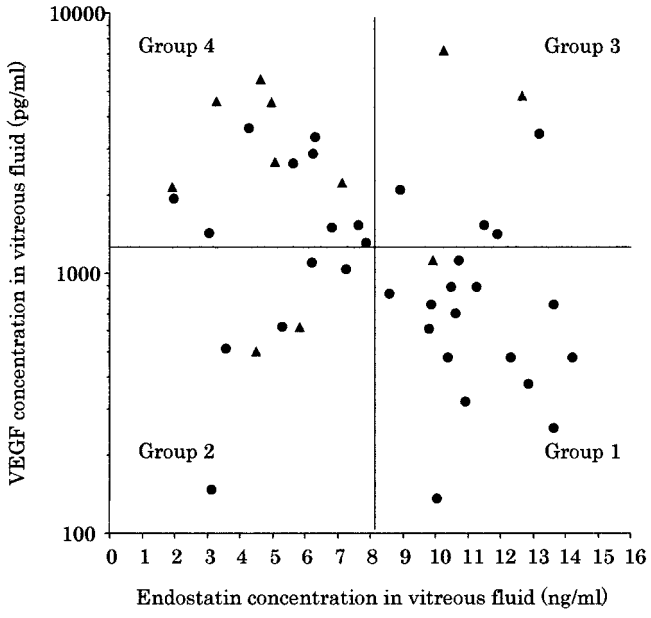

Figure 3. Eyes with no improvement or progression of PDR and eyes with regression of PDR stratified according to the vitreous fluid concentrations of both VEGF and endostatin. Group 1: eyes with a vitreous VEGF level less than 1213 pg/mL and an endostatin level 8.2 ng/mL or more. Group 2: eyes with a vitreous VEGF level less than 1213 pg/mL and an endostatin level less than 8.2 ng/mL. Group 3: eyes with a vitreous VEGF level 1213 pg/mL or more and an endostatin level 8.2 ng/mL or more. Group 4: eyes with a vitreous VEGF level 1213 pg/mL or more and an endostatin level less than 8.2 ng/mL. (●) Regression of PDR. (▲) No improvement or progression of PDR.

Table 6. Odds Ratios for Progression of Retinopathy in Four Groups Based on the Vitreous Fluid Levels of VEGF and Endostatin

	Odds Ratio (95% CI)	*P*
Group 1 ($n = 16$)	1.00	
Group 2 ($n = 7$)	6.00 (0.443–81.195)	0.1776
Group 3 ($n = 6$)	7.50 (0.534–105.278)	0.1349
Group 4 ($n = 15$)	10.00 (1.030–97.043)	0.0470

Group 1: VEGF <1213 pg/mL and endostatin ≧8.2 ng/mL; Group 2: VEGF <1213 pg/mL and endostatin <8.2 ng/mL; Group 3: VEGF ≧1213 pg/mL and endostatin ≧8.2 ng/mL; Group 4: VEGF ≧1213 pg/mL and endostatin <8.2 ng/mL. CI, confidence interval.

It has been reported that vitreous fluid levels of VEGF are significantly elevated in patients with active PDR when compared with patients with quiescent PDR[8,9] and that VEGF plays a key role in the process of angiogenesis in eyes with PDR. Endostatin is a 20-kDa proteolytic fragment of collagen XVIII, which has been shown to inhibit VEGF- and fibroblast growth factor (FGF)–induced vascular endothelial cell migration and proliferation in vitro.[10] In the eye, collagen XVIII is localized to the inner retinal limiting membrane and the retinal pigment epithelium.[11] Local production of endostatin may occur during corneal wound healing, because the relevant enzymes and the substrate (collagen XVIII) are present in the basement membrane during this process. Endostatin inhibits VEGF-induced endothelial cell migration in vitro and inhibits tumor growth in vivo.[12] However, it has been unclear whether endostatin is involved in the regulation of retinal and choroidal neovascularization. Recently, Mori et al.[13] reported that intravenous injection of adenoviral vectors containing an expression construct for endostatin significantly reduced laser-induced choroidal neovascularization (CNV) in mice, and they found a strong negative correlation between the area of CNV and the serum level of endostatin. Based on these reports, we selected VEGF and endostatin to predict the outcome of vitreous surgery.

As the first step toward predicting the outcome of vitrectomy, we stratified the patients into four groups according to the vitreous fluid levels of VEGF and endostaitn. We found that group 4 (high VEGF and low endostatin levels) had a significantly greater risk of progression of PDR after vitreous surgery than did group 1 (low VEGF and high endostatin levels). These results suggest that both a high vitreous fluid level of VEGF and a low level of endostatin stimulate neovascularization, whereas

a high endostatin level and a low VEGF level inhibit neovascularization in PDR-affected eyes, and a change in the balance between VEGF and endostatin may influence the progression and/or regression of PDR after vitreous surgery.

The present study confirmed that endostatin is produced in proliferative membranes and is not directly related to new vessel formation, because vitreous fluid levels of endostatin correlated with the grade of NVE but not with the grade of NVD. In fact, the level of endostatin was lower in eyes with NVD than in eyes with NVE alone. To further clarify the clinical significance of endostatin, we must investigate the regulation of its expression and its possible inhibitory effect on neovascularization at the molecular level, including cross talk with VEGF and other stimulators of angiogenesis.

An important finding in this study is that patients with panretinal photocoagulation before surgery had higher endostatin levels than did patients without previous photocoagulation, but had lower VEGF levels than did the patients without photocoagulation. The latter result was in agreement with previous reports.[8,14,15] Pournaras et al.[16,17] demonstrated that scatter photocoagulation reverses tissue hypoxia in miniature pigs with experimental vasoproliferative microangiopathy[16] and that hyperoxia reduces VEGF production in ischemic retina.[17] Retinal photocoagulation induces the regression of neovascularization and has been shown to be associated with a decrease in neovascularization.[18] Presumably, the effect of photocoagulation itself and the reduction of retinal ischemia after photocoagulation lead to increased expression of angiogenesis inhibitors, such as pigment epithelium-derived factor (PEDF), angiostatin, and transforming growth factor-β_2 (TGF-β_2).[14,19,20] However, it has not been clarified whether expression of endostatin is increased by photocoagulation and/or by reduction of retinal ischemia. Taken together, these results suggest that the vitreous fluid levels of endostatin and VEGF may be related to the extent of retinal ischemia and to the area of previously photocoagulated retina.

In conclusion, in the present study, vitreous fluid levels of VEGF and endostatin correlated with the activity or severity of PDR and that the balance between VEGF (a stimulator of angiogenesis) and endostatin (an inhibitor of angiogenesis) was closely related to the progression or regression of PDR after vitreous surgery. Accordingly, evaluation of the balance between VEGF and endostatin in the eye may have the potential to predict the outcome of vitreous surgery.

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