ELSEVIER

Stimulation and Inhibition of Angiogenesis in Diabetic Retinopathy

Hideharu Funatsu*, Hidetoshi Yamashita†,
Hidetaka Noma*, Erika Shimizu*, Tetsuji Yamashita‡ and Sadao Hori§

*Department of Ophthalmology, Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan; †Department of Ophthalmology, Yamagata University School of Medicine, Yamagata, Japan; ‡Mitsubishi Chemical Bio-Clinical Laboratories, Tokyo, Japan; §Department of Ophthalmology, Tokyo Women's Medical University, Tokyo, Japan

Purpose: To investigate the role of stimulators and inhibitors of angiogenesis in the pathogenesis of diabetic retinopathy.

Methods: Undiluted vitreous samples and simultaneous paired plasma samples were obtained from 30 diabetic patients (35 eyes) undergoing vitreous surgery. The levels of vascular endothelial growth factor (VEGF), endostatin, and platelet factor-4 (PF-4) were measured simultaneously in each specimen by enzyme-linked immunosorbent assay. The severity of diabetic retinopathy was evaluated according to the modified Early Treatment Diabetic Retinopathy Study retinopathy severity scale.

Results: Vitreous levels of VEGF and endostatin were significantly correlated with the severity of diabetic retinopathy ($\rho = 0.52$, $\rho = 0.48$, respectively), but the vitreous level of PF-4 was not ($\rho = 0.12$). Vitreous levels of VEGF, endostatin, and PF-4 were not significantly correlated with their plasma levels. The vitreous level of VEGF was significantly correlated with that of endostatin ($\rho = 0.42$). The VEGF concentration was significantly higher in the vitreous than in the plasma, while the endostatin concentration was not.

Conclusions: The present study showed that VEGF and endostatin were expressed in the vitreous of patients with diabetic retinopathy and may be involved in the pathogenesis of this condition. **Jpn J Ophthalmol 2001;45:577–584**

Key Words: Diabetic retinopathy, endostatin, platelet factor, vascular endothelial growth factor.

Introduction

New vessel formation in patients with diabetic retinopathy causes visual loss, and is the main target of treatment for this condition. Numerous angiogenic factors have been implicated in the pathogenesis of proliferative diabetic retinopathy.[1,2] There is now considerable evidence that various growth factors are involved in initiating and perpetuating the process of neovascularization.[1,2] Because blood vessel formation is a complex, multi-step process, several angiogenic factors may operate under different circumstances or various factors may act synergistically. Clinically, retinal neovascularization is associated with retinal capillary closure and retinal ischemia, leading to the hypothesis that angiogenic factors diffusing from the ischemic retinal tissue may be responsible for neovascularization in diabetic retinopathy.[1–4]

Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen in vitro, and it induces an increase of vascular permeability and angiogenesis in vivo.[5,6] Numerous retinal cells produce VEGF.[7–9] In addition, its upregulation by hypoxia has been demonstrated, making VEGF an appealing candidate for

Received: January 18, 2001
Correspondence and reprint requests to: Hideharu FUNATSU, MD, Department of Ophthalmology, Diabetes Center, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

Published by Elsevier Science Inc.

0021-5155/01/$–see front matter
PII S0021-5155(01)00420-8

Jpn J Ophthalmol
Vol 45: 577–584, 2001

mediating ocular angiogenesis.[10] High-affinity VEGF receptors have been identified on retinal endothelial cells and pericytes.[11,12] The normally quiescent vasculature can be activated to sprout new capillaries (angiogenesis), a morphogenic process that is controlled by an angiogenic switch mechanism.[13] In some tissues, the absence of angiogenesis inducers may ensure that the switch stays off, while angiogenesis inducers may be present in other tissues but held in check by higher levels of angiogenesis inhibitors. Thus, either reducing the concentration of angiogenesis inhibitors or increasing the level of inducers may alter the balance and switch on angiogenesis, leading to the formation of new blood vessels.[13] It therefore seems important to investigate the balance between stimulators and inhibitors of angiogenesis, but the state of this balance in patients with proliferative diabetic retinopathy remains unknown. Most studies have only measured angiogenic factors in vitreous samples.[14–19]

A growing number of endogenous inhibitors of angiogenesis have been reported.[20–23] We selected endostatin and PF-4 as important potential angiogenesis inhibitors. Amino acid sequence analysis has revealed that endostatin is a C-terminal fragment of collagen XVIII.[23] Endostatin specifically inhibits endothelial cell proliferation and potently inhibits angiogenesis and tumor growth.[23] Endostatin also inhibits the VEGF-stimulated migration of human umbilical vein endothelial cells in a dose-dependent manner.[24] PF-4 is synthesized by megakaryocytes and is normally sequestered inside platelets.[25] PF-4 is reported to be an effective inhibitor of angiogenesis in the chicken chorioallantoic membrane and a specific inhibitor of growth factor-stimulated endothelial cell proliferation in vitro.[20] Based on these reports, we have been investigating the role of endostatin and/or PF-4 in the pathogenesis of proliferative diabetic retinopathy.

In the present study, we investigated the balance between stimulators and inhibitors of angiogenesis in patients with diabetic retinopathy. Endostatin seems to be significantly associated with the severity of retinopathy in addition to VEGF, but this is the first study to investigate the relationship between this angiogenesis inhibitor and diabetic retinopathy.

Materials and Methods

Patients

We obtained samples of vitreous fluid from of 30 diabetic patients (18 men and 12 women) (35 eyes) undergoing vitreous surgery. Their mean age was 54.9 years (range, 25–77 years) and the mean duration of diabetes was 13.2 years (range, 1–30 years). The mean Hb_{A1c} value was 7.2% (range, 4.8–12.4%). Vitreous fluid samples, 10 from nondiabetic patients with ocular disease, were obtained. The nondiabetic patients included 8 with macular hole and 2 with epiretinal membrane; none of these 10 patients had associated proliferative vitreoretinopathy. Undiluted vitreous fluid samples and plasma samples were obtained simultaneously from the patients. The vitreous surgery and sampling were carried out at the Diabetes Center of Tokyo Women's Medical University. All patients gave informed consent. None of the patients had undergone prior ocular surgery and none had a history of intraocular inflammation.

The preoperative and operative findings were recorded. Clinical data, including the severity of diabetic retinopathy, were obtained from the surgeon at the time of the operation on standard forms and were confirmed by standardized fundus color photography and fluorescein angiography (FAG). The severity of diabetic retinopathy was graded according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity scale.[26,27] In particular, the severity of soft exudates, intraretinal microvascular abnormalities (IRMA), venous beading, venous loops, 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.[26] The stage of diabetic retinopathy was classified as panretinal photocoagulation (PRP) *for* treated eyes and non-PRP *for untreated* eyes.

Vitrectomy was performed on 30 diabetic patients (35 eyes) for the following conditions; 10 eyes had vitreous and/or preretinal hemorrhage, 7 eyes had retinal detachment, 7 eyes had macular heterotropia, 10 eyes had macular edema, and 1 eye had a macular hole.

The baseline assessment included an interview, physical examination, and laboratory tests, as well as assessment of the best-corrected visual acuity, intraocular pressure, and ocular fundus evaluation by ophthalmoscopy, 10-field nonsimultaneous color fundus photography, and FAG.

Sample Collection

After the sclerotomy ports were inserted, a vitreous cutter was introduced and a sample of undiluted vitreous (0.3–0.7 mL) was aspirated manually into a disposable tuberculin syringe before turning on the infusion and completing the surgical procedure. Vitreous samples were stored at −80°C until assay.

Plasma samples were also obtained from 30 of the patients. Blood samples were immediately placed on ice, clarified by centrifugation at 3,000 *g* for 5 minutes at 4°C, and rapidly frozen at −80°C until assay. Our institutional review board approved the protocol for sample collection.

Measurement of VEGF, Endostatin, and PF-4

The concentrations of VEGF, endostatin, and PF-4 were measured by enzyme-linked immunosorbent assay (ELISA) using immunoassay kits for human VEGF, endostatin, and PF-4 (R&D Systems, Minneapolis, MN, USA; Cytimmune Sciences, Baltimore, MD, USA, and Roche Diagnostics, Tokyo, respectively).[28–30]

The VEGF kit used could detect 2 of the 4 VEGF isoforms ($VEGF_{121}$ and $VEGF_{165}$). Assays were performed according to the manufacturers' instructions. The minimum detectable concentrations were 15.6 pg/mL, 0.95 ng/mL, and 1.0 pg/mL for VEGF, endostatin, and PF-4, respectively, which meant that the levels of these factors in vitreous fluid and plasma were within the detectable range

Statistical Analysis

Analyses were performed with SAS Software (SAS Institute, Cary, NC, USA).[31] Results are presented as mean ± SD or as the geometric mean ± SD for data shown on a logarithmic scale. To assess the relationship between each angiogenic factor and the ETDRS retinopathy severity, Spearman's rank-order correlation coefficient and the 95% confidence interval were calculated. Two-tailed probability values of <0.05 were considered to indicate statistical significance.

Results

Severity of Retinopathy and Vitreous Angiogenic Factors

Vitreous levels of VEGF, endostatin, and PF-4 in diabetic patients were 812.0 ± 635.0 pg/mL, 7.24 ± 4.42 ng/mL, and 16.9 ± 12.5 pg/mL, respectively. Vitreous VEGF levels were significantly correlated with the clinical severity of diabetic retinopathy ($\rho = 0.52$, $P < .01$, Figure 1A). Vitreous endostatin levels were also significantly correlated with the clinical severity of diabetic retinopathy ($\rho = 0.48$, $P < .01$, Figure 1B). Although PF-4 was detected in the vitreous fluid, vitreous PF-4 levels were not significantly correlated with the clinical severity of diabetic retinopathy ($\rho = 0.12$, $P = .59$, Figure 1C). Vitreous levels of VEGF, endostatin, and PF-4 in nondiabetic patients were 27.2 ± 25.0 pg/mL, 5.84 ± 3.25 ng/mL, and 17.4 ± 10.6 pg/mL, respectively. The mean vitreous level of VEGF in diabetic patients was much higher than its mean level in nondiabetic patients. The mean vitreous level of endostatin and PF-4 in diabetic patients was not higher than their mean level in nondiabetic patients.

Fundus Findings and Vitreous Angiogenic Factors

Among the fundus findings, the vitreous VEGF level was significantly correlated with the grade of soft exudates, IRMA, venous beading, venous loops, NVE, and NVD (Table 1). In addition, the vitreous endostatin level was significantly correlated with the grade of IRMA, venous beading, NVE, and FPE (Table 1). In contrast, there was no significant relationship between the vitreous PF-4 level and any of the fundus findings (Table 1).

Vitreous and Plasma Angiogenic Factors

Plasma levels of VEGF, endostatin, and PF-4 were 55.0 ± 44.8 pg/mL, 7.38 ± 5.94 ng/mL, and 95.9 ± 9.98 pg/mL, respectively. Vitreous levels of VEGF, endostatin, and PF-4 were not significantly correlated with the plasma levels of these factors ($\rho = 0.29$, $P = .26$, $\rho = 0.04$, $P = .92$, $\rho = 0.27$, $P = .45$, respectively). The mean vitreous level of VEGF (812.0 ± 635.0 pg/mL) was much higher than its mean plasma level (55.0 ± 44.8 pg/mL). The vitreous levels of endostatin were higher than the plasma levels in all the patients with active proliferative diabetic retinopathy (9.26 ± 2.33 ng/mL). The mean vitreous level of PF-4 (16.9 ± 12.5 pg/mL) was much lower than its mean plasma level (95.9 ± 9.98 pg/mL) in all the diabetic patients.

Balance Between Angiogenic Factors

The vitreous level of VEGF was significantly correlated with the vitreous level of endostatin ($\rho = 0.42$, $P < .01$, Figure 2A). The ETDRS retinopathy severity ratings are stratified according to the vitreous levels of VEGF and endostatin in Table 2. When the vitreous level of VEGF was low (<800 pg/mL), the ETDRS retinopathy rating was also low in eyes with low vitreous levels of endostatin (<7 ng/mL), while it was high in eyes with high vitreous levels of endostatin (≥7 ng/mL). On the other hand, when the vitreous level of VEGF was high (≥800 pg/mL), the ETDRS retinopathy rating was high for eyes with both low and high vitreous levels of endostatin.

Jpn J Ophthalmol
Vol 45: 577–584, 2001

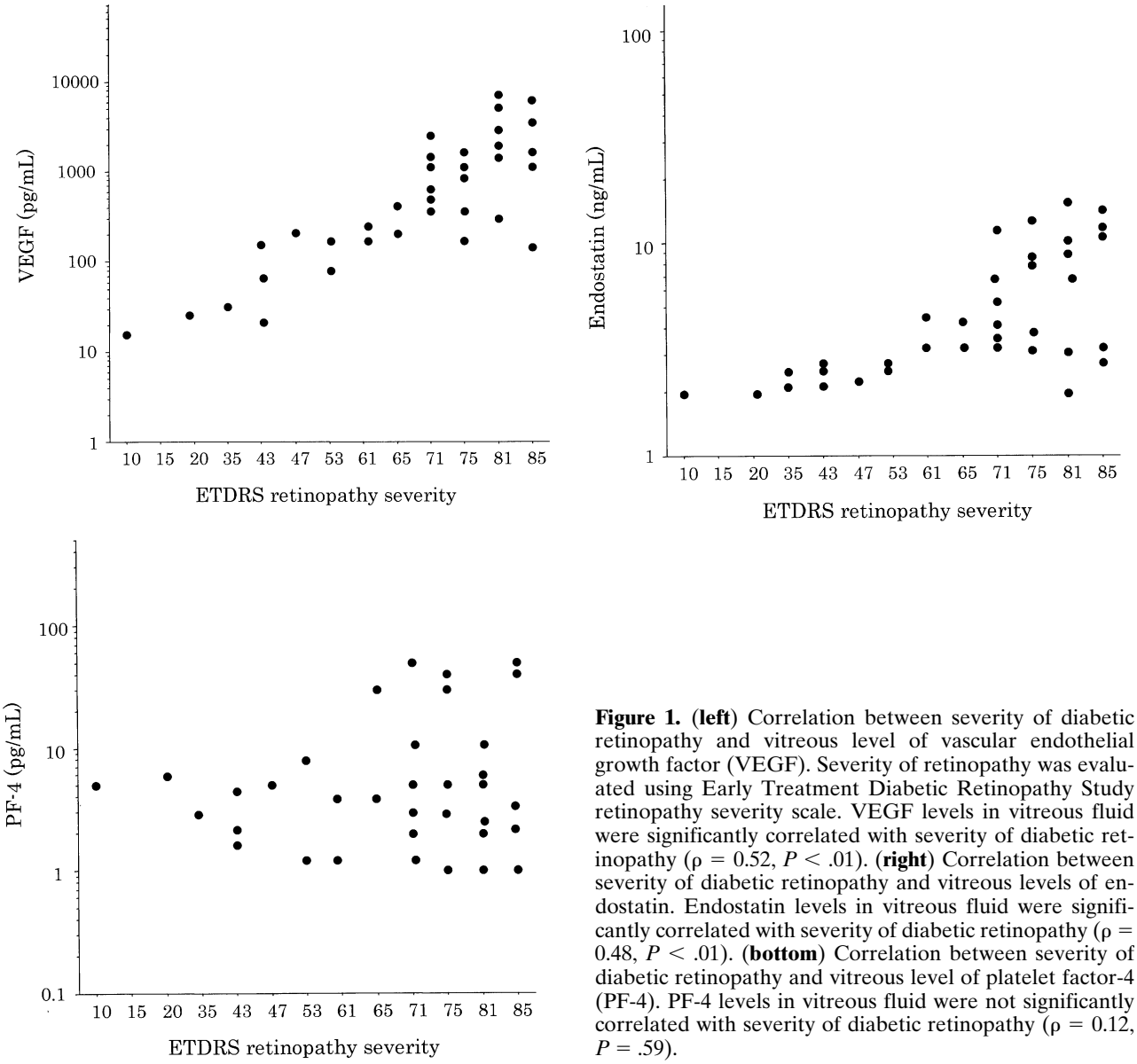

Figure 1. (**left**) Correlation between severity of diabetic retinopathy and vitreous level of vascular endothelial growth factor (VEGF). Severity of retinopathy was evaluated using Early Treatment Diabetic Retinopathy Study retinopathy severity scale. VEGF levels in vitreous fluid were significantly correlated with severity of diabetic retinopathy ($\rho = 0.52$, $P < .01$). (**right**) Correlation between severity of diabetic retinopathy and vitreous levels of endostatin. Endostatin levels in vitreous fluid were significantly correlated with severity of diabetic retinopathy ($\rho = 0.48$, $P < .01$). (**bottom**) Correlation between severity of diabetic retinopathy and vitreous level of platelet factor-4 (PF-4). PF-4 levels in vitreous fluid were not significantly correlated with severity of diabetic retinopathy ($\rho = 0.12$, $P = .59$).

Vitreous levels of endostatin were not significantly correlated with vitreous levels of PF-4 ($\rho = -0.08$, $P = .73$, Figure 2B). There were no differences in the ETDRS retinopathy ratings for eyes with low and high vitreous levels of endostatin and PF-4 (data not shown). Vitreous levels of VEGF were also not significantly correlated with vitreous levels of PF-4 ($\rho = -0.21$, $P = .35$, Figure 2C). Likewise, there were no differences in the ETDRS retinopathy ratings between eyes with low and high vitreous levels of PF-4 and VEGF (data not shown).

Vitreous levels of VEGF in non-PRP treated eyes (1232.8 ± 864.0 pg/mL) were significantly higher than those in PRP treated eyes (612.6 ± 427.4 pg/mL) ($P < .05$). On the other hand, vitreous levels of endostatin in PRP-treated eyes (8.24 ± 4.88 ng/mL) were not significantly higher than those in non-PRP treated eyes (5.86 ± 3.47 ng/mL) ($P = .055$).

Systemic Factors and Vitreous Angiogenic Factors

The vitreous levels of VEGF were not significantly correlated with age, duration of diabetes mellitus (DM), and Hb_{A1c} ($\rho = 0.12$, $P = .38$, $\rho = 0.21$, $P = .12$, $\rho = 0.24$, $P = .08$, respectively). The vitreous levels of

Table 1. Relationship Between Retinopathy Grade and Vitreous Levels of Vascular Endothelial Growth Factor (VEGF), Endostatin, and Platelet Factor 4 (PF-4)

	VEGF		Endostatin		PF-4	
Characteristic*	ρ	*P*	ρ	*P*	ρ	*P*
Soft exudates	0.44	.04	0.26	.20	0.18	.41
IRMA	0.51	.01	0.49	.01	0.02	.92
Venous beading	0.51	.01	0.41	.04	0.21	.33
Venous loops	0.65	.001	0.30	.14	0.02	.91
NVE	0.45	.02	0.62	.001	0.09	.67
NVD	0.62	.001	0.35	.08	0.10	.63
FPE	0.35	.09	0.71	.0001	0.01	.96
Vitreous hemmorrhage	0.35	.09	0.29	.16	0.14	.52
Retinal detachment	0.21	.30	0.32	.15	0.14	.52

IRMA: intraretinal microvascular abnormalities, NVE: new vessels elsewhere, NVD: new vessels on or within 1 DD of the disc, FPE: fibrous proliferation elsewhere.

endostatin were also not significantly correlated with the systemic factors ($\rho = 0.18$, $P = .24$, $\rho = 0.19$, $P = .22$, $\rho = 0.20$, $P = .18$, respectively).

Discussion

Neovascularization is the most important event in the development of proliferative diabetic retinopathy. Prevailing evidence suggests that changes in the relative balance of inducers and inhibitors of angiogenesis may activate the angiogenic switch during tumorigenesis.[13] In some tissues, the absence of angiogenesis inducers may ensure that the switch remains off, while inducers may be present in other tissues but may be blocked by high levels of angiogenesis inhibitors. Thus, either reducing the levels of inhibitors (eg, endostatin and PF-4) or increasing the level of inducers (eg, secondary to induction of VEGF by hypoxia) could alter the balance and activate angiogenesis, leading to the growth of new blood vessels.[13] According to previous studies, a number of angiogenesis inducers are implicated in the pathogenesis of proliferative diabetic retinopathy.[1,2,7,9] However, the role of the balance hypothesis and the participation of inhibitors in the pathogenesis of proliferative diabetic retinopathy have not been investigated.

We simultaneously measured the vitreous and plasma levels of VEGF, which is one of the most important angiogenesis stimulators, as well as the levels of endostatin and PF-4 (angiogenesis inhibitors) in diabetic patients. As a result, we demonstrated that the vitreous levels of VEGF and endostatin were significantly correlated with the severity of diabetic retinopathy. Previous studies have shown that the levels of many potent angiogenic factors are elevated in the vitreous of patients who have proliferative diabetic retinopathy when compared with patients who do not have proliferative retinopathy.[14–19] For example, the vitreous level of VEGF is elevated in patients with proliferative diabetic retinopathy.[16–18] However, this was the first study, to our knowledge, to detect both VEGF (an angiogenesis stimulator) and endostatin (an angiogenesis inhibitor) in the vitreous, and to show that the vitreous levels of these factors were correlated with the severity of diabetic retinopathy.

Vitreous levels of VEGF were significantly correlated with the grade of fundus findings such as soft exudates, IRMA, venous beading, venous loops, NVE, and NVD. Vitreous levels of endostatin were also significantly correlated with the grade of IRMA, venous beading, NVE, and FPE. However, the vitreous levels of VEGF and endostatin were not significantly correlated with the plasma levels of these factors. Vitreous levels of VEGF were much higher than the plasma VEGF levels, but the vitreous level of endostatin was similar to its plasma level. In diabetic retinopathy, VEGF production is induced by local hypoxia, which is caused by occlusion of retinal vessels.[7,8,10] VEGF stimulates the growth of new vessels and is believed to be involved in the pathogenesis of proliferative diabetic retinopathy. On the other hand, the main inhibitor of angiogenesis in patients with ischemic eye disease has not yet been determined. As far as we know, this is the first study to show that endostatin (an angiogenesis inhibitor) is significantly correlated with fundus findings such as retinal ischemic changes (IRMA and venous beading) and proliferative changes (NVE and FPE), making it a strong candidate as an angiogenesis inhibitor in proliferative diabetic retinopathy. Endostatin inhibits VEGF-induced endothelial cell migration in vitro and inhibits tumor growth in vivo.[24] It seems possible that the severity of diabetic retinopathy is

Jpn J Ophthalmol
Vol 45: 577–584, 2001

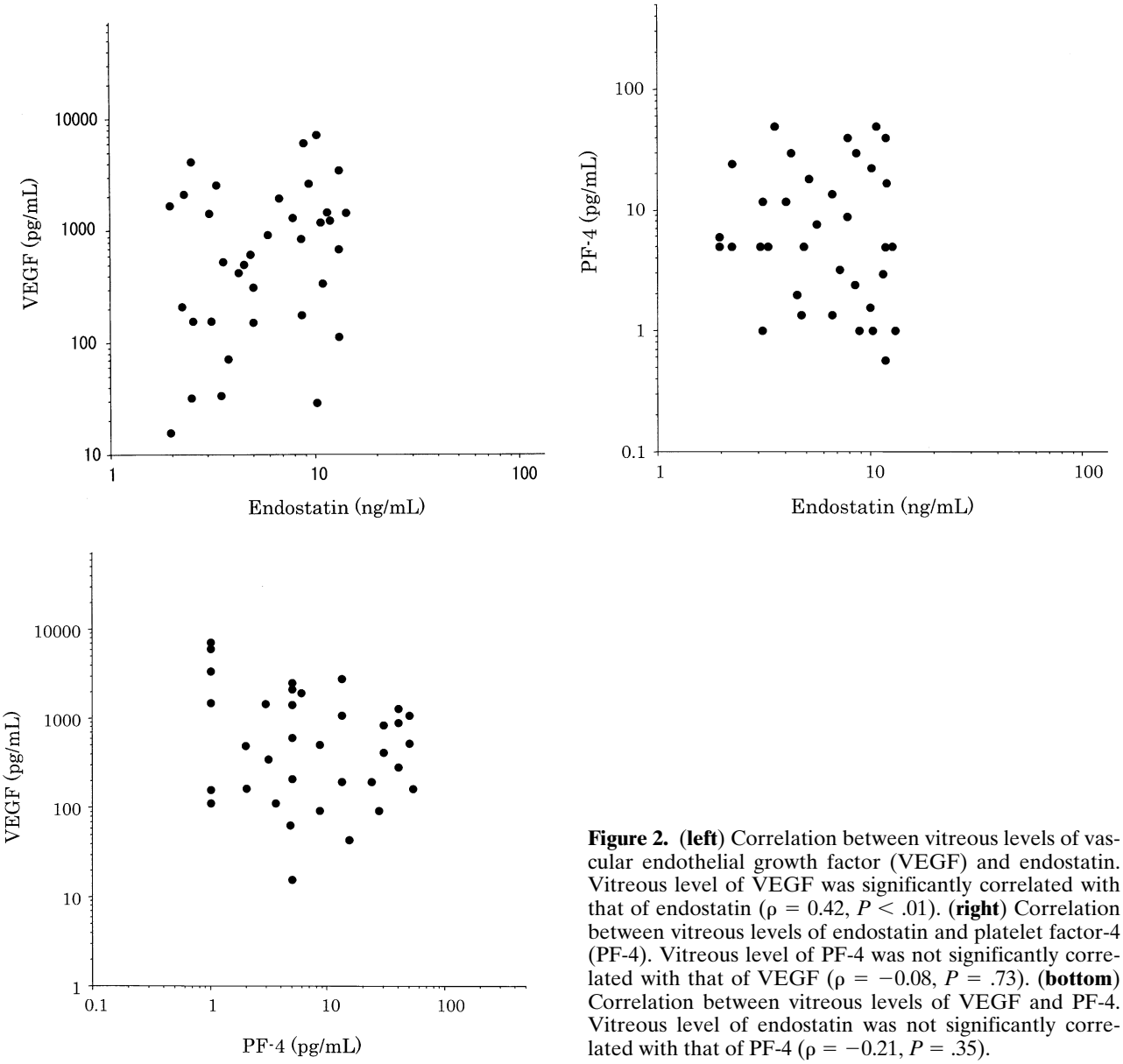

Figure 2. (**left**) Correlation between vitreous levels of vascular endothelial growth factor (VEGF) and endostatin. Vitreous level of VEGF was significantly correlated with that of endostatin ($\rho = 0.42$, $P < .01$). (**right**) Correlation between vitreous levels of endostatin and platelet factor-4 (PF-4). Vitreous level of PF-4 was not significantly correlated with that of VEGF ($\rho = -0.08$, $P = .73$). (**bottom**) Correlation between vitreous levels of VEGF and PF-4. Vitreous level of endostatin was not significantly correlated with that of PF-4 ($\rho = -0.21$, $P = .35$).

also related to the endostatin level in the vitreous fluid; since endostatin blocks at one or more steps in the VEGF-induced migration of cultured human umbilical vein cells and inhibits the growth of human renal carcinoma in vivo at levels that are 1,000-100,000-fold lower than those previously reported to be required for its activity.[24,32] In the present study, there was no significant correlation between the vitreous and plasma levels of VEGF, a finding that was in agreement with an earlier report.[33]

Vitreous levels of VEGF were significantly correlated with those of endostatin, and the vitreous level of endostatin was higher in patients with severe proliferative retinopathy than in those with mild proliferative retinopathy. Furthermore, diabetic retinopathy was mild in eyes with both a low vitreous level of VEGF and a low level of endostatin, while retinopathy was severe in eyes with high vitreous levels of VEGF regardless of the level of endostatin. These results suggest that the vitreous VEGF level increases above the endostatin level in severe diabetic retinopathy. It is possible that a change in the balance between VEGF and endostatin is implicated in the pathogenesis of diabetic retinopathy.

There are several possibilities with respect to this relationship: (1) both VEGF and endostatin may in-

Table 2. Relationship Between Vitreous Concentrations of Vascular Endothelial Growth Factor (VEGF) AND Endostatin

		VEGF Concentration			
		<800 pg/mL		≥800 pg/mL	
Endostatin Concentration	ETDRS Severity	n	%	n	%
<7 ng/mL	10–53	9	56.2	0	0
	61	2	12.5		
	65	2	12.5	0	0
	71	3	18.8	1	16.7
	75	0	0	1	16.7
	81	0	0	2	33.3
	85	0	0	2	33.3
	All	16	100.0	6	100.0
≥7 ng/mL	10–53	0	0	0	0
	61				
	65	0	0	0	0
	71	0	0	2	18.1
	75	1	50.0	3	27.3
	81	1	50.0	3	27.3
	85	0	0	3	27.3
	All	2	100.0	11	100.0

fluence the development of diabetic retinopathy; (2) vitreous endostatin levels may *contribute secondarily* to an increase of VEGF expression or secretion; (3) VEGF activity may be much higher than endostatin activity in severe proliferative diabetic retinopathy; and (4) vitreous endostatin levels may show two patterns in severe proliferative diabetic retinopathy (either a high concentration or a low concentration). It is unknown whether the increase of endostatin in the vitreous fluid is secondary to enhanced production in extraocular tissues and/or in the eye itself. It is also unclear whether or not endostatin plays a role in the pathogenesis of diabetic retinopathy.

In the present study, there was no relationship between the vitreous level of PF-4 and the severity of diabetic retinopathy. PF-4 levels were not significantly correlated with the fundus findings, including the severity of vitreous hemorrhage. Furthermore, the vitreous PF-4 level was lower than the plasma level. Finally, we found no relationship between the vitreous levels of PF-4 and VEGF. PF-4 is synthesized by megakaryocytes and is usually sequestered inside platelets.[25] It displays anti-angiogenic activity in vivo and can inhibit tumor growth without affecting the proliferation of cancer cells.[20] N-terminal processed PF-4 exhibits a 30- to 50-fold greater inhibition of endothelial cell growth than native PF-4,[21] and PF-4 has been shown to inhibit the VEGF-induced proliferation of vascular endothelial cells.[22] The molecular weights of PF-4, VEGF, and endostatin are 29 kD, 23 kD, and 20 kD, respectively. Despite these findings, it seems that PF-4 is not involved in the pathogenesis of diabetic retinopathy.

In conclusion, the present study showed that the vitreous levels of VEGF and endostatin were correlated with the severity of diabetic retinopathy and with the fundus findings, but were not correlated with the plasma levels of these factors. These findings suggest that the balance between VEGF (an angiogenesis stimulator) and endostatin (an angiogenesis inhibitor) may determine whether or not angiogenesis occurs in proliferative diabetic retinopathy.

This study was supported by Health Science Research Grant (#10060101, Dr. Funatsu, Dr. Hori, Dr. Yamashita) from the Ministry of Health and Welfare, Research on Eye and Ear Sciences, Immunology, Allergy and Organ Transplantation in Japan.

The authors thank Dr. Shigehiko Kitano, Dr. Yasuhiro Konno, Dr. Kozue Ohara, Dr. Kaori Sekimoto, Dr. Rie Takeda, Dr. Koji Makita, Dr. Tatsuya Mimura, Dr. Kensuke Haruyama, Dr. Shinko Nakamura, and Dr. Maho Hirai for assistance with ophthalmological examinations and the collection of vitreous samples. We thank Dr. Yasuhiko Iwamoto and Dr. Naoko Iwasaki for assistance in the internal medical examinations. We wish to thank Katsunori Shimada for his assistance with statistical analysis.

References

1. D'Amore PA. Mechanisms of retinal and choroidal neovascularization. Invest Ophthalmol Vis Sci 1994;35:3974–9.
2. Casey R, Li WW. Factors controlling ocular angiogenesis. Am J Ophthalmol 1997;124:521–9.
3. Shimizu K, Kobayashi Y, Muraoka K. Midperipheral fundus involvement in diabetic retinopathy. Ophthalmology 1981;88:601–14.

Jpn J Ophthalmol
Vol 45: 577–584, 2001

4. Davis M. Diabetic retinopathy. A clinical review. Diabetes Care 1992;15:1844–74.
5. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983–5.
6. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306–9.
7. Pe'er J, Shweiki D, Itin A, Hemo I, Gnessin H, Keshet E. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. Lab Invest 1995;72:638–45.
8. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LEH. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci USA 1995;92:905–9.
9. Lutty GA, McLeod DS, Merges C, Diggs A, Plouét J. Localization of vascular endothelial growth factor in human retina and choroid. Arch Ophthalmol 1996;114:971–9.
10. Aiello LP, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular endothelial growth factor in retinal cells. Arch Ophthalmol 1995;113:1538–44.
11. Thieme H, Aiello LP, Takagi H, Ferrara N, King GL. Comparative analysis of vascular endothelial growth factor receptors on retinal and aortic vascular endothelial cells. Diabetes 1995;44:98–103.
12. Takagi H, King GL, Aiello LP. Identification and characterization of vascular endothelial growth factor receptor (Flt) in bovine retinal pericytes. Diabetes 1996;45:1016–23.
13. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86: 353–64.
14. Sivalingham A, Kenny J, Brown GC, Benson W, Donoso L. Basic fibroblast growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch Ophthalmol 1990;108:869–72.
15. Asar AMAE, Maimone D, Morse PH, Gregory S, Reder AT. Cytokines in the vitreous of patients with proliferative diabetic retinopathy. Am J Ophthalmol 1992;114:731–6.
16. Adamis AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 1994; 118:445–50.
17. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480–7.
18. Katsura Y, Okano T, Noritake M, et al. Hepatocyte growth factor in vitreous fluid of patients with proliferative diabetic retinopathy and other retinal disorders. Diabetes Care 1998;21:1759–63.
19. Hirase K, Ikeda T, Sotozono C, Nishida K, Sawa H, Kinoshita S. Transforming growth factor β2 in the vitreous in proliferative diabetic retinopathy. Arch Ophthalmol 1998;116:738–41.
20. Maione TE, Gray GS, Petro J, et al. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 1990;247:77–9.
21. Gupta SK, Hassel T, Singh JP. A potent inhibitor of endothelial cell proliferation is generated by proteolytic cleavage of the chemokine platelet factor 4. Proc Natl Acad Sci USA 1995;92:7799–803.
22. Gengrinovitch S, Greenberg SM, Cohen T, et al. Platelet factor-4 inhibit the mitogenic activity of VEGF121 and VEGF165 using several concurrent mechanisms. J Biol Chem 1995;270: 15059–65.
23. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin. Endogenous inhibitor of angiogenesis and tumor growth. Cell 1997;88:277–85.
24. Yamaguchi N, Anand-Apte B, Lee M, et al. Endostatin inhibits VEGF-induced endothelial cell migration and tumor growth independently of zinc binding. EMBO J 1999;18:4414–23.
25. Ravid K, Beeler DL, Rabin MS, Ruley HE, Rosenberg RD. Selective targeting of gene products with the megakaryocyte platelet factor 4 promoter. Proc Natl Acad Sci USA 1991;88:1521–5.
26. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs. An extension of the modified Airlie House classification. ETDRS Report Number 10. Ophthalmology 1991;98:786–806.
27. Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy. RTDRS Report Number 12. Ophthalmology 1991;98:823–33.
28. Hyodo I, Doi T, Endo H, et al. Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. Eur J Cancer 1998;34:2041–5.
29. Hefler L, Tempfer C, Kainz C, Obermair A. Serum concentrations of endostatin in patients with vulvar cancer. Gynecol Oncol 1999;74:151–2.
30. Bellon JL, Castellanos C, Acevedo L, Amiral J. Measurement of beta-thromboglobulin and platelet factor 4 to follow up patients with artificial heart valves. Semin Thromb Hemost 1993;19(Suppl 1):178–82.
31. SAS Institute. SAS/STAT Software: changes and enhancements through release 6.12. 1–1167, 1997.
32. Dhanabal M, Ramchandran R, Volk R, et al. Endostatin: yeast production, mutants, and antitumor effect in renal cell carcinoma. Cancer Res 1999;59:189–97.
33. Burgos R, Shimo R, Audi L, et al. Vitreous levels of vascular endothelial growth factor are not influenced by its serum concentrations in diabetic retinopathy. Diabetologia 1997;40: 1107–9.