Serum Concentrations of Bevacizumab (Avastin) and Vascular Endothelial Growth Factor in Infants With Retinopathy of Prematurity

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• PURPOSE: To determine the serum concentrations of bevacizumab and vascular endothelial growth factor (VEGF) in infants with retinopathy of prematurity (ROP) who received intravitreal bevacizumab; and to determine whether the changes in the serum concentration of bevacizumab were significantly correlated with the serum concentration of VEGF after intravitreal bevacizumab.

• DESIGN: Case series.

• METHODS: Eleven infants (4 girls and 7 boys) with ROP were studied. They received 0.25 mg or 0.5 mg of intravitreal bevacizumab to either 1 eye (unilateral cases) or both eyes (bilateral cases) with vascularly active ROP. Serum samples were collected before and 1 day, 1 week, and 2 weeks after the intravitreal bevacizumab. The serum concentrations of bevacizumab and VEGF were measured by enzyme-linked immunosorbent assay, and the correlation in the serum levels between the 2 was determined.

• RESULTS: The serum concentration of bevacizumab before and 1 day, 1week, and 2 weeks after a total of 0.5 mg of intravitreal bevacizumab was 0 ng/mL, 195 ± 324 ng/mL, 946 ± 680 ng/mL, and 1214 ± 351 ng/mL, respectively. The serum bevacizumab level before and 1 day and 1 week after a total 1.0 mg of intravitreal bevacizumab was 0 ng/mL, 248 ± 174 ng/mL, and 548 ± 89 ng/mL, respectively. The serum concentration of VEGF before and 1 day, 1 week, and 2 weeks after a total of 0.5 mg intravitreal bevacizumab was 1628 ± 929 pg/mL, 427 ± 140 pg/mL, 246 ± 110 pg/mL, and 269 ± 157 pg/mL, respectively. There was a significant negative correlation ($r = -0.575$, $P = .0125$) between the serum concentration of bevacizumab and VEGF when a total of 0.25 mg or 0.5 mg of bevacizumab was injected.

• CONCLUSIONS: These results indicate that bevacizumab can escape from the eye into the systemic circulation and reduce the serum level of VEGF in infants with ROP. Continued extensive evaluations of infants are warranted for possible effects after intravitreal bevacizumab in ROP patients. (Am J Ophthalmol 2012; 153:327–333. © 2012 by Elsevier Inc. All rights reserved.)

Accepted for publication Jul 22, 2011.

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RETINOPATHY OF PREMATURITY (ROP) IS THE LEADing cause of infant blindness, especially in developed countries. Retinal photocoagulation of the peripheral avascular retina is commonly used to treat eyes with ROP without retinal detachment, and scleral buckling or vitrectomy is used in ROP eyes with retinal detachment. Recently, early vitrectomy has been used to treat eyes with ROP to obtain favorable functional and structural outcomes.[1] However, some of the ROP eyes have high vascular activity, and vitrectomy in these eyes usually results in poor surgical outcomes.[2]

For such cases with high vascular activity, we have performed vitrectomy combined with a preoperative intravitreal injection of an antibody against vascular endothelial growth factor (VEGF).[3] VEGF is the main growth factor responsible for angiogenesis and is considered to be the primary angiogenic factor that mediates retinal neovascularization in eyes with ROP.[4] Studies of patients with stage 4 ROP showed that the vitreous concentration of VEGF in eyes with vascularly active ROP was significantly higher than in eyes with vascularly inactive ROP,[5–7] and anti-VEGF therapy has been shown to be effective in reducing the angiogenic activity in eyes with ROP.[3,8,9]

Bevacizumab (Avastin; Genentech Inc, South San Francisco, California, USA) is a recombinant humanized monoclonal antibody that is directed against all isoforms of VEGF. Many studies have reported on the effectiveness of intravitreal bevacizumab on neovascular disorders, for example, age-related macular degeneration,[10] proliferative diabetic retinopathy,[11] neovascular glaucoma,[12] and ROP.[3,8,9] In addition, the results of a randomized clinical trial that compared intravitreal bevacizumab as monotherapy with laser therapy in the treatment of ROP have been published.[13] As intravitreal bevacizumab was shown to be of significant benefit compared to laser therapy in zone I stage 3+ ROP, the use of intravitreal bevacizumab in the treatment of ROP is likely to be more common in the near future. However, there are also studies that have

0002-9394/$36.00
doi:10.1016/j.ajo.2011.07.005
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TABLE 1. Demographics of Infants with Retinopathy of Prematurity

Patient	Sex	Eye/Stage	Intravitreal Bevacizumab (mg)	Gestational Age (Weeks)	Body Weight at Birth (g)	Postmenstrual Age at Intravitreal Bevacizumab (Weeks)	Body Weight at Intravitreal Bevacizumab (g)	Time of Intravitreal Bevacizumab to Vitrectomy (Days)
1	Male	Right/5	—	24	753	—	1820	Not applicable
		Left/4A	0.5			36		2
2	Male	Right/4B	—	23	611	—	2490	1
		Left/4A	0.5			39		1
3	Female	Right/4A	0.5	23	492	37	1354	3
		Left/4A	0.5			37		Not applicable
4	Male	Right/5	0.5	24	332	38	1384	2
		Left/4A	0.5			38		2
5	Male	Right/4A	0.5	23	686	41	2098	2
		Left/3	0.5			41		Not applicable
6	Female	Right/3	0.5	26	826	33	1214	Not applicable
		Left/3	0.5			33		Not applicable
7	Female	Right/3	0.25	25	768	41	2600	Not applicable
		Left/4B	—			—		0
8	Male	Right/3	0.25	27	454	51	2151	Not applicable
		Left/3	0.25			51		Not applicable
9	Male	Right/3	0.25	26	828	35	1476	Not applicable
		Left/3	0.25			35		Not applicable
10	Female	Right/3	0.25	23	472	38	940	6
		Left/5	0.25			38		6
11	Male	Right/3	0.25	27	1042	32	1398	Not applicable
		Left/3	0.25			32		Not applicable

reported that intravitreal bevacizumab had adverse systemic effects.[14,15] These adverse effects (for example, systemic thrombotic events and hypertension) are similar to the ones reported after intravenous administration of bevacizumab for cancer treatments. Although no systemic adverse event has been reported after intravitreal bevacizumab in eyes with ROP,[3,8,9,13] the serum concentration of bevacizumab after intravitreal bevacizumab has not been determined.

Thus, the purpose of this study was to determine the serum concentrations of bevacizumab and VEGF in ROP infants who received intravitreal bevacizumab.

METHODS

THE FUNDUS OF INFANTS WITH ROP WAS EXAMINED WITH a slit lamp and contact lens (Volk Quad Pediatric Lens; Volk Optical Inc, Mentor, Ohio, USA) under general anesthesia. During the examinations, fundus photographs and fluorescein angiograms were taken with a RetCam 120 digital fundus camera (Clarity Medical Systems, Inc, Pleasanton, California, USA). The stage of the ROP was based on the International Classification of Retinopathy of Prematurity.[16] The ROP eyes were also classified into 3 groups according to the vascular activity: highly vascular-active ROP, moderately vascular-active ROP, and mildly vascular-active ROP.[17] The eyes with highly vascular-active ROP initially received 0.25 mg or 0.5 mg of intravitreal bevacizumab and underwent 23-gauge pars plicata vitrectomy without cannula system. The surgery was performed within 1 week after the injection when considered to be necessary.[3]

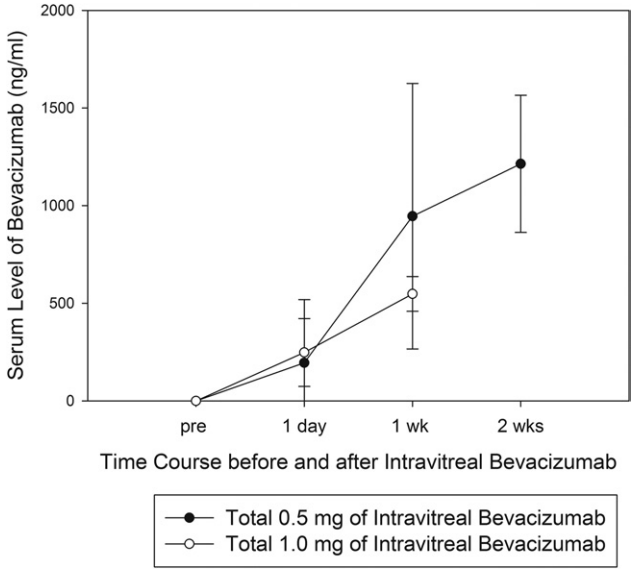


FIGURE 1. Time course of serum level of bevacizumab in infants with retinopathy of prematurity who received intravitreal bevacizumab. The abscissa represents the time before and after intravitreal bevacizumab and the ordinate represents the serum level of bevacizumab.

TABLE 2. Serum Levels of Bevacizumab (Avastin) and Vascular Endothelial Growth Factor in Infants With Retinopathy of Prematurity

			Serum Level of Bevacizumab (ng/mL)				Serum Level of Vascular Endothelial Growth Factor (pg/mL)			
Patient/Sex	Eye/Stage	Total Dosage of Intravitreal Bevacizumab (mg)	Before Intravitreal Bevacizumab	1 Day After Intravitreal Bevacizumab	1 Week After Intravitreal Bevacizumab	2 Weeks After Intravitreal Bevacizumab	Before Intravitreal Bevacizumab	1 Day After Intravitreal Bevacizumab	1 Week After Intravitreal Bevacizumab	2 Weeks After Intravitreal Bevacizumab
1/Male	Right/5 Left/4A	0.5	0	23	NA	NA	NA	NA	NA	NA
2/Male	Right/4B Left/4A	0.5	0	31	81	NA	NA	NA	NA	NA
3/Female	Right/4A Left/4A	1.0	0	206	665	NA	NA	NA	NA	NA
4/Male	Right/5 Left/4A	1.0	0	396	513	NA	NA	NA	NA	NA
5/Male	Right/4A Left/3	1.0	0	19	560	NA	NA	NA	NA	NA
6/Female	Right/3 Left/3	1.0	0	372	453	NA	NA	NA	NA	NA
7/Female	Right/3 Left/4B	0.25	0	11	113	NA	418	303	301	NA
8/Male	Right/3 Left/3	0.5	0	33	1204	NA	603	227	106	NA
9/Male	Right/3 Left/3	0.5	0	36	610	844	1140	515	208	129
10/Female	Right/3 Left/5	0.5	0	841	1905	1255	2110	433	331	439
11/Male	Right/3 Left/3	0.5	0	209	928	1542	2660	533	337	239

NA = The serum levels of bevacizumab and vascular endothelial growth factor in the blank cells could be not measured because of the limited sample volumes.

Blood samples were collected before and 1 day, 1 week, and 2 weeks after the intravitreal bevacizumab. This schedule was based on the data from animal experiments demonstrating that the maximum blood level of bevacizumab is achieved about 1 to 2 weeks after the intravitreal bevacizumab.[18–20] The blood samples were collected in sterile tubes by an anesthesiologist or a neonatologist and centrifuged at 5000 rpm for 10 minutes until a clear separation between serum and the cell components was seen. The serum was transferred to sterile tubes and stored at −80 C until the assay.

The serum concentration of bevacizumab was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Protein Detector ELISA Kit; Kirkegaard & Perry Laboratories, Inc, Gaithersburg, Maryland, USA), according to the manufacturer's protocol and also according to an earlier report with slight modifications.[21] Briefly, microwell plates (Immuno 96 MicroCell solid plates; Nunc, Roskilde, Denmark) were coated with recombinant human $VEGF_{165}$ (PeproTech, Rocky Hill, New Jersey, USA) at a concentration of 1.0 μg/mL for 1 hour at room temperature (100 μL/well). After blocking the wells to reduce nonspecific binding, 100 μL of each sample and different concentrations of the standard were added to the plates. A standard curve was prepared with bevacizumab ranging from 1 ng/mL to 5000 ng/mL. The bound bevacizumab was made visible with 0.1 μg/mL of horseradish peroxidase–goat anti-human IgG (H+L) conjugate prepared by the ELISA kit. The optical density was determined at 405 nm with an absorption spectrophotometer ($ARVO_{MX}$; PerkinElmer Japan, Kanagawa, Japan). The background absorbance was subtracted from all values. This assay measures the free bevacizumab, and all measurements were performed twice according to the manufacturer's recommendation.

The serum concentration of VEGF was measured with an ELISA kit for human anti-VEGF (R & D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's protocol. The anti-VEGF kit can detect the 121 and 165 isoforms of VEGF. The minimum detectable level of the test was 9.0 pg/mL for VEGF. The optical density was determined at 450 nm with the absorption spectrophotometer with the correction wavelength set at 540 nm. The assay was also performed in duplicate.

Statistical analyses were performed using the SPSS software (Sigma Stat; Systat Software, Inc, San Jose, California, USA). Data are presented as the means and standard deviations. If the data were normally and equally distributed, 1-way repeated-measures analysis of variance was used to compare 3 or more matched groups, followed by the Holm-Sidak method to detect significant differences between each set of data. If the data were not normally or equally distributed, Friedman repeated-measures analysis of variance on ranks was performed to compare 3 or more matched groups, followed by Dunn's method to detect significant differences between each set of data. The significance of differences between 2 groups was determined by *t* tests if the data were normally and equally distributed and by the Mann-Whitney rank sum test if not normally distributed. The correlation between 2 parameters was determined by the Spearman rank order correlation because the residuals were not normally distributed with constant variance. A *P* value less than .05 was considered to be statistically significant.

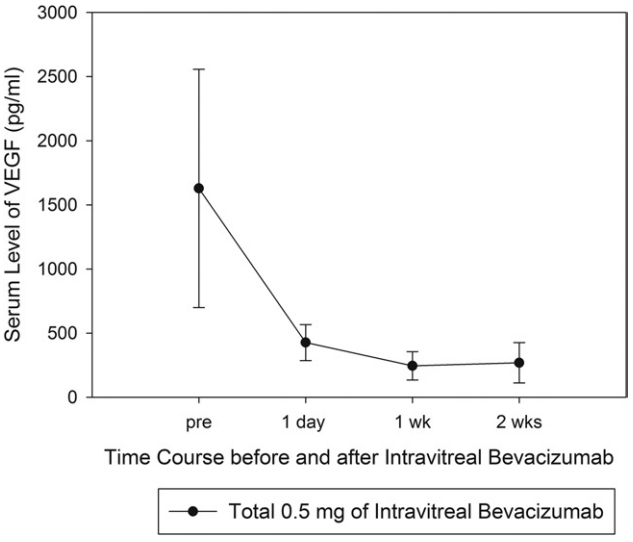


FIGURE 2. Time course of serum level of vascular endothelial growth factor (VEGF) in infants with retinopathy of prematurity who received a total of 0.5 mg of intravitreal bevacizumab. The abscissa represents the time before and after intravitreal bevacizumab and the ordinate represents the serum level of VEGF.

RESULTS

ELEVEN INFANTS (4 GIRLS AND 7 BOYS) WITH HIGHLY VASCULAR-active ROP were studied. The demographics of the patients are summarized in Table 1. Three patients received intravitreal bevacizumab in 1 eye and the other 8 received intravitreal bevacizumab in both eyes. The mean gestational age of the infants was 25 weeks (range, 23–27 weeks), and the mean body weight at birth was 660 grams (range, 332–1042 grams). All of the infants had received laser photocoagulation of the peripheral avascular retina before the intravitreal bevacizumab. The mean postmenstrual age of the infants at the time of intravitreal bevacizumab was 38 weeks (range, 32–51 weeks), and the mean body weight at the time of the intravitreal bevacizumab was 1720 grams (range, 940–2600 grams). In Patients 6, 8, 9, and 11 with stage 3 ROP, vitrectomy was not performed after the intravitreal bevacizumab because of the reduction of vascular activities. In the remaining patients, vitrectomy was performed 0 to 6 days after the intravitreal bevacizumab.

The average serum levels of bevacizumab before and 1 day, 1 week, and 2 weeks after a total of 0.5 mg of

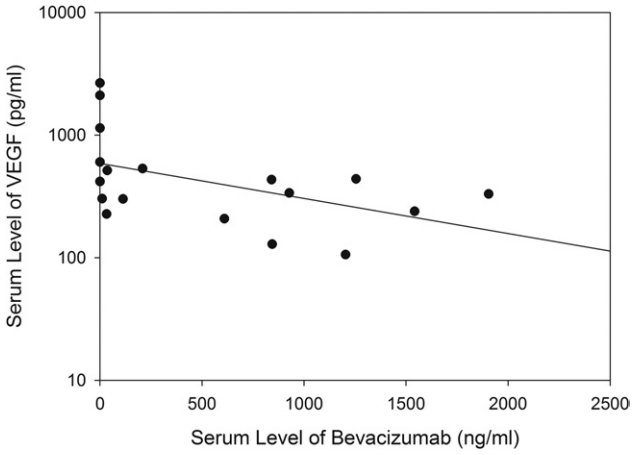


FIGURE 3. Correlation between bevacizumab and vascular endothelial growth factor (VEGF) levels in the serum of patients with retinopathy of prematurity (ROP). The abscissa represents the serum levels of bevacizumab and the ordinate represents the VEGF level in ROP infants. Statistical analyses were performed by Spearman rank order correlation ($r = -0.575$, $P = .0125$).

intravitreal bevacizumab were 0 ng/mL, 195 ± 324 ng/mL, 946 ± 680 ng/mL, and 1214 ± 351 ng/mL, respectively (Figure 1, Table 2). In Patients 2, 8, 9, 10, and 11, the serum bevacizumab levels were significantly different ($P = .008$) before and 1 day and 1week after the intravitreal bevacizumab, and the serum bevacizumab level at 1 week after the intravitreal bevacizumab was significantly higher ($P < .05$) than that before the intravitreal bevacizumab.

The average serum bevacizumab levels before and 1 day and 1 week after a total of 1.0 mg of intravitreal bevacizumab were 0 ng/mL, 248 ± 174 ng/mL, and 548 ± 89 ng/mL, respectively (Figure 1, Table 2). In Patients 3 through 6, the serum bevacizumab levels were significantly different ($P = .005$) before and 1 day and 1 week after the intravitreal bevacizumab. The serum bevacizumab level at 1 week after the intravitreal bevacizumab was significantly higher ($P < .05$) than that before the intravitreal bevacizumab. The differences in the serum bevacizumab levels after a total of 0.5 mg and 1.0 mg of intravitreal bevacizumab were not significant at any time points.

The average serum concentrations of VEGF before and 1 day, 1 week, and 2 weeks after a total of 0.5 mg of intravitreal bevacizumab were 1628 ± 929 pg/mL, 427 ± 140 pg/mL, 246 ± 110 pg/mL, and 269 ± 157 pg/mL, respectively (Figure 2, Table 2). The serum VEGF level in Patients 1 through 6 could not be measured because the volumes of the samples collected were too small. In Patients 8 through 11, who received a total of 0.5 mg of intravitreal bevacizumab, the serum VEGF levels were significantly different ($P = .005$) before and 1 day and 1 week after the intravitreal bevacizumab. The serum VEGF level at 1 week after the intravitreal bevacizumab was significantly lower ($P < .05$) than that before the intravitreal bevacizumab.

The correlation of serum levels of bevacizumab and VEGF was investigated in Patients 7 through 11, who received a total of 0.25 mg or 0.5 mg of intravitreal bevacizumab. The results showed that there was a significant negative correlation between the 2 levels ($r = -0.575$, $P = .0125$; Figure 3).

DISCUSSION

OUR RESULTS SHOWED THAT THE SERUM BEVACIZUMAB level was significantly higher at 1 week after than before the intravitreal bevacizumab in the ROP infants who received a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab. In addition, the serum VEGF level was significantly lower at 1 week after than before the intravitreal bevacizumab in the ROP infants who underwent a total of 0.5 mg of intravitreal bevacizumab. Our results showed that there was a significant negative correlation between the serum levels of bevacizumab and VEGF in the ROP infants who received a total of 0.25 mg or 0.5 mg of intravitreal bevacizumab.

With regard to the serum bevacizumab level, animal experiments showed that the blood level of bevacizumab peaked at about 1 to 2 weeks after intravitreal bevacizumab.[18–20] In our patients, the serum level of bevacizumab was significantly increased 1 week after the intravitreal bevacizumab after a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab, and the highest serum level of bevacizumab was achieved 2 weeks after the intravitreal bevacizumab after a total of 0.5 mg of intravitreal bevacizumab.

An in vitro experiment using human umbilical vein endothelial cells demonstrated that about 500 ng/mL of bevacizumab was able to completely block the VEGF activities.[22] Our results showed that the average serum level of bevacizumab exceeded 500 ng/mL at 1 week after the intravitreal bevacizumab in ROP infants who received a total of 0.5 mg or 1.0 mg of intravitreal bevacizumab. These results account for the decreased serum levels of VEGF after intravitreal bevacizumab in ROP infants. The results of an in vivo experiment (interview form for bevacizumab, Chugai Oncology, Tokyo, Japan; available only in Japanese) showed that a once-weekly intravenous injection of 2 mg/kg of bevacizumab in young macaque monkeys did not induce any obvious side effects 26 weeks after the beginning of the injections. The in vivo experiment demonstrated that the bevacizumab concentration in the serum 1 week after 1 intravenous injection of 2 mg/kg bevacizumab was over 10 μg/mL, which is much higher than the maximum serum level of bevacizumab in our study. Fortunately, the patients in our study did not show any systemic adverse events as far as our neonatologists

(K.W. and H.A.) could determine. However, a careful long-term study is necessary.

Our results also showed that the VEGF level significantly decreased 1 week after intravitreal bevacizumab, and the VEGF level was significantly correlated negatively with the serum bevacizumab level. These results suggest that bevacizumab escapes from the vitreous into the systemic circulation and reduces the VEGF concentrations in ROP infants after the intravitreal bevacizumab.

Data regarding the safe range of VEGF serum concentrations in premature infants with ROP have not been reported, although the systemic levels of VEGF in infants with or without ROP have been investigated.[23,24] Villegas-Becerril and associates[23] reported that at 4 to 6 weeks after birth, the mean serum VEGF concentration in premature babies with ROP was 708 pg/mL, which was significantly higher than the 511 pg/mL in premature babies without ROP. Pieh and associates[24] reported that the median plasma level of VEGF in ROP infants was 904 pg/mL at 32 weeks and 344 pg/mL at 36 weeks of postmenstrual age, and that in infants without ROP was 658 pg/mL at 32 weeks and 437 pg/mL at 36 weeks of postmenstrual age. The differences in the VEGF levels between ROP and non-ROP infants at both 32 and 36 weeks were not significant.[24]

The average serum VEGF level before the intravitreal bevacizumab in our ROP infants (Patients 7 through 11), whose average gestational age was 26.0 weeks, was 1386 pg/mL at an average postmenstrual age of 39.8 weeks. After the intravitreal bevacizumab, the average serum VEGF level was comparable to those of the 2 reports[23,24] for both ROP and non-ROP infants. Thus, the intravitreal bevacizumab did not induce an extreme inhibition of VEGF activities in ROP infants, although the serum VEGF level was significantly reduced 1 week after the 0.5 mg of intravitreal bevacizumab in ROP infants. Further studies are needed to determine the safe range of VEGF in ROP infants in order to establish the appropriate dose of intravitreal bevacizumab in ROP infants.

There are some limitations in this study. The number of patients was limited mainly because of the small number of infants with severe ROP. The number of blood samples at various time points was also limited because of the technical difficulties in obtaining blood samples from low-birth weight infants. These limitations made the statistical analyses difficult, and there is a possibility that the serum bevacizumab level may reach its maximal point more than 2 weeks after the intravitreal bevacizumab. Another limitation is that all of the infants had received laser photocoagulation to the peripheral avascular retina before the intravitreal bevacizumab. The laser photocoagulation may break down the retinal barrier.[25] Thus, although bevacizumab is a large molecule so that it has difficulty in escaping from the eye,[13] there is a possibility that the retinal photocoagulation led to the higher systemic levels of bevacizumab. In addition, the eyes with preoperative intravitreal bevacizumab received vitrectomy in Patients 1, 2, 3, 4, 5, and 10. Thus, the possible role of vitrectomy should be determined by either allowing the systemic diffusion of bevacizumab by opening the eye or decreasing it by washing out the intravitreal bevacizumab.

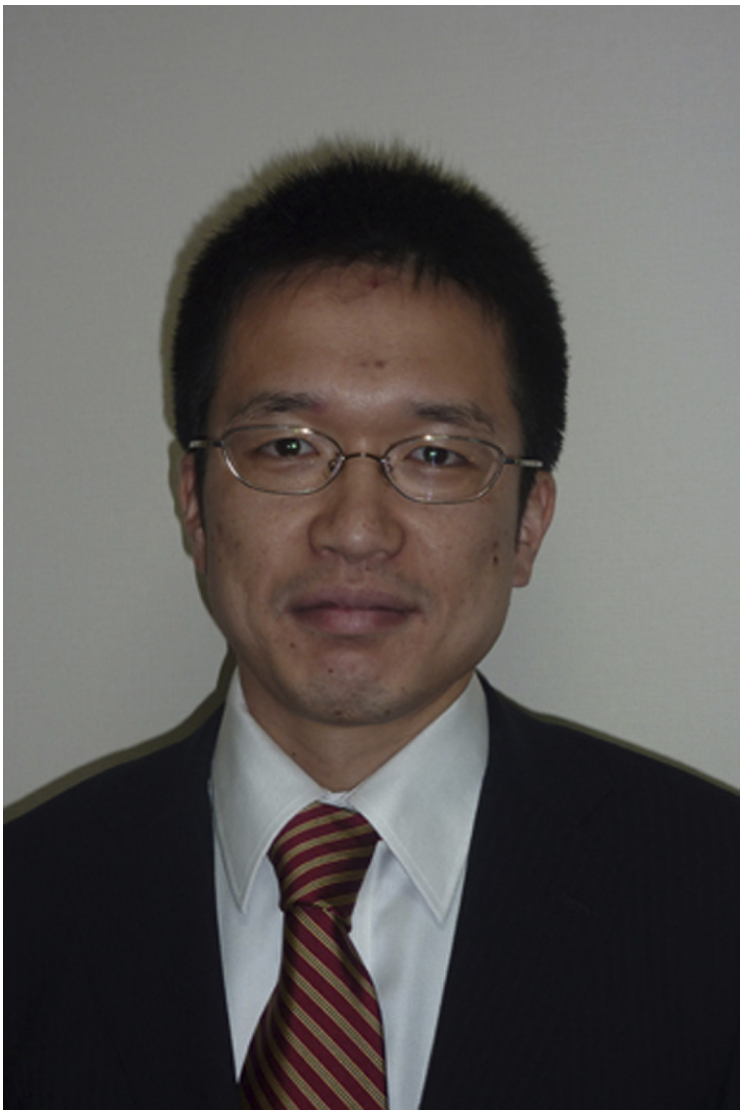
In conclusion, the serum levels of bevacizumab and VEGF were determined in vascularly active ROP infants who received intravitreal bevacizumab. The results suggest that bevacizumab escapes from the vitreous into systemic circulation and could suppress the VEGF concentration in infants with ROP after intravitreal bevacizumab. Although no systemic adverse events were observed in our patients, continued extensive evaluation of infants is warranted for possible effects after intravitreal bevacizumab in ROP patients.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF Interest. Publication of this article was supported by Grant-in-Aid 20592041 from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan. N. Kuno and K. Imoto are employees of Santen Pharmaceutical Co, Ltd. Involved in conception and design (S.K.); analysis and interpretation (T.S., S.K.); writing the article (T.S., S.K.); critical revision (S.K.); final approval (S.K.); data collection (K.W., H.A., C.I.-S.); provision of patients and resources (S.K.); statistical expertise (T.S., S.K.); obtaining funding (S.K.); literature search (T.S., S.K.); and technical support (N.K., K.I.). The sponsor or funding organization had no role in the design or conduct of this research. The procedures used in this study conformed to the tenets of the Declaration of Helsinki. The Institutional Review Board of Osaka University Hospital approved this retrospective study. The parents of all of the patients provided written informed consent after an explanation of the nature and possible consequences of this study.

REFERENCES

1. Azuma N, Ishikawa K, Hama Y, Hiraoka M, Suzuki Y, Nishina S. Early vitreous surgery for aggressive posterior retinopathy of prematurity. Am J Ophthalmol 2006;142(4):636–643.
2. Hartnett ME. Features associated with surgical outcome in patients with stages 4 and 5 retinopathy of prematurity. Retina 2003;23(3):322–329.
3. Kusaka S, Shima C, Wada K, et al. Efficacy of intravitreal injection of bevacizumab for severe retinopathy of prematurity: a pilot study. Br J Ophthalmol 2008;92(11):1450–1455.
4. Mechoulam H, Pierce EA. Retinopathy of prematurity: molecular pathology and therapeutic strategies. Am J Pharmacogenomics 2003;3(4):261–277.
5. Sonmez K, Drenser KA, Capone A Jr, Trese MT. Vitreous levels of stromal cell-derived factor 1 and vascular endothelial growth factor in patients with retinopathy of prematurity. Ophthalmology 2008;115(6):1065–1070.
6. Sato T, Kusaka S, Shimojo H, Fujikado T. Vitreous levels of erythropoietin and vascular endothelial growth factor in eyes with retinopathy of prematurity. Ophthalmology 2009;116(9):1599–1603.

7. Sato T, Kusaka S, Shimojo H, Fujikado T. Simultaneous analyses of vitreous levels of 27 cytokines in eyes with retinopathy of prematurity. Ophthalmology 2009;116(11): 2165–2169.
8. Mintz-Hittner HA, Kuffel RR Jr. Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina 2008; 28(6):831–838.
9. Wu WC, Yeh PT, Chen SN, Yang CM, Lai CC, Kuo HK. Effects and complications of bevacizumab use in patients with retinopathy of prematurity: a multicenter study in Taiwan. Ophthalmology 2011;118(1):176–183.
10. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 2006;113(3):363–372.
11. Avery RL, Pearlman J, Pieramici DJ, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. Ophthalmology 2006;113(10):1695–1705.
12. Oshima Y, Sakaguchi H, Gomi F, Tano Y. Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. Am J Ophthalmol 2006;142(1):155–158.
13. Mintz-Hittner HA, Kennedy KA, Chuang AZ; BEAT-ROP Cooperative Group. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N Engl J Med 2011; 364(7):603–615.
14. Fung AE, Rosenfeld PJ, Reichel E. The international intravitreal bevacizumab safety survey: using the internet to assess drug safety worldwide. Br J Ophthalmol 2006;90(11):1344–1349.
15. Shima C, Sakaguchi H, Gomi F, et al. Complications in patients after intravitreal injection of bevacizumab. Acta Ophthalmol 2008;86(4):372–376.
16. International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol 2005; 123(7):991–999.
17. Sato T, Shima C, Kusaka S. Vitreous levels of angiopoietin-1 and angiopoietin-2 in eyes with retinopathy of prematurity. Am J Ophthalmol 2011;151(2):353–357.
18. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 2007;114(5):855–859.
19. Miyake T, Sawada O, Kakinoki M, et al. Pharmacokinetics of bevacizumab and its effect on vascular endothelial growth factor after intravitreal injection of bevacizumab in macaque eyes. Invest Ophthalmol Vis Sci 2010;51(3): 1606–1608.
20. Nomoto H, Shiraga F, Kuno N, et al. Pharmacokinetics of bevacizumab after topical, subconjunctival, and intravitreal administration in rabbits. Invest Ophthalmol Vis Sci 2009; 50(10):4807–4813.
21. Zhu Q, Ziemssen F, Henke-Fahle S, et al. Vitreous levels of bevacizumab and vascular endothelial growth factor-A in patients with choroidal neovascularization. Ophthalmology 2008;115(10):1750–1755.
22. Wang Y, Fei D, Vanderlaan M, Song A. Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro. Angiogenesis 2004;7(4):335–345.
23. Villegas-Becerril E, Gonzalez-Fernandez R, Perula-Torres L, Gallardo-Galera JM. IGF-1, VEGF, and bFGF as predictive factors for the onset of retinopathy of prematurity (ROP). Arch Soc Esp Oftalmol 2006;81(11):641–646.
24. Pieh C, Agostini H, Buschbeck C, et al. VEGF-A, VEGFR-1, VEGFR-2 and Tie2 levels in plasma of premature infants: relationship to retinopathy of prematurity. Br J Ophthalmol 2008;92(5):689–693.
25. Sato Y, Berkowitz BA, Wilson CA, deJuan E Jr. Blood-retinal barrier breakdown caused by diode vs argon laser endophotocoagulation. Arch Ophthalmol 1992;110(2):277–281.



Biosketch

Tatsuhiko Sato, MD, received his medical degree from Osaka University Medical School, Osaka, Japan, in 2001. He completed residency at Osaka University Hospital. Now he is an attending staff in Ophthalmology at Osaka Rosai Hospital. His field of interest includes surgical treatment of vitreoretinal disease such as diabetic retinopathy, retinal detachment, and retinopathy of prematurity.