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RETINAL DISORDERS

Effects of intravitreal bevacizumab and laser in retinopathy of prematurity therapy on the development of peripheral retinal vessels

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Abstract
Objective To investigate laser photocoagulation and intravitreal bevacizumab efficacy and safety in patients with moderate-to-severe stage 3 retinopathy of prematurity (ROP), and to evaluate the effects of treatment on the development of peripheral retinal vessels.
Methods A retrospective chart review of 15 premature babies, all of whom were diagnosed with stage 3 ROP, was conducted. Patients with moderate-to-severe stage 3 ROP, thus with a vascular-active ROP, received intravitreal injections of bevacizumab (0.5 mg/0.02 ml) and laser photocoagulation, whereas those with relatively inactive ROP received laser photocoagulation only. Patients were examined 1, 2, 4, and 8 weeks after treatment, or until peripheral retinal vessel growth over the laser scar was noted.
Results Both eyes of 15 patients diagnosed with moderate-to-severe stage 3 ROP were evaluated. Eight patients (n=16 eyes) received intravitreal bevacizumab injection and laser photocoagulation, and seven patients (n=14 eyes) received laser photocoagulation only. During the follow-up period, regression of plus disease and peripheral retinal vessel development appeared significantly more rapidly in patients who received both intravitreal bevacizumab injection and laser photocoagulation. Peripheral retinal vessel development over the laser scar was identified 1–2 weeks after treatment. No systemic or significant ocular complications, such as vitreous hemorrhage, retinal detachment, or endophthalmitis, were noted during follow-up after treatment.
Conclusions A combination of laser photocoagulation and intravitreal bevacizumab injection seems to be a safe and effective therapy in patients with moderate-to-severe stage 3 ROP, promulgating rapid development of peripheral retinal vessels.

Keywords Bevacizumab · ROP (retinopathy of prematurity) · Vasculogenesis

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Introduction

Retinopathy of prematurity (ROP) is a leading cause of childhood blindness. Although most cases resolve spontaneously, some patients require treatment to prevent the progression of fibrovascular proliferation and tractional retinal detachment. Significant progress has been made in the treatment of ROP, using laser photocoagulation or vitrectomy. However, a more effective modality is needed in some patients, to prevent aggravation of ROP prior to intervention using vitrectomy.

A number of angiogenic factors, including fibroblast growth factor (FGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF), have been shown to play a role in retinal vasculogenesis [1, 2]. VEGF is an important contributor to neovascularization in various retinal neovascular diseases, including ROP. Accordingly, blocking the action of VEGF might be expected to reduce the vascular activity associated with ROP. In contrast, treatments that involve the use of laser photocoagulation or cryotherapy disrupt cells, resulting in release of VEGF in the peripheral retina [2, 3].

Bevacizumab (Avastin; Genentech, San Francisco, CA, USA) is a humanized, recombinant monoclonal antibody against full-length VEGF that inhibits the biological activity of all forms of the molecule. Although originally approved by the FDA for treatment of metastatic colorectal cancer, bevacizumab is now widely used to treat angiogenic disorders and to increase vasopermeability in conditions including age-related macular degeneration, proliferative diabetic retinopathy, neovascular glaucoma, and macular edema related to retinal vein occlusion. Recent small-scale clinical studies using intravitreal bevacizumab to treat ROP have yielded promising results, with reduced neovascularization in the absence of apparent ocular or systemic adverse effects [4–8]. These results indicate that bevacizumab could be useful in the treatment of severe ROP or to prevent ROP aggravation. However, Spitzer and colleagues [9] reported that bevacizumab only moderately inhibited the growth of pig choroidal endothelial cells, and that high-dose bevacizumab may be harmful to the human retinal pigment-synthesizing cell line ARPE-19 in vitro.

Because VEGF expression is essential for maintaining a balance between normal angiogenesis and vascular permeability [10], suppression of VEGF synthesis could produce unexpected side-effects, especially in a premature baby, in whom VEGF inhibition might retard normal growth of the developing retinal vasculature. The present study investigated the efficacy and safety of combined intravitreal bevacizumab and laser photocoagulation as a treatment paradigm for newborns with moderate-to-severe stage 3 ROP. In addition, we specifically evaluated the effects of VEGF suppression on the normal development of the peripheral retinal vasculature.

Methods

We retrospectively reviewed the clinical charts of premature babies diagnosed with moderate-to-severe stage 3 [11] ROP at the Department of Ophthalmology of the Asan Medical Center, Seoul, Korea. Our study was a retrospective, consecutive, comparative case series, and was approved by the Institutional Review Board of our Center. Patients with moderate-to-severe stage 3 ROP that was vascular-active received intravitreal bevacizumab (0.5 mg/0.02 ml) injections, and laser photocoagulation using an 810 nm laser indirect ophthalmoscope (LIO); patients with relatively inactive vascular ROP were treated with laser photocoagulation only. The decision on whether a lesion was vascular-active or not depended on the extent of extraretinal fibrovascular tissue infiltrating the vitreous, and the presence of active tunica vasculosa lentis. Patients were followed-up 1, 2, 4, and 8 weeks after treatment, or until peripheral retinal vessel growth over the laser scar was confirmed.

At first examination, and during each follow-up, the fundus was examined by indirect ophthalmoscopy and, if indicated, wide-field photography. A RetCam (Clarity Medical Systems, Pleasanton, CA, USA) was employed to record the severity of ROP, the regression of new vessels, and the development of peripheral retinal vessels after treatment.

Informed consent was obtained from all parents after careful explanation of the expected risks and benefits of intravitreal bevacizumab treatment in addition to conventional laser photocoagulation. All procedures were performed under general anesthesia. After photocoagulation of the peripheral avascular retina using a diode laser, intravitreal bevacizumab (0.5 mg/0.02 ml) was injected into both eyes using a 30-gauge needle.

Results

Both eyes of 15 patients diagnosed with moderate-to-severe stage 3 ROP were evaluated in the present study. Eight patients (n=16 eyes) received intravitreal bevacizumab injections and laser photocoagulation, and seven patients (n=14 eyes) were treated with laser photocoagulation only. There were no significant differences in birth weight or gestational age between the two groups. During the follow-up period, regression of plus disease and the development of peripheral retinal vessels appeared significantly more rapidly in patients who received both intravitreal bevacizumab injection and laser photocoagulation than laser photocoagulation alone (Table 1). All patients had moderate-to-severe stage 3 ROP. The demographics of patients are presented in Tables 2 and 3. Bevacizumab was used at a mean gestational

Table 1 Comparison between groups receiving LIO plus intravitreal bevacizumab, and LIO only

	LIO + bevacizumab (n=8)	LIO (n=7)	P-value*
Birth weight (g)	820.6±190.5	933.1±355.9	0.524
Gestational age (weeks)	25.7	26.9	0.417
Gender (M:F)	3:5	3:4	
Mean age at treatment (Gestational age, weeks + days)	39+1	36+3	0.201
Decrease in plus sign after treatment (weeks ± SD)	1.0±0	2.3±0.8	0.002**
Peripheral vessel growth after treatment (weeks ± SD)	2.0±0.5	2.9±0.7	0.02**

* Mann–Whitney U test; ** Statistically significant.

Table 2 Demographics of infants receiving intravitreal bevacizumab injection and laser photocoagulation

Patient	Gender	Gestational age (weeks + days)	Birth weight (g)	Zone	Stage	Age at treatment (gestational age; weeks + days)
1	F	25+2	870	3	stage 3	36+4
2	F	27+6	1,140	2	stage 3	36+1
3	M	24+1	555	1	stage 3	36+1
4	M	25+0	750	2	stage 3	37+2
5	M	24+6	780	2	stage 3	36+3
6	F	25+5	1,010	2	stage 3	38+2
7	F	28+6	830	2	stage 3	36+0
8	F	23+5	630	3	stage 3	34+6
Mean		25+5	820.6			36+4

age of 36.6 weeks (minimal gestational age: 34.9 weeks; Table 2).

In patients who received both intravitreal bevacizumab injection and laser photocoagulation, more rapid resolution of the plus sign and regression of the fibrovascular membrane were notable. Also, peripheral retinal vessel development over the laser scar was identified 1 or 2 weeks after treatment. Intravitreal bevacizumab injection did not prevent, but may rather have stimulated, peripheral retinal vessel development (Figs. 1, 2 and 3). In addition to regression of ROP, anterior tunica vasculosa lentis vessels diminished rapidly in number after intravitreal bevacizumab injection (Fig. 4). At last follow-up, both eyes of all 15 patients showed regression of ROP. In addition, no systemic or significant ocular complications, including vitreous hemorrhage, retinal detachment, or endophthalmitis, were noted during treatment or follow-up.

Discussion

Inhibition of VEGF has been shown to be effective in several angiogenic disorders. Bevacizumab, a humanized monoclonal antibody against VEGF, genetically engineered from a murine monoclonal anti-VEGF antibody, has been widely used as an off-label treatment for ocular angiogenic conditions. Because bevacizumab recognizes full-length VEGF and has two binding sites for the molecule, the antibody binds and inhibits all biologically active forms of VEGF. Although bevacizumab is a potent treatment modality for various ocular angiogenic disorders, concerns regarding the safety of the drug in ROP patients remain. VEGF is an essential component of normal vasculogenesis. Thus, if intravitreal bevacizumab inhibits retinal vascular development, premature babies may be at greater risk after bevacizumab treatment because retinal vasculogenesis continues to the full gestational term. In addition, VEGF is an important neurotrophic factor in the central nervous system, and is also involved in retinal development. Thus, bevacizumab may be toxic in rapidly developing newborn eyes. Recently, Wu and colleagues [12] reported that the serum level of bevacizumab was higher when intravitreal injection was performed at an early age. However, no long-term functional or morphologic changes in the retina were reported after single or multiple injections of 1.25 mg bevacizumab in the newborn rabbit.

In the present study, intravitreal bevacizumab injection did not inhibit peripheral retinal vasculogenesis. Our

Table 3 Demographics of infants receiving laser photocoagulation only

Patient	Gender	Gestational age (weeks + days)	Birth weight (g)	Zone	Stage	Age at treatment (gestational age; weeks + days)
1	M	24+5	1,020	2	stage 3	36+2
2	M	29+5	1,590	3	stage 3	43+2
3	F	24+4	550	3	stage 3	36+0
4	F	28+6	1,100	2	stage 3	36+0
5	F	26+4	872	2	stage 3	39+3
6	F	24+1	830	2–3	stage 3	43+6
7	M	29+0	570	2	stage 3	39+1
Mean		26+6	933.1			39+2

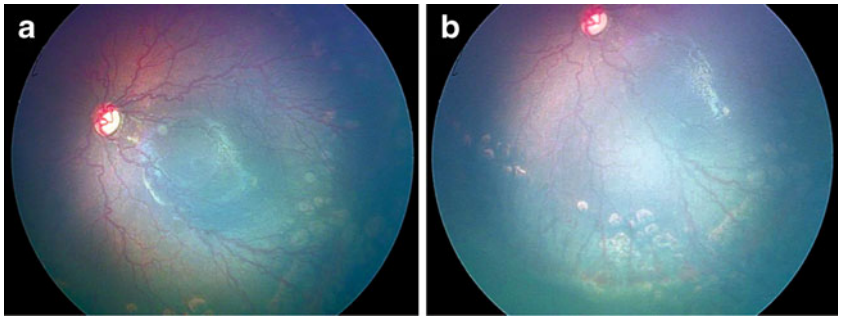

Fig. 1 Panels **a** and **b** show continued vascularization over the laser scar in the temporal retina of patient 3, 3 months after treatment

interpretation is that intravitreal bevacizumab may be safely used at a gestational age of 34+6 weeks. If bevacizumab is used earlier, before a gestational age of 32 weeks, peripheral retinal vasculogenesis may be inhibited. Mintz-Hittner [7] reported that the growth of normal retinal vessels after intravitreal injection of bevacizumab is often slower than the growth seen in very premature infants. The cited author used bevacizumab in infants of gestational age 32–38 weeks, thus younger than the children treated in the present study. Smith [13] reported that ROP demonstrated a diphasic pathologic neovascularization. The early phase I was hyperoxic, with decreased levels of VEGF, and late phase II hypoxia was associated with increased levels of VEGF. Thus, the cited author recommended that anti-VEGF therapy should be administered only during phase II.

Laser photocoagulation and cryotherapy are conventional treatments for threshold ROP without retinal detachment, but some patients with a severe form of ROP often progress to unfavorable outcomes, including tractional retinal detachment or severe vitreous hemorrhage. For this subset of patients, a more effective and rapidly acting treatment is required. The present study shows that combined treatment with intravitreally injected bevacizumab and laser photocoagulation results in rapid ROP regression and development of peripheral retinal vessels within 1–2 weeks. Our interpretation of the data is that intravitreal bevacizumab caused immediate regression of new vessel ingrowth and minimized vitreoretinal traction by the fibrovascular membrane, but allowed subsequent, peripheral, retinal vessel growth. By including intravitreal injection of bevacizumab as a component of initial therapy, changes were effected more rapidly than was possible using laser photocoagulation alone. If bevacizumab encourages peripheral retinal vessel growth, and minimizes vitreoretinal traction by regression of the fibrovascular membrane, the incidence of late complications of regressed ROP, such as tractional retinal detachment, can be decreased [14]. Mintz-Hittner [15] also reported that ROP ceases within 48 h after application of anti-VEGF therapy. This indicates that use of such therapy not only inactivates VEGF in the retina, but also that present in the vitreous. In a recent study, using a rat model of ROP, Hartnett and associates [16] reported that

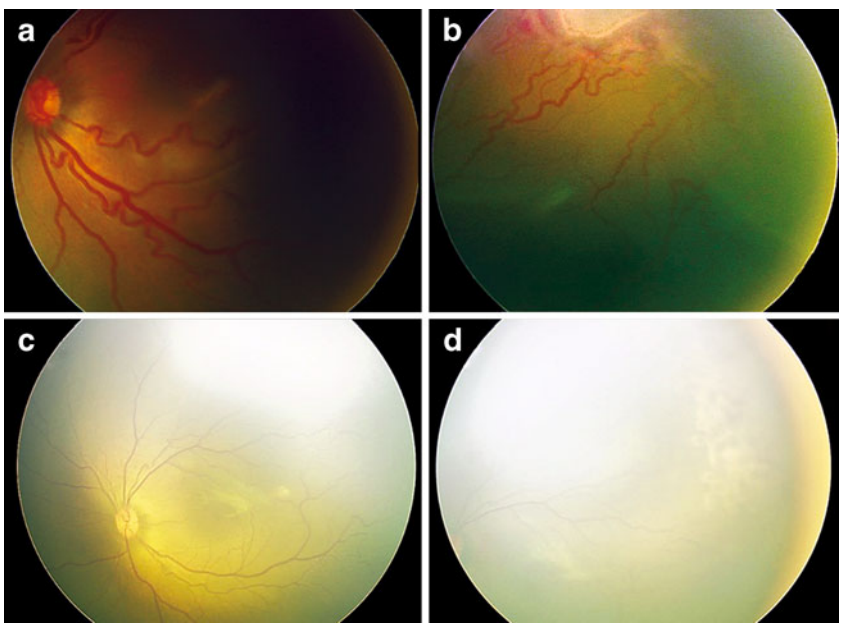

Fig. 2 Fundus images of patient 2. Fundus imaging before treatment shows (**a**) severe plus disease with posterior venous dilatation and arterial tortuosity, and (**b**) severe stage 3 ROP with massive proliferation of fibrovascular tissue. Fundus images obtained 1 month after treatment show (**c**) ROP regression with resolution of plus disease, and (**d**) resolution of fibrovascular tissue with good laser scar formation

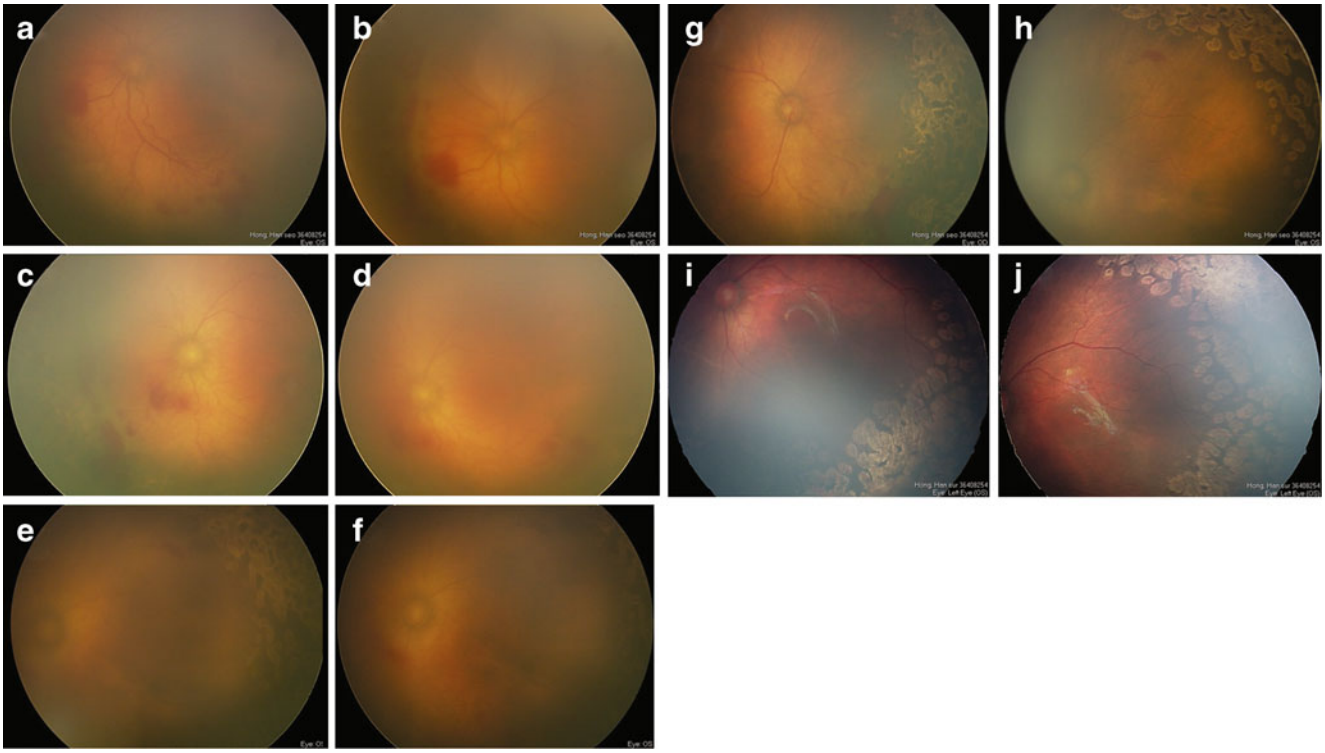

Fig. 3 Fundus images of patient 7. Fundus images before treatment show (**a**, **b**) severe stage 3 ROP with massive proliferation of fibrovascular tissue and retinal hemorrhage. Fundus images after treatment show ROP regression and resolution of fibrovascular tissue, and good laser scar formation. Fundus images **c**, **d**: 1 week after treatment, **e**, **f**: 2 weeks after treatment, **g**, **h**: 3 weeks after treatment. At 2 months after treatment (**i**, **j**), the images show continued peripheral vascularization over the laser scar in the temporal retina

an antibody neutralizing VEGF reduced intravitreous neovascularization and arteriolar tortuosity. The cited authors also showed that the direction of cell division in veins changed from a form favoring vessel widening to a mode encouraging vessel elongation. In addition, cell division in arterioles altered from use of a random cleavage plane to a process favoring vessel elongation, similar to that found during normal development. These findings support our hypothesis, and indicate that appropriate use of anti-VEGF antibodies can induce normal angiogenesis at the cellular level. Thus, intravitreal application of bevacizumab not only induces regression of new vessel ingrowth, but may also promote the rapid development of peripheral vessels over the laser scar. In recent clinical studies, promising results after use of intravitreal bevacizumab injection for ROP patients at high risk of progression have been reported [4, 7, 17]. In addition, the cited authors [7] reported rapid and marked diminution in the plus sign within 24–48 h.

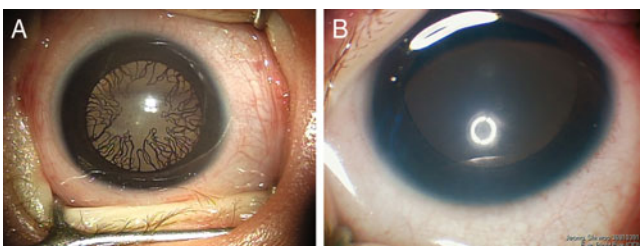

Fig. 4 Anterior segment photography of patient 8. **a** Severe, persistent anterior tunica vasculosa lentis (TVL) before treatment. **b** Diminished engorged vessel of the TVL 1 week after treatment

The present study had some limitations, including the retrospective nature of the work, small patient numbers, and limited follow-up duration. The patient population was not controlled with respect to co-existing systemic conditions. However, our results showed rapid regression of fibrovascular membrane, new vessel ingrowth, and development of peripheral retinal vessels, after intravitreal bevacizumab injection in all treated patients, in the absence of serious systemic or ocular complications. Together, the results indicate that an appropriate dose of bevacizumab is effective as an adjunctive treatment option for patients with moderate-to-severe stage 3 ROP, in particular those at potential risk of aggravation if treated with laser photocoagulation alone. Although our experience with intravitreal bevacizumab and laser photocoagulation is promising, additional large-scale prospective studies, with longer follow-up periods, should be conducted to confirm our findings.

In conclusion, all patients in the present study, suffering from moderate-to-severe stage 3 ROP, achieved rapid

peripheral retinal vessel development after treatment with a combination of bevacizumab and laser photocoagulation. In such patients, the combination may confer certain advantages, including rapid regression of fibrovascular membrane, new vessel ingrowth, retinal vessel development, and promotion of peripheral vasculogenesis. Importantly, intravitreal injection of a carefully calculated level of bevacizumab, within an appropriate therapy window, appears to be safe for treatment of premature babies.

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