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Early vitreoretinal surgery on vascularly active stage 4 retinopathy of prematurity through the preoperative intravitreal bevacizumab injection

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ABSTRACT.

Purpose: **To evaluate the effect of early vitreoretinal surgery on vascularly active stage 4 ROP through the preoperative use of intravitreal bevacizumab.**

Methods: **This was a retrospective study. Eighteen patients with vascularly active stage 4 ROP who underwent primary vitrectomy from April 2007 to March 2010 were enrolled. Twelve eyes from eight patients received one-time intravitreal injection of 0.625 mg bevacizumab 7 days prior to vitrectomy (bevacizumab group), and 11 eyes from 10 patients underwent the surgical procedure without bevacizumab (control group). Demographical information of all patients was recorded. The patients were followed up for 12–36 months after the surgery. The postmenstrual age at vitrectomy, surgical procedure, anatomical and visual outcome, adverse effects and surgical complications were compared.**

Results: **There was no statistically significant difference between the two groups in gender, birthweight and gestational age. The bevacizumab group showed remarkable regression of vascular activity after the injection. The mean postmenstrual age at the time of vitrectomy was significantly earlier in the bevacizumab group (40 versus 47 weeks, $p = 0.002$) compared with the controls. The mean surgery time was shorter in the bevacizumab group (74.81 versus 101.70 min, bevacizumab group versus control, $p = 0.002$). At the final follow-up, all patients in the bevacizumab group achieved anatomical retinal attachment, compared with 70% in the control group. Eighty-eight per cent patients in the bevacizumab group obtained pattern vision, while it was 30% in the control group ($p = 0.015$).**

Conclusion: **Intravitreal bevacizumab administrated prior to vitrectomy effectively reduced active neovascularization in vascularly active stage 4 ROP patients, thus advancing the timing of vitrectomy and facilitating pars plicata vitrectomy (PPV).**

Key words: bevacizumab – lens-sparing vitrectomy – neovascularization – retinopathy of prematurity

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Introduction

Retinopathy of prematurity (ROP) is a potentially blinding eye disease that develops in eyes with incomplete blood vessel development at birth (Steinkuller et al. 1999). With the improved survival of high-risk neonates, ophthalmologists are encountering more severe premature infants. Although laser ablation has proven to be very effective in the treatment of the vision-threatening forms of ROP, retinal detachment may occur even in those treated in a timely manner (ETROP 2003). Visual outcome of eyes with ROP-related retinal detachment is generally poor (Gilbert et al. 1996; CRYO-ROP 2001). Thus, the high percentage of good anatomical and visual outcomes favours the use of vitrectomy in early stages of detachment (Capone & Trese 2001; Moshfeghi et al. 2004; Prenner et al. 2004). The key to successful outcome of vitreoretinal surgery for ROP-related retinal detachment is to operate after the neovascular activity and plus disease have completely or almost completely regressed. The persistent presence of plus disease is a predictor of poor surgical outcome. Performing vitrectomy before regression of vascular activity may be associated with bleeding, increased exudation, or with continued proliferation, and contraction

after vitrectomy, which lead to the failure of surgery (Hartnett 2003; Lakhanpal et al. 2005; Azuma et al. 2006). Therefore, many surgeons chose to do the vitrectomy until the neovascular membranes became quiescent (Fuchino et al. 1995; Capone & Trese 2001; Prenner et al. 2004; Nonobe et al. 2009). In some instances, however, detachment may progress rapidly before the plus disease and neovascular activity have a chance to regress. As the time and the extent of detachment increase, the prognosis becomes less desirable. Once the eyes progress to total detachment, the likelihood of reattachment decreases dramatically (Quinn et al. 1996; Mintz-Hittner et al. 1997). Therefore, more effective management should be carried out as soon as possible to reduce the vascular activity for the vitrectomy, which poses a major ongoing challenge.

Vascular endothelial growth factor (VEGF) plays an important role in neovascularization and vascular permeability of ROP (Smith 2004). Elevated levels of VEGF have been found in subretinal fluid (Lashkari et al. 2000) and vitreous fluid (Sonmez et al. 2008) of eyes with vascularly active stage 4 ROP. Accordingly, blocking the action of VEGF is expected to reduce the vascular activity associated with ROP and improve surgical outcomes. Bevacizumab (Avastin; Genentech Inc., South San Francisco, CA, USA) is an anti-VEGF antibody that was approved for the treatment of colorectal cancer and has been off-label used in the treatment of severe and advanced forms of ROP in small-scale clinical studies (Mintz-Hittner & Kuffel 2008; Nonobe et al. 2009). A recent prospective randomized trial showed that intravitreal bevacizumab monotherapy for zone I stage 3+ ROP resulted in a better anatomical outcome compared with laser treatment (Mintz-Hittner et al. 2011). Bevacizumab injection is now being used more often as a first-line treatment for advanced ROP (Honda et al. 2008; Mintz-Hittner & Kuffel 2008; Spandau et al. 2013).

The purpose of our study was to compare the outcomes of vitreoretinal surgery with and without preoperative use of intravitreal bevacizumab for vascularly active stage 4 tractional retinal detachments caused by ROP.

Methods

This was a retrospective, consecutive and comparative case series. The study was approved by the institutional ethics committee and was performed in accordance with the Declaration of Helsinki. The stages of ROP were classified according to the International Classification of Retinopathy of Prematurity (ICROP 2005), and the vascular activity was classified as active if the eye had (i) plus disease, (ii) new vessels growing into the vitreous at the ridge of a tractional RD area or (iii) combined effusive and tractional retinal detachment.

Patients

A total of 23 eyes from 18 infants with vascularly active stage 4 ROP who came to our hospital were enrolled in this study. All patients had previously undergone peripheral laser ablation, but did not receive any intraocular surgery prior to the vitrectomy. They were followed for at least 12 months after vitreoretinal surgery. Eleven eyes from 10 patients who received vitrectomy without preoperative therapy from April 2007 to May 2008 were defined as the control group. Twelve eyes from eight patients who received intravitreal bevacizumab injection (IVB) prior to vitrectomy from June 2008 to March 2010 were defined as the bevacizumab group. The clinical histories of all patients were obtained from their medical records.

Treatment protocol for vascularly active stage 4 ROP

For the control group, atropine sulphate eye gel and tobramycin dexamethasone eye drops were given and fundus examination was taken weekly to observe the regression of neovascular activity. Vitrectomy was not performed until the neovascular membranes became quiescent. However, there were four patients who had progressive retinal detachment. Therefore, the surgery was performed before vascular activity had regressed.

For the bevacizumab group, as the neovascular activity persistently existed in all eyes in 2–4 weeks after laser ablation, intravitreal bevacizumab was given. Colour wide-field fundus photographs were taken with a RetCamII-digital fundus camera (Massie Research Laboratories, Inc., Pleasanton, CA, USA) on Day 5 after intravitreal bevacizumab to observe the vascular activity. Seven days after IVB, vitrectomy was performed. The off-label use of the drug and its potential risks and benefits were discussed in detail with parents of the bevacizumab group, and written consent was obtained.

Intravitreal injection of bevacizumab

The injection was given to patients under general anaesthesia in the operating room. After instillation of 10% povidone-iodine and insertion of a lid speculum, a dose of 0.625 mg (0.025 ml) bevacizumab was injected into the vitreous cavity using a 30-gauge needle inserted through the superotemporal quadrant of pars plicata, 0.5–1.0 mm posterior to the limbus. After the injection, central retinal artery perfusion was confirmed by indirect ophthalmoscopy. The affected eye was given one drop of 0.3% ciprofloxacin 4 times a day until the day of vitrectomy. All patients were observed 24 hr in NICU for any possible systemic conditions.

Vitreoretinal surgery

The vitreoretinal surgical technique was the same for both groups. Three-port pars plicata vitrectomy using 20- or 23-gauge instrumentation was performed by the same surgeon (P. Z.). Sclerotomies were made 0.5 or 1.0 mm posterior to the limbus through the pars plicata. The cannula for the infusion line was placed inferotemporally unless the configuration of the tractional retinal detachment precluded placement in that quadrant. If so, the infusion port was placed away from the anteriorly displaced retina. Lens-sparing vitrectomies were used whenever possible; however, in cases with significant retinal-lenticular touch, a lensectomy was performed. The Binocular Indirect Ophthalmic Microscope (BIOM) and the paediatric contact lens were used for wide-angle viewing. The aim of the vitrectomy was to release vitreous adhesions between the ridge and pars plicata, the ridge and lens, and the ridge and optic nerve. The Accurus vitrectomy

machine (Alcon, Fort Worth, TX, USA) was used for vitrectomy and membrane peeling with vacuum levels of 100–300 mmHg and cutting rates of 1000–1500 cuts per minute. Gas-forced infusion settings for intraocular pressures were set at 30 mmHg unless intraocular bleeding required higher pressure. The horizontal and/or vertical scissors were used for membrane dissection. After the completion of membrane peeling, balanced salt solution (BSS) or sodium hyaluronate was used for postoperative tamponade. If iatrogenic retinal breaks developed, fluid–air exchange and laser photocoagulation would be performed. Perfluoropropane or silicone oil was used as a long-acting tamponade.

Assessment of surgical results

The anatomical outcomes, according to the CRYO-ROP criteria (CRYO-ROP 1988), were evaluated by binocular ophthalmoscopy and fundus photographs taken with the RetCamIIdigital fundus camera during follow-up visits.

For the evaluation of visual development at the last follow-up, visual results were divided into categories of no light perception (no perceived reaction to light), light perception (attraction or aversion response to the light), fixing and following behaviours, and pattern vision (recognition of targets on the Teller acuity cards).

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 17.0 (IBM Corporation, Armonk, NY, USA). A p value of <0.05 was considered statistically significant.

Results

The baseline characteristics of both groups are summarized in Table 1. There was no statistically significant difference between the two groups in gender, birthweight and gestational age at birth.

The surgical details of all patients are shown in Table 2. In the bevacizumab group, bevacizumab was injected at the median gestational age of 38 weeks (range, 37–40 weeks). Significant regression in the vascular activity of the eyes was observed on Day 5 after IVB (Fig. 1). The dilation and tortuosity of the retinal vessels and elevation of the ridge transiently decreased to some extent. Between intravitreal injection and vitrectomy, no complications, such as endophthalmitis, uveitis, ocular hypertension, or any other obvious ocular or systemic adverse events developed in any patients. Five days after IVB, a change from vascular to fibrous tissue was observed in two patients (25%) in this group (Fig. 2), but there was no rhegmatogenous retinal detachment after bevacizumab injection.

The median gestational age at the time of vitrectomy in the bevacizumab group was younger than the control group (40 versus 47 weeks). The surgical time was shorter in the bevacizumab group (74.81 min) compared with the control group (101.70 min). In the bevacizumab group, 11 eyes from seven patients (87%) had a lens-sparing vitrectomy, and only one eye (13%) underwent combined vitrectomy and lensectomy. While in the control group, eight eyes from seven patients (70%) had a lens-sparing vitrectomy, and three eyes from three patients (30%) underwent combined vitrectomy and lensectomy. In terms of tamponade agents, the percentage of using perfluoropropane or silicone oil was significantly higher in the control group (40%), while that was zero in the bevacizumab group. All the patients were followed up for at least 12 months, with the mean follow-up time of 21 months (range, 12–33 months).

The anatomical and visual outcomes of the surgery are shown in Table 3. In the bevacizumab group, all eyes were anatomically reattached at the final follow-up examination (Fig. 3), of which 10 (83%) had a 4a stage and two (17%) had a 4b stage. Eight eyes from seven patients in the control group were anatomically reattached when checked during the last visit. Of the eight successfully reattached eyes, 5 (63%) had a 4a stage and 3 (38%) had a 4b stage.

The visual outcome of the bevacizumab group was much better than that of the control group. Pattern vision was obtained in 10 eyes from seven patients (88%) in the bevacizumab group, compared with only three eyes from three patients (30%) in the control group. In the eyes that

Table 1. Patient demographics.

	Bevacizumab group: vitrectomy with preoperative IVB (8 patients, 12 eyes)	Control group: vitrectomy without preoperative IVB (10 patients, 11 eyes)	p value
Male (%)	4 (50)	5 (50)	1.000*
Birthweight, g (range)	1310 (860–1740)	1376 (950–1800)	0.672†
Gestational age, weeks (range)	30.50 (29–33)	30.00 (26–32)	0.341†
Multiple births (%)	3 (38)	2 (20)	0.410*
Stage 4a (%)	6 (75)	5 (50)	0.280*

IVB = intravitreal bevacizumab; * Pearson chi-square test; † *t*-test.

Table 2. Surgical details.

	Bevacizumab group: vitrectomy with preoperative IVB (8 patients)	Control group: vitrectomy without preoperative IVB (10 patients)	p value
Postmenstrual age at vitrectomy, weeks	39.63 ± 1.19	47.20 ± 5.79	0.002†
Mean surgical time, minutes	74.81 ± 13.41	101.70 ± 17.94	0.002†
Lensectomy (%)	1 (13)	3 (30)	0.375*
Iatrogenic retinal breaks, *N*	0 (0)	4 (40)	0.043*
Tamponade with BSS or sodium hyaluronate (%)	8 (100)	6 (60)	0.043*
Tamponade with Perfluoropropane (%)	0 (0)	3 (30)	0.090*
Tamponade with silicone oil (%)	0 (0)	1 (10)	0.357*
Follow-up, months	17.88 ± 5.57	22.70 ± 5.93	0.095†

IVB = intravitreal bevacizumab; † *t*-test; * Pearson chi-square test.

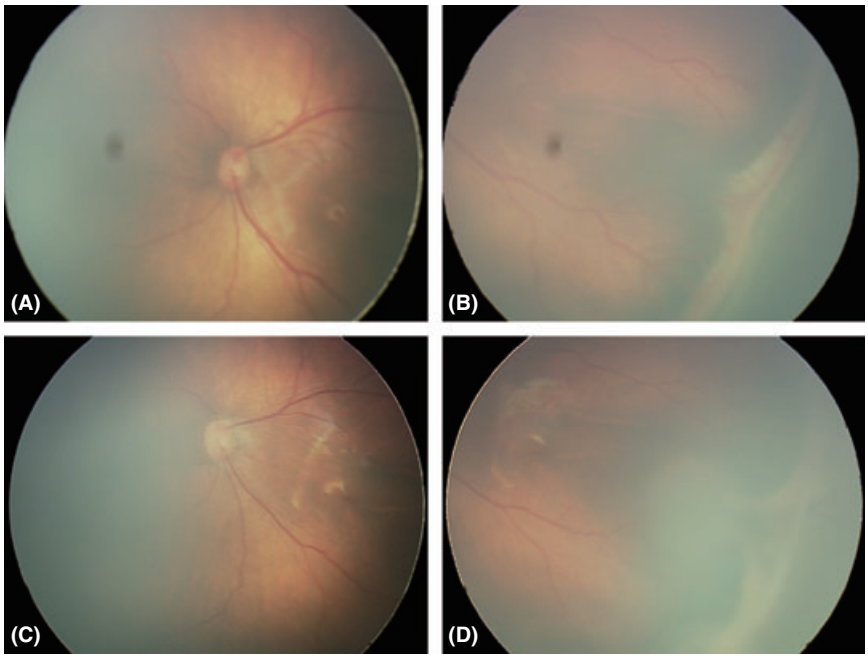

Fig. 1. Fundus photographs of the left eye of a case in the bevacizumab group. A fundus image obtained before intravitreal bevacizumab shows vascular tortuosity and dilatation in the posterior retina (A) and a tractional retinal detachment with neovascular membranes in the temporal peripheral retina (B). A fundus photograph of the same eye obtained 5 days after intravitreal bevacizumab shows decreased vascular tortuosity and dilatation (C), mild contraction and whitening in the neovascular membranes (D).

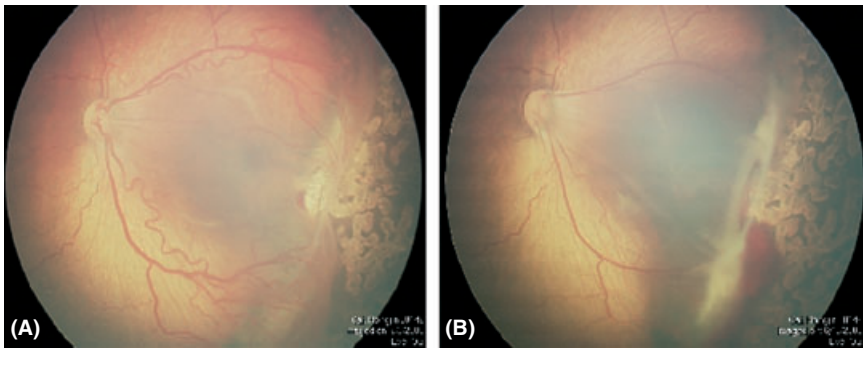

Fig. 2. Fundus photographs of the left eye of another case in the bevacizumab group. A fundus image obtained before intravitreal bevacizumab shows retinal detachment with neovascular membranes in the temporal peripheral retina, vascular tortuosity and dilatation in the posterior retina (A). A fundus image obtained 5 days after intravitreal bevacizumab shows a change from vascular to fibrous tissue (B).

Table 3. Anatomical and visual outcomes.

	Bevacizumab group: vitrectomy with preoperative IVB (8 patients)	Control group: vitrectomy without preoperative IVB (10 patients)	p value*
Retina attached (%)	8 (100)	7 (70)	0.090
Retina partial attached (%)	0 (0)	3 (30)	0.090
Normal posterior pole (%)	7 (88)	3 (30)	0.015
Pattern vision (%)	7 (88)	3 (30)	0.015
Fixation and following light (%)	1 (13)	4 (40)	0.196
Light perception (%)	0 (0)	2 (20)	0.180
No light perception (%)	0 (0)	0 (0)	/
Uncertain (VEP positive) (%)	0 (0)	1 (10)	0.357

* Pearson chi-square test.

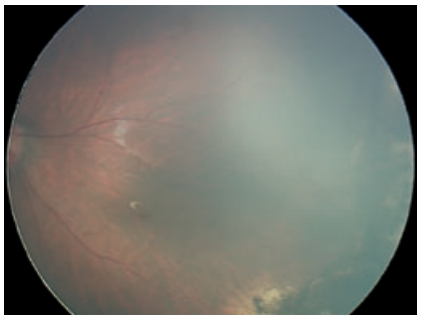

Fig. 3. A fundus image of the Fig. 1 eye obtained 3 months after vitrectomy shows complete retinal reattachment.

underwent Teller cards acuity check, the averaged visual acuity was 2.14 ± 1.06 and 1.67 ± 0.58 cycles/cm for the bevacizumab group and control group, respectively. In the control group, the visual result was uncertain in one patient (10%) because of the inability to measure reliably, but the visual evoked potential was positive.

Surgical complications are listed in Table 4. There was a significantly higher incidence of preretinal haemorrhage in the control group (40%) compared with the bevacizumab group (13%). Other complications in the control group included reproliferation of the fibrovascular tissues (two patients), cataract (two patients), vitreous haemorrhage (two patients) and glaucoma (one patient). Two patients received lens surgery because of dense cataracts. The patient with glaucoma was controlled by topical medication, and no further surgical intervention was required. In the bevacizumab group, only one eye had preretinal haemorrhage and the haemorrhage was spontaneously absorbed within 1 month after the vitrectomy.

Discussion

In this study, we were able to inhibit neovascular activity in vascularly active ROP patients by a single injection of bevacizumab. This resulted in the early vitrectomy surgery at postmenstral age of 40 weeks in the bevacizumab group. We found that the bevacizumab group has less intraoperative bleeding, reduced use of endodiathermy during the surgery, less surgery time, higher percentage of lens preservation, higher percentage of anatomical reattachment, less

Table 4. Surgical complications.

	Bevacizumab group: vitrectomy with preoperative IVB (8 patients)	Control group: vitrectomy without preoperative IVB (10 patients)	p value*
Reproliferation (%)	0 (0)	2 (20)	0.180
Vitreous haemorrhage (%)	0 (0)	2 (20)	0.180
Preretinal haemorrhage (%)	1 (13)	4 (40)	0.196
Cataract (%)	0 (0)	2 (20)	0.180
Glaucoma (%)	0 (0)	1 (10)	0.357
Total no. of patients with complications (%)	1 (13)	4 (40)	0.196

* Pearson chi-square test.

postoperative complications and better vision recovery.

In our current study, the regression of vascular activity was observed in bevacizumab group eyes on Day 5 after IVB. The rapid regression of the vascular activity induced by bevacizumab might contribute to operability on vascularly inactive rather than on active eyes without the drawback of a long wait for natural regression of the neovascular activity. The median postmenstrual age at vitrectomy of the bevacizumab group was 40 weeks, which was significantly earlier than that of the control group ($p = 0.002$). Nonobe et al. (2009) found that vitrectomy could be performed on the eyes treated with bevacizumab without severe bleeding during membrane removal at postmenstrual age of 40.3 weeks, which is earlier than the surgical time without bevacizumab.

Vitrectomy during active vascular phase for severe ROP is not recommended because it is often associated with sustained bleeding during surgery, increased risk of iatrogenic damage and poor retinal reattachment (Hartnett 2003; Lakhanpal et al. 2005; Azuma et al. 2006). Yu et al. (2006) reported that progressive retinal detachment with plus disease was a cause of the failure of retinal reattachment and intra- or postoperative vitreous haemorrhage. In this study, we were able to avoid the aforementioned complications in the bevacizumab group. We found that in the bevacizumab group, membranes could be peeled using blunt dissection, the frequency of intra-operative bleeding decreased, and endodiathermy was less employed, because of the regression of retinal new vessels. However, in the control group, during the vitrectomy, several tools were needed to coagulate, to aspirate the blood, and to use scissors or vitrectomy cutter to remove membranes, which made the surgery longer. On the other hand, it is extremely important to avoid iatrogenic breaks during the paediatric vitreous surgery. This is often difficult because the retina in patients with ROP is extremely thin and fragile. In our study, no iatrogenic retinal breaks occurred in the bevacizumab group. The improvement is most likely attributable to bevacizumab-induced reduction in the vascular permeability and the increased tissue resistant to traction. Moreover, the shorter duration of the surgery, the less probability of intra-operative damage.

Lens preservation is important to prevent deprivation amblyopia and promote visual development (Taylor & Hoyt 2005). Early surgery reduces the chance of the fibrovascular tissue reaching the posterior lens surface and extends to the vitreous base. Surgical intervention before the anterior extension of fibrovascular growth with traction that pulls the retina towards the lens is technically easier and more likely permits the surgeon to spare the lens in vitrectomy. In the bevacizumab group, we were able to reduce lensectomy to 13%, largely due to the fact that these patients received surgery at a much younger age.

In the control group, we were able to achieve 73% anatomical reattachment, which was comparable to data published by other groups (Yu et al. 2006). However, the reattachment in the bevacizumab group was 100%, which was comparable to those obtained from quiescent stage 4 ROP (Capone & Trese 2001; Moshfeghi et al. 2004; Lakhanpal et al. 2005). Eighty-eight percent patients in the bevacizumab group achieved normal posterior pole and pattern vision, while only 30% in the control group ($p = 0.015$). The visual outcomes of the bevacizumab group were much better than those of the control group, as the early intervention prevented detachment of the fovea, which has a devastating effect on visual acuity. These results highlight the importance of timely and early intervention against vascularly active stage 4 ROP.

If the plus disease presents as a severe condition, there will be a higher probability of postoperative complications, and the surgical prognoses should be guarded (Yu et al. 2006). In our case series, it is noteworthy that apparent complications, such as vitreous haemorrhage and reproliferation, were not observed in the bevacizumab group. Only one eye had preretinal haemorrhage, and the haemorrhage was spontaneously absorbed in 1 month after the vitrectomy. The use of bevacizumab made neovascular membranes quiescent, allowed a more complete dissection at the vitreoretinal interface and achieved limited extensive bleeding and less recurrence of proliferation.

We noticed that two eyes from two patients had progression of tractional retinal detachment with contraction and whitening of the neovascular membranes 5 days after bevacizumab injection. Similar observation was made by another group (Honda et al. 2008). We assume that the use of bevacizumab accelerated the contraction of fibrous membrane which worsened the retinal detachment. From this perspective, surgery at earlier time after the bevacizumab injection might be more beneficial.

As VEGF plays a crucial role in the physiological development of the retinal vasculature and photoreceptors (Smith et al. 1994; Saint-Geniez et al. 2008), the control of proper dosage is critical. Previous studies have used doses ranging from 0.375 to 1.25 mg, all exhibiting effectiveness in the inhibition of retinal neovascularization (Kong et al. 2008; Mintz-Hittner & Kuffel 2008; Dorta & Kychenthal 2010; Nazari et al. 2010; Harder et al. 2011; Mintz-Hittner et al. 2011). One point twenty-five milligram of bevacizumab is the widely accepted adult dose. As the preterm eye is about 50% smaller than the adult eye, we chose the dosage of 0.625 mg, which

was used by several other groups (Mintz-Hittner & Kuffel 2008; Dorta & Kychenthal 2010; Nazari et al. 2010; Mintz-Hittner et al. 2011). Another issue associated with bevacizumab delivery was the time of injection. In our study, the timing of the bevacizumab injection was median postmenstrual age of 39 weeks (range, 37–40 weeks). By then the development of physiological vessels was almost completed, and the peripheral avascular retinas had already been treated with laser treatment. Previous studies reported intravitreous injection at 31–40 weeks (Kong et al. 2008; Mintz-Hittner & Kuffel 2008; Dorta & Kychenthal 2010; Nazari et al. 2010). The earliest time of injection we found was at 31 weeks, which did not cause interruption of retina differentiation or other evidence of ocular damage (Kong et al. 2008). Therefore, a further study titrating the best dosage and time is indeed necessary.

Experimental data indicated that blocking all isoforms of VEGF can inhibit physiological neovascularization (Ishida et al. 2003). In addition, anti-VEGF agents escape from the vitreous into the general circulation and reduce systemic VEGF concentrations for weeks to months (Hoerster et al. 2013; Sato et al. 2012), and therefore, systemic side-effects cannot be excluded. In our limited study, a single, low dose of bevacizumab given to immature infants by intravitreous injection did not show systemic or local toxicity. However, a separate study on mouse model of ROP showed that a bevacizumab dose of up to 2.5 μg did not increase retinal cell apoptosis, indicating a limited toxicity of the antibody to retinal cell biology (Akkoyun et al. 2012). Therefore, it remains important to be vigilant in the continued search for systemic complications and to conduct necessary clinical testing to identify any systemic complication in its early stages.

This study has several limitations worthy of consideration. The series is neither randomized nor prospective. The cohort size is relatively small, and the follow-up time varied in a number of patients. Despite these limitations, the results suggest that bevacizumab effectively reduced the neovascular activity, which makes it possible the early intervention with vitreous surgery for vascularly active stage 4 ROP and contributes to better anatomical and visual results.

In conclusion, our study showed that anti-VEGF-mediated neovascular activity regression in vascularly active stage 4 ROP patients enables us to perform vitrectomy at an early age. This is in turn associated with reduced surgery time and postoperative complications, increased lens preservation and better vision recovery.

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