



Original article

Effects of ranibizumab on very low birth weight infants with stage 3 retinopathy of prematurity: A preliminary report[☆]Chun-Ju Lin^{a,b,c,*}, San-Ni Chen^{a,b}, Chien-Chi Tseng^a, Yao-Chung Chang^a, Jiunn-Feng Hwang^{a,b}^aDepartment of Ophthalmology, Changhua Christian Hospital, Changhua, Taiwan^bSchool of Medicine, Chung Shan Medical University, Taichung, Taiwan^cDepartment of Optometry, Chung Hwa University of Medical Technology, Tainan, Taiwan

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ABSTRACT

Background/Purpose: To report the effects of ranibizumab on very low birth weight (VLBW) infants with retinopathy of prematurity (ROP).**Methods:** A retrospective, noncomparative, consecutive, interventional case series was conducted. Patients with stage 3 ROP were identified and evaluated from August 2011 to February 2012. All patients with ROP received one intravitreal ranibizumab injection (0.25 mg/0.025 mL) under topical anesthesia as the initial treatment.**Main outcome measures:** Regression of ROP and the complications associated with the intravitreal ranibizumab injection.**Results:** A total of 23 eyes of 12 patients (four boys and eight girls) were included in this study. All of the patients had a history of supplemental oxygen and mechanical ventilation use. The mean gestational age was 26.33 ± 2.06 weeks (range: 24–30 weeks), and the mean birth weight was 821.58 ± 297.63 g (range: 507–1480 g). The mean postmenstrual age during the intravitreal administration of ranibizumab injection was 35.08 ± 2.07 weeks (range: 32–39 weeks), and the mean follow-up period was 5.83 ± 1.64 months (range: 3–8 months). All eyes received one intravitreal ranibizumab injection (0.25 mg/0.025 mL) as the primary therapy. None of the eyes needed conventional laser photocoagulation or cryotherapy as adjuvant therapy and no systemic complications were noted. No cataracts, endophthalmitis, or retinal detachment occurred postoperatively. Preretinal hemorrhages were found in four eyes of three patients (17.39%), but all were absorbed without sequelae.**Conclusion:** Intravitreal ranibizumab injections seem to be effective and well tolerated in VLBW infants with stage 3 ROP. Only self-limited preretinal hemorrhages were noted, and no short-term systemic or major ocular side effects were identified.

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1. Introduction

Very low birth weight (VLBW) is defined as a birth weight of less than 1500 g in human infants. The survival rate of VLBW infants has improved with the widespread use of surfactant agents, maternal steroids, and advances in neonatal technologies.^{1,2} Retinopathy of prematurity (ROP) is a disease of the premature infant retina that has not yet fully vascularized.³ Changes in oxygen exposure have been postulated to cause a disruption in the natural course of vascularization. In the late stages of ROP, neovascularization (NV)

occurs because of retinal immaturity. NV may lead to retinal traction, retinal detachment, hemorrhage, funnel configuration of the retina, and eventually, blindness. It is mainly attributable to the expression of vascular endothelial growth factor (VEGF), the levels of which have been demonstrated to be highly elevated in the vitreous fluid of patients with ROP.^{4,5}

The treatment modalities of stage 3 ROP are cryotherapy (established in a clinical trial in 1988),⁶ laser therapy (established in a clinical trial in 2003),⁷ and intravitreal administration of bevacizumab (Avastin, Genentech Inc., South San Francisco, CA; established in a clinical trial in 2011).⁸ However, cryotherapy involves scarring of the full ocular thickness and laser therapy scarring of the retinal thickness. In addition, the main concern following these peripheral retinal ablative procedures is inflammation of the eyes and induced high refractive errors or astigmatism.

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Although intravitreal bevacizumab only scars when a needle is used near the limbus, bevacizumab is a humanized murine anti-VEGF-A monoclonal antibody, developed primarily to target cancerous cells, and is only designed for intravenous administration. In addition, it is well-known that larger amounts of intravitreal bevacizumab reach the systemic circulation more than ranibizumab (Lucentis, Genentech Inc., South San Francisco, CA),^{9,10} raising concerns of unwanted side effects on the infant's developing organs.

Ranibizumab, an antibody fragment (Fab) of humanized anti-VEGF monoclonal antibody, was initially designed specifically to treat age-related macular degeneration by intravitreal administration.^{11,12} In 2009, ranibizumab was approved for treating age-related macular degeneration in Taiwan. After one intravitreal injection, the serum concentration of ranibizumab is lower than that of bevacizumab,^{9,10} and in theory, the risk of systemic adverse events and complement-mediated toxicity is reduced by using ranibizumab to treat ROP. The aim of this study was to investigate whether it is beneficial to treat ROP with ranibizumab.

2. Methods

We conducted a retrospective, noncomparative, consecutive, interventional case series of VLBW infants with zone I or zone II stage 3 ROP. Patients with stage 3 ROP received only one intravitreal ranibizumab injection as the initial treatment, and follow-up visits for more than 3 months were evaluated retrospectively from August 2011 to January 2012. Patients with a history of laser treatment, cryotherapy, or intravitreal bevacizumab injections were excluded from the study. Informed oral and written consent was obtained from all parents.

The pupils were dilated with 0.5% tropicamide/0.5% phenylephrine (Mydrin-P, Santen OY, Finland), and the topical antibiotic levofloxacin (Cravit, Santen Pharmaceutical Co., Osaka, Japan) was applied before the administration of intravitreal injections. We did not sedate the infants due to concerns of respiratory distress. Topical anesthesia was achieved using 0.5% proparacaine hydrochloride (Alcaine, Alcon Pharmaceuticals), which is administered three times with 2-minute intervals before the surgery. Each eye was prepared in a sterile manner using 5% povidone/iodine. About 0.25 mg/0.025 mL ranibizumab was injected intravitreally via the pars plicata (2 mm away from the limbus). A nurse helped to hold the infant during the injection. After the injection, intraocular pressure and retinal artery perfusion were checked, and the patients received topical levofloxacin four times daily for 7 days.

The patients were followed-up after 1 day and then every week to monitor the regression of the disease. If the NV regressed, then we examined the infant every month. We recorded data including patients' gestational age, birth weight, fundus findings, and follow-up period. The effects and complications associated with the treatment were analyzed. Positive responses included the dilation of the pupils, disappearance or decrease in retinal vessel tortuosity and NV, and vascularization toward the peripheral retina. Fundus photographs (Clarity Medical System, Pleasanton, CA) were obtained only in some cases, especially in those with postoperative preretinal hemorrhages.

3. Results

A total of 23 eyes of 12 patients (four boys and eight girls) were included in this study, of which 6 eyes were in zone I stage 3 ROP and 17 were in zone II stage 3 ROP. All of the patients had a history of supplemental oxygen and mechanical ventilation use. The mean gestational age was 26.33 ± 2.06 weeks (range: 24–30 weeks), and the mean birth weight was 821.58 ± 297.63 g (range 507–1480 g).

The mean postmenstrual age during the administration of intravitreal ranibizumab injection was 35.08 ± 2.07 weeks (range: 32–39 weeks) and the mean follow-up period was 5.83 ± 1.64 months (range: 3–8 months) (Table 1). All eyes received one intravitreal ranibizumab injection (0.25 mg/0.025 mL) as the initial therapy.

The time of plus disease response to the injection was about 1 week, and the time of NV regression was about 1 month. Because the follow-up period was short, the mean time of complete vascularization toward the retinal periphery was inconclusive. Maybe the far peripheral retina does not fully vascularize in those very immature infants. However, no one had active components of NV at the end of follow-up. None of the eyes needed conventional laser therapy as adjuvant therapy, and no systemic complications were noted. No cataracts, endophthalmitis, or retinal detachment occurred postoperatively. Preretinal hemorrhages were found in four eyes of three patients (17.39%) at the border of the vascular and avascular retina; however, they were all absorbed after 11–14 weeks without sequelae (Table 2).

3.1. Case presentation

Patient 3 was a female infant with a birth weight of 758 g at a gestational age of 25 weeks. At a postmenstrual age of 35 weeks, she presented with zone 2, stage 3 ROP in both eyes. She was treated with one intravitreal ranibizumab injection in each eye. Preretinal hemorrhages were found in the right eye (Fig. 1), but they were absorbed after 14 weeks without sequelae.

4. Discussion

We found that intravitreal 0.25-mg ranibizumab injection used as a primary treatment for ROP led to NV regression in all patients with stage 3 ROP. The plus sign decreased after injection. Preretinal hemorrhages were found in some eyes after the injections; however, they were absorbed without sequelae. No apparent systemic adverse effects related to the injection were noted, and no eye needed conventional laser photocoagulation or cryotherapy as an adjuvant therapy.

The levels of vitreous fluid VEGF is highly elevated in patients with ROP,⁵ and NV is mainly attributable to the expression of VEGF. Treatment with an anti-VEGF agent, such as bevacizumab, seems to be effective and well tolerated in the majority of cases with ROP, especially in cases of stage 3 ROP.¹³ Favorable results were also seen in a randomized, prospective study with a concurrent control group.⁸ Nevertheless, a long-term study is necessary to clarify whether long-term ocular or systemic side effects can occur. The indications and relative contraindications of intravitreal bevacizumab in ROP are still being clarified.^{8,14–16}

A number of potential ocular and systemic complications related to intravitreal anti-VEGF injections can occur in pediatric patients. The procedure can cause intraocular infections and lens trauma,

Table 1
Demographics of the very low birth weight infants receiving ranibizumab treatment for stage 3 retinopathy of prematurity.

Eyes (patients)	23 (12)
Male/female	4/8
Mean gestational age (wk)	26.33 ± 2.06
Mean birth weight (g)	821.58 ± 297.63
Zone 1/zone 2	6/17
Mean injection time (postmenstrual age, wk)	35.08 ± 2.07
Number of eyes (%) with regression after ranibizumab treatment alone	23 (100)
Number of eyes (%) with postoperative preretinal hemorrhage	4 (17.39)
Mean follow-up period (mo)	5.83 ± 1.64

Table 2

Very low birth weight infants with stage 3 retinopathy of prematurity treated with ranibizumab.

Case	Gender	GA (wk)	BW (g)	PMA at injection (wk)	Stage/zone	Eye	Postoperative hemorrhage	FU (mo)
1	F	30	969	36	3/II	OU	OU	8
2	M	28	810	36	3/II	OU	OD	7
3	F	25	758	35	3/II	OU	OD	7
4	F	25	770	35	3/II	OU	—	7
5	F	24	537	33	3/I	OU	—	7
6	F	26	507	33	3/I	OU	—	7
7	F	24	554	32	3/I	OU	—	6
8	F	30	1480	36	3/II	OU	—	6
9	M	27	1080	39	3/II	OD	—	4
10	F	26	521	38	3/II	OU	—	4
11	M	26	1133	34	3/II	OU	—	4
12	M	25	740	34	3/II	OU	—	3

BW = birth weight; F = female; FU = follow-up; GA = gestational age; M = male; OD = oculus dexter (right eye); OU = oculus unitas (both eyes); PMA = postmenstrual age.

and infants may be more vulnerable to VEGF blockade than adults.¹⁷ The complete effect of anti-VEGF on normal developing vessels is not well understood, and the long-term systemic adverse effects of the anti-VEGF antibody are also a concern.

The effect and safety of anti-VEGF agents on developing retinas and neural tissues of newborns are important issues that need to be addressed. It has been proposed that VEGF acts as a protective factor for neurons and glial cells, particularly in developing neural tissues.^{18,19} In a study conducted by Fusco et al, bevacizumab appeared to alter programmed cell death patterns and promote gliosis in the developing retinas of rabbits. The authors concluded that bevacizumab should be used with caution in developing eyes.²⁰

After an intravitreal injection, the serum concentration of bevacizumab is greater than that of ranibizumab.^{9,12} In addition, ranibizumab has several safety-related advantages, including a shorter systemic half-life^{21,22} and the antibodies lack a crystallizable fragment (Fc).²³ In theory, the risk of systemic adverse events and complement-mediated toxicity is reduced by using ranibizumab.

Although the majority of eyes respond well to intravitreal bevacizumab injections, 10% of stage 3 ROP eyes require additional laser treatment.¹³ Recent studies have revealed that VEGF is not the only growth factor upregulated in the eye, and insulin-like growth

factor-1, angiopoietin-1, and angiopoietin-2 have also been identified.^{24,25} Vascular growth factors have compensatory mechanisms in ROP, and therefore, the inhibition of VEGF expression alone may be unable to induce regression in all ROP cases.

Four eyes of three patients in the current study had preretinal hemorrhages at the border of the vascular and avascular retina after the ranibizumab injection. Ranibizumab can lead to the regression of new vessels. These involutionary forces exerted on the NV may lead to local bleeding. However, the hemorrhages did not cause significant damage to the eyes and were absorbed after 11–14 weeks without sequelae. The rate of preretinal hemorrhage in our series was 17.39%, which is higher than that reported by Wu et al (9.76%).¹³ The possible influence of slight eye movement under topical anesthesia is trivial, because the hemorrhages were only seen at the junction of the vascular and avascular retina. Nevertheless, the actual mechanism should be studied further.

Data regarding the effects and complications associated with intravitreal injections of ranibizumab for zone 1 ROP with plus disease remain scarce. One case of delayed-onset retinal detachment after intravitreal ranibizumab injection for zone 1 ROP with plus disease has been reported.²⁶ Fundus photography and fluorescein angiography performed 3 months after laser photocoagulation and intravitreal injection of ranibizumab revealed regression of extraretinal fibrovascular proliferation, but incomplete normal vascularization toward the peripheral retina. In such cases, more frequent and prolonged weekly examinations are very important for timely intervention.¹⁴

One prospective, experimental, longitudinal, and open study included newborns with either a gestational age less than 32 weeks or birth weight less than 1500 g with threshold–prethreshold retinopathy or “plus disease.”²⁷ All cases received combined laser–ranibizumab therapy and were followed-up for 3 years. In total, there were 34 eyes of 17 patients whose gestational age was 29.9 ± 2.6 weeks and birth weight was 1120 ± 253 g, and regression of retinopathy was noted in all cases.²⁷ Nevertheless, 17.6% of the patients showed persistence of vascular tortuosity without vascular dilatation, and 11.7% revealed vitreous membrane development. All of our cases showed regression of ROP without vascular tortuosity or vitreous membrane formation, which may be complicated by laser therapy, as laser therapy scars the peripheral retina and causes intraocular inflammation.

In a VLBW infant, management of ROP, which can range from frequent examinations to intravitreal anti-VEGF injections, laser surgery, cryotherapy, or even vitrectomy, is dictated by ROP stage and location. The presence of significant plus disease or tortuosity of the retinal vessels is a poor prognostic sign and requires immediate treatment. The VLBW infants who do not have ROP or in whom ROP has resolved should undergo a follow-up eye examination at the age of 6 months or until complete vascularization toward the peripheral retina occurs. Besides, after the intravitreal injection of anti-VEGF, one of the important complications is recurrence of ROP after the initial inactivation of ROP.⁸ Therefore, long-term follow-up of these patients is needed following such treatment.

The limitations of this study are its retrospective nature and the lack of a concomitant control group. In addition, fundus photographs were not available in all cases. The main disadvantage of this new treatment is the relatively high expense. Nevertheless, these results are encouraging.

In conclusion, we found that intravitreal ranibizumab injections were effective and well tolerated in VLBW infants with stage 3 ROP. All of our cases needed only one injection of ranibizumab to regress ROP. Only self-limited preretinal hemorrhages were noted, and no short-term systemic or major ocular side effects were identified. Studies with more cases and a longer follow-up period are warranted.

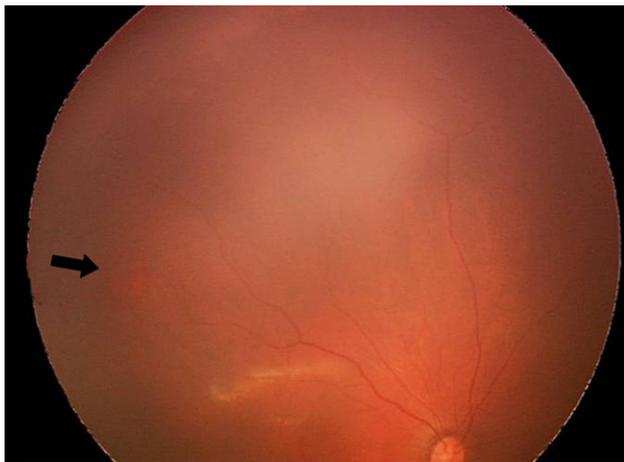


Fig. 1. Patient 3 was a female infant with a birth weight of 758 g at a gestational age of 25 weeks. At a postmenstrual age of 35 weeks, she presented with zone 2, stage 3 retinopathy of prematurity in both eyes. She was treated with one intravitreal ranibizumab injection in each eye. Preretinal hemorrhages were found in the right eye.

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