• Clinical Research •

Effect of anti-VEGF treatment on retinopathy of prematurity in Zone II Stage 3+

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Abstract

• AIM: To evaluate the effect of intravitreal ranibizumab injection for retinopathy of prematurity (ROP) in Zone II Stage 3+.

• METHODS: Data was collected for ROP patients with Zone II Stage 3+ who received intravitreal ranibizumab injections between October 2014 and January 2017 at the Department of Ophthalmology in our hospital. No prior laser or other intravitreal treatment was done. Prior to the intervention and at each follow-up visit, fundus examination was performed. Gestational age at birth, sex, birth weight, ROP zone, ROP stage, post menstrual age (PMA) at treatment, and follow-up period were recorded. The final clinical status of the retina was evaluated for each patient. The primary outcome measures included ROP recurrences requiring re-treatment, complete or incomplete peripheral vascularization.

• RESULTS: Eighty-six eyes of 46 premature infants with Zone II Stage 3+ ROP were enrolled in the study. The mean gestational age at birth was 28.18±1.67 (range: 25 to 33)wk and the mean birth weight was 1070.57±226.85 (range: 720.00 to 1650.00) g. The mean PMA at treatment was 38.32±2.99 (range: 32.29 to 46.00)wk. Seventy-one eyes (82.56%) were treated successfully with intravitreal ranibizumab as monotherapy. Fifteen eyes (17.44%) developed recurrent disease. The mean interval between the treatment and retreatment was 5.96±3.22 (range: 1.86 to 11.71)wk. All eyes vascularized into zone III at the end of the study and among them 62 eyes (72.09%) achieved complete vascularization.

• CONCLUSION: Intravitreal ranibizumab injection is an effective treatment in Zone II Stage 3+ ROP patients. More patients with longer follow-up duration are necessary to confirm the safety and efficacy of this treatment.

• **KEYWORDS:** retinopathy of prematurity; ranibizumab; plus disease

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INTRODUCTION

Retinopathy of prematurity (ROP) remains one of the main causes of childhood blindness in the world, despite advances in treatment. ROP is a neovascular disorder that occurs in premature infants, which leads to retinal detachment and eventually blindness if not treated in time[1-2]. Similar to other retinal vascular disorders, vascular endothelial growth factor (VEGF) is a potent pro-angiogenic factor in the pathologic angiogenesis of ROP, and blocking the action of VEGF could be expected to reduce vascular activity. Hence, anti-VEGF strategy might be a promising prospect in the current treatment of ROP[3-4]. Recently, a prospective randomized controlled stratified multicenter trial compared bevacizumab monotherapy and laser therapy for Zone I or Zone II posterior Stage 3+ ROP (BEATROP). They found that bevacizumab showed a significant benefit for Zone I but not Zone II disease, with continuation of peripheral retinal vessel growths after treatment[5]. The aim of this study was to evaluate the effectiveness of intravitreal ranibizumab to treat Zone II Stage 3+ ROP.

SUBJECTS AND METHODS

Data Collection The study was carried out in our Department of Ophthalmology. ROP was defined according to the International Committee for Classification of ROP protocol[6]. Forty-six Zone II Stage 3+ ROP patients from October 2014 to January 2017 were included in our study. The study followed the principles in Declaration of Helsinki and no participants receive any stipend. After a written informed consent was obtained, an initial intravitreal injection of 0.25 mg/0.025 mL ranibizumab (IVR; Lucentis, Novus, USA) was given to each eye. Intravitreal injection was performed as previously described[7]. The injections were carried out bilaterally if necessary during the same treatment session. The ocular surface was anesthetized with unpreserved oxybuprocaine eye drops. Eyelids and conjunctiva were sterilized with 5%

povidone iodine for disinfection. A sterile eyelid speculum was inserted and 0.25 mg/0.025 mL ranibizumab was injected into the vitreous cavity *via* the temporal lower quadrant of the pars plana. All treated infants received at least 3mo of follow-up.

Inclusive criteria: 1) Zone II Stage 3^+ ROP patients received the initial ranibizumab injection; 2) the patients followed up at least for 3mo. Exclusive criteria: patients received any other treatment such as laser photocoagulation or cryo-therapy before the injection.

Eye Examination, Follow-up and Definition of Recurrence Experienced ophthalmologists identified the location and sequential retinal changes of ROP and performed all examinations. Patients' pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine drops 1h before examination. Indirect ophthalmoscopy was routinely performed after topical anesthesia. We took RetCam photographs to document the retinal findings. ROP findings included the preoperative ROP zone and stage, plus disease, iris neovascularization, therapy method, and recurrence time.

Patients were examined 1, 3, 7d after the injection to monitor for signs of intraocular infection, then every one or two weeks until complete regression of the active ROP (regression of plus disease as well as of any proliferation present at time of treatment), followed by bi-weekly visits within the first 3mo and then at least every 1 or 3mo up to 12-15mo of age. Patients who received retinal laser photocoagulation were seen at least one or two weeks up to complete regression of active ROP, then every 1 or 3mo up to 12-15mo of age.

The recurrence was characterized by any worsening signs, including an aggravated ridge or plus signs after the initial regression. Recurrences requiring additional treatment after injection followed the rules of Retinopathy of Prematurity (ETROP) protocol. According to the Early Treatment for ETROP protocol, treatment was indicated in patients with type I ROP, threshold ROP and aggressive posterior ROP (AP-ROP)[8].

Statistics Statistical analysis was performed with statistical software SPSS (StatLab, SPSS for Windows, version 16.0; SPSS Inc., Chicago, Illinois, USA).

RESULTS

The study comprised 27 females and 19 males. The mean gestational age (GA) at birth was 28.18±1.67wk (range: 25 to 33)wk and the mean birth weight was 1070.57±226.85 (range: 720.00 to 1650.00) g. All eyes showed Zone II Stage 3^+ ROP. The mean post menstrual age (PMA) at treatment was 38.32±2.99 (range: 32.29 to 46.00)wk. One week after intravitreal injection, 32 eyes (37.21%) had achieved complete regression of ROP (Figure 1A, 1B), and a partial regression of ROP was observed in 54 eyes (62.79%; Figure 1C, 1D). Seventy-one eyes (82.56%) were treated successfully with intravitreal ranibizumab as monotherapy. Fifteen eyes (17.44%) developed recurrent disease. Six out of the 15 relapsed eyes

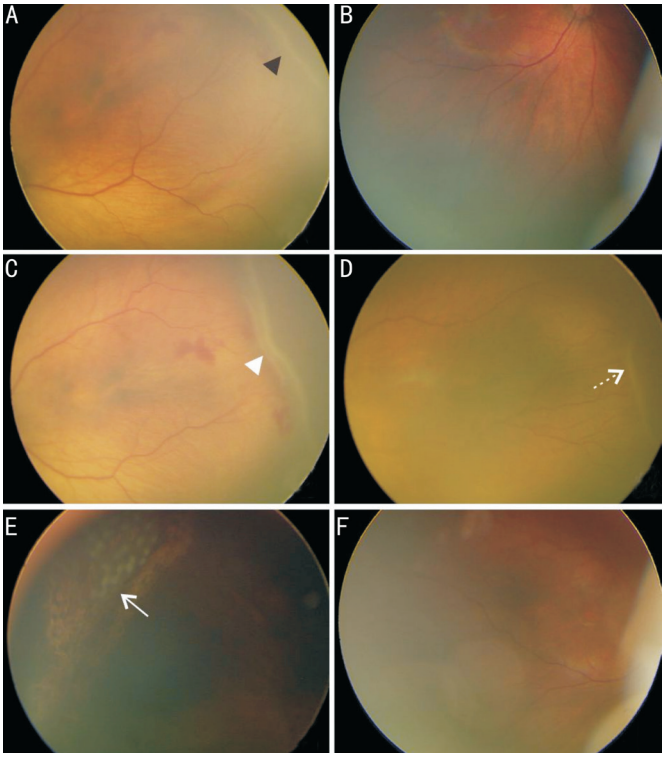

Figure 1 Anti-VEGF treatment for Zone II Stage 3^+ ROP patients Patient 1 information: male, GA: 30wk, BW 1570 g, PMA 34^{+4}wk, Zone II Stage 3^+ ROP before treatment (black arrow, A) and 1wk after intravitreal ranibizumab injection with complete regression (B); Patient 2 information: female, GA: 27^{+2}wk, BW 900 g, PMA 37^{+1}wk, Zone II Stage 3^+ ROP before treatment (white arrow, C) and partial regression after intravitreal ranibizumab injection (discontinuous arrow, D); Patient 3 information: female, GA: 26wk, BW 1070 g, PMA 37wk, ROP retreated with laser (E) and complete regression of retinopathy and full retinal vascularization (F), the same as patient 1.

(40.0%) required secondary ranibizumab treatment and 7 eyes (46.67%) received photocoagulation (Figure 1E). Only 2 eyes received combination therapy. The mean follow-up time was 6.61±4.82 (range: 3.0 to 25.93)mo, the mean interval between the treatment and retreatment was 5.96±3.22 (range: 1.86 to 11.71)wk (Table 1). In this series, all eyes vascularized into Zone III by the end of the study and among them 62 eyes (72.09%) achieved complete vascularization (Figure 1F). All of the cases had attached retinas with no macular dragging.

There were no other ocular complications such as cataract, ocular inflammation or endophthalmitis. Subsequent follow-up visits after the injection revealed no unexpected untoward events such as cardiopulmonary distress, renal insufficiency, or stroke, as reported by the neonatologist.

DISCUSSION

Peripheral retinal laser photocoagulation remains the current standard method for treatment-requiring ROP in Zone II[9-10]. More recently, anti-VEGF drug injection was emerging as successful treatment for ROP and was mainly used for Zone I ROP, AP-ROP, and failed ROP after laser treatment[11-12]. Recently

Table 1 Baseline characteristics of infants with ROP treated with intravitreal injection of ranibizumab

Parameters	Data
Number of patients/eyes	46/86
M/F	19/27
BW, mean±SD (range), g	1070.57±226.85 (720.0-1650.0)
GA, mean±SD (range), wk	28.18 ±1.67 (25-33)
PMA at treatment, mean±SD (range), wk	38.32 ±2.99 (32.29-46.0)
Follow-up time, mean±SD (range), wk	6.61±4.82 (3.0-25.93)
Mean interval between the treatment and retreatment, wk	5.96±3.22 (1.86-11.71)
Number of retreated eyes	15

BW: Birth weight; GA: Gestational age; PMA: Post menstrual age.

more Zone II ROP patients showed good response to the anti-VEGF treatment. However the safety and the efficacy diverse greatly. The BEAT ROP trial presented favorable results of bevacizumab in Zone I but not in Zone II disease compared to laser therapy[5]. However, in a case series of 11 eyes treated with bevacizumab, systemic VEGF levels were attenuated for at least 7wk post-injection[13]. Considering the sample size and the higher mortality rate of children 6.6% in the bevacizumab treated group *vs* 2.6% in the laser group[11], the treatment strategy needed to be further evaluated. It has been estimated that ranibizumab showed the least reduction in the plasma free-VEGF level in adults when compared with another two anti-VEGF drugs, aflibercept and bevacizumab[14]. It was reported that intravitreal ranibizumab did not induce prolonged systemic VEGF inhibition with reduced plasma VEGF levels 1d after injection and recovered until 1wk in infants with ROP. This data points in the direction of a better safety profile for ranibizumab[15-16]. Moreover, ranibizumab has a shorter half-life in human non-vitrectomized eyes (7.15d versus 9.82d of bevacizumab)[17]. These concerns led us to the idea of treating Zone II ROP with intravitreal ranibizumab instead of bevacizumab.

Intravitreal injection of ranibizumab is effective in this study; however, successful treatment cannot be achieved in all eyes with a single injection. In our study, seventy-one eyes (82.56%) showed rapid regression of ROP after single injection of 0.25 mg ranibizumab. Fifteen eyes (17.44%) needed additional treatment either by intravitreal injection or by laser treatment. The mean interval between the treatment and retreatment was 5.96±3.22wk (range: 1.86 to 11.71wk). The reason for recurrence might be associated with the location of disease and the severity of the disease. In our previous study we found that Zone I ROP had higher recurrence rate compared to Zone II ROP and Stage 3 ROP needed more treatments than Stage 1 or 2 ROP[18]. Further study are still needed to clarify the reason. Feng *et al*[19] reported that the incidence of recurrence that needed treatment was 39.0% which included type 1 pre-threshold ROP and AP-ROP. The recurrence rate was higher in Zone I ROP (61.6%) than in Zone II ROP (31.0%). The total recurrence rate of Zone I and posterior Zone II disease was 6% in the BEAT-ROP study[5]. Hu *et al*[20] estimated that the rate of recurrence for ROP in Zone I Stage 3^+ in their study was 26.2%. These difference might be explained by a lower sample size in the present study and in addition, the previous study recruited patients with several stages of ROP for analysis. Another study found that the incidence of disease relapse was higher in eyes that received ranibizumab compared with bevacizumab in the treatment of type 1 ROP[21]. This can be explained by a much longer systemic half-life with bevacizumab than that with ranibizumab in adult patients (20d versus 2h). A shorter half-life and a lower systemic absorption may decrease possible side effects, one the other hand, the rapid clearing of the vitreous and serum levels of VEGF often suggests a high chance of recurrence. As to the comparison between laser treatment and anti-VEGF treatment considering the recurrence rate there is no data available yet so far. In our previous study we found the recurrence rate for Zone II ROP patients was 4.55% (4/88) who received laser photocoagulation[18]. Further study are needed to compare intravitreal ranibizumab and laser photocoagulation as treatment for Zone II Stage 3^+ ROP patients.

In our cases each eye received 0.025 mL (0.25 mg) ranibizumab treatment. There is still ongoing discussion about the dosage of anti-VEGF drug required to treat ROP sufficiently with one single injection. Sears[22] proofed that approximately 0.5-1.0 mg of bevacizumab is 10 000 times the concentration needed to neutralize the highest measured concentration of VEGF. Most of the ophthalmologist choose a half dose of anti-VEGF when treating the ROP patients. However considering the vitreous volume, the retinal surface area and even the body weight of an infant[11], the dosage of anti-VEGF should be further optimized. An encouraging report from Lin *et al*[23] published a series of cases of ROP receiving ranibizumab treatment and showed good response to the treatment with no short-term systemic side effects or major ocular side effects during 3y follow-up. Castellanos *et al*[24] presented a case series of six eyes treated with one injection of ranibizumab and followed patients over 3y. All patients gained complete retinal vascularization with full regression of ROP. After 3y, average visual acuity (Snellen equivalent) was 20/30, which implies normal ocular growth and function after ranibizumab treatment in these cases. As currently there is no valid dosage data available and the risk of

under-treatment should be avoided and together with the good results from a long follow-up observation, in this study we took the half dose of adult as one single treatment.

Our study was limited as a retrospective and nonrandomized controlled trial. There was also a lack of fluorescein angiography in the evaluation of ROP patients. Further prospective studies with larger study populations are required to evaluate the safety and effectiveness of ranibizumab for ROP treatment. Our case series has not the power to assess safety of ranibizumab treatment. Even though 72.09% of the treated eyes showed complete retinal vascularization after treatment by funduscopy, but since no fluorescein angiography was performed, it remains unknown whether vascularization was normal or abnormal. Based on our own observations, follow up should be at least every 1 to 3mo until vascularization is full completed.

We conclude that IVR treatment is as effective as monotherapy for ROP and does not seem to be associated with serious ocular or systemic adverse events. However, the increased risk of recurrence and shorter interval until recurrence relative to the reported results with bevacizumab necessitates a longer follow-up period with more frequent monitoring.

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