Intravitreal Bevacizumab versus Laser Treatment in Type 1 Retinopathy of Prematurity

Report on Fluorescein Angiographic Findings

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Purpose: To compare the structural outcome at 9 months of eyes treated with intravitreal injection of bevacizumab with fellow eyes treated with conventional laser photoablation in zone I type 1 retinopathy of prematurity (ROP).

Design: Single randomized controlled trial.

Participants: All inborn babies with type 1 zone I ROP at a single institution were included in the study. One eye was randomized to receive an intravitreal injection of 0.5 mg bevacizumab; the fellow eye received conventional laser photoablation.

Methods: Digital fundus photographs and fluorescein angiography (FA) using the RetCam (Clarity Medical Systems Inc., Pleasanton, CA) were performed before treatment and 9 months after treatment.

Main Outcome Measures: Presence of retinal and choroidal abnormalities on FA at 9 months.

Results: Thirteen infants were enrolled; 1 died 3 months after birth. One laser-treated eye progressed to stage 5 retinal detachment. The remaining 23 eyes had favorable structural results at the 9-month follow-up and provided FA results. At 9 months of age, all eyes treated with a bevacizumab injection were noted to have abnormalities at the periphery (large avascular area, abnormal branching, shunt) or the posterior pole (hyper-fluorescent lesion, absence of foveal avascular zone). These posterior and peripheral lesions were not observed in the majority of the lasered eyes.

Conclusions: This study documents significant vascular and macular abnormalities of eyes in the bevacizumab group. Long-lasting implications of these abnormalities for visual function of the child need to be studied. Ophthalmology 2014;121:2212-2219 © 2014 by the American Academy of Ophthalmology.
VEGF receptor binding and signaling: Its epitope overlaps (although it is not identical to) the epitopes of VEGF receptor-1 and -2. The drug has been approved by the US Food and Drug Administration for use in humans as a cancer therapeutic since 2004.

In 2007, the first 3 reports of the use of bevacizumab for ROP appeared in the literature,16–18 followed by numerous others, including the use of bevacizumab as monotherapy,19,20 in combination with laser,21,22 or as rescue therapy after failed laser photocoagulation,23 and in combination or before vitrectomy.24 Although the use of anti-VEGF drugs in eyes with abnormal vasculogenesis has rapidly spread around the world, concern about safety for both adults25 and premature babies has arisen.26,27 Adverse ocular effects thus far have been mainly due to fibrotic reaction after intravitreal injection.28,29 Many authors urged caution about using an antiangiogenic drug in an infant because of its potential adverse systemic effects in developing neurons and the lung.26,27 This concern is reinforced by the data from Sato et al30 documenting that bevacizumab “escape(s) from the eye into systemic circulation” and reduces the serum level of VEGF in infants with ROP.

On the basis of the results of a randomized multicenter clinical trial of eyes with stage 3 ROP with plus disease in zone I or posterior zone II, Mintz-Hittner et al31 reported a significant reduction of ROP recurrence requiring treatment in zone I eyes compared with eyes treated with conventional laser therapy (6% vs. 42%).31 Although this could represent an important improvement in the therapy of severe ROP, concerns about long-term local and systemic adverse effects arose.26,27 We report a series of fluorescein angiography (FA) studies in a case series of babies who developed type 1, zone I ROP in both eyes. For each infant, 1 eye was randomly assigned to receive bevacizumab and conventional laser photocoagulation in the fellow eye within 24 hours of diagnosis of type 1 ROP, as defined by ETROP criteria.4

Methods

This is a single-center, randomized, controlled trial conducted at the Catholic University in Rome. All babies born in this clinical center who developed type 1 zone I ROP in both eyes were eligible to be included in the study.

Within a maximum interval of 24 hours after diagnosis, all babies underwent general anesthesia for evaluation and treatment. Before treatment, fundus images were obtained using a RetCam II (Clarity Medical Systems Inc., Pleasanton, CA) and video-digital FA was performed using a bolus of 10% fluorescein solution intravenously administered at a dose of 0.1 ml/kg, followed by an isotonic saline flush.3 For treatment, 1 eye was randomly selected (using a random number series) to undergo conventional laser photocoagulation of the peripheral avascular retina; the fellow eye received an intravitreal injection of 0.5 mg bevacizumab in a 0.02 ml balanced salt solution. The eye assigned to conventional laser peripheral ablation was treated first. The FA was examined by the treating physicians before conducting laser photocoagulation to provide more detailed information about the status of the eye and to indicate areas of the retina that might be treated. The eye randomized to receive bevacizumab was then prepared using 5% povidone/iodine and topical antibiotic, and 0.5 mg (0.02 ml) of bevacizumab was injected intravitreally through the pars plicata. After the injection, intraocular pressure and retinal artery perfusion were checked, and patients received topical tobramycin in both eyes for 3 days.

After treatment, binocular indirect ophthalmoscopy and RetCam imaging were performed every 3 days, and FA was performed every 2 weeks until discharge. After discharge from the neonatal intensive care unit, binocular indirect ophthalmoscopy was performed every 2 weeks until 52 weeks’ postmenstrual age and then monthly until 1 year of age. Babies underwent FA at 9 months of age under general anesthesia.

Angiograms were examined retrospectively by 2 experienced ophthalmologists (D.L. and F.M.) for the following characteristics described by Lepore et al.2 Abnormalities at the junction of vascular and avascular retina were considered present if at least 1 quadrant of the eye showed irregular arteriolar branching or naked arteriovenous shunts. The same criteria were used to assess the capillary loss within the vascularized retina and the posterior pole. The macula was considered abnormal if any or all of the following were present: (1) absence of foveal avascular zone, (2) presence of hyperfluorescent lesions, and (3) pigment epithelium abnormalities of the macular region. If only large linear choroidal vessels without choriocapillaries were observed in the early FA phases, a linear filling pattern was recorded.

The institutional review board at the Catholic University of the Sacred Heart of Rome approved the study protocol, and the trial was registered with the EudraCT as number 2009-012609-20, protocol number 343/09 April 24 2009. Group differences were examined using the Fisher exact test.

Results

From September 1, 2009, to December 31, 2010, 127 preterm babies with a gestational age (GA) of 32 weeks or less or a birth weight (BW) less than 1500 g were referred for ROP examinations at the Neonatal Intensive Care Unit at the “Agostino Gemelli” University Hospital. Thirteen infants (10.2% of the screened population) had developed type 1 ROP in zone I in both eyes and required treatment, according to ETROP criteria. These 13 were enrolled in this study. Six eyes (3 babies; mean BW, 693 g; BW range, 615–710 g; mean GA, 25.0 weeks; GA range, 24–27 weeks) were classified as zone I stage 3 with plus disease; 20 eyes (10 babies; mean BW, 705 g; BW range, 450–750 g; mean GA, 25.7 weeks; GA range, 23–29 weeks) were classified as zone I stage 3 without plus. All of these eyes had flat neovascularization typical of severe ROP.

One eye (8.5%) with a stage 3, zone I ROP with plus disease treated with conventional laser progressed to a complete retinal detachment 4 weeks after treatment. A second eye also with stage 3 zone I ROP with plus disease and treated with conventional laser progressed to stage 4a and underwent lens-sparing vitrectomy with a favorable structural outcome. Furthermore, 1 infant died because of pulmonary complications at 3 months of age.

Pretreatment FA scans from the 13 patients were available for evaluation for this report by 2 different ROP experts, along with FA scans from 12 eyes from the bevacizumab-injected eyes and 11 from the laser-treated eyes. The FA results before treatment and 9 months after treatment are presented in Tables 1 and 2.
Vascular Features of the Vascular-Avascular Junction

Before treatment, all eyes in this study showed hyperfluorescence (popcorn abnormalities, focal capillary dilatation, cotton-wool capillary tufts, rosary-bead–like arterioles); hypofluorescent lesions (capillary loss, periarteriolar capillary-free zone); and irregular branching starting at various distances from the optic disk (Table 1, Fig 1A, C). A less common feature, observed in 87.0% (20/23 eyes), was a circumferential vessel that ran along the vascular-avascular junction.

Fluorescein angiography 9 months after the treatment showed that the bevacizumab eyes had extensive areas of avascular retina (Fig 1B), whereas the lasered eyes all showed the typical retinocochoroidal atrophy expected from the treatment (Fig 1D). In the bevacizumab eyes, the normal dichotomous branching pattern was absent and abnormal tangles were observed in 11 of 12 eyes (Table 1, Fig 2A). All but 1 of the bevacizumab eyes showed a circumferential vessel along the junction between vascular and avascular retina. The so-called naked arteriovenous shunt seems to be a long-lasting characteristic of the eyes injected with bevacizumab (Figs 2B and 3B).

Abnormalities within the Vascularized Retina

As shown in Table 2, a massive loss of retinal capillary bed at the posterior pole or in the periphery within the vascularized retina is a common finding not only, as expected, in the pretreatment FA scans (Fig 4A, C) but also 9 months after treatment in 11 of 12 eyes treated with bevacizumab (Fig 4B). In contrast, just 3 of the 11 eyes treated with conventional laser showed hypofluorescent lesions within the junction of vascularized and avascular retina. The so-called naked arteriovenous shunt seems to be a long-lasting characteristic of the eyes injected with bevacizumab (Figs 2B and 3B).

Choroidal Filling Pattern

Details on choroidal filling pattern are provided in Table 2. Before treatment, the majority of eyes (91.30%, 21/23 eyes) showed a linear choroidal filling pattern (Fig 4A, C). This pattern was still clearly visible in 6 of 12 eyes injected with bevacizumab at 9 months’ follow-up (Fig 4D); on the other hand, a lobular pattern, as is commonly observed in premature babies 9 months post-term in our clinical experience, was noted in 8 of 11 eyes (72.73%) treated with laser.

Discussion

The results of this study confirm that there may be serious and lasting ocular structural abnormalities in eyes with severe ROP treated with bevacizumab.32 These abnormalities include both vascular changes, such as branching abnormalities and capillary dropout, and macular changes. Findings such as abnormal choroidal filling are noted in both the bevacizumab and the laser groups and also suggest that abnormalities of blood flow, which we have previously noted in severe ROP eyes before treatment, may be long lasting.

When considering the results of this study, it is also important to recall that masking of images when assessing treatment effects can be done only for posterior pole views because when assessing the more peripheral changes, laser scars are clearly evident juxtaposed to the peripheral vascular changes. Further, because bevacizumab is found in the serum after intravitreal injection,30 it is reasonable to conclude that some of the features observed in the laser-treated eye were affected by the injection of bevacizumab in the fellow eye. However, both of these circumstances would bias against finding differences between laser- and bevacizumab-treated eyes, but that is not the case.

Table 1. Branching Abnormalities in Bevacizumab versus Laser Groups: Number and Percentage of Eyes with Abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Tangles</th>
<th>Naked Shunts</th>
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<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>9 Months After Treatment^</td>
</tr>
<tr>
<td>Avastin (Genentech, South San Francisco, CA) (n = 12)</td>
<td>12 (100%)</td>
<td>11 (91.6%)</td>
</tr>
<tr>
<td>Laser (n = 11)</td>
<td>11 (100%)</td>
<td>3 (27.3%)</td>
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</tbody>
</table>

^P < 0.001.

Table 2. Lesions within Vascularized Retina in Bevacizumab versus Laser Groups: Number and Percentage of Eyes with Abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Macular Abnormalities (Absence of Foveolar Avascular Zone or Hyperfluorescent Lesions)</th>
<th>Capillary Bed Loss (Central or Peripheral)</th>
<th>Linear Choroidal Filling Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>9 Months After Treatment^</td>
<td>Before Treatment</td>
</tr>
<tr>
<td>Avastin (n = 12)</td>
<td>12 (100%)</td>
<td>9 (75.0%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Laser (n = 11)</td>
<td>11 (100%)</td>
<td>4 (36.4%)</td>
<td>11 (100%)</td>
</tr>
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</table>

^P < 0.05.

^P < 0.001.

^Not significant.
One of the concerns that has been voiced about the use of anti-VEGF drugs to treat severe acute ROP is that systemic effects may be observed later in life in this vulnerable population.\textsuperscript{26,27} This small case series in which 1 eye was treated with bevacizumab and the fellow eye with laser photocoagulation now raises concerns about the longer-term ocular effects in the use of these drugs. There were not only effects found in macular development in the bevacizumab group but also more peripheral vascular abnormalities noted in this group at 9 months. Such abnormalities may have long-lasting implications for the visual function of the child. This study also highlights advances in imaging that are transforming our understanding of angiogenesis-related pathogenesis of ROP and that techniques such as FA could play a central role in understanding ROP.

![Figure 1. Fluorescein angiographic images before treatment and 9 months after treatment showing the junction between the vascularized and avascular retina in an infant born at 24 weeks' gestational age (A and B, bevacizumab injected; C and D, laser treated). There is persistent leakage and irregular branching in both eyes at the time of treatment (A and C). In the eye injected with bevacizumab at 9 months' follow-up (B), there is persistent avascular retina (black circles) together with hypofluorescent areas (white circles), capillary tufts (white arrow), and peripheral shunt at the junction between vascularized and avascular retina. D, The chorioretinal scar 9 months after conventional laser treatment is shown.](image)

![Figure 2. Fluorescein angiograms of 2 different eyes treated with a single injection of bevacizumab. On the left (A), irregular retinal branching is present with massive leakage. On the right (B), there is a prominent peripheral arteriovenous shunt. Abnormal branching and hypofluorescent areas within the vascularized retina are also evident.](image)
In the conventional management of severe ROP using laser photocoagulation, the retina peripheral to the retinopathy is ablated, and there is a low further risk for abnormal angiogenesis. On the other hand, after bevacizumab treatment, there were large avascular areas peripheral to the location of acute-phase retinopathy. Are these areas likely to require further treatment or are they likely to lead to retinal tears or holes? The fact that abnormal arteriolar branching was noted in bevacizumab-treated eyes likely indicates continued abnormal angiogenesis, and the implications of this in the long term are unknown. It may be that the ocular effects noted in bevacizumab-treated eyes in this series would not be observed if a different dose of bevacizumab had been chosen, a different anti-VEGF drug had been used, or the vehicle or methods of delivery of the drug had been different.

The ROP status of the eyes in this study is not identical to the eyes described in Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity (BEAT ROP) study because all eyes in BEAT ROP were diagnosed with plus disease. Still, the eyes in this study fulfilled the ETROP criteria for treatment as type 1 ROP eyes, and the long-term ocular results of their treatment are relevant to the care of premature infants today.
The current standard of peripheral retinal ablation does have untoward effects, including at least some loss of visual field in eyes that respond to the treatment, and the field defects may be more dramatic in eyes with very posterior disease. It is yet uncertain what the effect on visual field extent will be after the use of anti-VEGF agents.

In conclusion, there is a need for a masked randomized trial to investigate the use of anti-VEGF drugs for ROP, as well as a registry to record the long-term findings of infants who are treated. While waiting for further information on the important issue of systemic and ocular effects of the use of anti-VEGF drugs in type 1 ROP in zone I, we should err
on the side of caution until we understand the implications of these findings on the visual system and the overall development of the child.

References

Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
- BW = birth weight; ETROP = Early Treatment for Retinopathy of Prematurity;
- FA = fluorescein angiography; FAZ = foveal avascular zone;
- GA = gestational age; ROP = retinopathy of prematurity;
- VEGF = vascular endothelial growth factor.

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