Retinal fluorescein angiographic changes following intravitreal anti-VEGF therapy

Andree Henaine-Berra, MD,[a] Gerardo Garcia-Aguirre, MD,[a] Hugo Quiroz-Mercado, MD,[b] and Maria Ana Martinez-Castellanos, MD[a]

PURPOSE To describe the retinal vascular morphology in eyes injected with intravitreal bevacizumab for treatment-requiring retinopathy of prematurity (ROP).

METHODS In this prospective, nonrandomized case series, fundus photographs and fluorescein angiography in patients diagnosed with stage 3 threshold or prethreshold ROP, were obtained immediately before and 1 month after injection of 0.03 cc (0.75 mg) of intravitreal bevacizumab using wide-field digital pediatric imaging system.

RESULTS A total of 47 eyes of 26 patients were included. Before treatment, fluorescein angiography showed vascular abnormalities, including capillary nonperfusion throughout and shunting in the vascularized retina, demarcation line, limited vessel development, new vessels leakage, avascular periphery and absence of foveal avascular zone. After intravitreal bevacizumab, fluorescein angiography showed involution of the neovascularization, flattening of the demarcation line and subsequent growth of vessels to the capillary-free zones. During the following weeks large areas devoid of microvessels were seen as well as vascular remodeling with uneven spacing of the retinal capillaries and vascular loops in the areas that were previously devoid of vessels. In some patients, retinal vessels in the far periphery never developed: patients with these findings did not subsequently develop pathological neovascularization.

CONCLUSIONS In this study cohort, patients showed improvement of their abnormal vascular findings after intravitreal bevacizumab, however even when the vascular pattern remained abnormal, there was creation of small vessels, establishment of directional flow, maturation of retinal vessels, and adjustment of vascular density. (J AAPOS 2014;18:120-123)

The formation of the retinal vascular system is a complex process involving the creation of large and small vessels, the establishment of directional flow, the association with mural cells (pericytes and smooth muscle cells), and the adjustment of vascular density to meet the nutritional requirements of the surrounding tissue.[1-3] The purpose of this study was to describe retinal vascular changes after the inhibition of vascular endothelial growth factor (VEGF) with intravitreal bevacizumab in treatment-requiring retinopathy of prematurity (ROP).

Subjects and Methods

The study protocol was reviewed and approved by the Ethics Committee and Institutional Review Board of the Association for the Prevention of Blindness in Mexico (Asociación para Evitar la Ceguera en México). Patients diagnosed with stage 3 threshold or prethreshold ROP as defined in the International Classification of ROP[4] and the ETROP Study were recruited from January 2008 to December 2012.[5] Informed parental consent was obtained prior to any study procedure.

Treatment was performed under topical anesthesia in an office setting. Topical povidone iodine was applied in the conjunctival cul-de-sac and in the periocular skin. A lid speculum was placed, and 0.03 cc (0.75 mg) of bevacizumab was injected 1 mm posterior to the corneoscleral limbus using a 27-gauge needle. This dose was used because it was approximately half the adult dose, and our study was begun before publication of the BEAT-ROP study protocol in 2011. Topical antibiotic (ciprofloxacin) was applied for 4 days after the procedure.

Under topical anesthesia, fundus photographs and fluorescein angiography of the posterior pole and nasal and temporal fields were obtained using a lid speculum and a wide-field digital imaging pediatric system (RetCamII, Clarity Medical Systems, Pleasanton, CA). For the angiograms, 0.1 ml/kg of sodium fluorescein was injected intravenously, and photographs were recorded in early, middle, and late phases. All treated infants were kept under strict observation and monitoring by their respective treating neonatologists with detailed systemic evaluations. Infants were evaluated for any adverse events the day after treatment and angiogram then weekly for 1 month and once a month thereafter. Fluorescein

Author affiliations: [a]Retina Department, Asociacion Para Evitar la Ceguera en Mexico, I.A.P. Hospital "Dr. Luis Sanchez Bulnes", Coyoacan, Mexico City; [b]Ophthalmology Department, Denver Health Medical Center, School of Medicine, University of Colorado, Denver

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Correspondence: Maria Ana Martinez-Castellanos, MD, Vicente Garcia Torres, No. 46 San Lucas, Coyoacan, Mexico City, P.C. 04030 (email: mamc@dr.com).

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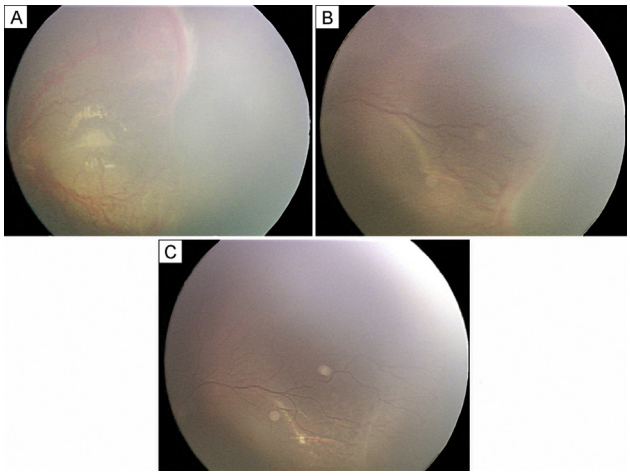

FIG 1. Fundus image showing ROP stage 3 in zone I with plus disease. A, Before treatment. B, 2 weeks after treatment. C, 3 months after treatment. Regression of neovascularization and progressive growth of normal retinal vasculature beyond the demarcation line is evident after treatment.

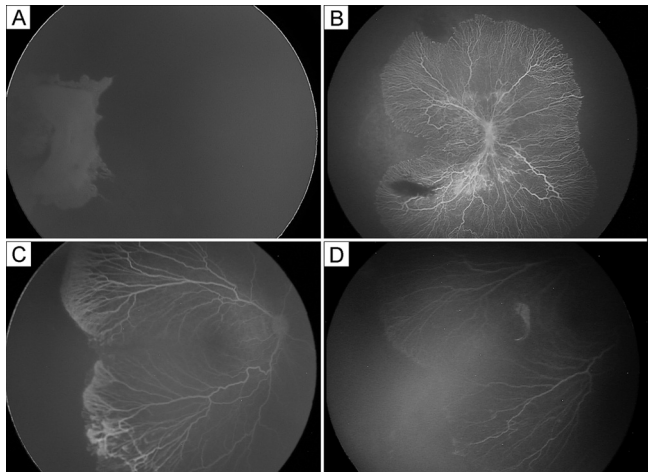

FIG 2. Fluorescein angiography showing the progression of retinal vasculature after intravitreal bevacizumab treatment. A, At 24 seconds, showing minimal vascularization and a neovascular proliferation near the optic disk at 6 weeks of age (38 weeks postmenstrual age); this eye was treated the same day. B, At 31 seconds, showing growth of the retinal vasculature, hyperfluorescence due to vascular incompetence, formation of vascular loops, absence of the foveal avascular zone (FAZ), and presence of a preretinal hemorrhage 6 weeks after injection. C-D, showing further growth of the retinal vasculature at 20 and 24 weeks after treatment and the formation of the FAZ.

angiograms and fundus photographs were obtained at the same session just before treatment and 1 month after treatment and additionally if advised by the treating physician. Patients were followed until vascularization reached zone III; average follow-up was 6 months.

Results

Results are summarized in e-Supplement 1 (available at jaapos.org). A total of 47 eyes of 26 patients (16 males) were included. The mean gestational age was 29.3 weeks (range, 26-32 weeks). Mean birth weight was 1216.4 g (range, 780-2400 g).

At baseline, vascular tortuosity was observed in all eyes, as was absence of the foveal avascular zone (FAZ), capillary nonperfusion throughout the vascularized retina, and presence of a demarcation line with leakage secondary to neovascularization (Figures 1A-B, 2A).

One month after treatment, there was a marked decrease of vascular tortuosity in 45 eyes (96%) (Figures 1C, 2B-D). Angiographic evidence of a foveal avascular zone was observed in 25 eyes (53%), beginning at 1 month after injection (Figures 2C, 3), and continuing throughout the follow-up period. Marked regression of neovascularization, followed by flattening of the demarcation line and subsequent growth of vessels to the capillary-free zones was observed in all 47 eyes (100%) at 1 month (Figures 2-4). However, in 33 eyes (70%) vascular loops were seen in the areas where demarcation lines regressed of eyes (Figures 3-5). These loops were present in some eyes at 1 month and in all eyes at 2 months. In 14 eyes (30%) there were persistent areas of capillary closure posterior to the demarcation line (Figure 6), which disappeared at approximately 3 months after treatment. In 39

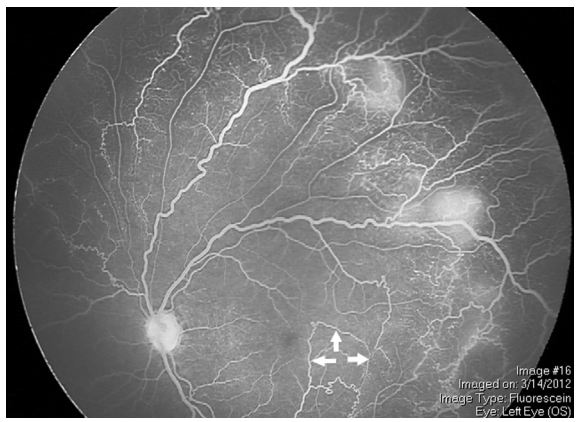

FIG 3. At 1 minute 48 seconds, abnormal development of the macular vessels with the formation of vascular loops in the posterior pole (arrows) and presence of persistent perivascular leakage can be observed 3 months after treatment.

eyes (83%) perivascular leakage was observed (Figure 3). This leakage persisted in most of these eyes through the end of the follow-up period, although it decreased gradually. In 10 eyes (22%) terminal dilatation of capillaries was observed (Figure 7), disappearing completely by the second month after treatment. In some patients the retinal vessels in the far periphery did not develop during the follow-up period (Figure 2D).

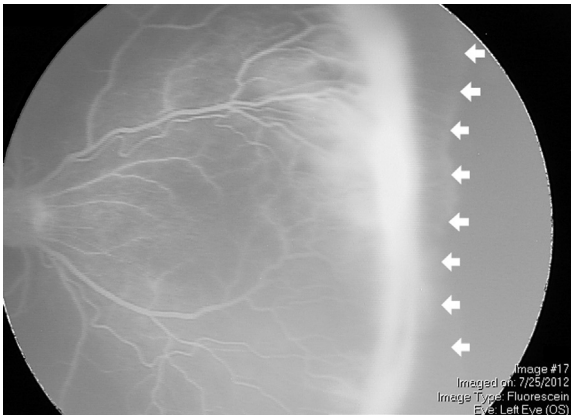

FIG 4. Fluorescein angiography, at 1 minute 34 seconds, showing growth of vessels beyond the demarcation line to the capillary-free zone (arrows) 2 weeks after intravitreal bevacizumab.

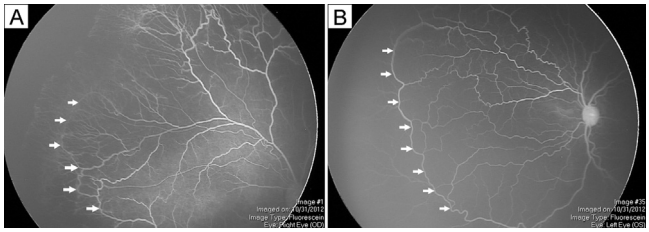

FIG 5. Fluorescein angiography of the right eye at 14 seconds (A) and left eye at 2 minutes 58 seconds (B) 3 months after intravitreal bevacizumab treatment showing abnormal development of the retinal vasculature with the formation of shunts (vascular loops [arrows]).

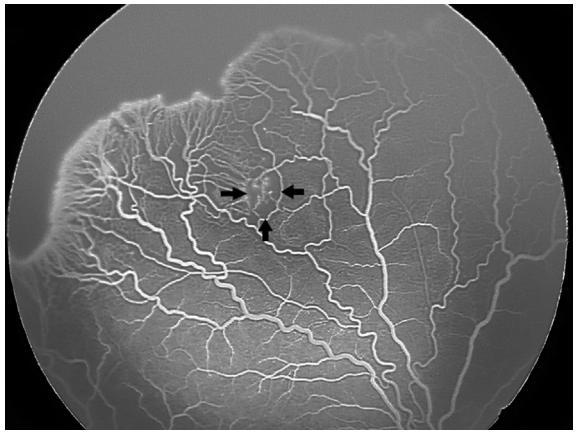

FIG 6. Persistent areas of capillary closure posterior to the demarcation line can be observed, at 26 seconds, after 2 months of intravitreal bevacizumab treatment (arrows).

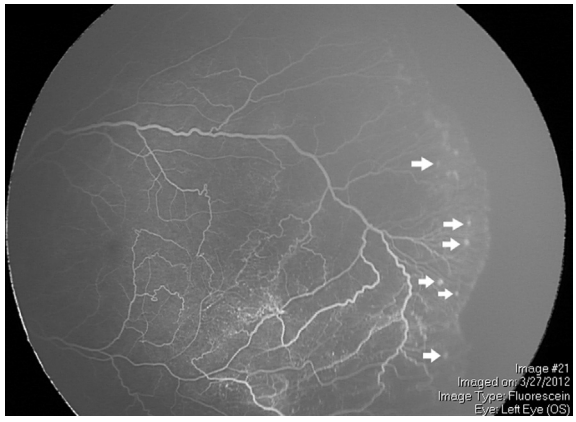

FIG 7. Fluorescein angiographic image, at 2 minutes 4 seconds, showing terminal dilatation of capillaries (arrows) after 2 months of intravitreal bevacizumab treatment.

Discussion

Anti-VEGF therapy with bevacizumab has been applied in patients with treatment-requiring ROP to prevent vascular proliferation and promoting regression of the disease.[6-9] VEGF is essential for retinal blood vessel formation in the embryo and protects retinal vessels from hyperoxia-induced obliteration. However, although VEGF is required to sustain immature retinal vessels, the production of VEGF is suppressed under hyperoxia.[3,10] VEGF is also critical for the survival of "immature/remodeling" blood vessels and independence from VEGF is a molecular hallmark of maturation.[1] Our findings show that VEGF blocking with intravitreal bevacizumab causes regression of abnormal neovascularization, followed by growth of retinal vessels toward the periphery in a cascade of events described below.

In the present study, the first event seen 1 month after intravitreal anti-VEGF therapy was the regression of neovascularization. This was followed by flattening of the demarcation line and subsequent growth of vessels to the capillary-free zones. During the following weeks, large areas devoid of microvessels were seen. Afterward, vascular remodeling was seen, with uneven spacing of the retinal capillaries and vascular loops in the areas that were previously devoid of vessels. Retinal vasculature continued to develop toward the periphery in all patients, however, in some patients the vascularization did not reach the ora serrata. Subsequently, these patients did not develop pathological neovascularization within the follow-up period.

Regarding the development of the FAZ, two theories have been proposed. Gariano and Provis[11] hypothesized that in macaque monkeys and humans the foveal region is not vascularized during any stage of development and that the diameter of the FAZ may change during development as neurons of the inner retina migrate laterally to form the foveal pit. Mintz-Hittner and colleagues[12] hypothesized that the FAZ in developing humans is initially densely vascularized with a fine meshwork of inner retinal vessels during vasculogenesis, and that subsequently this vascular meshwork undergoes regression by apoptosis. They describe that infants born at ≥36

weeks' gestational age develop a normal FAZ by this mechanism, while infants born at ≤30 weeks' gestational age develop a smaller or absent FAZ. In the present study, we observed angiographic evidence of capillaries in the foveal zone, and subsequent involution of these vessels to form the FAZ in 25 eyes (53%). An FAZ developed in 10 of 16 of patients born at ≤30 weeks' gestational age (56%) and did not develop in 4 of 8 of patients born at ≥36 weeks (50%), which differs from the findings of Mintz-Hittner and colleagues[12]; however, only treated patients were included, and therefore comparisons with that study should be made with caution.

Lepore and colleagues[13] reported angiographic findings in patients before treatment for ROP. Among their findings, they describe extreme variability in both retinal circulation and choroidal filling pattern, different patterns of vessels branching at the junction between vascular and avascular retina (V–Av junction), hypoperfused retinal areas with or without hyperfluorescent "cotton-wool-like" or "popcorn-like" lesions due to dye leakage, focal dilatation of capillaries, capillary tuft formations, and rosary-bead-like hyperfluorescent lesions inside the vessels as well as macular abnormalities, including absence of the FAZ.[13] These findings were observed in our patients as well in pretreatment fluorescein angiograms. The notable differences in our study were in the post-treatment fluorescein angiograms, where rapid and complete regression of neovascularization was observed in 100% of cases, and the fact that vascularization continues to grow toward the periphery.

One question that arises about anti-VEGF treatment in ROP is, why is the vascularization to the periphery resumed, especially if the VEGF is blocked in its entirety? One possible explanation is that VEGF levels decrease sharply when treatment is applied and subsequently begin to increase, but in amounts closer to physiological levels needed for the development of the vasculature toward the periphery. The second possible explanation is that bevacizumab blocks only isoform A of VEGF but that there are other isoforms that contribute to the continuation of vascularization while isoform A reaches physiological levels. A third possibility is that bevacizumab blocks most but not all molecules of VEGF A, allowing some molecules to reach their receptor and have enough effect to continue the vascularization.

The present study was limited by the lack of a control group. Additionally, the timing at which the fluorescein angiography was performed was not uniform among all patients after the first month; thus some angiographic findings could have been missed.

In conclusion, our study shows that even when the vascular pattern is abnormal after intravitreal anti-VEGF therapy, the creation of small vessels, the establishment of directional flow, the maturation of retinal vessels, and the adjustment of vascular density have been accomplished. Longer follow-up is needed of prospective multicenter studies to determine the safety profile for the use of intravitreal bevacizumab in treatment-requiring ROP.

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