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Anti-Angiogenic Therapy in the Management of Retinopathy of Prematurity

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Abstract

Retinopathy of prematurity (ROP) is a vitreoretinal abnormality that significantly affects premature babies with low birth rates. Despite improved screening and management of these infants, a subset will progress to retinal detachment and permanent visual impairment. Current treatment consists of peripheral laser ablation and subsequent surgical intervention if a detachment occurs. We sought to evaluate the vitreous biochemistry of eyes that progress despite appropriate laser intervention. Additionally, a limited trial of an anti-VEGF (vascular endothelial growth factor) therapy was used in one eye of infants with persistent Plus disease and neovascularization. The anti-VEGF treatment successfully decreases abnormal angiogenesis but does not decrease the proliferative changes associated with retinal detachment. Biochemical analysis of the vitreous of stage 4 ROP eyes shows significantly elevated VEGF and transforming growth factor (TGF-β) concentrations, and normal levels of other angiogenic factors.

Background

Retinopathy of prematurity (ROP) continues to be a leading cause of blindness in children in developed countries around the world, and an increasing cause of blindness in developing countries. The current standard of treatment is ablation of the peripheral avascular retina. Screening and intervention is now directed by the criteria established by the ETROP Study [1]. The ETROP Study demonstrated superior results compared to the Cryo-ROP Study but also recommends earlier treatment with laser ablation. In the children requiring laser treatment the peripheral retina is ablated and destroyed for future use. The ablated retina is not functional and is not amenable to regeneration. In addition, morbidity from laser can include cataract and vitreous hemorrhage as well as anterior segment ischemia, which can lead to phthisis bulbi.

The mechanism driving the development of ROP is in large part dependent on vascular endothelial growth factor (VEGF) [2–5]. The normal biochemistry of the

developing eye is altered due to the change in environment when a baby is premature. During the second trimester the developing fetus enters phase 1 of vascular development. During phase 1 the endogenous VEGF levels of the fetus are elevated to promote angiogenesis and vascular maturation. In contrast, the relatively hyperoxic environment that the premature baby is introduced in to results in the converse reaction, with decreased production of VEGF leading to delayed retinal maturation. Phase 2 occurs during the third trimester and during this phase the developing fetus will normally reduce VEGF production [6]. VEGF production becomes dysregulated in the premature child, presumably due to large areas of avascular retina creating tissue hypoxia, resulting in abnormally high levels of VEGF. This biochemical shift heralds the pathological changes seen in ROP and explains why the peak incidence of stage 3 ROP occurs between a postmenstrual age of 36–38 weeks [3].

Recently, a subset of patients developing a severe form of ROP has been identified [1]. This group develops an aggressive form of zone 1 disease, designated as aggressive posterior ROP (APROP). APROP develops between postmenstrual age (PMA) 32–34 weeks and is seen in very low birth weight infants generally with a GA less than 26 weeks. It has the poorest outcome with many eyes progressing to retinal detachment despite complete and timely laser ablation of the avascular retina [7].

Vitreous Biochemistry

Vitreous samples were taken at the time of surgery for stage 4 ROP eyes (± pegaptinib treatment). The fluid was analyzed by ELISA assay for total VEGF, TGF-β (isomers 1 and 2), hepatocyte growth factor (HGF) and insulin growth factor (IGF-1). We found significantly elevated levels of VEGF and TGF-β in eyes with ROP-related retinal detachment (fig. 1). The average age of surgery was postmenstrual age 42 weeks, a time point that represents the most frequent onset of tractional retinal detachment in ROP eyes that have undergone laser ablation. Vitreous VEGF concentrations were 576 pg/mg protein in vascularly active eyes and 56 pg/mg protein in nonactive ROP eyes, compared to 12 pg/mg protein in control eyes. The VEGF was most elevated in eyes that demonstrated persistent vascular activity characterized as Plus disease or neovascularization at the time of surgery [2]. It is interesting that despite appropriate and timely laser ablation elevated levels of VEGF persist in the ROP eyes. This finding indicates that the biochemical disturbance is not due to avascular retina alone.

There are a number of other angiogenic factors that have been associated with neovascularization in various eye diseases. HGF and IGF-1 have been associated with proliferative diabetic retinopathy [8–10], although there have been discrepancies regarding the intravitreal HGF levels [11]. We evaluated the vitreous samples for other factors implicated in angiogenesis (HGF and IGF-1) and found that they were not elevated in eyes with ROP compared to controls. From these studies, it appears that VEGF is the primary factor driving Plus disease and neovascularization in ROP.

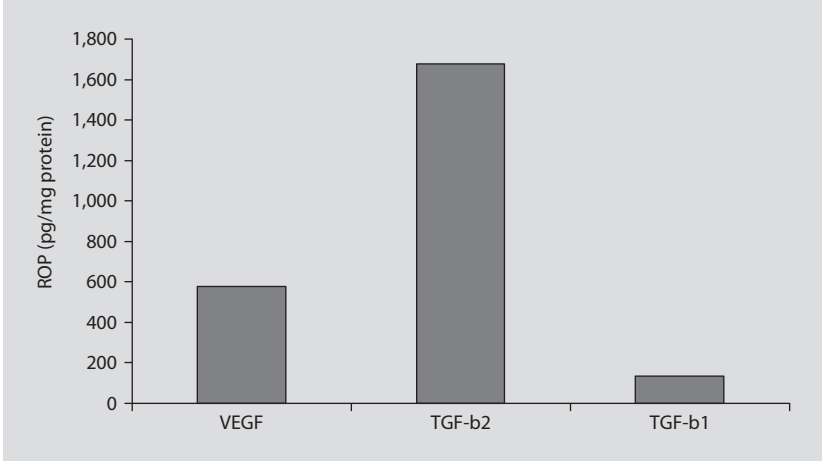

Fig. 1. Graphic depiction of intravitreal growth factor concentrations in stage 4 ROP.

Transforming growth factor has an anti-angiogenic activity and also increases preretinal proliferative changes. It has been noted that TGF-β levels increase near the due date of premature infants and may play a role in the final suppression of intraocular angiogenesis. The majority of infants with ROP requiring laser ablation do not go on to develop a retinal detachment. Interestingly, those infants that do develop a tractional retinal detachment following appropriate laser ablation do so shortly after their due date (highest incidence at PMA 42 weeks). The vitreous samples of stage 4 ROP eyes were evaluated for the concentrations of the two predominant intraocular isomers of TGF-β (1 and 2). TGF-β2 is the predominant form, accounting for nearly 90% of the total TGF-β. Although TGF-β1 only accounts for approximately 10%, it may play a greater role in proliferative events due to its control of pericyte growth [12]. TGF-β1 averaged 145 pg/mg protein in ROP eyes compared to 4 pg/mg protein in control eyes. TGF-β2 averaged 1,744 pg/mg protein in ROP eyes compared to 470 pg/mg protein in control eyes. When compared to controls these are significantly higher concentrations. However, the TGF-β levels for both isoforms are also significantly higher than those seen in rhegmatogenous retinal detachment and proliferative diabetic retinopathy (fig. 2, 3). This finding supports the suggestion that TGF-β levels increase with increasing elevations of VEGF and likely accounts for the small subset of ROP eyes that progress to tractional retinal detachment despite timely and complete laser ablation of the avascular retina.

Anti-Angiogenesis Treatment

With the advent of FDA approved drugs for anti-VEGF treatment, the possibility of treating ROP with anti-VEGF agents has become possible. Drugs that are available

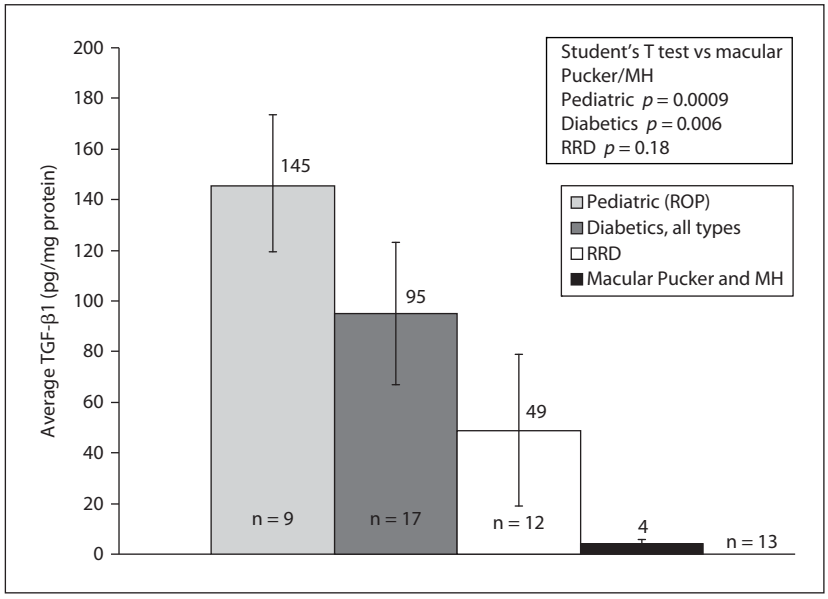

Fig. 2. Graphic depiction of TGF-β1 concentrations.

include the drug pegaptinib (Macugen) for partial blockage of VEGF-A, or complete blockage of VEGF-A with drugs such as ranibizumab (Lucentis) and bevacizumab (Avastin). Some clinicians have evaluated the usefulness of bevacizumab (Avastin) in ROP. These were retrospective case reports or series. All reports demonstrated safety and tolerance of an intravitreal anti-VEGF treatment and all investigators found a rapid resolution of Plus disease and/or neovascularization [13, 14]. One report, however, did find severe progression of fibrosis and retinal detachment with a funnel detachment ensuing [15]. Of note, this particular case received the treatment when proliferative changes were noted and an early detachment was already occurring. Many clinicians have seen similar tractional changes following anti-VEGF treatment of patients with proliferative diabetic retinopathy [16, 17].

In a prospective limited trial, our institution evaluated the effect of partial VEGF inhibition using pegaptinib. VEGF is required in the developing retina for normal angiogenesis and neurogenesis [4] and it was felt that partial VEGF blockade may be beneficial to the developing retina and its vasculature. Pegaptinib was developed to inhibit the $VEGF_{163}$ isomer only, with the theory that this particular isomer represents the pathological changes seen with increased VEGF concentrations. The goal of intravitreal anti-VEGF treatment was to block the excessive levels of VEGF trapped within the overlying vitreous and maintain a normal vanguard of intraretinal VEGF, promoting continued vascular maturation. Eyes requiring surgery had biochemical analysis of their vitreous fluid in order to evaluate other potentially important agents in the development of ROP and progression of retinal detachment.

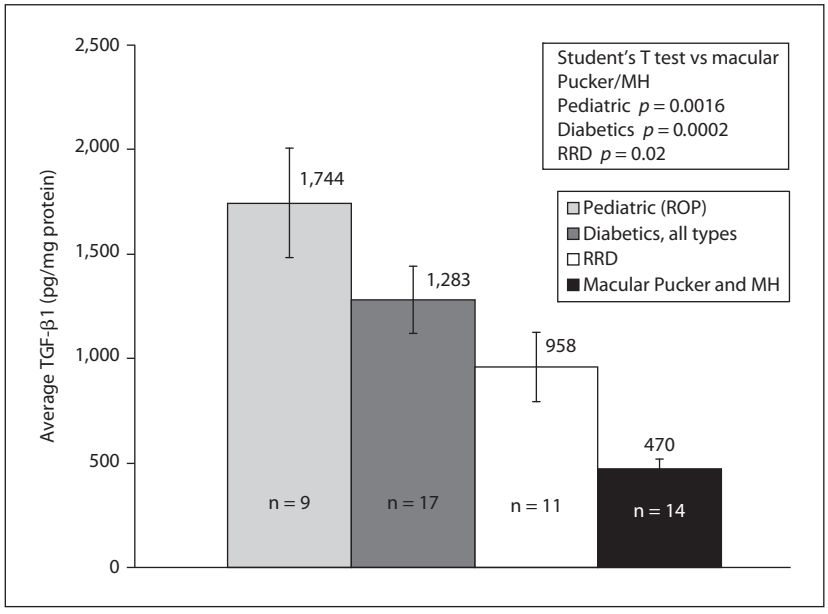

Fig. 3. Graphic depiction of TGF-β2 concentrations.

Premature babies with zone 1 ROP (fig. 4) requiring treatment per ETROP criteria underwent standard care using peripheral laser ablation. Infants with zone 1 disease who demonstrated continued vascular abnormalities (persistent Plus disease or neovascularization) despite appropriate and complete laser ablation and were less than 36 weeks of gestational age were offered rescue therapy with pegaptinib. A total of 5 infants participated and each infant had one eye treated with intravitreal anti-VEGF agent. The study eye received two injections of 1/2 dose pegaptinib (0.05 ml) 1 week apart. The fellow eye was managed by standard care with laser ablation and surgical intervention if progression to tractional retinal detachment was noted. Dilated fundus examination with fundus photography was performed weekly. Surgery was performed if a tractional retinal detachment developed in either eye.

Three infants progressed to bilateral retinal detachment despite treatment (laser ablation of both eyes; one eye received pegaptinib). Of note, all of the pegaptinib-treated eyes demonstrated prompt resolution of Plus disease and regression of the tunica vasculosa lentis 1 week after the initial intravitreal injection (fig. 5) (1/2 dose), consistent with the findings of other investigators. The eyes remained quiet for the remainder of the study. Also, of interest, the pegaptinib-treated eyes that went on to tractional retinal detachment developed the detachment 1–2 weeks after the laser – only eye developed detachment, indicating that decreasing vascular permeability delays the onset of retinal detachment. Once retinal detachment began, however,

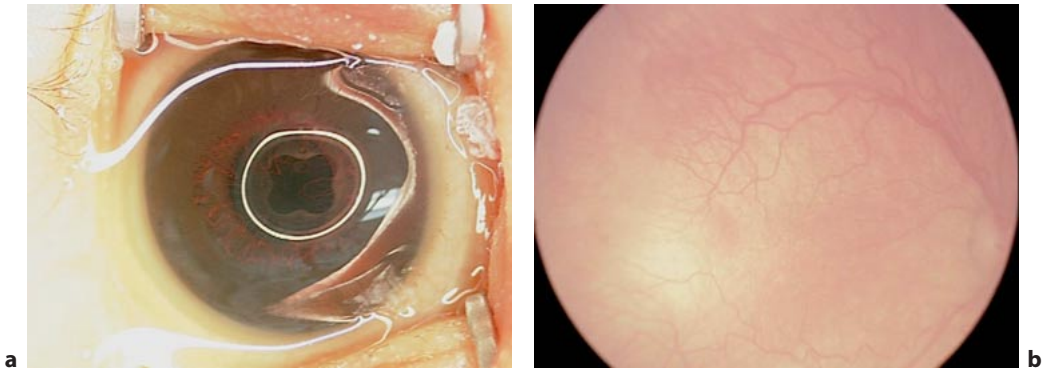

Fig. 4. **a** Persistent tunica vasculosa lentis with poor pupil dilation. **b** Zone 1, stage 3, early Plus. Both eyes had symmetrical disease.

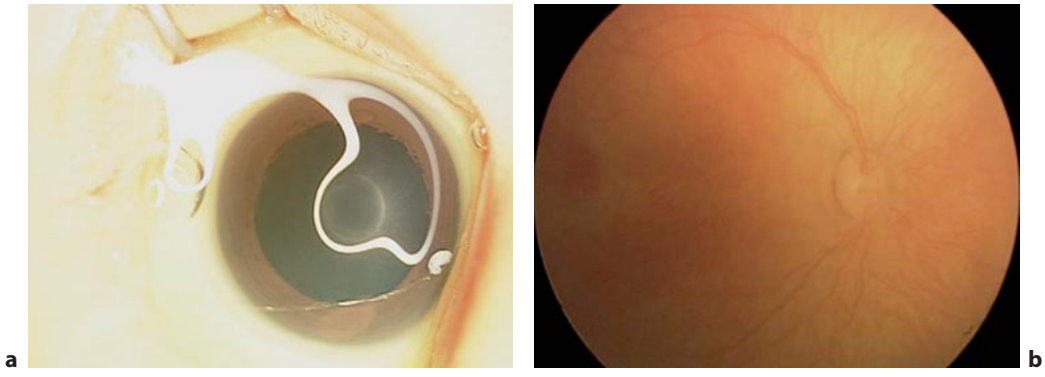

Fig. 5. Zone 1, stage 3, early Plus OU; 1 week after treatment. **a** Right eye: treated with laser ablation and pegaptinib. Note resolution of Plus disease. **b** Left eye: treated with laser only. Note persistent Plus disease.

the pegaptinib-treated eyes had an aggressive fibrosis and proliferation that led to an accelerated detachment progression (fig. 6). Despite the anti-VEGF treatment not preventing retinal detachment, early surgical intervention at the first sign of fibrosis and/or retinal detachment leads to an improved long-term result, both structurally and functionally, compared to the laser-only fellow eye (fig. 7).

The two pegaptinib-treated eyes that did not progress to tractional retinal detachment received the first intravitreal injection at the time of laser, and therefore, at the youngest age (post-menstrual age 33 weeks) in this study group. Presumably, this early anti-VEGF inhibition results in early reversal of the pathologic effects of VEGF accumulation in the vitreous and stabilizes the intraocular biochemistry. Blockage of VEGF accumulation when the fetus is entering phase 2 may improve the anti-VEGF treatment response and reverse the pathological angiogenesis.

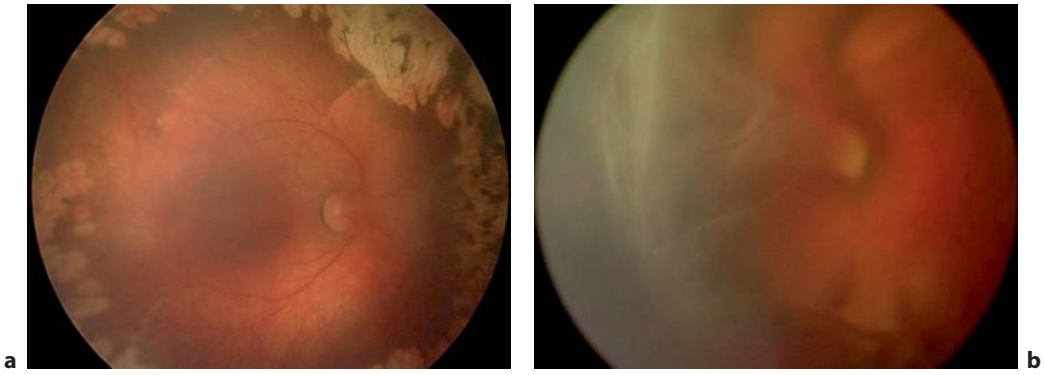

Fig. 6. Postmenstrual age 42 weeks; bilateral retinal detachment. **a** Right eye: early nasal retinal detachment with posterior pole distortion s/p pegaptinib. **b** Left eye: extensive nasal retinal detachment with folding of the temporal retina.

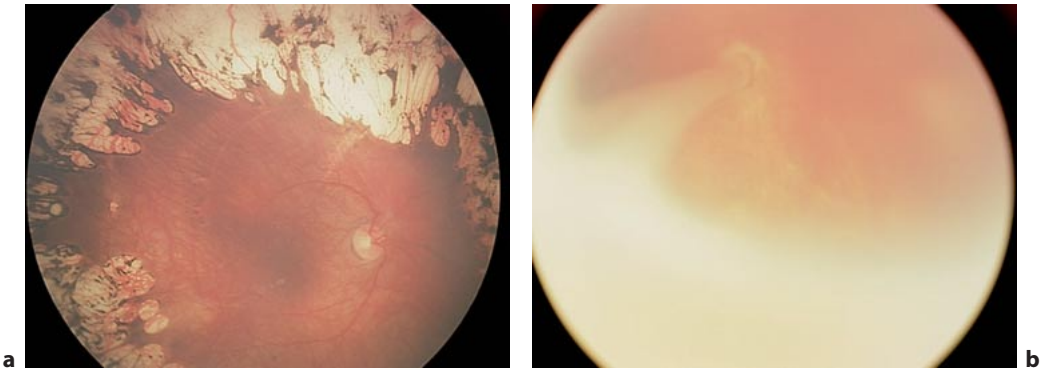

Fig. 7. 2 months s/p vitrectomy OU. **a** Right eye: excellent anatomical and visual outcome (20/40). **b** Left eye: poor anatomical outcome with light perception only.

As noted previously, it has been suggested that TGF-β levels endogenously upregulate near the due date and that the level of TGF-β corresponds to the levels of VEGF present at that time. Binding of VEGF does not appear to alter the concentration or effects of TGF-β. TGF-β plays a role in proliferative changes affecting the retina and the rapid binding of VEGF may allow unopposed TGF-β to have a more dramatic proliferative response. Support of this theory is seen in the vitreous sample of infants with stage 4 ROP, where the levels of TGF-β are elevated significantly above those seen in other proliferative diseases (fig. 2, 3). Additionally, the intravitreal concentrations of TGF-β are greatly elevated compared to the VEGF concentrations in eyes with stage 4 ROP at the time of surgery (fig. 1). These findings indicate that early VEGF inhibition is key to successful anti-VEGF treatment in ROP.

Conclusion

The biochemical constituents of patients with stage 4 ROP indicate that abnormally high levels of VEGF and TGF-β are present and appear to be the predominant factors driving pathological angiogenesis and retinal detachment. HGF and IGF-1 have been implicated in angiogenesis, especially in diabetic patients [9, 10, 18]. HGF has also been implicated in neuronal differentiation [11, 19] and is lower than expected in these samples. Both HGF and IGF-1 are expressed at low levels and do not appear to play a significant role in ROP pathology. The surprisingly low levels of IGF-1 in the vitreous of ROP infants may be an indication of delayed infant maturation or further biochemical abnormalities in the premature eye.

Inhibition of VEGF effectively decreases the vascular pathology and results in stabilization of the vasculature [4]. It does not, however, prevent the progression to retinal detachment. The significantly elevated levels of TGF-β likely contribute to the tractional changes seen in theses eyes. TGF-β may be abnormally elevated as a secondary response to high intravitreal VEGF concentrations. Possibly, the blocked VEGF leads to unopposed TGF-β activity and promotes proliferation of cells and tractional retinal detachment. Additionally, the incomplete regression of primary vasculature and vitreous may provide a platform of increased cellular substrate for TGF-β to act upon, creating greater tractional forces. Earlier intervention with an anti-VEGF agent may avoid this late complication and further studies will need to be conducted to answer this. Prompt surgical intervention, however, results in a favorable outcome. The anti-VEGF treatment quiets the vascular abnormalities and creates less intraoperative bleeding, allowing for early surgical intervention if necessary. Alternatively, a TGF-β inhibitor may be helpful in preventing retinal detachment in these patients.

Continued investigation into the pathology of ROP and the use of biochemical modulation with timely surgical intervention may lead to improved visual outcomes for these infants.

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