REVIEW ARTICLE

Retinopathy of Prematurity –Promising Newer Modalities of Treatment

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Retinopathy of prematurity (ROP) is a disorder of neonatal retinal vascularization. The incidence is increasing in developing countries like India in view of the rising numbers of preterm deliveries and improved neonatal care. Traditional modalities of treatment included cryotherapy and laser therapy, which were laborious and required special training. Hence, research is on way to find novel treatment modalities directed at various levels of pathogenesis for this blinding disease. We reviewed the published and unpublished literature on newer methods of ROP management. The pathogenesis of ROP has been studied with respect to the mediators of angiogenesis. Anti vascular endothelial growth factor (Anti-VEGF) therapy has been extensively studied and the studies have demonstrated its promising role early stages of ROP. The role of Insulin like growth factor (IGF), Granulocyte colony stimulating factor (GCSF), and June kinases (JNK) inhibitors are being studied by various researchers across the world. Gene therapy holds promise in the reversal of ROP changes.

Key words: *Anti-VEGF, IGF, GSCF, Gene therapy, JNK1 inhibitor, Retinopathy*.

Retinopathy of prematurity (ROP) is a developmental disorder that occurs in the incompletely vascularized retina of premature infants and is an important cause of blindness in children in both the developed and the developing countries. Progress in neonatal intensive care in recent years has led to an increased survival of extremely low birth weight (ELBW) infants weighing ≥1000 g at birth and, subsequently, to an increasing incidence of ROP [1]. Lack of a screening program in India has led to larger number of babies developing retinal detachment, because the retina in ROP eyes does not seem to grow with the growing eyeball [2]. It gives way at a vulnerable point because it is stretched thin causing recurrent retinal detachment [3]. Re-attachment of retina also does not contribute to improved vision in these infants.

Cryotherapy emerged as the standard treatment for acute phase ROP in the 1980's, following publication of the results of Cryotherapy for Retinopathy of Prematurity Cooperative Group (CRYO ROPCG) trial [4]. The study showed significant decline in the progression of threshold ROP at which stage the risk of blindness if untreated was 50%. Cryotherapy also significantly reduced the unfavorable structural outcome of threshold ROP to 49.3% at 3 months and 45.8% at 12 months [5]. Cryotherapy requires a general anaesthetic or sedation and ventilation. Conjunctival dissection is needed in posterior disease to enable access for the cryoprobe. Complications are post-operative pain, lid edema, laceration and hemorrhage of conjunctiva, preretinal and vitreous hemorrhage. Laser (light amplification by stimulated emission of radiation) therapy evolved as the primary modality of treatment in 1990's, with lesser complications, and provided blindness prevention as effectively as cryotherapy. However, the total cost of required equipment for laser therapy was much higher than for cryotherapy. This factor may have to be considered for the developing countries of the world, where the incidence of ROP is on the rise. The complications reported with laser are burns of the cornea, iris and lens, hyphema, retinal hemorrhages and choroidal rupture [6]. Hence, the need for newer modalities of treatment, which require less skill and equipment. Some of these novel modalities are discussed here.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Two theories exist on the pathogenesis of ROP. The mesenchymal spindle cells, exposed to hyperoxic extra uterine conditions, develop gap junctions. These gap junctions interfere with the normal vascular formation, triggering a neovascular response, as reported by Kretzer and Hittner [7]. Ashton theorized that 2 phases exist [8]. The first phase is at the time of premature birth, the infant retina becomes hyperoxic (even in room air) with decreased levels of VEGF. For a period of time, vessel

formation is halted at the interface between the vascular and avascular retina (Phase I, clinically 22–30 weeks postmenstrual age). As the eye grows, the avascular retina continues to increase in size without accompanying inner retinal vessels. This creates a peripheral area of hypoxic retina, resulting in increased levels of VEGF, which stimulates angiogenesis (pathological neovascularisation) at the interface between the vascular and avascular retina (Phase II, clinically 31-45 weeks postmenstrual age). This two phase process leads to vision-threatening ROP most frequently in extremely immature infants with other comorbidities of prematurity (risk factors for ROP). These new vascular channels are not mature and do not respond to proper regulation. Although many causative factors, such as low birth weight, prematurity, and supplemental oxygen therapy are associated with ROP, several indirect lines of evidence suggest the role of a genetic component in the pathogenesis of ROP [9]. Genetic polymorphism may alter the function of the genes that normally control retinal vascularization, such as VEGF, which may also be involved in the pathogenesis of ROP.

Anti-Vascular Endothelial Growth factor Therapy

Vascular endothelial growth factor A (VEGF-A) is a known promoter of angiogenesis and is upregulated by hypoxia. It interacts with endothelial cells via its two membrane-bound receptors, VEGFR-1 and VEGFR-2, which belong to the tyrosine kinase receptor family [10]. VEGFR-1 controls the assembly of tubes and functional vessels by endothelial cells. VEGFR-2 promotes the differentiation and proliferation of endothelial cells. Its expression is increased by hypoxia and potentiated by VEGF [11,12]. An association with ischemia-induced proliferative diseases has clearly been established [13]. VEGF-A plasma levels of infants born at term were reported to vary from 200 to 450 pg/mL during the first few weeks of life and decline rapidly to adult range levels of 10-110 pg/mL within a few months after birth [14]. Interestingly, plasma VEGF-A levels in preterm infants were found to be relatively low and stable during the first 7 days of life (48 pg/mL, SD 6) [15]. Similarly, in a mouse model of ischemia induced retinal revascularisation, immunohistochemistry for VEGFR-1 and VEGFR-2 revealed that the immunoreactivity of VEGFR-2 was increased in the vessels near the avascular area, whereas the pattern of VEGFR-1 expression in the hypoxic retina was almost the same as that of control animals [16]. VEGFR-2 expression was found to be mainly associated with pathological neovascularisation and less with physiological postnatal vessel development [17]. One study found VEGF-A to be elevated in the subretinal fluid in ROP stage 4 (mean 44.16 ng/mL, SD 18.72) and greatly reduced in stage 5 [18]. In contrast, another study investigated 38 cases of stage 5 ROP at the time of vitrectomy and found increased VEGF immunoreactivity in the vascularised regions of fibrovascular membranes [19].Thus the role of vascular endothelial growth factor (VEGF) in neovascularization and vascular permeability of ROP is established [20]. Hence, therapy for ROP is directed at treating the underlying pathogenesis by decreasing VEGF levels (specifically VEGF-A), either by completely ablating the peripheral avascular retina that produces the VEGF (LASER therapy) or by inactivating VEGF by binding it after its production (anti-VEGF therapy).

Bevacizumab, a humanized recombinant antibody, that inhibits the biological activity of VEGF, has been widely used as a off-label treatment for ocular angiogenesis disorders, including age-related macular degeneration [21], proliferative diabetic retinopathy (PDR) [22] and neovascular glaucoma [23]. It is a complete antibody rather than an antibody fragment like ranibizumab.

The BEAT–ROP study (Bevacizumab eliminates the angiogenic threat of ROP) is a Phase II study (intravitreal bevacizumab injections versus conventional LASER surgery for ROP). In one study, after injection of bevacizumab as the initial treatment, reduced neovascular activity was seen on fluorescein angiography in 14 of 15 eyes under study. In three eyes, a tractional retinal detachment developed or progressed after bevacizumab injection. No other ocular or systemic adverse effects were identified [24]. Bevacizumab has been shown in a small case series to temporarily slow vasculogenesis and permanently halt angiogenesis, usually with a single intravitreal injection, when used for vision-threatening ROP stage 3 (acute phase ROP including AP–ROP) [25].

Bevacizumab, given to extremely immature infants by intravitreous injection as low dose monotherapy (0.625 mg in 0.025 mL of solution) or upto 0.75 mg, has not shown systemic or local toxicity. The most likely local complications of the injections are infectious and traumatic. Infections can be avoided by strict sterile technique followed by one week of appropriate antibiotic ophthalmic drops. Trauma may occur to the lens because the injection is given too anteriorly. No systemic complications have been encountered to date whether bevacizumab given alone, or in combination with LASER surgery (when the retinal barrier has been breached).

Thus, bevacizumab alone for ROP stage 3 may

become not just an adjunct to laser therapy or vitrectomy, but primary treatment replacing laser therapy as standard of care, if efficacy and safety are validated by evidence-based data. Laser eventually may be contraindicated because it is a very destructive therapy that is never completely without residual effects and targets the same pathogenic substance: VEGF. ROP stages 4 and 5 will continue to occur due to late diagnosis of acute disease from less than adequately screened nurseries and in these desperate cases vitrectomy will be required, perhaps with bevacizumab as an adjunctive therapy.

This novel drug therapy is being tried by many neonatologists in developing countries where facilities for laser or cryotherapy may not be available. Moreover, bevacizumab is a relatively easy and inexpensive treatment. In developing countries like India, where the per capita GDP is USD 543 (March 2006), the majority of patients cannot afford to pay USD 730 for a single vial of bevacizumab [26]. Therefore, efforts were made to make 20 fractions of 0.2 mL from a single vial, thus decreasing the cost to USD 38 per injection [27].

Another Anti-VEGF drug researched is Pegaptanib sodium. The initial experience using pegaptanib sodium to treat ROP suggest that the medication is well tolerated, and helps to quieten the vascular activity in eyes with severe posterior disease, but does not prevent the development of retinal detachment. This thus is still under study as an alternate anti VEGF therapy. We would advocate caution; however, before introducing this new, potentially exciting therapy, especially since large randomized clinical trials are still to be conducted [28].

INSULIN LIKE GROWTH FACTOR – I

It was shown that lack of insulin-like growth factor I (IGF-I) in knockout mice prevents normal retinal vascular growth, despite the presence of vascular endothelial growth factor, important to vessel development [29]. Results from studies in premature infants suggest that if the IGF-I level is sufficient after birth, normal vessel development occurs and retinopathy of prematurity does not develop [30]. When IGF-I is persistently low, vessels cease to grow, maturing avascular retina becomes hypoxic and vascular endothelial growth factor accumulates in the vitreous. As IGF-I increase to a critical level, retinal neovascularization is triggered [31]. These data indicate that serum IGF-I levels in premature infants can predict which infants will develop retinopathy of prematurity and further suggests that early restoration of IGF-I in premature infants to normal levels could prevent this disease [32]. A study from Sweden [33] studied the pharmacokinetics and dosing of intravenous insulin-like growth factor-I and IGF-binding protein-3 complex in preterm infants. The recombinant human IGF-I (rhIGF-I/rhIGFBP-3) equimolar proportion was effective in increasing serum IGF-I levels and administration under study conditions was safe and well tolerated. IGFBP-3 is a protein that binds to IGF-I and regulates the availability and activity of IGF-1. Mecasermin rinfabate mimics the effects of this natural protein complex (IGF-1/IGFBP-3) in the bloodstream and is able to stay in the body for a longer period. The drug is under study and no studies to prove its efficacy have been published yet. The cost efficacy of this drug is quite disappointing when compared to the other modalities.

GRANULOCYTE COLONY–STIMULATING FACTOR

Granulocyte colony-stimulating factor (GCSF), a biologic at commonly used to increase leukocyte counts in neutropenic adults and children might also have a regulatory effect on vasculogenesis and thus prevent ROP. GCSF has been shown to increase levels of insulin-like growth factor-1(IGF-1), which supports the normal, measured, calm vascularisation of the retina. Conversely, falling levels of IGF-1 appear to set off a disorderly, aggressive vascularisation of the retina. In mouse oxygen-induced retinopathy model, G-CSF significantly reduced vascular obliteration (P <0.01) and neovascular tuft formation (P <0.01). G-CSF treatment also clearly rescued the functional and morphologic deterioration of the neural retina [34]. So GCSF, which is given to many premature infants, might be used to prevent ROP, particularly in infants with falling IGF-1 levels. The beauty of this approach, as opposed to, say, using bevacizumab, is that it doesn't destroy VEGF. This hunch led to a retrospective chart review of 213 infants who, for non ophthalmic reasons, received GCSF in the neonatal intensive care unit at the University of Louisville. Fifty infants with low birth weight and a gestation of 32 weeks and under were matched to a control group that did not receive GCSF. Only 10% of the infants who received GCSF required laser treatment, compared with 18.6% of the controls. Further, those babies in the GCSF group who required laser had an exceptionally low average birth weight and had received relatively low doses of GCSF [35].

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. It's potential role in the prevention of ROP is now being studied, hence the dose required and side effects are still not documented. As this drug is more easily available in India with costs ranging from 2500 – 3000 INR for a 300 mcg/mL vial, this would be a

beneficial adjunct to the treatment of ROP in a developing country like ours.

JUN KINASES (JNK) INHIBITORS

The Jun kinases (JNK) belong to the mitogen-activated protein kinase (MAPK) family [36]. These kinases, which are encoded by three separate loci, *Jnk1-3*, regulate key cellular processes such as cell proliferation, migration, survival, and cytokine production. In cell culture studies, a nonspecific JNK inhibitor was shown to affect VEGF mRNA stabilization [37]. Hence, JNK1 is a critical factor in hypoxia induced retinal VEGF production and that it promotes hypoxia induced pathological angiogenesis. JNK1 deficiency or JNK inhibition results in reduced pathological angiogenesis and lower levels of retinal VEGF in an experimental model of ROP [38]. Intravitreal injection of a specific JNK inhibitor decreases retinal VEGF expression and reduces pathological retinal neovascularization without obvious side effects. These results strongly suggest that JNK1 plays a key role in retinal neoangiogenesis and that it represents a new pharmacological target for treatment of diseases where excessive neoangiogenesis is the underlying pathology [39]. D-JNKi – the specific JNK-1 inhibitor is the drug that is under study for the prevention of ROP.

GENE THERAPY

A series of small studies have investigated the association of gene and severe ROP or failure of treatment [40]. They have implicated mutations and polymorphisms in the *Norrie disease pseudoglioma* (*NDP*) gene, *Endothelial nitric oxide synthetase* (*eNOS*) gene and *Vascular endothelial growth factor* (*VEGF*) gene. Unfortunately, most studies do not show a significant association of genetic abnormalities and ROP. The influence of genes on the occurrence, progression and severity of ROP warrants further investigation in various populations and in larger cohorts [41]. The answer may lie in manipulating transcription factors and alternative splicing of putative genes involved in ocular NV, tipping the microenvironment to an anti-angiogenic state [42]. We believe that through techniques in gene therapy, alternative splicing and RNA interference, we may meet with greater success in restoring ocular 'angiogenic privilege'. Chowers [43] gave his view of the future by demonstrating that gene transfer into blood vessels is possible in a rat model of retinopathy of prematurity. In their studies, Chowers tested retrovirus, adenovirus and herpes virus based vectors, shuttling a galactosidase reporter gene for expression in retinal blood vessels in rodents undergoing oxygen induced retinal neovascularisation. Interestingly, they found that adenovirus offered the best efficiency in expression in retinal blood vessels compared with all the other vectors. Moreover, the adenovirus expression was specific to the blood vessels of the inner retina and did not appear to be expressed in the deeper neural retina. Hence, gene therapy has a definite future in this blinding eye disease.

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