·Review·

Treatment of retinopathy of prematurity: a review of conventional and promising new therapeutic options

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Abstract

• **Retinopathy of prematurity (ROP), a retinal vascular disease of premature infants, continues to be a major cause of preventable childhood blindness all over the world. The incidence of ROP varies among countries, being influenced by the quality of the level of neonatal intensive care. Here, we discuss the potential treatments that are now available or will soon or probably be available for ROP. Although ablation of the avascular retina with laser photocoagulation remains the current gold standard and well established therapy for ROP, some new therapeutic options including angiostatic therapies are being explored based on our knowledge of the pathophysiology of the ROP and complications and efficacy of laser treatment. However, prevention of the development of severe ROP and screening for ROP seem to be the best strategy in avoiding visual impairment caused by ROP in premature infants. New therapeutic interventions including vascular endothelial growth factor antibody administration, gene therapy and supplemental therapies should be supported with evidence-based data for the treatment of ROP.**

• **KEYWORDS:** retinopathy of prematurity; laser; retina; vascular endothelial growth factor; propranolol
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INTRODUCTION

Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the eye affecting premature infants. It is a leading cause of possibly avoidable childhood visual impairment and blindness in the developing and developed world despite current treatment strategies [cryo, light amplification by stimulated emission of radiation (laser) or vitreous and retinal surgery treatments]. Incidence and risk factors of ROP may differ according to the progress in perinatal care, race, country, various populations and screening criteria [1-8]. Its control is given high priority in the World Health Organization's "Vision 2020 Programme" [9]. Potential therapeutic treatment strategies for ROP with vascular endothelial growth factor antibodies (anti-VEGF), as well as possible promising future therapies are discussed in this review.

PATHOGENESIS

Normal Retinal Vascular Development Blood vessels that supply the inner portion of the eye are continually undergoing development. Initially, the inner part of the eye is metabolically supported by the choroid hyaloid vasculature, an arterial network in the vitreous, and choroid. The choroid begins to develop at about gestational age of 6 weeks and becomes almost completely organized by the 8th week. Retina remains avascular as the choroid hyaloid vasculature and the underlying choroid provide the nutrients to the developing retina during the first 4 months of gestation. In the latter stages of development the hyaloid vasculature is replaced by the retinal vasculature since the choroidal circulation alone cannot meet all the needs of the retina [10,12]. As the hyaloid vascular system regresses, retinal vascular network in the form of superficial and deeper capillary plexuses begins to develop at 16 weeks of gestation from mesenchymal spindle cells and blood vessels precursors, emerging from the optic nerve head giving rise to the retinal vasculature [10-14]. As mesenchymal spindle cells lead to endothelial proliferation, retinal vessels grow out of the optic disc as a wave of mesenchymal spindle cells and capillary formation follows. Formation of the superficial capillary plexus of the retinal vasculature occurs on the retinal surface within the ganglion cell layer. Retinal vessels growing out of the optic disc reach the ora serrata nasally at approximately 32nd week of gestation and temporally shortly after birth. At 25th week of gestation, glial cells such as astrocytes cover much of the retina, at a time when the spindle cells (astrocyte precursor lineage) are virtually nonexistent [14-16]. During retinal vascular development, astrocytes form a template for endothelial cell migration within the retina rather than into the vitreous and may also serve as an

important source of vascular endothelial growth factor (VEGF) [17-21]. Although cell type-specific functions of VEGF isoforms in development and homeostasis of the retinal vasculature have not been completely identified, hypoxia-regulated VEGF has been demonstrated to play a critical role in this process as well as pathological neovascularization [21]. Recently, it has been reported that astrocytes in the retina have highly divergent roles during developmental, physiological angiogenesis and ischemia-driven, pathological neovascularization, and astrocyte hypoxic response has been reported to be essential for pathological but not developmental angiogenesis of the retina [22]. The deeper plexus of the retinal vasculature (outer plexus) develops independently of retinal astrocytes by sprouting from the preexisting retinal vessels (the superficial capillary plexus) [23]. At the end, the branches from the central retinal circulation form two distinct capillary plexuses within the ganglion cell layer (the superficial capillary plexus) and inner nuclear layer (the deeper capillary plexus). In a prematurely born infant, the retina is immature and incompletely vascularized depending on the gestational age.

A number of angiogenic factors including insulin-like growth factor-1 (IGF-1) [24], VEGF [17-20], basic fibroblast growth factor (FGF) [25], transforming growth factor beta (TGF-β) [26], platelet derived growth factor (PDGF) [27], and hepatocyte growth factor [28] have been shown to play various roles in retinal vascular development. VEGF, a hypoxia-inducible vasoactive cytokine, is a potent mitogen for vascular endothelial cells, necessary for the physiological angiogenesis [29,30]. VEGF is mainly secreted in the maturing avascular retina, and is regulated by tissue hypoxia. The VEGF gene is responsive to oxygen tension; generally hypoxia stimulates VEGF transcription and hyperoxia decreases VEGF transcription [17,30]. Retinal development creates increased metabolic need and a local relative (physiological) hypoxia in advance of the developing retinal vessels. Oxygen-regulated VEGF plays a part in all stages of vascular development and works in conjunction with other factors, such as non-oxygen-regulated IGF-1 [31]. VEGF is secreted in response to physiological hypoxia in the maturing avascular retina by mesenchymal spindle cells and astrocytes [17,19,29-32].

Two phases of ROP and VEGF ROP is a biphasic disease; the first phase is a hyperoxic vessel obliteration and the second is a hypoxic neovascularization phase [19,32,33]. Neonatal hyperoxia, experienced by premature infants soon after birth and during the first days of postnatal life, causes shut-down of sections of the retinal vasculature, by apoptosis and excessive capillary regression, resulting in interruption of normal vascularization and later ischemia of the retina (phase I; hyperoxia-vasocessation). This first phase of ROP occurs from birth to approximately 30-31 weeks of gestation. Administration of supplemental O_2 in this phase may also aggravate hyperoxia, increasing the stage for vaso-obliteration of existing vessels. As the metabolic demand of the developing retina increases, the immature nonperfused area of the retina becomes more hypoxic and may stimulate an overproduction of VEGF pathologically, resulting in abnormal neovascularization of the retina, known as ROP (phase II; hypoxia-vasoproliferation). This phase begins around 31-32 weeks of gestational age. So, regulation of VEGF appears to play a key role in normal angiogenesis during embryogenesis and in abnormal neovascularization of the retina in pathological conditions after birth. VEGF levels are elevated when the retina is hypoxic, and VEGF mRNA has been expressed in the avascular zone of the human infant eye with threshold ROP [18,34]. IGF-1 is another important growth factor, levels of which correlate very well with the birth weight and gestational age [7,8,19,33,35]. It is also a key factor in normal vessel development, and regulates retinal neovascularization by controlling the activation of VEGF regardless of oxygenation. When IGF-1 is low, vessels do not grow. Fetal IGF-1, levels of which rise in the second and third trimester, is secreted by fetal tissues; the fetal liver and placenta. It is dependent on nutrient supply and controlled by unknown mechanisms at the maternal-fetal interface. Oxygen-independent IGF-1 and oxygen-dependent VEGF are synergistic. A low serum IGF-1 may predict ROP but IGF-1 is expressed by many tissues and a low serum level therefore could be a general marker of a sick baby at risk of ROP rather than a specific marker for a retinal disease [31,35]. All these explanations describe why hyperoxia is important in the initial phase and how vascular shutdown is the consequence of oxygen-induced VEGF downregulation [24,32,33,35-37].

There are some other angiogenic cytokines that have different roles for retinal vascular development [24-28,32-35,38]. Planning treatment strategies of ROP according to the cytokines that have different roles and regulatory effects in both phases of ROP may influence pathologic neovascularization in ROP. Furthermore, there is a suggested genetic predisposition to ROP [39-43], and progression of ROP to threshold ROP or the risk for proliferative ROP in very low birth weight infants may be influenced by genetic polymorphisms in VEGF production [42,43]. Genetic changes in the wingless/int1 (Wnt) receptor signaling pathway during retinal development are considered as probable risk factors for advanced ROP [40,41]. In the future, blocking the effects of these genes (NDP, LRP5, FZD4, and TSPAN12) may have an influence on the development of ROP.

TREATMENT OF ROP

Ablative Therapy The choice of treatment for ROP has shifted from cryotherapy to diode laser photocoagulation after clinical studies showing the superiority of laser therapy to cryotherapy [44,45]. Laser therapy can be applied both transpupillary or trans-sclerally. Indications of laser therapy for ROP have been described [1,46]. Over time, the timing of

treatment has moved to an earlier stage of the disease, as documented by the Early Treatment for Retinopathy of Prematurity (ETROP) study [46]. Although ablation of the peripheral retina with laser reduced the progression and incidence of the disease in the ETROP study, patients still had poor visual outcomes after treatment, especially for zone I ROP. Despite the advances, progression to retinal detachment occurred in 12% of eyes in the ETROP study with adequate peripheral ablation. This may be related with either the individual skills of the surgeon who applies laser treatment, or vitreal VEGF levels which cannot be reduced by retinal laser photocoagulation. Although VEGF is mainly secreted in the avascular retina, vitreal macrophages as a second source of VEGF may be responsible for the lack of the effectiveness of laser therapy for ROP [47].

Additionally, laser therapy has some disadvantages including corneal edema, anterior chamber reaction, intraocular hemorrhage, cataract formation, intraocular pressure changes. Significant decrease in peripheral vision due to ablation of peripheral retina, strabismus, glaucoma, severe myopia and retinal detachment are some of the long-term side effects probably related to disease severity rather than laser therapy itself. However, the only evidence-based therapy for ROP is still laser photocoagulation, depending on the severity, in combination with vitreoretinal surgery.

Future Therapeutic Strategies Although laser photocoagulation of avascular retina remains currently as the gold standard and well established therapy for ROP, there are some new treatment modalities for ROP, which are being evaluated in ongoing or planned studies. New pharmocological treatment concepts such as anti-VEGF agents [48-53], systemic propranolol [54,55], gene therapy [56,57], supplementation with omega-3 fatty acids or IGF-1 [58-60] also represent interesting pharmacological approaches to the management of ROP.

Antivascular Endothelial Growth Factor Therapy VEGF is a potent mitogen for vascular endothelial cells and necessary for the physiological angiogenesis and it is regulated by tissue hypoxia [6,17,19,32,33]. It also promotes pathologic angiogenesis. Blocking the action of VEGF might be expected to reduce the vascular activity associated with ROP [17,19,61-63]. Current treatment for ROP is retinal ablation either with cryo or laser photocoagulation that indirectly reduces VEGF levels by completely ablating the peripheral avascular retina that produces the VEGF [18,34,61]. In contrast, anti-VEGF drugs offer the major advantage of limiting tissue destruction by decreasing VEGF levels both in the retina and vitreous [47,62,63]. Available drugs include pegaptanib sodium (Macugen®) for partial blockage of VEGF-A or drugs such as ranibizumab (Lucentis®), bevacizumab (Avastin®) and aflibercept (Eylea®) for pan-VEGF-A blockage. Three anti-VEGF drugs (pegaptanib sodium, ranibizumab and aflibercept) are approved by the US Food and Drug Administration (FDA) for intraocular use in the treatment of some ocular neovascular diseases in adults. In contrast, bevacizumab is approved by the FDA for only intravenous use in the treatment of colorectal, breast, lung and renal cell cancers.

Pegaptanib sodium was the first anti-VEGF drug licensed for the treatment of age related macular degeneration. It may be a safer option with respect to normal development in premature newborns as it is a selective VEGF-165 inhibitor without blocking all VEGF isoforms. There are a few reports describing the use of intravitreal pegaptanib in the management of ROP and Autrata *et al* [64] recently reported promising results with the administration of intravitreal pegaptanib in the management of stage 3+ ROP without any systemic or ocular complications during 19.3 months. Ranibizumab has a shorter serum half-life than bevacizumab which may theoretically reduce the risk of systemic complications in premature infants [65,66]. Aflibercept, a fusion protein that inhibits all isoforms of VEGF-A, has high binding affinity and longer intraocular half-life which may result in a longer duration of clinical action intraocularly than either ranibizumab or bevacizumab [66,67]. However, no randomized clinical tials regarding the effects of intravitreal ranibizumab and aflibercept injections yet exist. Even though bevacizumab is associated with an increased risk of hemorrhagic and thromboembolic events in adults with intravenous therapy [66,68], clinical experiences with intravitreal bevacizumab have shown that this agent is well tolerated and widely off-label used in the treatment of intraocular neovascularizations [48-53,69]. Lower doses of bevacizumab are used for the treatment of ocular conditions, however, use of anti-VEGF therapy in premature newborns is still controversial regarding safety [66,70,71].

Bevacizumab, a humanized recombinant antibody, binds all isoforms of VEGF-A [62,63,72,73]. This could potentially be disadvantageous because physiologic vascular development could be suppressed. However, its large molecular weight and being a full antibody combined with its half-life and the thick preterm vitreous allow a longer period of effectiveness. Penetration of bevacizumab through the monkey retina after the intravitreal injection has been reported [74], and Sato *et al* [75] have recently reported high serum bevacizumab concentrations after intravitreal bevacizumab injection in infants with ROP. However, its low cost and promising results have led to a widespread off-label use of bevacizumab in the treatment of ROP in the last decade. While ablative therapy requires training, special equipment and facilities, the drug is inexpensive and can be administered at the bedside, if necessary, by an ophthalmologist trained for the treatment of ROP [53,72,73]. But, bevacizumab is a cancer drug for intravenous use and has neither been approved for use in eyes nor in infants yet.

Bevacizumab and the other anti-VEGF drugs have already

been in use for some ocular neovascular diseases in adults. So far, there have been various case reports or small case series of bevacizumab use in ROP [48-53]. Currently, there are two ongoing and planned studies which are evaluating efficacy of bevacizumab by comparing with standard laser therapy: Pan-VEGF Blockade for the Treatment of ROP (BLOCK-ROP) study (clinicaltrials.gov Identifier: NCT01232777; unfunded and not yet open for participant recruiment) and Bevacizumab Eliminates the Angiogenic Threat of ROP (BEAT-ROP) study (clinicaltrials.gov Identifier: NCT00622726). Recently, Mintz-Hittner *et al*[53] (BEAT-ROP group) have reported on the study investigating the efficacy of intravitreal bevacizumab for stage 3+ ROP in zone I. The study consisted of a prospective, controlled, randomized, multicenter trial to assess intravitreal injection of bevacizumab for zone I or zone II posterior stage 3+ (*i.e.*, stage 3 with plus disease) ROP. Infants were randomly assigned to receive intravitreal bevacizumab (0.625mg) or conventional laser therapy, bilaterally. The primary ocular outcome was recurrence of ROP in one eye or both eyes requiring retreatment before 54 weeks' gestational age. The results showed a strong benefit from intravitreal injection of bevacizumab as compared with conventional laser therapy in infants with stage 3+ ROP. Bevacizumab injection had a recurrence rate of 6% in combined retinal zones I and II compared with a 26% recurrence rate in laser treatment. The rate of recurrence with zone I disease alone was significantly higher with conventional laser therapy than that with intravitreal bevacizumab (42% *vs* 6%). However, the rate of recurrence with zone II posterior disease alone did not differ significantly between the laser-therapy group and the bevacizumab group (12% *vs* 5%). So, a significant treatment effect was reported for zone I ROP (P=0.003) but not for zone II disease (P=0.27). The authors also reported 6 cases of ROP with recurrence after intravitreal bevacizumab monotherapy. These findings suggest the importance of careful follow-up and choice of combined treatment (laser+intravitreal bevacizumab). There are various reports using bevacizumab for ROP simultaneously with laser therapy or separately, but these reports do not include evidence-based data [48-53]. Correct injection timing of the bevacizumab is important. Trese [76] reported that due to the interaction of an anti-VEGF drug and retinal vascular development, the timing of anti-VEGF therapy should be later than 30 weeks of postconceptional age. There are some other cytokines that have various roles for retinal development [25-28,31,38,77,78]. Transforming growth factor-beta (TGF-β), a natural antagonist of VEGF, which plays an important role in wound healing and scarring, rises in concentration during retinal development, between 36 and 40 weeks of postmenstrual age [76-80]. TGF-β becomes unopposed when VEGF is blocked through treatment and this may end up exacerbating proliferative tissues and cause tractional changes [76]. If anti-VEGF drugs are given late in the course or in cases with retinal detachment, increased concentrations of TGF-β may contribute to a tractional retinal detachment. The use of anti-VEGF therapy in stages 4 or 5 may accelerate retinal detachment due to a rapid neovascular involution with accelerated fibrosis and contraction of fibrous membranes in response to decreased levels of VEGF. Bevacizumab seems to be useful in ROP stage 3 cases with especially rigid pupils due to iris neovascularization, with intravitreal hemorrhage or zone I disease [80,81]. The use of anti-VEGF therapy seems as an efficacious treatment for severe ROP, but correct injection timing of the bevacizumab might be an important limitation for its use.

Another important consideration in the use of bevacizumab for ROP is the potential risk of local and systemic complications. Serious systemic complications may occur when bevacizumab is administered intravenously with high doses [68,71,82]. Bevacizumab is originally an anticancer drug for intravenous use; it has no regulatory approval for use in eyes or in infants. However off-label use of intravitreal bevacizumab for ROP increases despite the lack of studies on safety and dosage in growing babies. Systemic absorption has been considered negligible in adults; however, serum levels following intravitreal bevacizumab injection have not yet been determined in premature infants. Intravitreal bevacizumab enters the general circulation, suppresses plasma VEGF levels and remains in the blood for more than 8 weeks in primates [75,83]. There is no study reporting serious local and systemic complications of intravitreal bevacizumab injection [48-53,63,69,73]. However, there are various reports describing the role of VEGF in the normal development of human kidney, brain and lung [83-86]. Recently, Sato *et al*[75] reported that bevacizumab can escape from the vitreous into the systemic circulation and reduces the VEGF concentrations in infants with ROP following intravitreal bevacizumab injection to either one or both eyes. All of the 11 infants in that study received laser photocoagulation to the peripheral avascular retina before bevacizumab injection, and serum bevacizumab level one week after the injection was significantly higher than that before the intravitreal injection. So, possible adverse effects on VEGF dependent development should be considered. However, the BEAT-ROP study did not make any comment about the safety of the drug as the sample size of the trial was too small to assess the safety of the drug [53]. Infants of the BEAT-ROP study randomly received either intravitreal bevacizumab injection or conventional laser therapy, bilaterally. Study designs and dosage of intravitreal bevacizumab injections are not similar in these studies [53,75]. After publication of the BEAT-ROP study, some reviews, editorials and commentaries raised questions about the efficacy and safety of bevacizumab [83,87,88]. Thus, efficacy and safety of bevacizumab are still important issues to be solved,

and should be validated by evidence-based data.

Most recently, the American Academy of Pediatrics (AAP), the American Academy of Ophthalmology (AAO) , the American Association for Pediatric Ophthalmology and Strabismus (AAPOS) and the American Association of Certified Orthoptists (AACO) revised the previous statement for screening of premature neonates for ROP which was published in 2006 and advised to consider intravitreal injection of bevacizumab for the treatment of infants with zone I, stage 3+ ROP even though bevacizumab is not currently approved by the US FDA for the treatment of ROP [89]. Also they strictly advise to use bevacizumab only after obtaining a detailed informed consent, and long-term follow-up for the potential problems of the drug.

As a conclusion, further controlled studies with long-term follow-up and adequate sample size are needed for such potentially dangerous growth factor inhibitors, anti-VEGF agents, which may be considered as "a miracle rescue" for the treatment of ROP in immature infants.

Propranolol Propranolol has been used for the treatment of infantile hemangiomas, and the effect of the propranolol was hypothesized to be the reduction of VEGF levels depending on systemic propranolol application [54]. There are a few reports describing the effect of this β-adrenergic receptor blocker in regulation of retinal angiogenesis. Recently, systemic administration of propranolol has been reported to reduce retinal VEGF and IGF-1 expression, retinal neovascularization, and vascular leakage in a mouse model of oxygen-induced retinopathy [90]. A pilot study, the Safety and Efficacy of Propranolol in Newborns with ROP (PROP-ROP) [54], was planned to compare safety and efficacy of propranolol with conventional laser treatment alone for stage 2 ROP in zone II or III without plus disease in premature infants. The study group hypothesized that VEGF overexpression in ROP might be induced by beta2-adrenoreceptor stimulation, and propranolol as a non-selective beta-adrenoreceptor blocker administered in preterm newborns could reduce the progression of ROP. However, this study was halted because of increased mortality in the treatment arm. Although the use of prophylactic propranolol for oxygen-induced retinopathy in mice model reported promising results,Chen *et al* [91] recently reported that propranolol treatment with a dose up to 30 times the standard human dose failed to suppress retinopathy development in mice. This recent finding raised the question about the effect of propranolol as an appropriate therapeutic approach for treating ROP. Propranolol has also serious side effects such as bradycardia, heart block, hypotension, bronchospasm, hypoglycemia and dyslipidemia [92,93]. The efficacy of this drug may be quite disappointing in a vulnerable preterm infant. The relevance of all these results, benefits and safety of propranolol to the developing preterm infant is uncertain, and further animal and prospective clinical Trials should be conducted.

Gene therapy Targeting and regulation of various cytokines and growth factors known to be involved in the pathogenesis of ROP by gene therapy seems to be a realistic goal for developing newer and better ROP treatments. In an exciting publication by Chowers *et al*[56], it was demonstrated that intravitreal gene transfer was possible in a rat model of ROP. Local gene transfer, that is, the intraocular delivery of recombinant viruses carrying genes encoding angiostatic proteins and small interfering RNA against various cytokines and their receptors, offers the possibility of targeted, sustained, and regulated delivery of angiostatic proteins and other angiogenic regulators to the retina [56,57]. Although gene therapy has been reported to show good results in animal studies, the safety of gene application therapy and its possible effects to a developing preterm infant are uncertain and have yet to be fully evaluated.

Supplemental Therapies

Omega –3 polyunsaturated fatty acids (PUFAs) In a ROP mouse study, it was found that an increased retinal omega-3 and omega-6 PUFA ratio had 50% protective effect against pathologic neovascularization due to increased regrowth of vessels after vessel loss [94]. The same protective effect against retinal neovascularization or phase II retinopathy was seen with treatment of mouse model of oxygen-induced retinopathy by supplementing omega-3 PUFA intake [58]. Their protective action against retinal neovascularization was reported to be correlated with the suppression of tumor necrosis factor-alpha (TNF-α) [59]. This inflammatory cytokine was found in a subset of microglia that was closely associated with retinal vessels. These findings indicate that increasing the sources of omega-3-fatty acids could help protect against pathological angiogenesis by reduction of TNF-α [58,59].

Omega-3 and 6 PUFAs are transferred from mother to the fetus during the 3rd trimester of pregnancy. Like IGF-1, omega-3 and omega-6 PUFAs are non-oxygen-regulated angiogenic factors [58,59,95]. Their placental transfer is missed by the prematurely born infant. These lipids are essential fatty acids and must be obtained through diet or total parenteral nutrition (TPN) in prematures. TPN, given to very premature infants, does not provide sufficient omega-3 fatty acids except for some products such as Omegaven® and Smoflipids® containing fish derived omega-3. Supplementing omega-3-PUFA intake may be of benefit in preventing ROP and this may be an interesting pharmacological approach. However, larger controlled trials are required to validate the benefits and safety of omega-3-PUFA supplementation therapy in prematures.

Erythropoetin Although erythropoetin (Epo) has been reported as an oxygen-regulated retinal angiogenic growth factor like VEGF in animal studies, the role of Epo in normal vascular development and angiogenesis is largely

unknown [96,97]. Recently,Sato *et al* [98] reported that Epo levels were significantly elevated in the vitreous of infants with stage 4 ROP, suggesting Epo as a contributor to ROP in addition to VEGF. Recombinant Epo (rhEPO) was used to treat anemia in premature infants. There are various studies suggesting a significant association between administration of rhEPO and ROP in premature infants, however, they are not specifically designed clinical trials.Suk *et al* [99] compared retrospectively cohorts of newborn infants admitted to their unit in two different periods and found that the incidence of ROP (any stage) and threshold ROP in the infants treated with rhEpo was significantly higher than that in untreated infants (58% and 26.6% *vs*42.9% and 13.4%, respectively; P=0.014 and P=0.009).Additionally, there are controversial results about the relationship between rhEPO treatments and the incidence and severity of ROP in premature infants [100,101]. So, further controlled studies should be designed to validate the efficacy and safety of the administration of EPO in prematures, and to elucidate the causal relationship between rhEPO and ROP.

IGF -1 Fetal development during pregnancy is also dependent on the IGFs (IGF-1 and -2) [102]. IGF-1 has been reported as a critical non-oxygen-regulated retinal angiogenic growth factor for normal retinal vascular development through regulation of VEGF signaling. IGF-1 levels rise significantly in the 3rd trimester of pregnancy, but fall after preterm birth since it is synthesized in placenta and fetal liver in nutrient dependent processes. In preterm infants, low levels of IGF-1 have been associated with impaired growth and development of ROP [31,35]. Suppression of retinal neovascularization by an IGF-1 receptor antagonist was reported by Smith *et al* [102].IGF-1 treatment may help normal retinal vascular development and prevent abnormal vascular proliferation in prematures [7,19,32,33,102]. Although there is no study describing the results of IGF-1 treatment in premature infants, a recent study has described the safety and pharmacokinetics of the administration of recombinant IGF-1 (rhIGF-1) with its binding protein 3 to premature infants [60]. Additionally, there is an ongoing clinical trial (clinicaltrials. gov identifier: NCT01096784) to investigate the preventive effect of IGF-1 for ROP development in premature infants.

Granulocyte colony-stimulating factor (GCSF) GCSF, a biologic cytokine to increase leukocyte counts, has been shown to increase levels of IGF-1, which supports the normal vasculogenesis [103]. So, GCSF may promote angiogenesis in ischemic retina without any known negative effect on VEGF [104-106]. In a retrospective chart review of all neonates who received GCSF, Bhola *et al* [104] reported that need for laser treatment in patients who received GCSF decreased, but the observed differences were not statistically significant. However, its potential role in the prevention of ROP has not been studied, and the dose required, side effects and safety are still not documented.

Vitamin E The antioxidant system is functionally immature in premature infants, and premature infants are susceptible to oxidative stress resulting in oxygen-radical disease, one of which is ROP [107]. Even meta-analyses of randomized studies of vitamin E supplementation have produced conflicting results [108,109], vitamin E supplementation other than standard doses given in TPN in order to decrease the risk of ROP is not currently suggested and even carries an increased risk of developing sepsis and necrotizing enterocolitis in premature infants with rapid intravenous administration [110].

SUMMARY

The incidence of ROP and severe ROP has shown differing rates (decreasing in some studies but increasing in others) among various reports over the last two decades [1-8]. This may be related with the advances in neonatal intensive care by improving the survival rates of the very premature infants who previously did not survive. Improved survival of very premature infants has resulted in an increasing rate of ROP requiring close screening and treatment. Prevention of the development of severe ROP is the best strategy available at present to avoid visual impairment and blindness caused by ROP. Oxygen control and improvements in nutrition are the most important preventative strategies in avoidance of ROP-dependent blindness. ROP screening performed by an experienced ophthalmologist remains the most important pillar, on the other hand, in the management of ROP. The current gold standard for treatment of proliferative ROP depending on the severity is still panretinal laser photocoagulation. Although it usually works well, it is not ideal. Various novel pharmacological treatment approaches to suppress the neovascularization have not been sufficiently evaluated with evidence-based data. However, ongoing studies investigating the safety and efficacy of antiangiogenic therapies, especially anti-VEGF drugs, in premature infants seem to provide valuable and encouraging information for ROP treatment in the near future.

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