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Pharmacologic interventions for the prevention and treatment of retinopathy of prematurity

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ABSTRACT

Retinopathy of prematurity (ROP), a significant morbidity in prematurely born infants, is the most common cause of visual impairment and blindness in children and persists till adulthood. Strict control of oxygen therapy and prevention of intermittent hypoxia are the keys in the prevention of ROP, but pharmacologic interventions have decreased risk of ROP. Various drug classes such as methylxanthines (caffeine), VEGF inhibitors, antioxidants, and others have decreased ROP occurrence. The timing of pharmacologic intervention remains unsettled, but early prevention rather than controlling disease progression may be preferred. These drugs act through different mechanisms, and synergistic approaches should be considered to maximize efficacy and safety.

Introduction

Retinopathy of prematurity (ROP) is a developmental vascular disorder characterized by abnormal growth of retinal blood vessels in the incompletely vascularized retina of extremely low gestational age neonates (ELGANs) who are <1250 g, <28 weeks gestation.[1–3] In the United States, ROP afflicts about 16,000 ELGANs annually[1] and remains the third leading cause of childhood blindness (14%) with much higher rates in developing countries.[5] Incomplete retinal vascularization due to prematurity and oxygen are key factors in ROP; however, the etiology of this "new" form of ROP is multivariate and complex and involves hypersensitivity of the immature retina to changes in oxygen.[4,6,7]

Pathophysiology of ROP

In humans, the retina develops in utero where tissue oxygen is low.[7] Vascular precursor cells are laid from 12 to 21 weeks gestational age creating a scaffold for future vessel development. The vessels emerge from the optic disk and follow a VEGF template established by astrocytes which populate the retina before the vessels.[8] Angiogenesis begins at approximately 16–17 weeks gestational age, with new vessels budding from existing vessels. The metabolic demands of the developing retina exceed the oxygen supplied by the choroidal circulation resulting in "physiologic hypoxia," and thus stimulate angiogenesis.[7] Vasoactive factors, such as insulin-like growth factor (IGF)-1, vascular endothelial growth factor

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(VEGF), and erythropoietin (Epo), in addition to maternally derived factors, stimulate new vessel formation. The vessels reach the nasal ora serrata by 36 weeks and the temporal ora serrata by 40 weeks. In ELGANs, the retinal vasculature is immature and thus vulnerable to oxidative damage. Early studies by Ashton et al.[9] demonstrated that exposure to oxygen causes vaso-obliteration and vasoproliferation when room air breathing was resumed. Those early studies led to a two-phase hypothesis of ROP: (1) phase 1 or vaso-obliteration begins at preterm birth with the transition from an intrauterine to extrauterine environment causing a rise in PaO_2 of 30–35 mmHg to 55–80 mmHg and loss of placental and maternal growth factors. During this phase, exposure to supplemental oxygen, required for treatment of respiratory distress syndrome, further suppresses retinal growth factors that are already compromised due to preterm birth and poor nutrition,[10] thus leading to arrest and retraction of the developing retinal vessels, or vaso-obliteration; and (2) phase 2 or vasoproliferation begins at approximately 32–34 weeks.[11] As the infant matures, the avascular retina becomes metabolically active, inducing a second phase, or retinal neovascularization.[3] This phase of ROP is driven by hypoxia and subsequent upregulation of VEGF and IGF-I which leads to abnormal vascular overgrowth into the vitreous, retinal hemorrhages, retinal folds, dilated and tortuous posterior retinal blood vessels, or "Plus" disease, and retinal detachment. ELGANs with chronic lung disease experience numerous alterations in their O_2 saturations or apneas.[12,13] Infants who experience the greatest fluctuations in their PaO_2 seem to be at a higher risk for the development of threshold ROP.[6,13] In these infants with "new" ROP, intermittent hypoxia (IH) occurs during supplemental oxygen treatment or phase 1, thus worsening the outcomes during phase 2. Indeed, this was demonstrated in a rat model that utilized brief episodes of hypoxia during hyperoxia, simulating apnea of prematurity.[14–17] The fluctuating oxygen model also shows a higher incidence of intravitreal neovascularization[18] with corresponding high levels of retinal VEGF[19] and vitreous fluid growth factors.[14,15] The pattern of IH may also play a role in the development of ROP[13] and OIR.[14–17] Clustering IH episodes resulted in a more severe form of OIR with increased retinal hemorrhages, vascular tufts, leaky vessels, vascular tortuosity, and vascular overgrowth, compared to dispersed IH episodes. This may be due to differences in exposure time of the retina to hypoxia at a given time point. Clustering episodes of brief hypoxia or grouping of desaturations with minimal time for recovery between episodes causes the retina to remain hypoxic for a longer period of time, thus leading to a more exaggerated increase in VEGF resulting in characteristics consistent with "Plus" disease.[14] In light of these new findings, the phase 1/phase 2 hypothesis of ROP originally proposed in 1954 by Ashton et al.[9] may need to be redefined with respect to "new" ROP and IH.

Oxygen

Oxygen is the most commonly used drug in neonatal care for respiratory support.[20] The widespread use of unrestricted oxygen in preterm infants began in the early 1940s in response to observations that inspired oxygen improved the irregular breathing pattern of premature infants.[21,22] This led to the first epidemic of ROP, described in 1942 by Terry.[23] and then known as retrolental fibroplasia or fibroblastic overgrowth behind the crystalline lens. In 1951, it was suggested that oxygen use was associated with ROP.[24] This was confirmed in 1952 in humans[25] and later in animals.[26] By 1953, approximately 10,000 infants worldwide were blinded.[21] The first multicenter randomized clinical trial to study ROP started in 1953 and involved 18 centers. The study enrolled infants <1500 g in two arms: (1) $FiO_2 \geq 50\%$ for 28 days and (2) $FiO_2 < 50\%$. In 1954, one of the centers reported that blindness was prevented if oxygen did not exceed 40%. However, 6 years later, review of autopsies revealed that curtailed use of oxygen increased the incidence of mortality such that for every eye sight gained, 16 lives were lost.[27] Despite the introduction of transcutaneous oxygen monitoring and pulse oximetry in the 1960s–1980s, and many nonrandomized and randomized clinical trials, the optimum range of oxygenation in preterm infants remains elusive and controversial. The phase 1 (hyperoxia)/phase 2 (hypoxia) hypothesis of ROP led to the premise that administration of oxygen during phase 2 would increase tissue oxygen, decrease VEGF, and curtail vessel overgrowth. This hypothesis was tested in the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP) randomized clinical trial in 2000.[28] The study randomized 649 infants with prethreshold ROP and O_2 saturation $<94\%$ at 35 weeks to oxygen saturation targets of 89–95% or 96–99%. The STOP-ROP study failed to decrease progression of the disease or reduce the number of infants requiring peripheral ablative surgery and showed no differences in the development of threshold ROP. ELGANs experience continuous fluctuations in arterial O_2 saturation; therefore, increasing inspired oxygen may not be an appropriate approach.[29,30] Instead, low and stable oxygen therapy may be more beneficial.[2] The Australian Benefits of Oxygen Saturation Targeting (BOOST) randomized, double-blind, multicenter trial involving 358 preterm infants, kept the saturation ranges at 91–94% or 95–98%. The incidence of severe ROP was comparable between the groups.[31] Five large multicenter, masked, randomized, control trials (collectively known as The Neonatal Oxygen Prospective Meta-Analysis or NeOPRoM Collaboration) enrolled approximately 5000 ELGANs <28 weeks, compared 85–89% versus 91–95%. In the United States, the Surfactant Positive Pressure and Pulse Oximetry (SUPPORT) trial reported that SpO_2 levels of 85–89% was associated with increased mortality and a higher incidence of severe ROP was found in the 91–95% group.[32] This was reflected in the BOOST II trial (Australia, New Zealand, and United Kingdom) after interim analysis, and enrollment was stopped.[33] The Canadian Oxygen Trial (COT) reported no difference in mortality or severe ROP.[34] A recent meta-analysis of all published randomized trials evaluating the effect of restricted versus liberal oxygen exposure in preterm infants shows no difference in ROP.[35] After over 70 years of oxygen use, and despite large multicenter, randomized clinical trials, there is still no consensus regarding optimal oxygen therapy for ELGANs. Oxygen remains the most commonly used drug in neonatal intensive care units worldwide[36] and the incidence of severe ROP has not appreciably declined.

Pharmacologic interventions in ROP

In addition to better control of oxygen therapy, numerous pharmacologic interventions have been tried, and some appears promising for the prevention of ROP and stopping its progression. These pharmacologic agents are discussed.

Antioxidants

Oxygen used in excess has damaging effects particularly when used in ELGANs. In normal respiration, oxygen is reduced in the mitochondria to two molecules of water during the process of aerobic energy metabolism, or oxidative phosphorylation (OXPHOS), which produces more than 90% of our cellular energy.[37] During OXPHOS, reactive oxygen species (ROS) are produced as a byproduct. ROS are highly reactive chemical molecules that react with lipids to initiate lipid peroxidation and DNA damage.[38] Major ROS produced in the mitochondria are superoxide anion, hydrogen peroxide (H_2O_2), hydroxyl radical, and hydroperoxyl radical. Reactive nitrogen species (RNS) such as nitric oxide (NO), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and peroxynitrite, are also produced. The primary endogenous scavengers of ROS, or antioxidants, are superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx). SOD acts on superoxide anion to produce hydrogen peroxide (H_2O_2), catalase converts H_2O_2 to water, and Gpx detoxifies peroxides and hydroperoxides. Other antioxidants include peroxiredoxins (which catabolize H_2O_2 to water), peroxiredoxin, heme oxygenase, glutaredoxin and thioredoxin, and nonenzymatic agents such as β-carotene, retinol, vitamin C, and vitamin E. When endogenous antioxidant systems are inadequate or overwhelmed by ROS, many pathologies including hypoxia/reperfusion injury and ROP develop. The retina is rich in mitochondria and has a high rate of OXPHOS, thus rendering it a target for ROS and oxidative damage.[39,40] Excessive ROS production contributes to mitochondrial damage and vascular dysfunction, particularly in ELGANs with immature antioxidant systems.[41–43] Therefore, many investigators tested the hypothesis that antioxidants are beneficial for treatment and/or prevention of ROP.

Vitamin E was the first antioxidant to be used for treatment and prevention of ROP.[44] Several later trials of supplementing preterm infants with vitamin E for preventing or limiting ROP have been conducted with conflicting results.[45–51] A randomized trial of 755 VLBW infants found no significant decrease of severe ROP although the progression from moderate to severe ROP was less frequent following vitamin E treatment.[47] Phelps et al.[52] and Rosenbaum et al.[53] found increased retinal hemorrhages and an increased rate of grades 3 and 4 IVH in the vitamin E group. Two meta-analyses reported beneficial effects of Vitamin E for reducing the risk of ROP and blindness. The earlier report analyzed six clinical trials and found a 52% reduction in the incidence of stage 3+ ROP.[54] The later report analyzed 26 randomized clinical trials and a reduction in severe ROP and blindness.[55] A third report found no effect at all.[56] In this report, the authors analyzed nine randomized controlled trials and concluded that not more than about 4% of all very low-birthweight infants are likely to benefit from routine vitamin E supplementation. Vitamin E was the most commonly used antioxidant for ROP spanning over 4 decades. Its use has now been largely abandoned secondary to increased risks of morbidity with no conclusive evidence for protection against severe ROP.

Other antioxidants used to treat/prevent ROP include: *D-penicillamine (DPA)* an antioxidant and suppressor of VEGF bioavailability.[57] DPA was introduced to prevent ROP in the 1980s. In a prospective controlled trial, DPA was administered to preterm infants at 26–35 weeks gestation and found to be effective for ROP with no serious adverse effects[58] and at 1-year follow-up.[59] Later trials refuted those findings[60,61]; *superoxide dismutase (SOD)* dismutates the extremely toxic superoxide anion into H_2O_2 and H_2O. SOD is the first line of defense against oxidative stress in the mitochondria, with MnSOD located in the inner mitochondrial matrix. A multicenter trial of intratracheal rhSOD showed a reduction in severe ROP above stage 2 in rhSOD-treated infants born at <25 weeks[62]; *lutein and zeaxanthin* are antioxidants in the eye. A single-center, double-blind randomized controlled trial comparing lutein administration versus placebo, showed no differences in the incidence of ROP at any stage.[63] These findings were later confirmed[64,65]; *vitamin A* is one of the most important micronutrients necessary for growth and differentiation of tissues.[66] Vitamin A supplementation reduces infant mortality.[67] Retinal vitamin A allows adequate rhodopsin availability for phototransduction and protects the photoreceptors from the harmful effects of hypoxia and hyperoxia.[68] Studies show a trend for decreased incidence of threshold ROP in ELBW infants treated with 10,000 IU intramuscular vitamin A three times a week (0%) compared with 16% in those who received half this dose.[69] A recent double-blind, randomized controlled trial of early high-dose intramuscular vitamin A supplementation for infants at risk of ROP showed improved retinal function at 36 weeks PMA.[68] A meta-analysis of the three studies suggests a trend toward reduced incidence of ROP in vitamin A supplemented infants.[70] Although promising, there is a paucity of studies examining the benefits of vitamin A for severe ROP. Considering its antioxidant properties and possible beneficial effects on the photoreceptors, further studies are warranted, and *allopurinol* is a xanthine oxidase and superoxide anion inhibitor. A randomized, controlled, clinical trial involving 400 preterm infants who received either allopurinol or placebo showed no benefit.[71]

Cyclooxygenase inhibitors

In preterm infants who are exposed to supraphysiologic levels of oxygen and have compromised antioxidant systems, the imbalance of oxidant/antioxidants in favor of oxidants lead to oxidative stress and activation of proinflammatory mediators, including prostanoids.[72,73] The primary pathway through which prostanoids are activated is through arachidonic acid (AA), a long-chain polyunsaturated fatty acids released from phospholipids through the action of phospholipase A1 (PLA_2).[74] AA is the precursor of the eicosanoids, including prostanoids, through the cyclooxygenase (COX) enzyme, and leukotrienes, through the lipoxygenase (LOX) pathway. The two main COX isoforms are the constitutively expressed COX-1, responsible for maintaining basal

prostanoid levels, and the inducible COX-2 which is generally absent and is induced in inflammation.[75,76] Both isoforms transform AA into prostaglandin H_2, which is then transformed by synthases into prostaglandins (PGs) and thromboxanes. A third isoform, COX-3 was identified in 2002[77] as a variant of COX-1. This variant is more sensitive to acetaminophen.[78] The role of prostanoids in ROP has been demonstrated.[73,79–81] Other prostanoid compounds, the F-isoprostanes, are generated by lipid peroxidation of AA, independent of COX.[82,83] One of these isomers, 8-epi-prostaglandin$F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), is a potent mitogen and constrictor of vascular smooth muscle and is highly correlated with oxidative stress.[83] Studies have demonstrated that 8-epi-$PGF_{2\alpha}$ is functionally linked to, and activates the thromboxane A_2 (TxA_2) receptor[84] and may be involved in retinal vasoconstriction and ischemic retinopathies.[85] The importance of the prostanoid cascade in ROP and the role of COX-2 in inflammation led to the premise that COX inhibition could have potential benefits. Nonselective COX inhibitors, such as indomethacin and ibuprofen, improve OIR and ocular biomarkers of angiogenesis in animal models.[86–90] However, there are only two clinical studies involving the use of COX inhibitors for prevention of ROP. One controlled study administered topical ketorolac (a nonselective COX inhibitor) to 59 infants (<30 weeks gestation, <1250 gbirth weight) every 8 h in each eye compared to 53 untreated preterm infants. Ketorolac reduced the risk of developing severe ROP with no significant adverse side effects.[91] A later study of 47 preterm infants (<29 weeks, <1000 g) who receive ketorolac in one eye and placebo in the other eye showed that two infants did not develop ROP, six showed different ROP staging between the two eyes, four of which had a better outcome in the eye receiving ketorolac and no significant treatment-related side effects occurred.[92] Dexamethasone is a selective COX-2 inhibitor with anti-inflammatory effects that has been demonstrated to reduce the incidence of ROP.[93,94] However, its use is associated with significant side effects,[95–100] and its efficacy in preventing ROP is controversial. Despite overwhelming evidence for the role of COX metabolites in oxidative stress and inflammation, only one prospective randomized, controlled multicenter clinical trial to investigate the benefits of COX inhibitors for prevention of ROP has been initiated and is currently underway (Aranda JV, Clinicaltrials.gov Identifier: NCT02344225).

Inositol

Inositol is a nonglucose carbohydrate that is present in high amounts in the umbilical cord and maternal serum at term.[101] Inositol is also present at high concentrations in human breast milk, particularly colostrum suggesting an importance for postnatal growth.[102] In 1992, Hallman et al.[103] showed a reduction in ROP in inositol-treated infants (13%) versus placebo (26%), and none of the infants given inositol had stage 4 disease compared to seven in the placebo group. These findings were later confirmed by Friedman et al.[104] In 2015, a meta-analysis of four clinical trials showed reduced stage 3 ROP in two of the four trials.[105] A large size multicenter, randomized, controlled trial is currently ongoing to further investigate the benefits of inositol for ROP (Clinicaltrials.gov Identifier: NCT01954082).

Propranolol

Propranolol is one of the newest proposed treatments for ROP despite weak and contradictory animal preclinical data. Propranalol is a nonselective β-adrenoreceptor blocker shown to reduce the growth of infantile capillary haemangiomas.[106] The mechanism of action is likely due to suppression of VEGF. Studies have shown that polymorphisms of the β-adrenergic receptor in many black infants may be responsible for the lower incidence of ROP.[107] Clinical trials have shown reduction in the incidence of ROP with oral propranolol with few side effects.[108–110] Clinical trials in Israel and Italy are being conducted to evaluate efficacy and safety of this beta blocker for ROP prevention in preterm neonates (ClinicalTrials.Gov: NCT01238471, NCT02014454, and NCT02504944). One Italian Trial using propranolol eye drops of 0.5 mg/kg oral, every 6 h was discontinued due to serious adverse effects attributed to the pharmacologic actions of *propranolol* such as severe hypotension, bradycardia, or bronchospasm (ClinicalTrials.Gov NCT01079715).

Caffeine

Caffeine is the most widely used drug worldwide.[111] It is a methylxanthine that acts to antagonize adenosine receptors and block the release of endogenous adenosine. Caffeine also stimulates dopaminergic activity by removing the negative modulatory effects of adenosine at dopamine receptors.[112] Caffeine was first demonstrated to be effective for treatment of apnea of prematurity (AOP) in the 1970s by Aranda et al.[113] After over 40 years, caffeine is now the drug of choice for AOP. It is one of the most commonly prescribed drugs for use in ELGANs.[114,115] It is a respiratory stimulant in ELGANs and is effective for reducing the duration of mechanical ventilation.[116] The Caffeine for Apnea of Prematurity (CAP) trial found that caffeine was also beneficial for reducing the incidence of bronchopulmonary dysplasia (BPD), improving long-term neurodevelopmental outcome, and reducing the incidence of severe ROP.[117] The mechanism of caffeine effects on severe ROP remains to be determined, but may involve effects on regulators of angiogenesis such as VEGF, sonic hedgehog matrix metalloproteinases (MMPs), and oxidative stress.[118–121] Additionally, caffeine potentiates the anti-inflammatory effects of COX inhibitors in activated microglia which may also occur in the retina.[122] Caffeine has been used in combination with non-steroidal anti-inflammatory drugs (NSAIDs) for many decades to enhance their analgesic effects, suggesting that caffeine may be an effective adjuvant to NSAIDs.[122–124] Due to its significant impact on major acute neonatal morbidities including AOP, BPD, PDA, and ROP, caffeine is now recognized as the “wonder drug in neonatology”[125] and the “silver bullet in neonatology.”[126] Notwithstanding, only one prospective randomized, controlled multicenter clinical trials to investigate the benefits of caffeine for prevention of ROP has been initiated (Aranda JV, Clinicaltrials.gov Identifier: NCT02344225).

Angiogenic factors

ROP is characterized by the formation of new and aberrant retinal vasculature, suggesting that angiogenic factors play a major role. The role of angiogenic factors in retinal development was first hypothesized 1948.[127] In 1983, Senger et al.[128] discovered an angiogenic growth factor present in tumor cells which he named vascular permeability factor due to its ability to induce vascular leakage. In 1989, Ferrara and Henzel[129] identified, purified, and characterized a heparin-binding angiogenic growth factor specific for vascular endothelial cells which they named VEGF. Several other angiogenic factors have been identified, including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), the angiopoietin family, insulin-like growth factor (IGF), and the Ephrin family, all of which work together to promote functional vascular systems.[130] VEGF is a vascular permeability factor. Consistent with this characteristic is its ability to induce the expression of plasminogen activators and MMPs to increase vascular permeability[131,132] which may be the first step in angiogenesis.[133] It is now widely accepted that the VEGF family is the most potent inducer of angiogenesis and ROP. Levels of VEGF in the vitreous humor correlate with the degree of neovascularization.[134,135] Plasma levels of preterm infants in the first week of life were found to be low (48 pg/mL) compared to term infants (200–450 pg/mL) and adults (10–110 pg/mL).[136] VEGF levels in the aqueous humor of stage 4 (1109 pg/mL) and stage 5 (3520 pg/mL) eyes were considerably higher than control eyes (158 pg/mL).[137] Infants with stage 4 ROP had elevated VEGF levels in the subretinal fluid and decreased levels in stage 5.[138] Induction of VEGF is also associated with generation of ROS, inflammatory cytokines and prostanoids, possibly via inducible COX-2.[139–141] VEGF is now considered the most important target for therapeutic interventions for prevention of severe ROP.

VEGF inhibitors

Several anti-VEGF drugs have been developed. The first anti-VEGF drug to be approved by the FDA for cancer use was bevacizumab (Avastin).[142] Bevacizumab is a recombinant humanized antibody that binds all forms of VEGF-A. Although it is used off-label, it is not formulated for use within the eye.[142] However, off-label use by ophthalmologists demonstrated that Avastin was beneficial when injected into the vitreous of adults with macular degeneration. Later, a truncated form of Avastin called ranibizumab (Lucentis) was FDA-approved for ocular use. The first FDA-approved drug for use in ocular neovascularization was pegaptanib (Macugen). Aflibercept (Eyelea), a human soluble VEGF receptor, which acts as a decoy to trap VEGF, was recently FDA-approved for ocular use. Bevacizumab is the first anti-VEGF drug to be used for treatment of ROP in 2007.[143] Since then, many single-center trials and case reports have been published making bevacizumab the most widely used drug for treatment of severe ROP. The first meta-analysis of bevacizumab use for ROP was published in 2009.[144] The authors analyzed all case reports, and retrospective and prospective trials in peer-reviewed journals reporting the use of bevacizumab in ROP, including nine articles, six case reports, one case series, and two retrospective studies of 48 infants administered doses from 0.4 to 1.25 mg and cautioned against potential systemic complications and long-term effects of intravitreal bevacizumab in preterm infants. From 2007 to 2009, all investigators reported favorable outcomes with bevacizumab use in ROP. However, the first report of adverse effects was published in 2010 demonstrating choroidal ruptures at 10 weeks post-treatment with laser.[145] Other reports of adverse events included vitreous or preretinal hemorrhage,[146] delayed bilateral retinal detachments at 1 month post-treatment,[147] and retinal detachment.[148] The first multicenter randomized, controlled trial was the BEAT-ROP study which demonstrated an advantage of intravitreal bevacizumab over laser therapy for zone I or zone II with stage 3+ ROP by improving structural outcomes, decreasing recurrence, and allowing continued development of peripheral retina.[149] In the BEAT-ROP study, however, assessments of local and systemic safety profile was not determined and follow-up was only 54 weeks postmenstrual age. Moshfeghi and Berrocal[150] estimated that 47.7% of recurrences would have occurred after the 54 weeks. This was demonstrated by Hu et al.[151] Studies have since tried to address safety of intravitreal bevacizumab use in ROP and demonstrated numerous acute and latent retinal adverse effects.[151–168] Interestingly, serum concentrations of bevacizumab concurrent with suppressed systemic VEGF levels lasted up to 60 days post-intravitreal administration,[169–172] and the levels were significantly lower than previously reported in preterm infants.[173] These reports demonstrate that bevacizumab escapes from the eye into the systemic circulation and has long-lasting effects on systemic VEGF levels in infants with ROP. A recent meta-analysis of eight prospective studies of 278 eyes of ROP infants and 15 retrospective studies involving 385 eyes of ROP infants treated with intravitreal bevacizumab showed reoccurrence and acceleration of fibrous traction.[174] Other anti-VEGF drugs used in ROP include ranibizumab which has a shorter half-life with the potential for decreased systemic toxicity. Treatment with ranibizumab was also associated with significant adverse outcomes.[147,175,176] Combination treatment of intravitreal pegaptanib or bevacizumab injection and laser photocoagulation showed significant benefits in the management of stage 3+ ROP.[177,178] Evidence now exist that intravitreal administration of anti-VEGF drugs can have prolonged suppressive effects on systemic VEGF in ELGANs. Considering the role of VEGF in the development of vital organs, more randomized, controlled multicenter trials should be conducted to evaluate dose–response effects and long-term safety profiles in this vulnerable population.[179–186]

Insulin-like growth factor (IGF)-1

Although VEGF plays an important role in the development of ROP, its inhibition does not completely prevent ROP. Another growth factor that is currently being studied for prevention of ROP is IGF-1. IGF-1 influences endothelial cell growth and angiogenesis,[187,188] and plays a critical role in the retinal vasculature development through its interaction with VEGF, acting as a permissive factor for maximum VEGF stimulation of angiogenesis.[189,190] Mean IGF-1 level has been shown as significantly and proportionately lower in postmenstrual age

matched babies with each stage of ROP than without ROP.[191–194] Work by Hellstrom et al.[191,194] have demonstrated that preterm infants who do not develop ROP have significantly higher circulating levels of IGF-I than those who do. Serum IGF-1 levels to predict ROP has been shown to be a reliable prognostic tool by some studies[195,196] but not others.[197–199] Only one small single-center clinical trial of Premiplex (rhIGF1/rhIGFBP3) pharmacokinetics in five preterm infants was conducted. None of the infants developed severe ROP.[200] A clinical trial to determine the dose of hIGF-1/rhIGFBP-3 given as a Continuous Infusion, to establish and maintain physiologic levels of serum *IGF-1* Levels to prevent ROP is ongoing (ClinTrials.gov NCT01096784).

Polyunsaturated fatty acids (PUFAs)

Omega-6 and omega-3 PUFAs are essential fatty acids that are important for normal growth, vision, and neurodevelopment. Omega-6 PUFAs are formed from linolenic acid (LA) which is converted by desaturation and chain elongation to arachidonic acid (AA), the precursor to eicosanoids (prostaglandins, thromboxane, and leukotrienes).[201] Omega-3 PUFAs are formed from alpha-linolenic acid (ALA) which is desaturated and chain elongated to docosahexaenoic acid (DHA).[202] PUFAs are important components of the phospholipid bilayer of cell membranes and contributes to cell structural integrity and function.[203] Deficiency of AA and DHA in the membrane alters the physical properties of the lipid bilayer and induces vascular leakage.[204] It is well established that omega-3 PUFAs play a key role in the protection against oxidative stress and inflammation. Among the benefits of omega-3 PUFAs is the ability to regulate eicosanoid metabolism. COX catalyzes the initial oxygenation of PUFAs to produce prostaglandin H (PGH_2), a short-lived intermediate, which is further metabolized to prostanoids.[205] DHA and its precursor, eicosapentaenoic acid (EPA), reduce the formation of COX-2-derived PGE_2,[206] a metabolite of omega-6 PUFA-derived AA. COX-2-derived PGE_2 increases VEGF-stimulated invasiveness and angiogenesis through NF-κB.[207] There is compelling evidence that EPA could function as a selective COX-2 inhibitor.[207–209] In the retina, PUFAs accumulate rapidly during the later stages of gestation and early postnatal life.[210,211] The retina contains the highest concentration of DHA of all tissues where it is cytoprotective and plays a key role in angiogenic regulation and neuroprotection.[212] Administration of fish oil to preterm infants from the first day of life showed a significantly lower incidence and severity of ROP and risk of laser therapy in the treated group.[213–215] However, two meta-analyses examined the potential benefits of lipid administration and found no significant benefits for the outcome of ROP.[216,217] Treatment with omega-3 fatty acids appears to be a promising therapy for prevention and/or treatment of ROP, but there is a paucity of multicenter randomized clinical trials.

Drugs associated with incidence of ROP

Surfactant

One of the earlier drugs found to be associated with ROP is surfactant, despite conflicting reports. In the 1990s, there were three report of a higher occurrence of ROP with surfactant use.[218–220] However, comparing the two commonly used surfactants at that time, Exosurf and Survanta, there was a higher occurrence of ROP in the Exosurf group.[220] A later meta-analysis involving 10 randomized clinical trials and over 2000 preterm infants confirmed those findings of a higher risk for ROP with Exosurf.[221] Numerous other clinical trials and meta-analyses showed no increased risk for ROP with surfactant therapy.

Erythropoietin (Epo) is routinely used to treat anemia of prematurity. Epo is a glycoprotein hormone, expressed mainly in the kidney and fetal liver. It is highly responsive to HIF-1α and hypoxia and is the main regulator of red blood cell production. Many observational and retrospective studies have reported that Epo is associated with an increased risk of ROP,[222–228] while other studies report no risk.[229–231] Early meta-analyses involving 23 studies and 2074 preterm infants in 18 countries showed a significant increase in the risk of stage >3 ROP in the Epo group.[232] An update in 2014 showed no significant increase in the rate of stage ≥3 ROP for studies that initiated EPO treatment at less than 8 days of age.[233] Another meta-analysis done in 2015 also concluded that there were no differences in the incidence of stage ≥3 ROP with Epo use.[234] Clearly, there are major conflicts regarding the role of Epo in the development of ROP, and the reasons for this remain unclear. However, it is well established that both Epo and VEGF are stimulated via HIF-1α during hypoxia, which then leads to angiogenesis,[235,236] therefore timing of administration may be crucial.

Steroid

In 1992, two conflicting retrospective studies were published regarding the incidence of ROP requiring laser therapy and postnatal dexamethasone. Batton et al.[237] reported that of the 21 factors examined, only the use of steroids was significantly associated with the need for cryotherapy. On the other hand, Sobel and Philip[238] showed a reduction in the need for cryotherapy with prolonged dexamethasone. Several other reports confirmed the findings of Baton et al.[237] of an increased risk of ROP with steroids,[239–244] while other studies showed no association.[245–249] However, meta-analyses showed that early exposure to steroids was not associated with a risk of severe ROP,[250,251] while exposure at >7days[252] and >3weeks[253] caused an increase in severe ROP. The use of antenatal steroids was associated with significantly less stage 2 or higher ROP.[93,254] Another study comparing antenatal betamethasone with antenatal dexamethasone showed a trend for greater risk of severe ROP associated with dexamethasone.[255] Due to its association with increased incidence of cerebral palsy,[98] the use of dexamethasone has been restricted.

Indomethacin

Indomethacin is a nonselective COX inhibitor used in neonatology for closure of a patent ductus arteriosus. While studies found no relationship between indomethacin and ROP,[256,257] one retrospective study of over 34,000 preterm infants from 162 sites showed a higher incidence of severe

ROP (stages 3 and 4) in the group treated with indomethacin on the first day of life.[258] Darlow et al.[259] reported that infants treated with indomethacin were 1.5 times more likely to have ROP than untreated infants. Another study involving 105 preterm infants <28 weeks gestation who received low versus high indomethacin showed a significant increase in the incidence of moderate/severe ROP which was directly related to the indomethacin serum concentrations.[260] Conversely, a retrospective chart review found that indomethacin use for PDA was protective for ROP.[261] It is suggested that the contradictory effects of indomethacin on ROP may be related to the infant's postnatal age.[262] This may indicate that the exact timing of treatment may be crucial, and further studies in this regard are needed.

Conclusions

ROP is a multifactorial disease, the cause of which remains largely unknown. Infants at risk suffer from numerous conditions and are exposed to many forms of polypharmacy which likely add to the etiology of the disease. Since its appearance in the 1940s, pharmacologic interventions for treatment and/or prevention of ROP have predominantly targeted oxidants and VEGF. Furthermore, an overwhelming majority of human and animal studies have focused on rescue treatment of ROP when damage to the retina has already occurred. Given the multifactorial characteristic of ROP and complexity of the disease, the use of a single therapeutic agent may not be prudent. No one therapy has proven to be effective without adverse effects. The timing of treatment must be considered as it is preferred to prevent any form of ROP rather than minimize its progression. There are conflicting reports regarding the benefits of many of the past and current therapies, most of which were initiated based on anecdotal/observational reports, and in some cases have resulted in severe adverse events to the detriment of the treated infant. Therefore, only data from well-designed, randomized, multicenter, placebo-controlled trials, as well as long-term follow-up studies should be the criteria for implementing a change in clinical practice to use off-label pharmaceuticals in these vulnerable patients.

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