A Paradigm Shift in the Prevention of Retinopathy of Prematurity

Talkad S. Raghuveer    Barry T. Bloom
Department of Pediatrics, University of Kansas Medical School-Wichita, and Pediatrix Medical Group, Wesley Medical Center, Wichita, Kans., USA

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Retinopathy of prematurity · Extremely low birth weight infants · Oxygen saturation · Vascular endothelial growth factor

Abstract
For more than 50 years it has been known that oxygen therapy can lead to retinopathy of prematurity (ROP). Recent clinical research has led many neonatologists to lower the target oxygen saturation alarm limits to 85–93% and to titrate the inspired oxygen in small increments. Despite efforts to optimize oxygen therapy, the number of cases of severe ROP remains high as more extremely low birth weight infants survive. Based on new insights into the pathogenesis of ROP, there are multiple interventions, in addition to optimizing oxygen therapy that may help decrease severe ROP. Interventions that have the potential to prevent phase I ROP (birth to ≤32 weeks PMA) include increasing retinal erythropoietin (exogenous rHuEPO) and serum IGF-1 (breast milk and/or exogenous IGF-1), maintaining serum glucose below 120 mg, and providing omega-3 supplements. Interventions with potential to prevent proliferative ROP in phase II (infants >32–34 weeks PMA) include treating anemia with a liberal policy of transfusion in premature infants with stage III ROP, photopic adaptation, vitamin E supplements (>34 weeks PMA), and omega-3 supplements. The WINROP algorithm has shown promise as a biomarker in the early identification of extremely low birth weight infants at high risk for proliferative ROP. As there is interplay of multiple factors in the causation of ROP, we suggest that the simultaneous application of some combination of multiple interventions, mentioned above, may reduce the burden of ROP in the most vulnerable infants. These concepts need study in well-designed randomized clinical trials before being incorporated into clinical practice.

Introduction

Retinopathy of prematurity, a retinal vascular disease of premature infants first described in 1942 [1], continues to be a major cause of childhood blindness in both developed and developing countries [2, 3]. Advances in neonatal intensive care have increased survival of extremely low birth weight (ELBW) infants and the incidence of severe ROP is high among the surviving ELBW infants [4–6] (table 1). The incidence of ROP and severe ROP has shown differing trends – decreasing in some studies but increasing in others [7, 8]. The incidence of more-severe ROP (pre threshold) increased from 27% in the CRYO-ROP study to 37% in the ETROP study (n = 6,998; October 2000 to September 2002) and the infants who developed prethreshold
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Manipulation of oxygen has been the mainstay of ROP prevention. This single intervention approach for a multifactorial disease may be one of the reasons that ROP continues to be a significant morbidity in surviving premature infants. Our knowledge of the pathogenesis of ROP is expanding, offering hope of additional prevention strategies, which, if successful, will change the preventive paradigm for ROP and is the focus of this review.

Pathogenesis and Prevention of ROP (fig. 1)

Using animal models, it is now well understood that pathogenesis of ROP involves two phases (I and II). Both phases of ROP have been reliably reproduced in animal models using oxygen-induced retinopathy protocols. The prevention of ROP can broadly be divided into:
(A) Prevention of Phase I ROP, and
(B) Prevention of Phase II ROP.

A. Prevention of Phase I ROP

Pathogenesis of phase I ROP leading to cessation of normal retinal vessel growth and vaso-obliteration involves suppression of both oxygen-mediated and non-oxygen-mediated factors and is triggered by preterm birth and may last until 32 weeks post-menstrual age (PMA) [16].

A.1 Oxygen-Mediated Factors and Phase I ROP

A.1(a) Suppression of Vascular Endothelial Growth Factor and Phase I ROP

During fetal life, retinal blood vessels grow from the optic nerve to the periphery of the retina. Vascular endothelial growth factor (VEGF) is necessary for normal retinal vessel growth. As the retina develops anterior to retinal vessels, there is increased oxygen demand from the developing neural tissue that creates localized hypoxia. VEGF is expressed in response to this hypoxia and blood vessels grow towards the VEGF stimulus. There is an increase in oxygen due to the formation of new vessels and this suppresses VEGF mRNA expression. Retinal vessels then grow toward the next stimulus from VEGF from a more distant area of hypoxia. Thus, the normal retinal vessel formation follows a wave of ‘physiologic hypoxia’ [17, 18].

This normal sequence is disrupted by premature birth, leading to phase I of ROP. In animal models, hyperoxia suppresses VEGF expression and the physiological wave of VEGF anterior to the growing retinal vessels resulting in cessation of retinal vessel growth and regression of existing vessels [19].

A.1(b) Oxygen Therapy and Oxygen Monitoring to Minimize Hyperoxia and Attenuate Phase I of ROP

The role of oxygen in causation of ROP has a long history of observations and randomized clinical trials.

(i) Era of Restricted Oxygen Use

The observations of Kinsey in 1949 and Campbell in 1951, implicated for the first time that oxygen was a ‘possible’ cause for ROP [20, 21]. Two controlled clinical studies showed that high concentrations could be toxic and result in increased ROP [22, 23]. This led to a multicenter, cooperative study that showed that ROP was significant-

Table 1. Shows incidence of severe ROP in ELBW infants (23–26 weeks’ gestation) from three different neonatal network databases

<table>
<thead>
<tr>
<th>Study</th>
<th>23 weeks’ GA</th>
<th>24 weeks’ GA</th>
<th>25 weeks’ GA</th>
<th>26 weeks’ GA</th>
<th>Mean incidence of severe ROP</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Express study (n = 707), 2004–2007, &gt;stage 2</td>
<td>62% (33/53)</td>
<td>48% (45/94)</td>
<td>32% (54/167)</td>
<td>19% (33/174)</td>
<td>40.25%</td>
<td>4</td>
</tr>
<tr>
<td>NICHD-NRN (n = 6,866), 2003–2007, ≥stage 3a</td>
<td>48%</td>
<td>42%</td>
<td>25%</td>
<td>14%</td>
<td>32.25%</td>
<td>5</td>
</tr>
<tr>
<td>Vermont Oxford Network (2005–2008)b</td>
<td>44.2%</td>
<td>35.5%</td>
<td>24.4%</td>
<td>13.2%</td>
<td>29.32%</td>
<td>6</td>
</tr>
</tbody>
</table>

*a 53% of infants from 2006 to 2007 had undetermined ROP status (not reached severe ROP or were still immature and required further examination). b Average incidence of severe ROP (≥stage 3) for the years 2005–2008.
a Normal retinal vessel development in utero

- 23 weeks in utero
- In utero: IGF-1, VEGF, EPO
- Normal retinal vessel development
- Term

b Prevention of phase I ROP: preterm birth (230/7–286/7 GA)

- In utero: IGF-1, VEGF, EPO
- Preterm birth: IGF-1, VEGF, EPO

- 230/7–286/7 GA
- Prevention of hyperoxia during resuscitation
  - Current: 100% oxygen
  - Proposed: 40% oxygen and titrate using pulse oximeter

- 31–32 weeks' GA
- Continued growth of retinal vessels
- No ROP
- Decreased growth of retinal vessels
- Onset of ROP

- Possible future intervention(s)
  - Exogenous IGF-1 (¿ duration)

- Intervention(s)
  - Current:
    - SaO2 85–93%, PaO2 >80 mm Hg/dl
  - Proposed:
    - SaO2 85–93%, PaO2 <80 mm Hg/dl
    - Exogenous rHuEPO (¿ first week)
    - Prevention of hyperglycemia (blood glucose <120 mg/dl)
    - Omega-3 supplements

- Prevention of hyperoxia during resuscitation
  - Current: 100% oxygen
  - Proposed: 40% oxygen and titrate using pulse oximeter

- SaO2 limits 95–98% or 96–99%
  - PRBC transfusion for anemia, and photopic adaptation to lower VEGF
  - Avoid rHuEPO
  - Omega-3 supplements
  - Vitamin E

- Regression of ROP
- Proliferation of ROP

- Possible future intervention(s)
  - Exogenous IGF-1 (¿ duration)
  - RAS inhibitors
  - Statins

- Intervention(s)
  - Current:
    - SaO2 limits 95–98% or 96–99%
    - PRBC transfusion for anemia, and photopic adaptation to lower VEGF
  - Proposed:
    - SaO2 limits 95–98% or 96–99%

- Onset of phase II ROP ≥32 weeks PMA

- ≥32 weeks PMA
- High VEGF
- High EPO
- High oxidative stress
- Mature retina with increased metabolic rate leading to retinal hypoxia with onset of ROP

- Regression of ROP
- Proliferation of ROP

- Possible future intervention(s)
  - Exogenous IGF-1 (¿ duration)
  - RAS inhibitors
  - Statins
ly higher in the routine (high concentration of oxygen) than the oxygen-restricted (≤40%) group; but the mortality in the oxygen-restricted group was 10% higher than in the high-oxygen group [24]. The results of the study led to recommendations against the routine use of oxygen in premature infants and restricting oxygen when used to less than 40%. There was immediate removal of the 'small float' in the air-oxygen intake assembly of the incubator responsible for maintaining high oxygen concentration and also change in clinical practice [25]. These actions led to the significant increase in early neonatal deaths over the next decade [26].

(ii) Era of Advances in Oxygen Monitoring in Neonates

The first advance was placing arterial lines and measuring intermittent arterial oxygen tension in premature infants, which was never shown in a controlled trial to decrease the risk of ROP [27]. This invasive procedure has potentially serious complications and the use of indwelling catheters is now limited to a few days.

Noninvasive and continuous monitoring of oxygen tension with transcutaneous monitors (TcPO_{2}) was developed in the 1970s. Clinical trials of TcPO_{2} monitoring did not show a reduction of ROP but suggested that ROP occurred more often when TcPO_{2} was ≥80 mm Hg in the first 4 weeks of life [28]. In addition, TcPO_{2} monitoring was limited by its heat-related adverse effects in ELBW infants at the highest risk of severe ROP.

The next advance for noninvasive oxygen saturation monitoring was the development of the pulse oximeter in 1974 [29]. After initial studies, it was shown that pulse oximeter provided continuous and reliable oxygen monitoring in neonates [30].

(iii) Current Era of Oxygen Therapy and Oxygen Monitoring to Prevent ROP

In the current era, oxygen therapy in ELBW infants is routinely monitored using the pulse oximeter from admission until the infant no longer needs supplemental oxygen. Tin et al. [31] showed that the incidence of ROP was 6, 14, 16 and 28% when target for oxygen saturation was 70–90, 84–94, 85–95 and 88–98%, respectively.

Subsequent studies have targeted oxygen saturation of 85–93% with a significant reduction in severe ROP [32, 33].

Following the publication of these and other studies, two reviews proposed that, with the current knowledge, targeting saturation of oxygen of 85–93% was optimal to prevent ROP [34, 35]. A recent meta-analysis showed that there was a 52% reduction of severe ROP in studies targeting low oxygen saturation (70–96%) in phase I (birth to ≤32 weeks PMA) [36]. In 3 of the 5 studies in the meta-analysis, there was no increase in mortality rate in the low-oxygen saturation group compared to the high-oxygen saturation group, and the other 2 studies did not report on mortality. A recent randomized trial (SUPPORT study, n = 1,310) compared oxygen target ranges of 85–89% to 91–95% and found that in the lower target range (85–89%) there was a reduced risk of severe ROP but an increased risk of in-hospital death. The absolute risk of death increased by 3.7 percentage points in the lower-oxygen saturation group compared to the high-oxygen saturation group, and the other 2 studies did not report on mortality.

The increase in mortality is difficult to explain as there was considerable overlap in the achieved oxygen saturation in the two groups, and the actual median oxygen saturation in the low-oxygen saturation group was 91%
compared to 94% in the high-oxygen saturation group. The upper limit of the lower-oxygen saturation group in this study was lower (89%) compared to previous studies (93%) that have not reported increased mortality.

Ongoing clinical trials such as BOOST II (Australia, ACTRN01260500055606), BOOST II NZ, Canadian Oxygen Trial (ClinicalTrials.gov number: NCT 00637169) and NeOProM (ClinicalTrials.gov number: NCT01124331) may confirm or refute the increased risk of death seen in the low-oxygen saturation group in the SUPPORT study.

It is difficult to achieve compliance with targeted alarm limits (83–85 to 92–94%) in clinical practice. In the study by Clucas et al. [38], the lower limit was set correctly 91% of the time, but the higher limit was set correctly just 23% of the time. Hagadorn et al. [39] found that SpO2 was above the target range 20–73% of the time and varied substantially among the centers. A prospective clinical trial (SAT01ROP) is underway to measure the time spent outside the target oxygen saturation range (ClinicalTrials.gov number: NCT00845624). As compliance with targeted alarm limits may be difficult to achieve because of ‘the human factor’, future clinical practice may involve automated adjustment of inspired oxygen (using closed loop devices interfaced with air oxygen blender and ventilator) as shown in a preliminary study by Claure et al. [40].

An important reason for early hyperoxia in ELBW infants is the use of 100% oxygen during resuscitation. A survey from 2005 showed that 90% of responders resuscitate premature infants using 100% oxygen [41]. Small clinical trials have compared the use of lower and higher concentrations of oxygen during resuscitation of premature infants and have evaluated short-term outcomes but not long-term outcomes such as ROP. Two countries have resuscitation guidelines for ELBW infants to begin with 21% oxygen [42]. The optimal oxygen concentration for resuscitating ELBW infants is not known, but by monitoring SpO2 and using a lower concentration of oxygen (~30 to 40%), it is possible to limit the degree of vaso-obliteration, from downregulation of VEGF, in the premature retina. The practice of using 30–40% oxygen for resuscitating ELBW infants and later targeting SpO2 limits of 85–93% in the NICU could potentially result in a significant decrease in ROP, but the safety and efficacy of this combined strategy is yet to be studied in a randomized clinical trial.

A.1(c) Erythropoietin and Phase I ROP

Erythropoietin (EPO) is a glycoprotein which is produced in the fetal liver and adult kidney and regulates erythropoiesis [43] and is involved in retinal vessel development. In the animal model of oxygen-induced retinopathy, it has been shown that there is suppression of retinal EPO leading to loss of retinal vessels and phase I ROP. In addition, early administration of exogenous EPO prevents retinal vessel loss in this animal model of ROP [44]. In the human fetal retina the levels of EPO mRNA and EPO protein increase with increasing gestational age [45]. After birth, there is possibly a fall in retinal EPO levels mirroring the well-documented fall in serum EPO.

The decrease in serum EPO after birth contributes to development of anemia in premature infants. Red cell transfusions are needed to treat anemia in more than 95% of ELBW infants [46]. Recombinant human erythropoietin (rHuEPO) has been used to decrease the number of transfusions in premature infants. Such use of rHuEPO in premature infants has been associated with increased incidence of ROP in some studies [47, 48] and no increase in the incidence of ROP in others [49, 50]. These conflicting results can possibly be explained from the studies in animal models of ROP that suggest early exogenous EPO may help attenuate phase I ROP but may exacerbate proliferative retinopathy in phase II (see below). The use of rHuEPO to prevent phase I ROP needs careful study in future randomized clinical trials.

A.2 Non-Oxygen-Mediated Factors and Phase I ROP

Insulin Growth Factor-1 and Phase I ROP

Retinal vascular development is dependent on optimal levels of VEGF, EPO and insulin growth factor-1 (IGF-1). Retinal vascular growth was significantly retarded in IGF-1 knockout mice compared to wild-type controls, suggesting that VEGF, in the absence of IGF-1, cannot stimulate normal retinal vascular growth. Low level of IGF-1 decreases retinal vascular growth by suppressing VEGF activation of protein kinase B (Akt) necessary for endothelial cell survival [51]. Children with genetic defects leading to low or absent IGF-1 activity in utero have abnormal retinal vessel morphology [52].

IGF-1 increases in the human fetus in the third trimester and correlates positively with gestational age and birth weight [53]. However, after preterm birth there is a fall in serum levels of IGF-1 [54]. In addition, IGF-1 is suppressed by poor nutrition, sepsis, and acidosis. In preterm infants with prolonged low serum levels of IGF-1 and slow weight gain there is an increased risk of ROP [55]. Human milk increases serum IGF-1 more than formula feeding [56]. This may be due to the presence of IGF-1 in human milk, especially early milk, and the presence of specific proteolyis of IGF/IGFBP-2 complexes that in-
crease the biologic availability of IGF [57]. As many premature infants have feeding intolerance in the first weeks after birth, there are now efforts to increase IGF-1 by parenteral administration.

Preliminary studies in premature infants have shown that infusion of fresh frozen plasma, which is a source of exogenous IGF-1, increased the serum level of IGF-1 [58] and infusion of rhIGF-1/rhIGFBP-3 increased the serum IGF-1 levels to in utero levels for a few hours [59]. The administration of rhIGF-1 in mice led to attenuation of oxygen-induced retinopathy [60]. A randomized, multicenter (phase II/III) clinical trial, of continuous infusion of rhIGF-1 from birth to 31 weeks PMA, is underway to study whether maintaining IGF-1 in the normal intrauterine range for the corresponding gestational age can prevent ROP in premature infants (ClinicalTrials.gov number: NCT01096784).

B. Prevention of Phase II ROP

The natural course of ROP is to regress in most premature infants. However, despite efforts at primary prevention of ROP and spontaneous regression, some infants progress to proliferative ROP (phase II). Pathogenesis of phase II of ROP, leading to neo-vascularization, involves elevated levels of both oxygen-mediated and non-oxygen-mediated factors. As the retina slowly matures and becomes metabolically active, the peripheral avascular retina becomes progressively hypoxic leading to the proliferative phase of ROP (phase II) that begins around 32–34 weeks PMA. [16]

B.1 Oxygen-Mediated Factors and Phase II ROP

B.1(a) Elevated Levels of VEGF and Phase II ROP

In the neonatal mouse model of ROP, initial exposure to hyperoxia results in vaso-obliteration of retinal vessels (phase I), and when these animals are subsequently returned to room air, the inner retina is hypoxic, stimulating the elevation of VEGF mRNA and protein levels and neovascularization. Muller cells and astrocytes in the inner retina are the source of increased VEGF [19].

In the transgenic mice model, it was shown that overexpression of VEGF results in retinal neovascularization [61]. Additional studies in animal models of ROP, including in nonhuman primates, have confirmed that an elevated level of VEGF is responsible for neovascularization [62]. Further, it has been shown that inhibition of VEGF, using VEGF-receptor chimeric proteins, decreases neovascularization by nearly 56% [63]. All this evidence suggests that an elevated level of VEGF, possibly in concert with other angiogenic factors, causes the proliferative phase (phase II) of ROP.

B.1(b) Role of Oxygen in Prevention of Progression of ROP in Phase II/Attenuation of Phase II of ROP – (Infants >32 Weeks PMA)

Just as there is elevation of VEGF from retinal hypoxia in phase II of ROP in animal models, there were significantly higher VEGF levels in the vitreous of premature infants with advanced ROP (‘vascular-active’ ROP) compared to controls without ROP [64]. Treating retinal hypoxia in a premature infant in phase II with supplemental oxygen could prevent the continued increase in retinal VEGF and may help prevent the progression of proliferative retinopathy and has been studied in two randomized clinical trials [Supplemental Therapeutic Oxygen for Pre-threshold Retinopathy of Prematurity (STOP-ROP) and Benefits of Oxygen Saturation Targeting (BOOST)].

(i) STOP-ROP Trial [65]

Premature infants (n = 649), with pre-threshold ROP receiving supplemental oxygen, were enrolled in the trial at a mean postmenstrual age of 35.4 ± 2.5 weeks (range 30–48 weeks). The conventional oxygen group and the supplemental oxygen group had oxygen saturation targets of 89–94% and 96–99%, respectively. Progression to threshold ROP was the primary outcome. The rate of progression to threshold ROP decreased significantly in infants who did not have plus disease at enrollment (46% in the conventional group vs. 32% in the supplemental group; p = 0.004), but overall (including infants with plus disease at enrollment), the rate of progression to threshold did not decrease significantly (48.5 vs. 40.9%; p = 0.032; not significant at the designed one-tailed α = 0.025). There was an increase in pulmonary morbidity in the supplemental oxygen group targeting high oxygen saturation (8.5 vs. 13.2%; p = 0.6). In this trial, the number of infants needed to treat with oxygen, to achieve higher oxygen saturation, to prevent progression to threshold disease in one infant, was 13.2 [66].

(ii) BOOST Trial [67]

Premature infants (22–29 weeks; n = 358) receiving supplemental oxygen at 32 weeks PMA were recruited into this study. The standard-saturation group had an oxygen saturation target of 91–94% and the high-saturation group had a target of 95–98%. ROP was a secondary outcome.
There was a trend towards a decrease in the rate of laser surgery for ROP in the subgroup of infants born less than 28 weeks of gestation (16% in the standard-saturation group vs. 8% in the high-saturation group; p = 0.06), but overall there was no difference in the need for laser surgery for infants in the study (11 vs. 6%; p = 0.09). Just as in the STOP-ROP study, there was an increase in respiratory morbidity in the high-oxygen saturation group with an increased proportion of infants requiring oxygen at 36 weeks (excess of 40%) and at discharge (excess of 78%). In addition, there was an increased incidence of death from pulmonary causes in the high-saturation group.

The meta-analysis by Chen et al. [36] showed a statistically significant risk reduction in progression to severe ROP in studies that targeted high oxygen saturation in infants >32 weeks PMA (RR: 0.54, 95% CI 0.35–0.82). Though two studies in their analysis (STOP-ROP and BOOST) did not show significant reduction in severe ROP, there was a significant risk reduction when they were pooled together (RR 0.84; 95% CI 0.71–0.99). The authors state that there is no published evidence that high oxygen saturation increases the risk of ROP in infants after 32 weeks PMA, and targeting oxygen saturation lower limit of ≥98% was significantly more beneficial to prevent proliferative ROP (RR 0.27; 95% CI 0.14–0.50), compared to targeting oxygen saturation lower limit of <98% (RR 0.72; 95% CI 0.54–0.96).

The ideal target for oxygen saturation, to decrease retinal hypoxia in ELBW infants >32 weeks PMA and prevent progression in phase II of ROP, without increasing pulmonary morbidity and mortality, is not known and needs further study.

### B.1(c) Elevated Levels of EPO and Prevention of Progression of ROP (Phase II ROP)

The retinal levels of both VEGF mRNA and EPO mRNA are increased significantly during the proliferative phase in a murine model of ROP [68]. Inhibition of retinal EPO, with small interfering RNA (siRNA), during the neovascular phase in a mouse model, significantly, but not completely, suppressed vessel proliferation [69]. It has also been shown, using the hyperoxia-normoxia ROP model, that wild-type mice developed neovascularization while HLF-knockdown mice (HLF<sup>kd/kd</sup>) did not, but developed neovascular buds when they received exogenous EPO [70].

A clinical study, confirming the observations from animal studies, showed that vitreous EPO was elevated, along with vitreous VEGF, in patients with proliferative ROP (n = 20) [64], and vitreous EPO was significantly higher (10-fold) than serum EPO, as reported by Bierer et al. [71], in infants with similar weight and gestational age (serum 26 ± 11 vs. vitreous 601.9 ± 233 mIU/ml). So it is possible that elevated retinal EPO is due to local production rather than diffusion from blood. Based on the evidence from animal and clinical studies, the next logical step would be to devise methods to decrease retinal EPO to stop the progression of proliferative ROP in phase II. As exogenous rHuEPO may increase retinal EPO, as it has been shown to do so in an animal model [72], it is best avoided in phase II ROP.

Anemia occurs concurrently in many premature infants who develop proliferative ROP with elevated retinal EPO (and VEGF) [64] from retinal hypoxia. Decreased oxygen carrying capacity of blood with anemia can potentially exacerbate retinal hypoxia and further increase retinal EPO. Correction of anemia with red cell transfusion may decrease retinal EPO. In premature infants with anemia and pre-threshold ROP, Gaynon’s group have observed regression of pre-threshold ROP with correction of anemia with red cell transfusion [73]. Other studies, though not specifically carried out during phase II ROP, suggest that red cell transfusion may increase the risk of ROP and that anemia is protective [74, 75]. Based on current understanding of the pathogenesis of ROP, it would seem that correction of anemia, with red cell transfusion, in patients with proliferative ROP (stage 3) would help by decreasing retinal EPO, but this needs further study in randomized clinical trials.

### B.2 Non-Oxygen-Mediated Factors and Phase II ROP

#### IGF-1 and Proliferative Retinopathy

In a mouse model, it has been shown that the IGF-1 receptor antagonist suppresses retinal neo-vascularization, without decreasing VEGF, suggesting that elevated retinal IGF-1 by enhancing VEGF-driven endothelial cell proliferation is involved in the pathogenesis of proliferative ROP [76]. Whether the IGF-1 receptor antagonist has a role in ELBW infants to prevent progression of proliferative ROP is yet to be studied.

#### Nutritional Interventions That May Be Beneficial in the Prevention of ROP

#### Vitamin E and ROP

The antioxidant system is functionally immature in premature infants due both to deficient antioxidant en-
zymes [77] and free radical scavengers, especially vitamin E [78]. As a result, premature infants are susceptible to oxidative stress resulting in oxygen-radical disease, one of which is ROP [79]. Supportive evidence for increased oxidative stress includes the finding of high concentration of hypoxanthine, a free oxygen radical generator, in the vitreous fluid of infants with ROP [80].

Vitamin E was the first intervention used in the 1940s to prevent ROP [81]. Subsequently, a number of clinical trials of vitamin E supplementation in premature infants were done to prevent ROP from the 1970s to the 1990s. Two meta-analyses of vitamin E studies showed that supplemental vitamin E significantly reduced the risk of severe (stage 3+) ROP in very low birth weight infants [82, 83]. However, there was an increased risk of NEC in one of the trials reviewed [84]. In addition, there was an increased risk of sepsis when serum vitamin E level exceeded 3 mg/dl [83].

Supplementing enteral vitamin E, to achieve high serum physiologic level of 2.5 mg/dl to prevent progression of proliferative ROP in ELBW infants who are >34 weeks PMA (to decrease the risk of NEC), needs further study.

Omega-3 Fatty Acid and ROP

In a mouse model of ROP, it has been shown that increased dietary content of omega-3 fatty acids increased retinal omega-3 and led to an increase in retinal vessel regrowth with subsequent decrease in the avascular area in the retina (attenuation of phase I ROP) and decreased neovascularization (attenuation of phase II ROP). The decrease in neo-vascularization was mediated by the bioactive metabolites of omega-3 fatty acids, neuroprotectin D1, resolvin D1, and resolving E1 by suppressing tumor necrosis factor-α (TNF-α) in the microglia close to retinal vessels (90% suppression of retinal Tnf mRNA and 30% suppression of TNF-α protein). In this mouse model of ROP, an intra-peritoneal injection of a TNF-α receptor fusion protein (etanercept), which sequesters TNF-α, achieved the same level of reduction of retinal avascular area and reduction of neovascularization as a diet rich in omega-3 fatty acids. This indicates that a diet rich in omega-3 fatty acids could help to protect against proliferative ROP by reduction of TNF-α [85].

From 2002 most infant formulas in the US are fortified with two long chain PUFAs (LCPUFAs) – docosahexaenoic acid (DHA, 22:6, n–3) and arachidonic acid (ARA, 20:4, n–6) [86]. Clinical studies of DHA fortification of infant formula (0.3% of total fatty acids) have shown improvement in visual acuity in term infants [86]. In premature infants, who are at greater risk for DHA deficiency, it has been shown that a higher DHA fortification (1% DHA instead of 0.3%) may be necessary to improve visual acuity [87]. The impact of fortification of infant formula with DHA (1 vs. 0.3%) or supplementation with omega-3 fatty acids to breast-feeding mothers on phase I or phase II ROP in ELBW infants needs further study.

Miscellaneous Interventions to Prevent ROP

Hyperglycemia and ROP

VEGF plays a major role in the pathogenesis of both diabetic retinopathy and ROP. In adult diabetics, hyperglycemia leads to elevated advanced glycosylation products (AGP) that upregulate VEGF leading to proliferative retinopathy [88]. Streptozotocin-induced transient hyperglycemia, in a rat pup model, led to a significant reduction in the retinal vascular area; though it is unclear if this was due to down-regulation of VEGF [89].

Hyperglycemia, defined as plasma glucose >150 mg/dl, occurs in 45% of infants <1,000 g and in 80% infants <750 g, and the risk of hyperglycemia in the first weeks of life is 18 times greater in infants less than 1,000 g compared to infants weighing more than 2,000 g [90, 91]. This is due to a defective processing of proinsulin to insulin and partial resistance to insulin leading to continuous hepatic glucose production [92]. Retrospective studies have shown that hyperglycemia increases the risk of ROP in ELBW infants [93, 94], and these studies raise the possibility of association between hyperglycemia and ROP, but they do not establish causality. Whether preventing hyperglycemia (or decreasing the duration of hyperglycemia) in ELBW infants would help decrease the incidence of ROP needs further study.

Phototopic Adaptation

Ambient light in the nursery was implicated in the causation of ROP [95], but this hypothesis was discredited as light exposure did not exacerbate proliferative retinopathy in mouse model of ROP [96], and a randomized clinical study (LIGHT ROP) showed that reduction of light did not reduce the incidence of ROP in ELBW infants [97]. Despite this evidence, in most NICUs in the US, premature infants are nursed in the dark by covering
their incubators with a thick quilt. There is new evidence, from a study in rat pups, that dark rearing decreases the oxygen-induced vaso-obliteration of retinal vessels [98]. So it would seem prudent to continue nursing ELBW infants in the dark per current practice, until they are 31 weeks PMA, as it may help attenuate phase I ROP.

There is also evidence from animal studies that dark adaptation significantly increases outer retinal oxygen consumption and can increase retinal hypoxia [99]. Exposing ELBW infants with proliferative ROP to light and allowing photopic adaptation by removing the light-blocking incubator cover may decrease retinal oxygen consumption and downregulate the hypoxic stimulus for VEGF. Gaynon and colleagues have used photopic adaptation in ELBW infants with prethreshold ROP at their center since 1991 [73]. Whether this practice of photopic adaptation in phase II ROP is beneficial needs further study.

Possible Future Interventions to Prevent ROP

Renin-Angiotensin System and Phase II ROP

The components of the renin-angiotensin system (RAS), which exist in the kidney, have now been identified in the human eye including mRNA for renin, angiotensin, angiotensin-converting enzyme (ACE) and angiotensigen [100]. In addition, angiotensin-1 (AT-1) and angiotensin-2 (AT-2) receptors have been localized to the retinal ganglion cells in the rat model [101]. There is evidence from a rat model for ROP that local ocular RAS may be involved in the pathogenesis of ROP as retinal neovascularization was associated with a rise of retinal renin, VEGF and VEGFR-2 (flk-1) mRNA. In addition, ACE inhibition prevented neovascularization and significantly decreased VEGF and VEGFR-2 mRNA [102]. Though RAS inhibitors have been shown to slow the progression of diabetic retinopathy [103], clinical trials to elucidate the role of RAS inhibitors in the prevention of phase II ROP in ELBW infants are yet to be done.

A preliminary study showed that in premature infants of 26–30 weeks’ gestation (n = 6) the serum prorenin was significantly higher in those with ROP compared to those without ROP (2,326 ± 334 vs. 1,164 ± 234 μg/ml; p = <0.05) [104]. It is to be determined whether serum prorenin has potential as a biomarker for the early identification of proliferative ROP.

HMG-CoA Reductase Inhibitors (Statins) and Prevention of ROP

Statins are a group of drugs that inhibit 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) and decrease cholesterol biosynthesis. In addition to lowering cholesterol, statins have cholesterol-independent anti-oxidant and anti-inflammatory effects [105]. A recent study in a mouse model of ROP has shown that treatment with high dose fluvastatin significantly reduced the area of the capillary-free zone in the retina and decreased the formation of neovascular tufts. These morphologic changes were associated with a decreased level of VEGF and ICAM-1 expression in the retina [106]. If the results of this study are replicated, then a careful clinical study of statins to prevent proliferative ROP in ELBW infants may be warranted.

Biomarkers for Predicting ELBW Infants at High Risk for Proliferative ROP

Current practice is to examine the retina periodically in ELBW infants to detect ROP and to diagnose proliferative ROP. There is now a biomarker that may help identify ELBW infants at high risk for proliferative ROP weeks earlier than a retinal exam.

WINROP Algorithm

This system comprises a two-step process using an online surveillance system to detect slowing of weight gain in premature infants or slowing of the rise of serum IGF-1 compared to a reference curve for each postnatal week [107]. Each weekly evaluation classifies the infants into three groups: (a) no alarm, (b) alarm at low risk (infants >29 weeks and/or birth weight >850 g), and (c) alarm at high risk (infants <29 weeks and/or birth weight <850 g). This algorithm has been validated in three different groups of premature infants in Sweden [108]. In the studies using the WINROP (Weight, Insulin-like growth factor, Neonatal ROP) algorithm, the sensitivity and specificity for detecting proliferative retinopathy were 100 and 54–84.5%, respectively [108, 109]. Infants were identified at a mean of 9–10 weeks before they needed laser eye surgery. WINROP has been simplified by eliminating measurement of IGF-1. Two retrospective studies of the WINROP algorithm, one from Sweden and the other from Boston, were done using the postnatal weight gain alone.
and have shown a sensitivity of 100% and were successful in early identification of all the premature infants who developed severe ROP \[109, 110\]. As postnatal weight gain in premature infants may vary from unit to unit based on day-to-day clinical care, WINROP algorithm needs further study using a larger sample across multiple centers.

Implications for Future Research

Despite careful day-to-day clinical care of ELBW infants with attention to oxygen saturation, respiratory support, nutrition, growth, and blood glucose, a significant number of ELBW infants go on to develop severe ROP (fig. 2). Therefore, it is important to study whether multiple interventions (reviewed above) can decrease severe ROP in ELBW infants as the likelihood that any one of these interventions will be independently successful is low. Similar to oncology clinical trials, we envision the first step would be an open-label, dose-finding, multicenter studies to evaluate the safety and optimal dosing of each new intervention in addition to targeting oxygen saturation. The next step would be to evaluate the efficacy of multiple combinations of these interventions in a RCT. The average incidence of severe ROP in ELBW infants between 23\(^{0/7}\) to 26\(^{6/7}\) weeks GA (table 1) is around 34%. If we projected that all of the combined therapies would decrease the incidence by 50%, with a power of 0.8, it would require over 500 infants to be enrolled into any one RCT. It would be important to track this target population's short-term and long-term rate of adverse events, side effects, and risks of drug interactions in addition to ROP outcome.

Conclusions

This is not an exhaustive review and so many factors that have been shown to increase the risk of ROP such as the influence of high pCO\(_2\), low pH \[111\] and others are not mentioned. In addition, a detailed analysis of the efficacy and safety of anti-angiogenic agents, such as intra-vitreal injection of bevacizumab, for treatment of infants with severe ROP is beyond the scope of this review. How-

FIG. 2. The hospital course of a 26-week gestation, 461 g (BW), female infant is shown. She was resuscitated with 40% oxygen and received prophylactic surfactant soon after birth. Phase I ROP (from preterm birth to 32 weeks PMA): the oxygen saturation target was set to 85–93%, the weight gain was \(-9.7\) g/day from birth to 32 weeks PMA despite best efforts at optimizing nutrition, multiple episodes of hyperglycemia (blood sugar \(\geq 120\) mg/dl) occurred and were promptly corrected with decrease in glucose infusion rate, supported with mechanical ventilation from birth to day 60 (34\(^{5/7}\) weeks PMA), and treated for sepsis at day 30. Phase II ROP (>32 to 34 weeks PMA): diagnosed with ROP stage 2 posterior disease at day 59 (34\(^{4/7}\) weeks PMA) at which time oxygen saturation target changed to 90–95% but progressed to plus disease on day 74 (36\(^{7/7}\) weeks PMA) and was treated with laser therapy. Regression of ROP was noted on day 81 (37\(^{5/7}\) weeks PMA). PMA = Postmenstrual age, BW = birth weight.
ever, there may be increased interest in anti-VEGF therapy as a randomized clinical trial (BEAT-ROP) has shown that intravitreal bevacizumab is superior to laser therapy in treating infants with stage 3 plus ROP in zone I [112].

Based on recent developments in the pathogenesis of ROP, it may be time to change the ROP prevention research paradigm. Rather than continued manipulation of supplemental oxygen in premature infants, it is time to develop multiple intervention trials based on the new scientific developments to prevent proliferative ROP. Prevention of phase I ROP would include strategies (fig. 1) to attenuate suppression of VEGF (oxygen restriction at birth and after admission to NICU), prevent decrease in EPO (early, short course exogenous EPO), increase IGF-1 (optimal nutrition), omega-3 supplements (to prevent deficiency), and prevention of hyperglycemia. Phase II ROP prevention strategies (fig. 1) could target reduction of high VEGF (supplemental oxygen to prevent hypoxia, correction of anemia with red cell transfusion, photopic adaptation), reduction of high EPO (avoid exogenous EPO), and omega-3 fatty acids (to suppress TNF-α?). For infants with stage III ROP (without plus disease), intervention would include use of vitamin E to limit oxidative stress. More information is needed for certain interventions such as exogenous IGF-1, RAS blockers and statins before use in randomized clinical trials. In addition to short-term outcomes, to evaluate the toxic effects of the interventions and impact, if any, on mortality, it is important to evaluate the long-term neurodevelopmental outcome.

As the incidence of proliferative ROP is increasing in countries where neonatal intensive care has been recently introduced, the need for separate randomized clinical trials involving premature infants in those countries is necessary and urgent.

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