A safety review of drugs used for the treatment of retinopathy of prematurity

Abstract

Introduction: Retinopathy of Prematurity (ROP) is a sight-threatening disease representing one of the main disabling diseases affecting premature newborns. Presently, ROP is treated by surgical interventions and drug therapies are limited to the off-label use of a little amount of molecules approved for other pathologies.

Areas covered: Many drugs that may potentially be used in treating ROP are recently proposed, in many cases after the demonstration of their effectiveness in preclinical studies. In this review, the authors discuss safety and effectiveness of the main proposed approaches in the pharmacologic treatment of the disease, including approaches based on oxygen therapy and nutritional interventions.

Expert opinion: Surgical approaches to ROP are not without side effects. However, most of the proposed pharmacologic interventions can also raise specific concerns. In particular, these approaches follow a curative paradigm and are proposed in patients once the disease has progressed, with an effectiveness that is often smaller than expected. A goal in the treatment of ROP would be moving the paradigm towards a preventive approach that could be potentially effective in treating extremely low birth weight preterm infants before ROP becomes manifest.

Key words: antioxidants, anti-VEGF, hyperoxia/hypoxia, IGF-1, NSAIDs, propranolol, steroids, VEGF

Article highlights

- ROP is a preventable neovascular retinal disease with a great impact on vision. Surgical therapies are the approved interventions to treat this disease.
- Surgical interventions show several adverse side effects that underlie the urgent need of pharmacologic therapies to treat ROP.
- Several drugs that may potentially be used in treating ROP have been proposed based on studies in preclinical models.
- Among pharmacologic interventions, anti-VEGF drugs are used off-label to treat newborns suffering from severe ROP. However, also anti-VEGF drugs raise several concerns. For instance, the neuroprotective role exerted by VEGF may be abrogated by sequestering VEGF.
- Propranolol may represent a valid approach to treat ROP although its safe profile needs to be better evaluated in appropriate clinical trials.
- Presently, pharmacologic approaches to ROP are mainly intended to treat ROP after the disease has progressed. A new goal would be the introduction of preventive approaches effective in treating extremely low birth weight preterm infants before ROP becomes manifest.

1. Introduction

Retinopathy of Prematurity (ROP) is a sight-threatening disease related to a pathological vascularization that occurs in the incompletely vascularized retina of preterm newborns. It still represents one of the main disabling diseases affecting premature babies.

In the human fetus, retinal vascularization begins during the fourth month of gestation and occurs in the hypoxic uterine environment. In preterm infants, the retina is incompletely vascularized and the exposure to the hyperoxic extra-uterine environment leads to the down-regulation of proangiogenic factors and to vessel regression characterizing the first phase of ROP. When the metabolic demand of the developing retina increases, the retinal environment becomes hypoxic and this moves ROP in its second phase, in which hypoxia triggers the up-regulation of proangiogenic factors that, in turns, stimulate the growth of pathological blood vessels. ROP is a typical multifactorial disease, with low oxygen tension triggering the expression of a variety of angiogenic growth factors. Among them, vascular endothelial growth factor (VEGF) is a master regulator of angiogenesis and its characteristic upregulation in ROP leads to the growth of abnormal blood vessels into the inner retina. Additional mechanisms, including oxidative stress and the activation of inflammatory signaling pathways, also play important roles in the disease [1]. Therefore, it is not surprising that numerous therapeutic and pharmacological interventions potentially capable in preventing ROP progression have been proposed for newborns with ROP.

ROP is classified in 5 stages, with stage 1 characterized by a mild disease and stage 5 representing the end stage of the disease with severe visual impairment. Treatment of premature infants with ROP, which is mainly based on surgical interventions, is considered when the disease develops to stage 3 [2].

Currently, the most effective strategy to reduce the incidence of ROP is certainly represented by a controlled and appropriate use of oxygen. However, many other pharmacological or nutritional strategies have been attempted in order to prevent or slow down disease progression, and others are currently being evaluated, with the awareness that a single therapeutic measure is unlikely to be sufficient.

Interventions to prevent the development of ROP must be carefully evaluated since prevention would certainly be the best strategy. However, every therapeutic attempt must be balanced by a careful evaluation of possible adverse events. The history of the different therapies tested in ROP confirms the importance of carrying out well-designed, randomized, multicenter, placebo-controlled trials with a large number of patients, as well as long-term follow-up studies before introducing pharmacological innovations into clinical practice.

2. Body of review

2.1 Oxygen

Oxygen is still the most commonly used “drug” in the treatment of preterm infants for respiratory support. However, an appropriate use of oxygen is recommended, avoiding dangerous periods of hypoxia and unnecessary high levels of oxygen that may trigger oxygen-related damages in developing organs.

The responsibility of uncontrolled use of oxygen in the development of ROP became clear in the 1940s when the first epidemic of ROP was followed by the diffusion of a widespread use of oxygen without restrictions and without controls in premature infants as the only form of respiratory support [3]. In the 1950s, the decisive role of oxygen in the development of ROP (at that time still

called “retrolental fibroplasia”) was demonstrated both with clinical observations [4] and animal studies [5]. A first multicenter randomized clinical trial demonstrated the positive association between oxygen exposure (concentration and time of exposition) and ROP development. In 1956, for the first time, an alert appeared on the use of oxygen concentration that “should not exceed 40%” [6]. However, this recommendation came into conflict with the observation that oxygen limitation increased the incidence of mortality, especially on the first day of life, demonstrating that for each case of blindness that was prevented, there was an excess of 16 deaths [7]. This study marked the beginning of a long research, not yet concluded, aimed at identifying the best strategy for oxygen supply. The introduction of transcutaneous oxygen monitoring and pulse oximetry has significantly improved the possibility to monitor plasma oxygen levels, but despite this, the optimal peripheral capillary oxygen saturation (SpO_2) for preterm newborns remains undefined.

Studies performed using animal models provided important information regarding the pathogenesis of ROP and demonstrated the biphasic oxygen-dependence of this disease. The first phase of ROP develops after the exposure to a hyperoxic environment that promotes the regression of the retinal vasculature through the down-regulation of retinal proangiogenic factors. However, this ischemic phase favors the progressive transition to the hypoxic phase where proangiogenic factors progressively increase. In essence, what happens in the first phase, following oxygen exposure, apparently mirrors what happens during the second phase, when the retina becomes hypoxic [1]. This specular dependence from oxygen (which is too high during the first phase and too low during the second phase) suggested the hypothesis oxygen supply during the proliferative phase might decrease VEGF levels and counteract vessel overgrowth. This possibility was tested in the STOP-ROP trial where newborns with prethreshold ROP were randomized to a target SpO_2 of 89-94% versus 96-99%. Contrary to the hypothesis, in the higher

saturation group the percentage of ROP progression to threshold stage was not reduced, even though a significant benefit was reported for infants in the high SpO_2 arm who did not have “plus disease”. However, in the higher saturation group an increased incidence of pulmonary adverse events was observed [8]. A few years later, the BOOST multicenter trial evaluated the clinical outcome of preterm newborns assisted, starting from the 32nd week of postmenstrual age, at two saturation ranges (91-94% versus 95-98%). Also in this study, no difference in severe ROP incidence was observed between the groups [9]. However, this line of research is not definitively closed, as confirmed by a recent retrospective cohort study in which a group of preterm infants with diagnosis of stage 2 ROP received oxygen supply in order to increase the SpO_2 over 97%; as a result, the progression of ROP to stage 3 was significantly reduced without an increased pulmonary morbidity [10]. Considering the numerous evidences demonstrating the role played by the different levels of SpO2 in survival rates and/or in the development of disabling diseases, a series of large, multicenter, randomized, control trials were executed to establish the ideal saturation target for preterm infants. The SUPPORT trial evaluated the clinical consequences of two different levels of SpO_2 (85-89% versus 91-95%). The study showed that the highest SpO2 levels were associated with a higher incidence of severe ROP, but with a lower death rate. Therefore, an increase in the incidence of ROP appeared an unavoidable consequence of targeting higher saturations to reduce mortality [11]. Also in the BOOST II trial the SpO_2 target 85-89% was associated with an increased risk of death if compared with the SpO2 target of 91-95%. Again, infants in the lower-target group for SpO_2 showed a reduced rate of ROP. Then, the study was stopped after the interim analysis showed an increased mortality in the lower SpO_2 arm at a corrected gestational age of 36 weeks [12]. The COT Study differed from the previous studies because differences neither in death nor in the rate of severe ROP were reported in the highest versus the lowest target saturation groups [13].

The meta-analysis of these five trials (known as NeOProM Collaboration) confirmed that the assignment to the lowest SpO_2 target range was associated with a higher risk of death and necrotizing enterocolitis, but a lower risk of developing a severe ROP. For every 1000 infants treated with a lower SpO_2 target, the average risk of death increased by 28% and of severe necrotizing enterocolitis by 23%, while the average risk of ROP treatment decreased by 40% [14]. Based on these data, the Committee on Fetus and Newborn of the American Academy of Pediatrics recommended that the ideal target range should settle between 90 and 95% [15], although there is no consensus regarding the optimal oxygen therapy, and the ideal SpO2 range for extremely low birth weight infants remains far from a definitive identification.

Recently, a study compared the incidence of severe ROP between a period preceding the SUPPORT study (when saturation was maintained at 85-92% for infants younger than 34 weeks of corrected gestational age and > 95% after the 34th week) and a period following the SUPPORT study (when saturation was kept at 90-95% constantly, regardless of age). This study revealed an increase in any ROP overall in the post-SUPPORT cohort, without difference in mortality, suggesting that a biphasic strategy might represent an intelligent approach in treating ROP, likely because it mirrors the 2 phases of the disease [16].

2.2 Pharmacological interventions

2.2.1 Erythropoietin

Erythropoietin (Epo) is a glycoprotein hormone, expressed in liver during fetal life, and then in kidney after birth. It is highly responsive to hypoxia through the hypoxia-inducible factor (HIF)-1 pathway, and is a main regulator of red blood cell production. Epo also has non-hematopoietic properties that includes enhancement of angiogenesis, similar to VEGF. Since the late 1980s

recombinant human Epo has been used to prevent anemia of prematurity in very low birth weight. Some observational and retrospective studies have reported a possible association between Epo use and an increased risk of ROP, related to the cumulative dose [17], or the timing of treatment, if started after 20 days of age [18]. However, these results were contradicted by other studies, where Epo was administered during the first week of life or after 2 weeks of life [19], even after the administration of a very high dose within the first 42 hours of life [20]. Contradictory results also emerged from meta-analyses, some demonstrating a significant increase in the risk of stage ≥3 ROP in the Epo group [21], other excluding a relationship between Epo and ROP [22].

2.2.2 Caffeine

Caffeine, is a methylxanthine that antagonizes both peripheral and central adenosine A1 and A2A receptors. Its main activity is to stimulate the medullary respiratory centers increasing the sensitivity to carbon dioxide and oxygen, and is currently considered the first-choice drug for the treatment of apneas of prematurity. Caffeine is often used in the neonatal intensive care units to facilitate extubation, and it has been demonstrated to reduce the duration of mechanical ventilation and the risk to develop bronchopulmonary dysplasia [23]. The Caffeine for Apnea of Prematurity (CAP) trial confirmed its efficacy against apneas, and the follow-up at 18-21 months showed a reduced incidence of severe ROP in the caffeine-treated group [24]. However, some adverse events have been reported in preterm newborns, such as tremors, tachycardia, tonic-clonic seizures, gastro-oesophageal reflux, vomiting or metabolic disorders such as hyperglycemia, hypokalemia, or jaundice [25]. A recent animal study demonstrated that caffeine attenuated not only the hypoxia-induced angiogenesis, but also hyperoxia-induced vaso-obliteration [26]. However, the effectiveness of a caffeine treatment in preventing ROP has not received unanimous consensus [27], thus making further studies necessary.

2.2.3 Antioxidants

The exposition of preterm infants to oxygen levels significantly higher than during intrauterine life favors the production of reactive oxygen species (ROS). ROS are highly reactive chemical molecules that react with lipids to initiate lipid peroxidation and DNA damage, and their role in ROP is well demonstrated [28]. Preterm newborns may be more susceptible to ROS-mediated damage due to a relative lack of antioxidants at birth. Therefore, many investigators tested the hypothesis that antioxidants may be beneficial for treatment and/or prevention of ROP.

2.2.3.1 Vitamin E

Studies performed about 50 years ago demonstrated that vitamin E supplementation was associated with a decrease in incidence and severity of ROP [29]. A first randomized trial confirmed a lower incidence, but not progression, of ROP in very-low birth weight-treated infants. However, this study showed an increased incidence of side effects among vitamin E-treated infants, such as sepsis and late-onset necrotizing enterocolitis [30]. Additional side effects, such as retinal hemorrhages [31] or grades 3-4 intraventricular hemorrhage [32], were reported after the supplementation of vitamin E. A meta-analysis concluded that, regardless of adverse events, vitamin E supplementation reduced the risk of severe ROP, even though the risk of sepsis was significantly increased [24]. For this reason, the use of vitamin E has been discontinued.

2.2.3.2 Vitamin A

Retinoic acid (vitamin A) is essential for vision, as it participates in the synthesis of the ocular light absorbing pigments. Extremely preterm infants have low levels of vitamin A because of the reduced transplacental transport from their mothers, inadequate intake from enteral feeding for several weeks after birth, and poor gastrointestinal absorption. A possible beneficial role of vitamin A in counteracting ROP has been studied using the mouse model of oxygen-induced

retinopathy, a model that, mirroring both the hyperoxic and the hypoxic phases that characterize human ROP, is widely used as a surrogate model for this disease [33]. Studies performed in this model demonstrated that vitamin A administration during the hyperoxic phase could increase endogenous VEGF production able to counteract the vaso-obliteration during the first phase of oxygen-induced retinopathy [34]. Some small clinical trials evaluating a beneficial role of vitamin A in decreasing the incidence of ROP yielded conflicting results. Indeed, a trial performed to evaluate the efficacy of vitamin A in preventing bronchopulmonary dysplasia in extremely low birthweight infants, having the evaluation of the effectiveness of vitamin A on ROP development as a secondary outcome, showed no difference in ROP incidence between vitamin A-treated and untreated infants [35]. However, a recent trial, whose primary outcome was the assessment of efficacy and safety of early vitamin A supplementation in extremely preterm infants, demonstrated that oral vitamin A was safe and decreased the incidence of ROP, suggesting that preterm newborns at high risk for ROP may take advantage from vitamin A supplementation [36]. In this respect, a meta-analysis evaluating the results of 4 clinical trials concluded that the odds of any ROP was significantly reduced when infants received vitamin A supplementation [37]. In conclusion, vitamin A supplementation appears promising, but further trials involving a larger number of patients are required to confirm the benefits of vitamin A in reducing ROP incidence.

2.2.3.3 Other antioxidants

In a first prospective controlled trial D-penicillamine, an antioxidant able to inhibit the effect of oxygen, appeared to be effective in reducing ROP incidence without serious adverse effects [38]. However, a meta-analysis did not confirm this positive effect of D-penicillamine [39].

Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of the extremely toxic superoxide radical into molecular oxygen and hydrogen peroxide. Its protective effect in reducing

the progression of oxygen-induced retinopathy was not confirmed by a multicenter clinical trial performed to evaluate the effect of intratracheal recombinant human SOD to prevent bronchopulmonary dysplasia [40]. However, an additional study suggested that in very low preterm infants the severity of ROP could be reduced by SOD administration [41].

In a series of clinical trials, lutein and its isomer zeaxanthin, two carotenoids with antioxidant effect in newborn infants, were shown to be unable to reduce ROP progression in preterm newborns [42,43]. A recent meta-analysis confirmed such results [44].

Allopurinol is a synthetic inhibitor of xanthine oxidase, an enzyme actively involved in ROS generation following hypoxia/ischaemia. The only randomized, controlled, clinical trial failed to demonstrate benefit in the group of ROP patients that received allopurinol. [45].

2.2.4 Nonsteroidal anti-inflammatory drugs (NSAIDs)

From the last years of the last century the hypothesis that NSAIDs could mitigate ROP development has been considered the basis of the possible role of inflammatory processes in ROP pathogenesis. This hypothesis was confirmed by first studies using the mouse model of oxygen-induced retinopathy [46] and by a little retrospective chart review [47]. On the other hand, indomethacin, an NSAID inhibiting prostaglandin synthase, has been reported to increase the risk of developing ROP [48]. It is possible that these contradictory effects may be related to the timing of drug administration. Indeed, precocious administration of indomethacin may induce vaso-constriction of retinal vessels, thus increasing ischemia in the first phase of ROP and, consequently, accentuating the subsequent proliferative phase [49]. Therefore, the exact timing of treatment may be crucial. Topical ketorolac, for example, appeared to reduce ROP progression without significant adverse side effects when administered after ROP diagnosis [50].

2.2.5 Steroids

While there is substantial consensus regarding the benefits of antenatal steroid administration in reducing neonatal morbidity and mortality in preterm births as well as in preventing ROP development and progression [51], results on the postnatal use are controversial. Some studies reported either no effect [52] or a reduced risk of developing ROP [53], but more numerous were the studies in which the postnatal administration of steroids appeared to be related to a greater progression of the disease [54, 55]. A recent meta-analysis demonstrated that steroids, when used early (within the first week of life) reduced the risk of ROP, including severe ROP [56], while when used later (after the first week of life) increased this risk [57]. The use of postnatal steroids in the first week of life raised many concerns about their safety in infants. In fact, gastrointestinal bleeding and intestinal perforation were important adverse effects, but also the risks of hyperglycemia, hypertension, hypertrophic cardiomyopathy, and growth failure increased. Moreover, long-term follow-up show an increased risk of abnormal neurological development and cerebral palsy [56]. Therefore, the benefits of early postnatal corticosteroid treatment do not outweigh the risk of associated adverse effects.

2.2.6 Insulin-like growth factor-1 (IGF-1)/Insulin-like growth factor binding protein 3 (IGFBP3)

IGF-1 plays an important role in fetal development influencing endothelial cell growth and angiogenesis, and is actively involved in ROP pathogenesis [58]. Longitudinal studies have reported that the deficiency of IGF-1 at birth in extremely preterm infants is positively associated with an increased risk of ROP [59]. Therefore, the hypothesis that a supplementation of extremely preterm infants with recombinant human IGF-1 complexed with its binding protein IGFBP-3 could reduce ROP development was evaluated in a prospective trial after the demonstration of its safety.

Unfortunately, the continuous intravenous infusion from the first day of life to 30 weeks of postmenstrual age did not affect ROP development [60].

2.2.7 Anti-VEGF drugs

VEGF has been found to play a major role in angiogenesis-driven ocular diseases and its inhibition through intravitreal injection of VEGF-sequestering drugs (mainly anti-VEGF antibodies or anti-VEGF traps) has been demonstrated to be effective in the treatment of some of these diseases, including neovascular age related macular degeneration, diabetic macular edema, and retinal vein occlusion. Having VEGF a main role in the pathogenesis of ROP, this disease has represented an attractive target for the use of anti-VEGF drugs. However, the use of anti-VEGF drugs in preterm infants may be more problematic than in adults. In fact, in preterm infants organogenesis is not fully completed and VEGF plays a major role in the normal development of several organs, including kidneys, lungs and brain in which angiogenesis is still active at the time when treatment with anti-VEGF drugs should initiate. Many infants with ROP have additional early morbidities such as bronchopulmonary dysplasia and the presence of these co-morbidities may confound the ability to detect an effect of anti-VEGF therapies on pulmonary function or neurodevelopment. Presently, the consequence of lowering systemic VEGF during organ development is not clear but this practice raises several concerns. On the other hand, a recent retrospective cohort study comparing respiratory outcomes in infants with ROP treated with laser therapy or with intravitreal anti-VEGF demonstrated that infants treated with anti-VEGF returned to their respiratory baseline faster than infants treated with laser suggesting that not only anti-VEGF did not affect pulmonary function but might have positive effect in recovering the respiratory status [61].

The first anti-VEGF drug used to treat ROP, and now the most widely used anti-VEGF drug in the treatment of severe ROP, was bevacizumab, a recombinant humanized antibody directed to VEGF

[62]. The BEAT-ROP study [63] investigated the effectiveness of bevacizumab treatment for zone I or posterior zone II stage 3+ ROP compared to standard laser photocoagulation. While 22% of the eyes treated with laser photocoagulation required retreatment, only 4% of the eyes receiving bevacizumab needed to be retreated, with an effect that was statistically significant for zone I, but not for zone II, ROP. Unfortunately, the trial was not sufficiently powered to evaluate systemic toxicity. Subsequent studies have assessed the safety of intravitreal bevacizumab demonstrating both retinal and systemic adverse effects [64]. In particular, bevacizumab moves from the eye to the systemic circulation, abnegating serum VEGF levels for up to 60 days after intravitreal administration [65]. Studies with ranibizumab, a truncated form of bevacizumab that has a shorter half-life and a potentially decreased systemic toxicity, has still demonstrated the presence of adverse side effects [66]. In contrast, the RAINBOW study, a multicenter clinical trial evaluating the efficacy and safety of intravitreal ranibizumab compared with laser therapy in the treatment of ROP, concluded that ranibizumab was as effective and safe in the treatment of active ROP as laser therapy [67]. Considering these inconclusive results, further randomized, controlled multicenter clinical trials are required to evaluate the safety profile of anti-VEGF drugs in the vulnerable cohort of ROP patients.

2.2.8 Propranolol

Propranolol is a non-selective beta adrenoceptor (ß-AR) blocker targeting ß1- and ß2-ARs and, besides its use in treating heart problems, it is a valuable and effective treatment option for proliferating infantile hemangiomas, the most common tumors affecting infants [68]. It is likely that this effect of propranolol is mediated by reduction of VEGF levels, which play a major role in hemangioma progression [69]. Based on this evidence, the effects of propranolol, either systemically delivered or used as eye drops, have been assessed in the mouse model of oxygen-

induced retinopathy, demonstrating that propranolol abrogates the hypoxia-induced upregulation of VEGF and reduces retinal neovascularization [70-72]. Of note, in the mouse model propranolol does not decrease VEGF below its control levels and it does not affect VEGF levels in the retina of control mice, suggesting that treatments based on beta blockers do not impact on VEGF physiologic levels. In addition, VEGF levels are not affected by propranolol in organs such as brain, heart and lungs. Additional studies performed in the mouse model of oxygen-induced retinopathy have also suggested that, among ß-ARs, ß2-ARs are likely to be the preferential targets of propranolol [73].

Based on preclinical results, a pilot randomized controlled clinical trial with oral propranolol at doses up to 2 mg/kg/day, the dose used to treat infantile hemangiomas, was performed in preterm newborns with stage 2 ROP [74,75]. This trial demonstrated that oral propranolol is effective in reducing ROP progression, as also confirmed by other studies [76-79]. However, although effective, oral propranolol aroused safety concerns in 5 newborns out of 26 treated with the drug [74].

Since in the mouse model of oxygen-induced retinopathy propranolol eye drops were demonstrated to be as effective as systemic propranolol in reducing retinal neovascularization [71], a second clinical study, an open-label trial, evaluated safety and efficacy of 0.1% propranolol eye drops in newborns with stage 2 ROP [80]. Results of this study demonstrated that propranolol is well tolerated although it was ineffective in reducing ROP progression. The plasma levels of propranolol were found to be about one order of magnitude lower than after oral administration and lower than 20 ng/mL, which is presently considered a safe cut-off value based on the evidence that serious adverse effects were observed in newborns treated with oral propranolol showing plasma propranolol concentration above 20 ng/mL [74]. This suggested that topical propranolol preparations may provide the retina with an adequate drug amount while avoiding the risks of

adverse side effects observed after oral propranolol. Based on this evidence, an additional open-label clinical trial evaluated safety and efficacy of 0.2% propranolol eye drops in newborns with stage 1 ROP [81,82]. This study demonstrated that this propranolol formulation is both safe and effective in reducing ROP progression. Overall, these clinical studies point to topical application of propranolol as an effective therapeutic approach to counteract ROP progression. This perspective needs a definitive assessment in randomized placebo-controlled clinical trials.

2.3. Nutritional interventions

2.3.1 Omega-3 polyunsaturated fatty acids (PUFAs)

With a premature birth, the mother-to-fetus placental passage of long-chain polyunsaturated fatty acids (LC-PUFAs), the structural and functional components of the phospholipid bilayer of cell membranes, is brusquely interrupted. Docosahexaenoic acid (DHA; 22:6 ω-3) and arachidonic acid (20:4 ω-6) are the most abundant LC-PUFAs in the central nervous system and in extremely preterm infants receiving standard care it is frequent to find low serum levels of these fatty acids. The attention of research in preterm infants has been focused mainly on the role of ω-3 LC-PUFAs, and in particular on DHA considering its role in promoting survival and differentiation of retinal photoreceptors during development [83]. In 2008, a first meta-analysis found that LC-PUFA supplementation did not result in a reduction of ROP incidence [84]. Subsequent studies demonstrated that newborns feed from the first day of life with an emulsion of soybean, olive oil, and fish oil (containing DHA) showed a significant lower incidence of severe ROP if compared with a historical population [85]. This retrospective study paved the way for two prospective trials that in which preterm infants fed with a parenteral fat emulsion containing fish oil developed less severe ROP, without significant adverse effects and with reduction of parenteral-related

cholestasis [86] or hypoglycemia [87]. In contrast, another prospective trial failed to find any reduction in ROP incidence [88].

2.3.2 Inositol

Inositol is a six-carbon sugar derivative actively produced by fetus, and detected in human breast milk, particularly colostrum, suggesting an important role in growth. Two little prospective studies planned to verify the effect of inositol supplementation in respiratory distress syndrome incidence, observed a lower incidence of ROP in infants receiving inositol [89]. These results were later confirmed by a study in which infants receiving inositol exhibited a lower incidence of severe ROP [90] and by a meta-analysis [22]. A recently published large multicenter randomized clinical trial did not confirm these data, but it reported that inositol administration resulted in a high incidence of serious adverse events, including necrotizing enterocolitis, hypotension, intraventricular hemorrhage, systemic infection, and respiratory distress [91]. An additional meta-analysis confirmed no benefits of inositol supplementation, while, on the other hand, it reported a trend toward an increase in mortality [92].

3. Conclusion

ROP is a preventable neovascular retinal disease with a great impact on vision and in ROP-related ocular morbidities laser ablation of newly formed, pathologic vessels represents the established treatment. A meta-analysis has estimated that in 2010 more than 180,000 preterm newborns per year developed ROP worldwide and about 30% of them developed severe, potentially vision-impairing diseases requiring treatment [93]. The main causes of ROP are related to immaturity of retinal vessels due to preterm birth and to oxygen therapy triggering the development of a

hyperoxic retinal environment. In this dysregulated environment, the upregulation of many proangiogenic factors, including VEGF, is likely to contribute to the risk of ROP development and to the severity of the disease. This indicates that ROP is a multifactorial disease in which a plethora of molecules represent possible targets for pharmacologic interventions. The pharmacologic therapies that are attempted to overcome the use of surgical interventions remain controversial and in some cases caused severe adverse side effects. This is a clear indication that possible changes in clinical practice need a solid background coming from well-structured clinical trials as well as from long-term follow up studies to evaluate fully the real potential of new drugs to prevent ROP progression.

4. Expert Opinion

Presently, there are no approved drugs for the specific treatment of newborns suffering from ROP, and the standard treatment for the advanced form of the disease relies on surgical approaches such as laser therapy or cryotherapy. However, surgical approaches are not without side effects; for instance, they save vision in most of the visual field but not in the periphery. The need of general anesthesia may represent an additional concern in preterm newborns. The limitations affecting surgical interventions and the increasing knowledge of ROP pathogenesis have encouraged investigations into pharmacologic therapies. In this respect, for several years drugs approved for different purposes have been used off target in combination with surgical approaches or as an alternative to them. This is the case of anti-VEGF therapies that are widely used to treat severe ROP. This class of drugs, when compared to the classical surgical approaches, has represented a seminal step forward in the treatment of neovascular retinopathies. However, limitations of the use of anti-VEGF drugs in patients suffering from ROP are particularly evident.

For instance, they may interfere with neuronal survival as VEGF is endowed with a neuroprotective activity and its partial or total deletion in the retina may alter the equilibrium of the neurovascular unit, resulting in retinal damage. In addition, diffusion of anti-VEGF drugs into the blood may suppress serum VEGF levels thus interfering with physiological angiogenesis or, more generally, with the development in target tissues. On the other hand, the optimal serum level of VEGF in preterm infants is unknown. In addition, although anti-VEGF has been used in ROP patients for over a decade, no studies have demonstrated that decreasing systemic VEGF levels results in damages to developing tissues or organs. Even, the RAINBOW study provided results indicating that 7 days after treatment the decrease in plasma VEGF after ranibizumab is similar to that after laser photocoagulation, without any evidence of deleterious effects on tissue development [67], as may be expected by the fact that laser treatments to the retina have never been shown negative effects in non-ocular tissues. Overall, if anti-VEGF may have negative systemic effects, these effects should be small.

Some of the concerns raised by the use of anti-VEGF may be overcome by drugs acting upstream of VEGF and targeting pathways modulating VEGF production. In this respect, propranolol is a promising drug. It needs to be more profoundly assayed in order to validate its safety profile in preterm newborns, but it appears to be very promising. Indeed, first, it is effective in preventing ROP progression when topically administered, thus avoiding the need of intravitreal injections and their related side effects. Second, in the retina propranolol does not deplete VEGF levels but it normalizes them to control levels, thus suggesting no side effects of the drug affecting the neurovascular unit. Third, in the mouse model of oxygen-induced retinopathy propranolol does not affect VEGF levels in organs other than the retina, thus suggesting that propranolol may have a safe systemic profile towards developing organs and tissues.

A major limitation of most of the pharmacologic approaches described above is that they target the second phase of ROP, when the increased production of proangiogenic factors driven by hypoxia triggers pathologic angiogenesis. This means that preterm newborns with ROP are treated once the disease has progressed, therefore the potential benefit of drugs such as anti-VEGF, propranolol and others are often smaller than expected. The possibility of intervening early during the disease, possibly before ROP becomes manifest, might be a significant improvement in the pharmacologic approach to ROP. The paradigm of ROP treatment should be moved from a curative approach (treatment during the second phase) to a preventive approach (treatment during the first phase). In this respect, preclinical studies have evidenced the effectiveness of the preventive approaches. For instance, this is the case of systemic administrations of retinoic acid in the mouse model of oxygen-induced retinopathy during the hyperoxic phase, which corresponds to the first phase of ROP [34]. As a consequence of retinoic acid administration, VEGF levels were stabilized during the hyperoxic phase thus preventing VEGF upregulation and retinal neovascularization during the hypoxic phase, which corresponds to the second phase of ROP. Similar results have been obtained with the administration of 17ß-estradiol [94]. Adenosine A_{2A} receptor antagonists seem to act with a different mechanisms, since their administration during the hyperoxic phase prevents retinal neovascularization likely by reducing the formation of reactive oxygen species [95]. Whatever the mechanism, these preventive approaches to ROP are based on the biphasic nature of the disease. The more pronounced is vaso-obliteration during the first, hyperoxic phase, the greater is the angiogenic drive during the second, hypoxic phase; therefore, reducing vaso-obliteration during the first phase is likely to be a strategy to decrease neovascularization (and the consequent visual damages) during the second phase. The potential of this approach (reducing the strength of the hyperoxic phase to decrease the impact of the disease) may be appreciated also in retinal diseases different from ROP. For instance, in a mouse model of

retinitis pigmentosa, the stabilization of HIF-1 (and the consequent normalization of VEGF levels) during the early phase of the disease, when rod degeneration leads to a hyperoxic environment, slows down retinal degeneration and recovers retinal function [96,97]. Although further studies in animal models are required and the safe profile of drugs acting on the first phase of ROP needs to be adequately studied in randomized clinical trials, the results obtained so far are encouraging and suggest that preventive approaches to ROP could be effective in treating extremely low birth weight preterm infants before ROP becomes manifest.

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Papers of special note have been highlighted as:

** of interest*

*** of considerable interest*

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