

Wei-Chi Wu
Wai-Ching Lam
Editors

A Quick Guide to Pediatric Retina

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 Springer

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Preface

This textbook on pediatric retina is our effort to provide an overview of the state-of-the-art management for pediatric retina patients. We hope that it is both practical and easy to understand. In this 33 chapters, grouped into two major sections, we tried to cover the essence in the understanding, diagnosis, and management of pediatric retina diseases. We hope this book will be a handy guide for the ophthalmic surgeons at all levels, from the novice, the occasional caregiver for the pediatric retina patients, to the expert leaders in this field.

Our aim is not only to cover the medical aspects but also to explore the surgical advances of these diseases, as well as not only just the clinical presentations but also the basic theory and hypotheses of the diseases, such as understanding of the foveal development.

Pediatric retina is not only a challenging field but also a lonely one. It is a specialty that has received little attention in the past, but has been gaining traction recently. However, we all know how important this field is, because it affects the vision of newborns and growing children. As physicians, we also know how time-consuming and difficult it can be to deal with a child patient.

Recognizing the cliché “kids are not small adults,” the anatomy, pathophysiology, and management philosophy in kids are very different from those in adults. In addition, their visual system is still developing. Therefore, while handling these cases especially during surgery, the surgical settings, the instruments used, and the surgical goals are very dissimilar from those of adults. Without such knowledge, a doctor can actually do far more harm than good for this unique population of pediatric patients.

In the past, there were very few people focusing on work related to this topic. Most of us work “part-time” in this field, thinking of it as a burden and praying that each case is the last. There were very few surgical instruments available for these small children. There are barely any dedicated textbooks available. Relevant topics at conferences were also scant. As a result, the surgical outcomes have been unsatisfactory, due to a lack of dedicated doctors, proper instruments, proper training, and information. But gradually, with the efforts of pioneers such as Dr. Mary Elizabeth Hartnett on the pathophysiology of ROP using animal models [1–3], Dr. Michael Trese in the vitrectomy for Stage 4 and 5 ROP [4–6], Dr. William V Good in the work of early treatment of ROP [7, 8], and Dr. HA Mintz-Hittner on “Bevacizumab Eliminates the Angiogenic Threat” (BEAT ROP) [9], we have seen much improved understanding and outcomes in the recent management of ROP patients. Young doctors may feel encouraged by the improved outcomes of the patients. Access to knowledge and information about pediatric retina has made significant strides; there are an increasing number of panels and symposia related to pediatric retina at the major international conferences. The industry has also started to pay more attention to this field. For instance, the launching of vitrectomies for the pediatric population (e.g., 25 + Short, Alcon), specifically designed for tiny eyes with thick vitreous and a thin sclera, is making ROP surgery safer.

We would like to express our sincere appreciation for the huge efforts from these world-renowned experts in contributing to this special issue. We are extremely lucky to know these scholars, great teachers, and close friends. We feel like standing on the shoulder of many giants so we could see much further and learn so much faster with their help. Dr. Lam and I also owe a great debt of gratitude to the professional team from Springer, especially the project

coordinator, Miss Kripa Guruprasad. Without their professional assistance, it would be impossible to achieve this remarkable outcome.

There are not many reference textbooks on pediatric retina. We hope this will be the one that people love to read and reference. For the young ophthalmologists or those new in this field, we hope they could get a good picture of this special subspecialty with the help of these contributing authors. If our readers could pick up some useful points for their practice in pediatric retina, it will be our joy and honor to see the changes this book has brought about. Finally, we hope to see more and more devoted young doctors get inspired to “fall in love” with this field and act as an invigorating force to propel this specialty into a better future in the care of the children.

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Acknowledgments

When I was a resident in ophthalmology, I could never dream of the career path I had for the next two decades: to focus on the research in pediatric retina. More often, I pictured myself to be a wealthy local medical doctor practicing an easy ophthalmic clinic. At that time, when I had to see the ROP patients in the NICU, I always felt this was a job that is not very pleasant, even painful because nobody could give me good guidance. I was like being thrown to the sea before learning how to swim. I was panicking because of the lack of support and proper guidance. Later, I was lucky to perform a couple of cases of laser photocoagulation in the NICU with a senior doctor. I felt happy to see the very positive effect after laser treatment for the patients. Then I was “assigned” to become a doctor specialized in this field by my retina section chief, Prof. Chi-Chun Lai, at that time. That have dramatically changed my life. That was a turning point for me because I have never seen any surgery for ROP associated with retinal detachment, including vitrectomy or scleral buckling. How could I start from ground zero?

I know I could not learn this knowledge by myself. I had to find a great teacher to guide me. I have heard of Dr. Michael Trese, who started vitrectomy for these premature babies with severe ROP, a giant in this field. But I did not have any connection nor my colleagues. How could I approach him?

I handed in my CV to Dr. Trese at an ARVO meeting after a pediatric retina session. I tried to chat with him about my admiration for him and expressed my hope to learn from him. But then I did not hear back from him for a couple of months. During those time, I was thinking, perhaps they did not like me and I might have to find somewhere else. One day, I got an email from Dr. Kimberly Drenser, saying they had a research fellow spot for me and asked if I could take it? I was exuberant in saying yes! Thank God! Later, I realized that it was Prof. Lai who contacted Prof. Stanley Chang, the teacher of Prof. Lai, who had helped to reach out to Dr. Trese to land the position. I owed Prof. Lai and Chang for their generous support.

At Associated Retinal Consultants (ARC), I was extremely lucky to learn from Dr. Michael Trese, Dr. Antonio Capone, and Dr. Kimberly Drenser. Please forgive my subjectivity, I think they are the best teachers in pediatric retina you could think of on this planet. Besides, you could also learn the best adult retina management from Dr. George Williams, MD. I really felt I could reach the sky because I was standing on the shoulder of these giants! Thanks so much for my teachers’ teaching.

So, with their help and introduction, I was able to meet these world-class experts in this field. Most of them are the contributing authors of this book. I am extremely lucky to work with them and learn from their knowledge. From my close observation, the doctors who are interested in pediatric retina are all special people with a kind heart and mind.

Finally, I would like to thank my families for their unwavering support. My parents, my wife, and my son are my pillars. It is a bit selfish to say: with their support, I have the luxury to do a lot of projects, including this one, without needing to worry about the things at home. I am deeply indebted to them.

—Wei-Chi Wu

It was a couple of years ago, during one of the international meetings, I met up with Dr Wei-Chi Wu. I believe we were presenting at one of the pediatric retina sessions. He was all excited to share the news that Springer has approached him to prepare a book on pediatric retina. He asked me to join him to do that together. I agreed on the spot without any hesitation. We then embarked on this wonderful journey together. This has been a journey of learning and excitements. His tireless effort to follow up with each and every author and his unwavering determination have made this project from a dream to reality. I am so thankful to have this opportunity to work with Wei-Chi.

I also want to thank Springer Nature and especially the projector coordinator Ms. Kripa Guruprasad for their support and guidance.

Of course, we are not only grateful but very much in debt to all the contributing authors for sharing their passion of knowledge, insights, expertise, and their personal collection of great illustrative pictures....

My children, Dawn and Alexander, are my inspiration for working on this book. I am so fortunate to have two healthy children, who constantly remind me the importance of what we do toward the well-being of our pediatric patients and their parents.

Finally, I want to thank my wife who is my soulmate and support. She is always there by my side supporting my every decision whether sensible or not. She has made everything possible and worthwhile.

—Wai-Ching Lam

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Part I

ROP

The Pathophysiology of Retinopathy of Prematurity

1

M. Elizabeth Hartnett

Abstract

The pathophysiology of retinopathy of prematurity (ROP) is evolving as advances in prenatal care allow extremely premature infants to survive. In addition, throughout the world, there is variability in the presentations of ROP. In regions that lack resources to support prenatal and perinatal care, ROP develops in infants of older gestational ages and larger birth weights and has potentially different pathophysiologic factors. This chapter will review the pathophysiology of ROP and factors involved in the presentation of ROP based on what is known through clinical studies and from experimental studies using animal models of oxygen-induced retinopathy (OIR), which are critical to study molecular mechanisms and cell–cell interactions. Although the main areas involve clinical study, it is impossible to discuss pathophysiology without including evidence from the most representative animal models of oxygen-induced retinopathy (OIR) and other models adapted to study maternal–fetal interactions. Understanding the role of vascular endothelial growth factor (VEGF) in the physiology of retinal and vascular development and survival and the pathology in stage 3 and plus disease of ROP is important in discussing pathophysiology. Thoughts regarding areas of future study will be briefly described.

Keywords

Retinal angiogenesis · Pathophysiology of ROP · Vascular endothelial growth factor (VEGF) · Reactive oxygen · Oxidative stress · Erythropoietin · ROP phases · Angiogenesis · Oxygen fluctuations · IGF-1

1.1 Introduction

ROP occurs because of a delay in physiologic retinal vascular development (PRVD) and damage to newly developed retinal vasculature often from high supplemental oxygen or other oxygen stresses. The initial two-phased hypothesis proposed by Ashton described oxygen-induced damage to newly developed retinal capillaries, called vaso-oblivation in Phase I [1]. Infants who survived premature birth during Ashton's time were only about 2 months premature so their retinas and retinal vasculature were more developed than premature infants at risk of treatment-warranted ROP today, who are born 3 or more months premature. In addition, at the time of Ashton, little was known about the effects of oxygen. Later clinical trials by Patz and Kinsey provided evidence of the damage to newly formed capillaries from high oxygen at birth [2, 3]. Today, however, the original Phase I “vaso-oblivation” no longer is accurate. Rather Phase I is better described as compromised physiologic vascularity and delayed physiologic retinal vascular development (PRVD), and both increase avascular retina. Although only the peripheral avascular retina is appreciated clinically on retinal examinations with indirect ophthalmoscopy or with retinal imaging using contact fundus cameras (see also Chap. 8). The peripheral avascular retina is described by the International Classification of ROP by zones [4]. Avascular areas within already vascularized retina are noted as reduced capillary density in experimental models but likely to be undetected on most clinical evaluations unless fluorescein angiography is performed [5, 6]. Once an infant is removed from high supplemental oxygen, then avascular retinal areas

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become hypoxic and cells within the retina are stimulated to express angiogenic growth factors. However, instead of causing angiogenesis to grow into the retina, it grows into the vitreous. This Phase II, vasoproliferation, leads to common causes of vision loss from ensuing retinal detachment or dragging of the retina.

The original two-phased hypothesis is based on animal models of oxygen-induced retinopathy (OIR). However, the current clinical stages of ROP (Fig. 1.1) have necessitated that the original hypothesis be refined (Fig. 1.2) [7, 8]. No longer is high oxygen at birth the main cause of ROP, but rather it is recognized that other stresses, including fluctuations in oxygenation are important [9]. Animal models will be covered extensively in Chap. 2. It must be emphasized that the most representative models of ROP are OIR models in the rat and beagle, but these species require newer techniques to study molecular mechanisms. The mouse OIR model may not be as representative of the oxygen stresses or of common presentations of ROP but it provides greater ease in testing molecular mechanisms through the use of knockout or transgenic mice. Nonetheless, all animal models share the limitation of using full-term newborn animals that develop retinal vasculature after birth. Therefore, as with any translational study of human pathophysiology, it is necessary to integrate evidence from multiple sources when developing a hypothesis.

In humans, there is a third phase of fibrovascular retinal detachment (Fig. 1.1) that is not well modeled in most animals (Fig. 1.2). Most treatment strategies have been to treat treatment-warranted, vascularly active ROP with stage 3 and plus disease [10, 11] to prevent retinal detachment, clinically known as stages 4 and 5 ROP. However, there are other causes of vision loss in premature infants beside retinal detachment. As premature infants survive at younger gestational ages, the vascular beds of the choroid or retina and retinal neurons and glia are incompletely developed [12]. Neural connections within the retina are also incomplete, and the interactions among neural, glial, and vascular cells and signaling cascades can affect physiologic function. Infants born at gestational ages below 28 weeks who never developed ROP have been shown to have reduced ERG functions at age 6 [13, 14], suggesting that extremely preterm birth itself affects the retina and visual function. Finally, prematurity can also cause cortical visual impairment, which broadly includes connections through the brain to the occipital cortex and can affect visual acuity and function.

Genetic studies have not found any variant associated with severe ROP, but many studies have been regional and studies have had small sample sizes [15, 16]. Infant characteristics also vary across studies and this can affect outcomes. If infants enrolled have already survived extreme premature birth, genetic variants associated with severe ROP in less

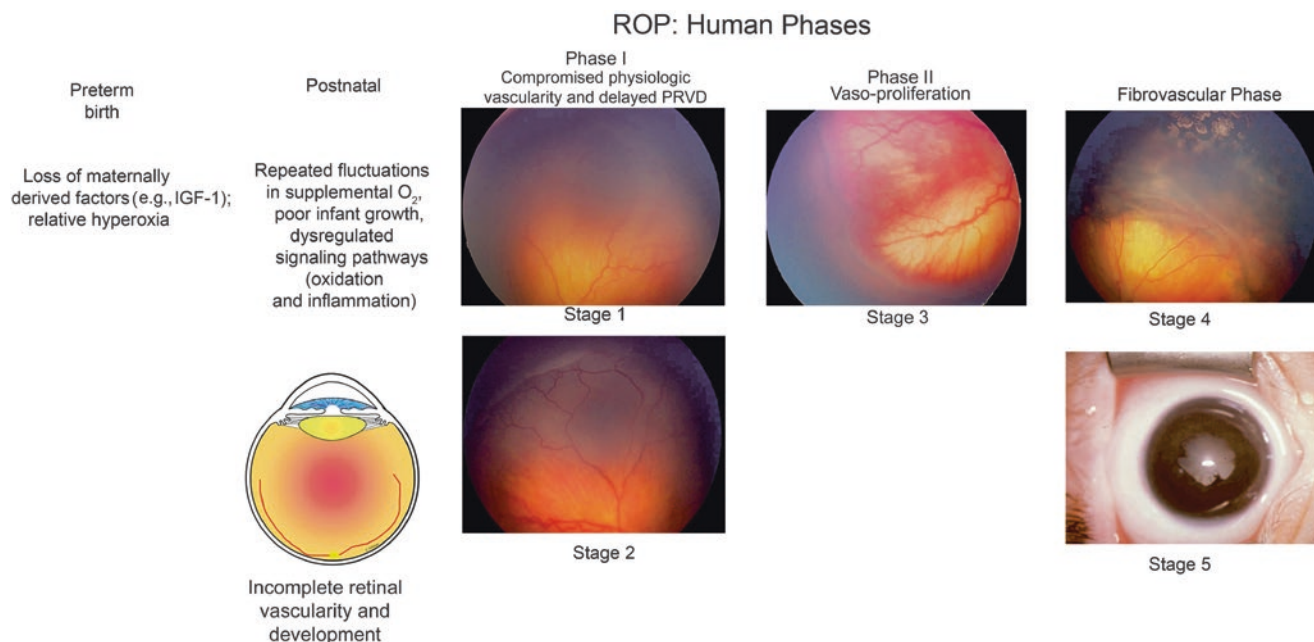


Fig. 1.1 ROP is characterized by zone of vascular development and the stage of disease. There are five stages. The stages relate to phases of OIR. Stages 1 and 2 ROP relate to Phase I OIR with compromised physiologic vascularity and delayed physiologic retinal vascular development (delayed PRVD). Stage 3 ROP relates to Phase II OIR with vasoproliferation in the form of intravitreal neovascularization, clinically known as Stage 3 ROP. Stage 1 has a line between the vascular-

ized and avascular retina. Stage 2 has a thickened ridge. Stage 3 has intravitreal neovascularization and Stage 4 has a partial retinal detachment. Stage 5 is a total retinal detachment and may appear like a white pupil in an infant eye (not shown). Drawing by James Gilman CRA, FOPS. Adapted from Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. *Ophthalmology* 2015;122:200–210, Figure 1

Phases of Oxygen-induced Retinopathy (OIR)

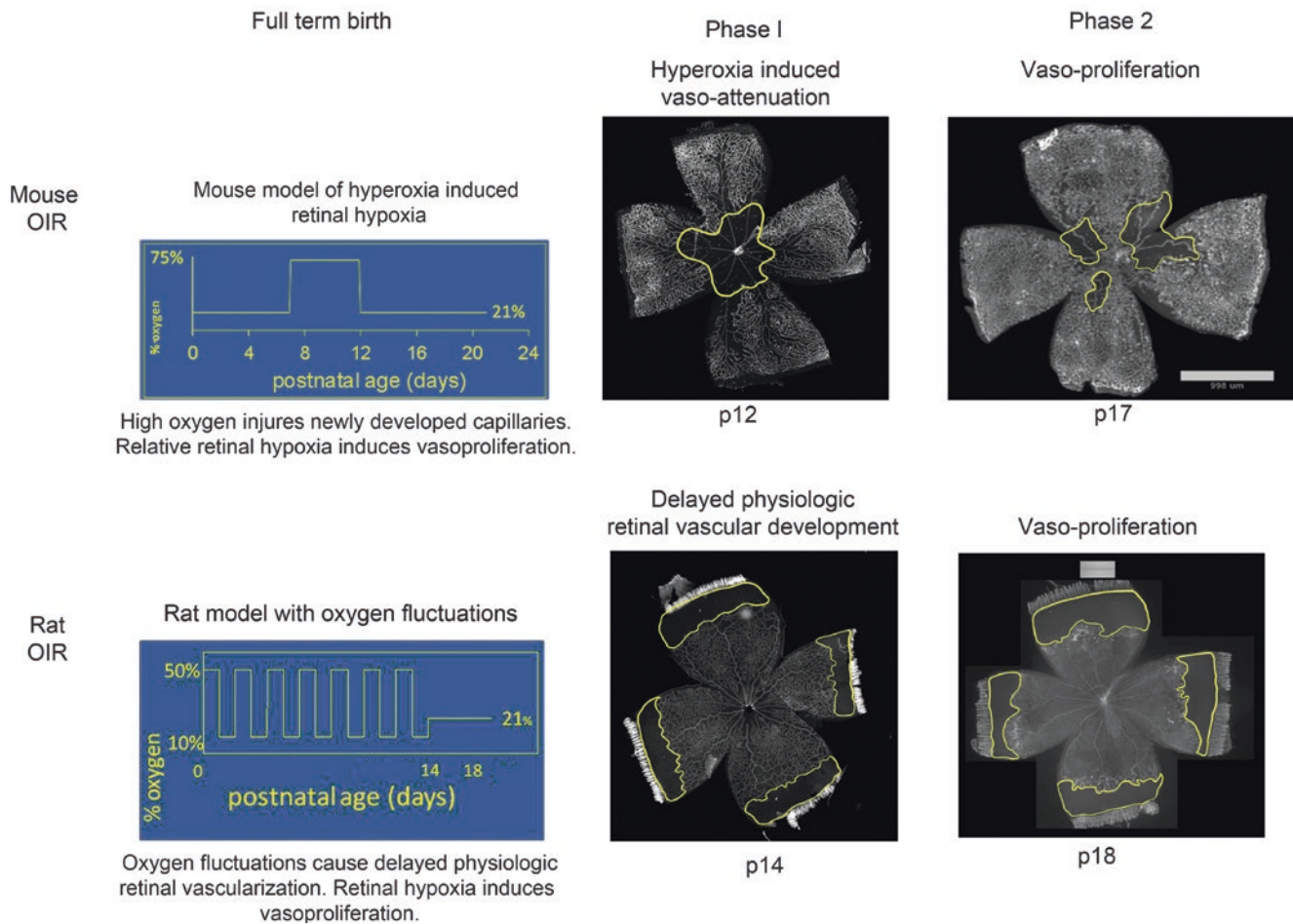


Fig. 1.2 The original two-phase hypothesis of ROP was based on animal models of oxygen-induced retinopathy OIR in kittens. The most often used models today are in mouse and rat. The rat OIR model is more representative of the pathophysiology of human. The mouse OIR model is easier to test genetic mechanisms involved in high oxygen-induced injury to newly formed capillaries. Phase I and Phase II are

shown for both OIR models. Yellow lines depict avascular nonperfused retina in Phases I and II OIR. Drawing by James Gilman CRA, FOPS. Adapted from Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. *Ophthalmology* 2015;122:200–210, Figure 2

hardy infants may not be present and, therefore, not be detected. One example from the Neonatal Research Network in the US tested blood spots from infants born <1000 g and discovered variants in brain-derived neurotrophic factor (BDNF) associated with severe ROP. Other studies in larger and older premature infants did not find *BDNF* variants involved. On the other hand, several factors have been reported associated with ROP in the Wnt signaling pathway [17, 18]. Such variants can also cause the variably expressed condition, familial exudative vitreoretinopathy (FEVR). FEVR is classically described as occurring in full-term infants but there can be overlap with ROP if infants with FEVR are born prematurely [19]. Evidence suggests that premature infants with known Wnt variants should be monitored for severe forms of ROP [18].

1.2 Pathophysiology Associated with Reduced Vascularity in Phase I and Events Leading into Phase II

Early clinical trials provided evidence that high oxygen at birth was associated with the loss of newly developed capillaries. Since then, efforts are made to avoid high oxygen at birth. However, in regions with insufficient resources to regulate or monitor oxygen, high oxygen-induced retinopathy has been reported [20].

There are maternal–fetal interactions that are believed to affect ROP. Infants at risk of developing ROP are usually born before the third trimester of gestation when maternal factors are transferred through the placenta to the fetus. Therefore, the premature infant has no benefit of maternally

derived factors and is often unable to produce them (Fig. 1.1). In addition, the process of premature birth is associated with the generation of oxidative compounds, but the premature infant has insufficient ability to quench reactive oxygen species. Generation of reactive oxygen species, particularly through the enzyme, NADPH oxidase, has been associated with vascular cell death in experimental models and in larger areas of peripheral avascular retina in Phase I [21]. Antioxidant treatment in experimental OIR models have reduced avascular retina [22, 23]. However, clinical studies have not shown a reduction in ROP, rather it has been associated with increased risks of sepsis in the use of exogenous vitamin E [24]. Deficiency of some compounds are believed to be involved in the pathophysiology of Phase I by not being available to promote angiogenesis or tissue protection, including insulin-like growth factor 1 [25], omega-3 fatty acids [26], erythropoietin (EPO) [27], lutein [28], and hypoxia-inducible factors [29] (Fig. 1.3). Studies are ongoing to determine if these factors would promote PRVD or protect newly developed capillaries from high oxygen and other stresses. However, current clinical trials have not led to definitive recommendations [28, 30].

1.2.1 Postnatal Growth and IGF-1

Poor postnatal growth has been associated with lower zone and worse ROP, and poor postnatal growth has become a biomarker for ROP [31, 32]. However, it remains unknown if interventions to improve growth will affect the development of ROP. Infant growth has been linked to IGF-1 levels and ROP [33, 34]. A hypothesis was developed based on the findings that premature infant IGF-1 levels fall rapidly after birth and remain low compared to in utero levels (Fig. 1.1). IGF-1 has been experimentally shown to permit VEGF to be angiogenic and have protective mechanisms against oxygen-induced retinopathy [25, 35, 36]. A recent multicenter phase 2 clinical trial tested the effect from an infusion of IGF-1/IGF-BP3 versus standard care to reduce ROP but did not find a reduction in ROP or an increase in infant growth parameters [37]. However, only 28 of 61 infants achieved target goals of serum IGF-1 levels. Nonetheless, there was a reduction in severe levels of bronchopulmonary dysplasia in treated infants compared to those with standard care, and more studies may provide greater sample size, optimized drug delivery, and additional information.

1.2.2 Oxygen

Besides early oxygen studies [2, 3, 38], a number of studies have been done regionally testing varying oxygen levels. The Supplemental Therapeutic Oxygen for Prethreshold ROP

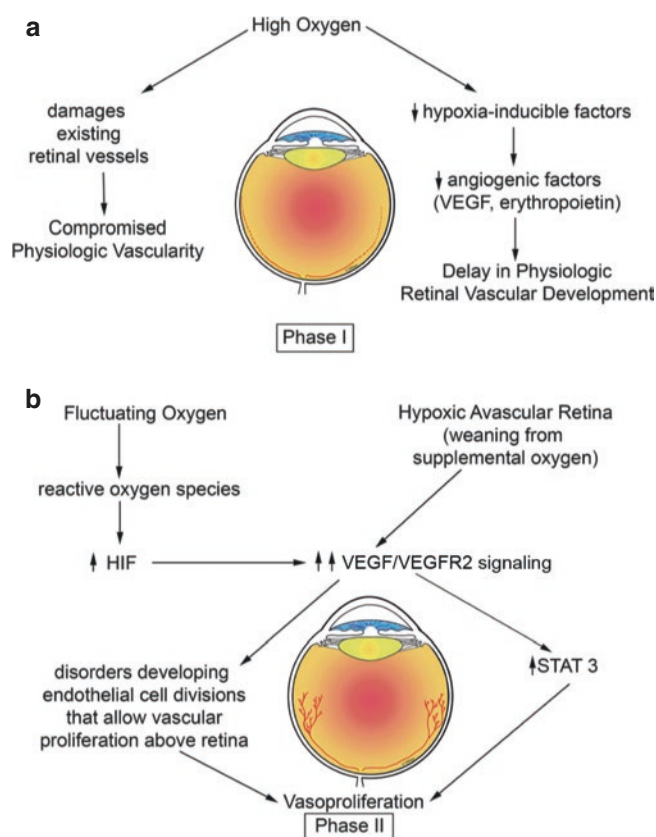


Fig. 1.3 Diagrams showing (a) the effects of high oxygen on damage of newly developed blood vessels and on reducing hypoxia-inducible factors, both of which lead to Phase I compromised physiologic vascularity and delay in physiologic retinal vascular development. Activation of VEGF/VEGFR2 disorders angiogenesis and interferes with PRVD in Phase I. (b) Fluctuations in oxygen that increase the generation of reactive oxygen species and lead to hypoxia-inducible factors while hypoxic avascular retina from Phase I can trigger VEGF expression when the infant is weaned from supplemental high oxygen. These events lead to vasoproliferation in Phase II. Activation of the transcription factor, STAT3, specifically in retinal endothelial cells by VEGF/VEGFR2 signaling contributes to vasoproliferation, but does not interfere with PRVD. Drawing by James Gilman CRA, FOPS

found no benefit or harm on the progression of prethreshold ROP in 96–99% oxygen saturations compared to 89–94% [39]. Several recent multicenter clinical trials have tested low oxygen saturation targets on the risk of ROP. The Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) and the Benefits of Oxygen Saturation Targeting II (BOOSTII) trials found a lower oxygenation target of 85–89% are associated with decreased ROP but also increased mortality compared to infants with a higher target of 91–95% [40, 41]. The Canadian Oxygen Trial did not find these associations [42].

Fluctuations in oxygenation have been associated with ROP clinically and in experimental models, most notably the rat OIR model [9]. Experimentally, there are several pathophysiologic events that occur in the retina in association with

oxygen fluctuations (Fig. 1.3). There is differential expression of VEGF splice variants, specifically greater expression of the prevalent VEGF164/165 variant that is most commonly found in pathologic angiogenesis in retinal diseases, compared to hypoxia alone [43]. There is also activation of the enzyme NADPH oxidase that generates ROS and triggers signaling events that lead to the death of vascular cells in Phase I [21] and to intravitreal neovascularization in Phase II [44]. In addition, intermittent hypoxemic episodes have been associated with treatment-warranted ROP [45, 46]. Some neonatologists recommend testing the effects of low oxygen saturation targets early followed by higher SaO₂ targets when ROP develops or at an older post-gestational age [47–49].

1.2.3 Light

The initial Light ROP study tested the effect of goggles to reduce ambient light to preterm infants upon birth on the risk of ROP, but there was no difference compared to control [50]. Experimental studies in mice reported that more light even through the abdomens of pregnant dams was important for physiologic intraretinal vascular development through a melanopsin dependent signaling pathway in the fetus [51]. Subsequent clinical studies have also suggested that higher average day length was associated with lower risk of severe ROP [52]. There is current interest in circadian studies on cycled light and the idea that infants might have better retinal vascular development and less severe ROP if kept on a circadian rhythm within neonatal nurseries, rather than be sheltered from light during daytime.

1.2.4 Erythropoietin and Role of Stress-Induced Factors in Infant

Erythropoietin has additional properties besides its role as a hematopoietic hormone, including in neuronal and tissue protection and angiogenesis [53]. The effect is believed to depend on the dimerization between the erythropoietin receptor (EPOR) and other receptors including the beta-common receptor (CD131) subunit for tissue-protective effects or the VEGFR2 receptor for angiogenesis [54]. EPO has been associated with the stabilization of vessels in Phase I but also with vascular activity in Phase II [27, 55]. Difficulty in experimental studies has been due to the inconsistency of EPO receptor (EPOR) antibodies that identify the location of the receptor in various tissues. Experimental studies are ongoing, but clinical studies have not identified the EPO promoter variant related to diabetic retinopathy to be present in infants with ROP [56]. Clinical trials testing supplemented EPO or derivatives for

protection have not resulted in clear recommendations regarding ROP [57]. The Preterm Erythropoietin Neuroprotection Trial (PENUT) did not increase risk of ROP. Experimental data has also led to the interesting premise that stress due to conditions surrounding prematurity may induce protective mechanisms that support vascularization of the retina during Phase I in certain animals [58]. Additional studies to identify such protective pathways are warranted.

1.3 Pathophysiology Related to Phase II

1.3.1 Vascular Endothelial Growth Factor Signaling

Aberrant angiogenesis manifesting as growth of blood vessels into the vitreous rather than into the retina is a hallmark of treatment-warranted ROP. Although a number of angiogenic factors are associated with aberrant angiogenesis, the one that has been studied the most and is likely a causative factor in treatment-warranted ROP is vascular endothelial growth factor (VEGF). VEGF is expressed as a result of hypoxia or reactive oxygen species, both stresses are associated with ROP, and there are feed-forward loops that can drive pathologic angiogenic signaling. VEGF-induced activation of receptor 2 (VEGFR2) causes disordered developmental growth of dividing endothelial cells allowing them to grow on top of one another into the vitreous rather than into the retina to extend vasculature to the ora serrata (Fig. 1.3). Experimental and clinical evidence has supported the hypothesis that overactive VEGF/VEGFR2 signaling causes intravitreal stage 3 and interferes with PRVD in ROP. (VEGF/VEGFR2 activation of the transcription factor, signal transducer, and activator of transcription 3 (STAT3) contributes to intravitreal neovascularization, but does not interfere with PRVD.) (Fig. 1.3) Neutralizing antibodies delivered into the vitreous that bind VEGF inhibited stage 3 ROP and extended physiologic retinal vascularization [59–61]. Additional experimental evidence using similar antibodies that bind rat VEGF in the rat OIR model has reduced the dilation of retinal veins and tortuosity of retinal arterioles, reducing plus disease [62]. Knockdown of VEGFR2 specifically in retinal endothelial cells using a novel gene therapy approach has reduced intravitreal neovascularization and extended physiologic retinal vascularization in the rat OIR model [63].

However, the molecular mechanisms and roles of other receptors are still being discerned as is the understanding of inhibition of VEGF ligand and its potential adverse effect to the developing stressed retina that has been found using experimental models [64]. In addition, neural guidance molecules and the role of glial, neural, and vascular interactions have been and are being studied [65].

1.3.2 Reactive Oxygen Species

Reactive oxygen species generated through the enzyme, NADPH oxidase, may affect Phase II through several signaling pathways [44, 63]. However, to date, the only interventions that have reduced vasoproliferation in Phase II are strategies to inhibit VEGF signaling.

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Animal Models of Retinopathy of Prematurity

2

Chi-Hsiu Liu and Jing Chen

Abstract

Retinopathy of prematurity (ROP) is a leading cause of blindness in preterm infants. It is a biphasic disease characterized by an initial phase of arrested vascular growth caused by hyperoxia exposure and loss of maternal–fetal interaction, followed by a second phase of hypoxia-induced neovascularization, which may lead to retinal detachment and vision loss. Animal models of ROP are crucial for studying the cellular and molecular mechanisms underlying ROP pathogenesis and for the development of potential therapeutics. Early animal models of oxygen-induced ROP were developed in the 1950s in multiple species including feline, canine, and murine, after the recognition of supplemental oxygen use as a risk factor. Refined mouse and rat models of oxygen-induced retinopathy were modernized in the 1990s with standardized oxygen protocols and improved methods for assessing retinopathy severity. More recently, murine models of hyperglycemia-associated neonatal retinopathy were developed to mimic neonatal hyperglycemia, a newly identified ROP risk factor. This chapter summarizes the basic concepts, advantages, and limitations of animal models of ROP, with a focus on the most widely used mouse model of oxygen-induced retinopathy.

Keywords

Retinopathy of prematurity · Animal models
Oxygen-induced retinopathy · Vaso-obliteration
Neovascularization

2.1 Introduction

Retinopathy of prematurity (ROP), a potentially blinding disorder caused by abnormal retinal blood vessel growth affecting premature infants, was first reported as “retrolental fibroplasia” by Theodore L. Terry during its epidemic in the 1940s [1]. In the 1930s and 1940s, oxygen administration became a common practice in neonatal care to improve the health of preterm infants with underdeveloped pulmonary function [2, 3]. The epidemic of ROP and ROP-associated blindness soon followed in the 1940s and early 1950s [4], which led to the recognition that unrestricted supplemental oxygen therapy was associated with ROP risk, as initially suggested by Kate Campbell [5] and confirmed by subsequent studies [6, 7]. This discovery of oxygen use as a key risk factor for ROP enabled early development of animal models of ROP in the 1950s. These models recapitulated the detrimental vascular effects of excess oxygen on retinal vascular growth in feline, canine, and murine pups, although inconsistencies remained. Modernization of murine ROP models in the 1990s [8, 9] standardized the protocols of oxygen exposure and methods for evaluation of ROP severity. Combined with the availability of genetically manipulated mice, these newer models greatly advanced research investigating the molecular basis of ROP pathogenesis and the development of potential therapeutics.

Decades of clinical and experimental ROP research have led to the consensus that ROP is a biphasic disease consisting of an initial phase of incomplete retinal vessel growth followed by a second phase of hypoxia-driven pathologic vessel proliferation [10–12]. Unlike full-term infants whose retinal vessels are almost completely developed, infants born prematurely have incompletely developed retinal vasculature with a peripheral avascular zone. After premature birth, the development of retinal vessels is delayed by the relatively hyperoxic postnatal room air environment (compared with in utero) and further worsened by the supplemental oxygen therapy. The first phase of ROP occurs between birth and approximately 30–32 weeks postmenstrual age. As the infant

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matures, the peripheral avascular retina becomes increasingly metabolically active, leading to tissue ischemia and hypoxia, which is often exacerbated by the termination of oxygen therapy [13–16]. Hypoxia stimulates upregulation of pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO), leading to uncontrolled vascular growth into the vitreous, occurring at the junction of avascularized and vascularized retina, and, in severe cases, tractional retinal detachment and blindness [10–12]. The second phase of ROP begins around 32–34 weeks postmenstrual age. Both ROP phases can be closely mimicked in the animal models of oxygen-induced retinopathy (OIR) to allow investigation on the mechanisms of vaso-obliteration, vascular regeneration, and pathological neovascularization processes.

Unlike humans, in whom retinal vessels develop fully before birth, in animal models of feline, canine, and rodent, retinal vessels develop and mature postnatally [16, 17]. Therefore, although these oxygen-induced ROP models reproduce the essential vascular features of ROP, they model only the oxygen influence without taking into account the prematurity itself, which is challenging to model in utero. Factors related to prematurity, such as essential fatty acids and insulin-like growth factors (provided in the third trimester in utero and often decreased or missing after premature birth), are commonly investigated experimentally using supplemental treatments in the OIR model [18, 19]. As additional risk factors (such as neonatal hyperglycemia, a common problem in extremely preterm infants) have been associated with severe ROP [20–23], new murine models of neonatal hyperglycemia-associated retinopathy [24, 25] have also been generated recently. This chapter mainly reviews the historical development of ROP models and focuses primarily on the most commonly used mouse and rat models [8, 9], with a brief discussion of hyperglycemia-associated neonatal retinopathy models [24, 25].

2.2 Experimental Models of ROP

The OIR models have been valuable tools with which to explore the role of oxygen and oxygen-related factors in the pathogenesis of ROP. These models were developed by exposing newborn experimental animals, including feline [26], murine [8, 9], canine [27–29], and piscine (fish) [30], to constantly high or cycling high and low oxygen environments. These animal models of OIR reproduce both the initial vaso-obliteration phase and subsequent ischemia- and hypoxia-induced neovascularization phase, resembling ROP in humans. Due to their simplicity in the induction of pathological neovessels and the ease of monitoring and quantification, the animal models of OIR are widely used for studying the mechanisms and evaluating new therapeutics for treating

the sight-threatening neovascularization in ROP and other ischemic proliferative retinopathies, albeit the consistency and reproducibility of neovascularization vary across different species.

2.2.1 Early Animal Models of OIR: The Feline and Canine Models

In light of the clinical observations linking oxygen with ROP, in the early 1950s Ashton et al. exposed kittens to 70–80% of oxygen for at least 4 days, causing retinal vascular closure and obliteration, then returned them to ambient air (21% oxygen), resulting in hypoxia-induced vaso-proliferation [26, 31]. This feline model of OIR is one of the earliest experimental models establishing the effects of altering oxygen concentration on retinal vascular development [16, 26]. The model successfully mimics the early stages of ROP closely and characterizes both the vaso-obliterative and the vaso-proliferative phases.

Attempts at using the canine OIR model for studying ROP were made at about the same time, in which the first model was developed by Patz's group, to determine the effects of oxygen on the immature retinal vasculature in puppies [29, 32], and modified in subsequent studies [27, 28, 33]. Beagle puppies were exposed to 95–100% of oxygen for 4 days to induce retinopathy. Similar to the kitten model, exposure to hyperoxia arrests the retinal vascular development, causing vaso-obliteration. After moving the puppies from hyperoxia back to room air, the resultant relative hypoxia leads to the vaso-proliferative stage [27, 34]. In addition, retinal detachment, which can be observed in the late, severe stage of human ROP, occurs in the canine but not feline or murine OIR model. Whereas feline and canine models are still occasionally used, achieving a statistically significant number of animals can be challenging. Usage of OIR models in mice and rats has been much more common, largely due to the standardization of the murine models and genetic manipulation in mice since the 1990s.

2.2.2 The Mouse Model of OIR

2.2.2.1 Postnatal Development of Mouse Retinal Vasculature

Mouse retinal vascular development follows similar morphological patterns as in humans [17, 35]. A major difference is the temporally delayed nature of retinal angiogenesis and neuron maturation in mice. In a human fetus, the retinal vasculature develops relatively early during the second trimester, whereas in mice it does not begin to develop until after birth [17, 36, 37]. Therefore, human full-term infants are born with mostly mature retinal vasculature while neona-

tal mice possess underdeveloped vasculature in the eyes in the first few postnatal weeks, allowing studies of physiological and pathological retinal angiogenesis. The development of the mouse retinal vasculature starts with sprouting from the optic nerve head, parallel to the regression of hyaloid vasculature. The hyaloid vasculature is a transient circula-

tory system providing oxygen and nutrient to the developing lens and inner retina as well as the primary vitreous in embryonic eyes [38–40]. During the first postnatal week, the retinal vessels expand radially, forming the superficial vascular plexus; the hyaloid vasculature starts to degenerate at the same time (Fig. 2.1a) [40]. From the second week onward,

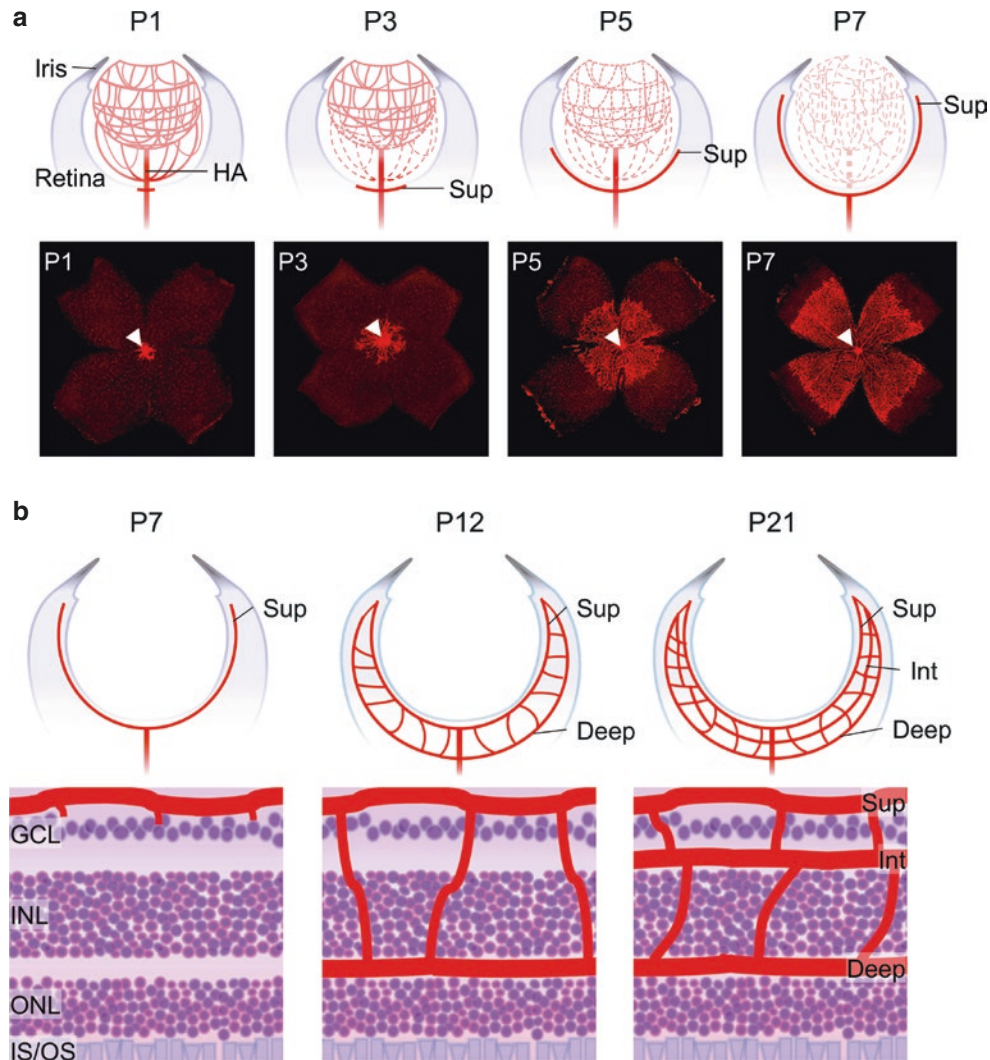


Fig. 2.1 Postnatal development of mouse retina vasculature. (a) Development of mouse retinal vessels occurs postnatally. The upper panels illustrate cross-section diagrams of mouse eyes with developing superficial retinal vascular plexus (Sup; indicated in red) from postnatal day (P) 1 to 7, parallel to regression of hyaloid vasculature (indicated in light red), which is a transient embryonic vascular bed providing blood supply to the developing eye. The lower panels are representative images of the retinal flat mounts from C57BL/6J mice. The vasculature was stained with isolectin (red). At P1, the mouse retina is nearly devoid of blood vessels. Originating from the optic nerve head (white arrow-heads), the superficial vascular plexus extends radially from the central retina and reaches the peripheral retina by the end of the first week of postnatal development. (b) Development of the deep and intermediate vascular plexus in mouse retina occurs from the second week postnatally onward. The upper panels are cartoons of eye cross-sections over-

viewing the organization of three retinal layers (at P7, P12, and P21) during the development of superficial, intermediate, and deep vascular layers. The lower panels are schematic images of retinal cross-sections showing the maturation process of the three retinal vascular plexuses (in red). During the first postnatal week, the superficial plexus reaches out to the edge of the peripheral retina. From the second week onward, the superficial vascular plexus starts to grow perpendicularly into the retina to form the deep vascular plexus between the INL and ONL, which is fully developed at P12. Further growth and maturation of the intermediate plexus continue between the GCL and INL and are mostly complete by P21. Deep, deep retinal vessels; GCL, ganglion cell layer; INL, inner nuclear layer; Int, intermediate retinal vessel; IS/OS, inner segment/outer segment of photoreceptors; ONL, outer nuclear layer; Sup, superficial retinal vessels. The retinal flat mount images in panel a were adapted and reprinted with permission from Stahl et al. [17]

the superficial capillaries grow perpendicularly into the inner retina, forming the deep and then the intermediate vascular plexuses (Fig. 2.1b). By the end of the third postnatal week, a complete retinal vasculature is developed, and hyaloid vessels are mostly regressed [37]. During this period, especially in the first week, the immature retinal vasculature and remnant hyaloid vessels in neonatal mouse pups resemble those of preterm human infants, having a largely avascular retina and highly susceptible to oxygen-induced retinopathy [17].

2.2.2.2 The Mouse OIR Model

In the 1950s, around the same time as the studies in the kitten OIR model, pathologic features resembling ROP were reported in mouse retinas exposed to high oxygen conditions [29, 41]. Subsequent studies in mice further examined the effects of varying oxygen conditions on reproducing ROP-like pathological ocular characteristics [42, 43], providing insights that helped shape the current standardized OIR models.

Coupled with advanced technology in the visualization of retinal vasculature, uniform and thorough assessment of retinopathy severity, as well as the advantage of genetic manipulation in mice, the current mouse model of OIR described by Smith et al. in the 1990s is one of the most commonly used OIR models [9, 44]. In this model, neonatal mice are exposed to $75\% \pm 2\%$ of oxygen for 5 days starting at postnatal day (P) 7, followed by 5 days in room air starting from P12 to P17 (Fig. 2.2a). Hyperoxia conditions from P7 to P12 result in central retinal vessel regression, leading to vaso-oblivation and mimicking the initial stage of ROP. The maximal amount of vessel loss is observed at P9, followed by slow revascularization into the vaso-oblivated areas [45, 46]. After returning to room air at P12, the retinal areas with vaso-oblivation become ischemic and hypoxic, leading to stimulation of hypoxia-induced pro-angiogenic factors, such as VEGF and EPO, resulting in both physiological revascularization of the vaso-oblivation area and also uncontrolled compensatory pathological neovascularization (Fig. 2.2b), resembling the proliferative stage of human ROP [9, 47–50]. These pathological neovessels, also known as preretinal tufts, are disorganized, small-caliber clumps of neovascularization growing at the border between vascular and avascular areas and protruding intravitreally (Fig. 2.2c, d). At P17, the typical endpoint of this mouse model, the extent of pathological neovessels reaches the maximal severity and is also accompanied by increased vascular leakage and breakdown of the blood–retinal barrier. After P17, abnormal preretinal neovascularization starts gradual regression spontaneously and completely disappears by about P25 [44].

The mouse retinal vasculature can be visualized by fluorescent angiography, which highlights the lumen of blood vessels, or (more efficiently) by staining with blood vessel markers such as isolectin in retinal flat mounts. Both meth-

ods allow for easy quantification of pathological vascular features with computer-aided imaging software. Both vaso-oblivation and neovascularization levels can be quantified as a percentage of total retinal areas in flat mounts [44, 51]. Moreover, preretinal neovascular tufts protruding into the vitreous cavity can also be visualized and quantified in cross-sections of eyes [9] (Fig. 2.2d). Vascular permeability can be visualized using fluorescent angiography and quantified by Miles assay [51–55]. Delayed retinal neuronal development or retinal thinning also occurs in OIR, mostly in the inner retina. Overall, the accessibility of genetic manipulation and the consistent and reliable induction of neovascularization make the mouse OIR model a popular one for studying ROP pathogenesis. In addition, OIR is a reliable model for the proliferative aspect of diabetic retinopathy and useful for investigating other diseases related to abnormal neovascularization.

The mouse OIR model reproduces vaso-oblivation and neovascularization consistently, contributing to its broad use in ROP research. Yet one limitation is that the area of vaso-oblivation locates in the central part of the retinas, unlike the peripheral avascular regions in humans. This geographic localization of the peripheral avascular region is recapitulated better in the rat OIR model with cycling oxygen exposure. In addition, there are strain-dependent differences in mouse angiogenic response and hence OIR susceptibility [56, 57]. In general, 129S strains with mixed background are more angiogenic and show higher levels of neovascular response in OIR than inbred C57BL/6 strains do, and the albino BALB/cByJ strain is even less angiogenic than C57BL/6. Moreover, venter-related substrain differences in OIR neovascularization may also exist, even within the same strain [17]. These strain considerations need to be taken into account when choosing appropriate OIR mouse strains.

Another important consideration for interpreting the OIR phenotype is postnatal weight gain of mouse pups during OIR. Postnatal weight gain is a reliable predictor for the severity of clinical ROP in infants [58–60]. Similarly, in the mouse OIR model, pups with poor postnatal weight gain (<5 g at P17) reveal dampened vascular response and prolonged phase of retinopathy compared with medium postnatal weight gain (5 g–7.5 g at P17) and extensive postnatal weight gain (>7.5 g at P17) pups [61]. Monitoring mouse weight gain and keeping them in the normal range is necessary for proper interpretation of OIR data. Runty pups (<5 g) with poor OIR responses should be excluded from data analysis. Other factors, such as the hypoxic effect on lactating mothers [42, 62], may affect maternal care of pups and the susceptibility and severity of the OIR phenotype, hence surrogate mouse mothers may be used when needed.

The mouse OIR model greatly facilitated the investigation of various molecular pathways in developmental and pathological retinal angiogenesis, particularly the discovery of

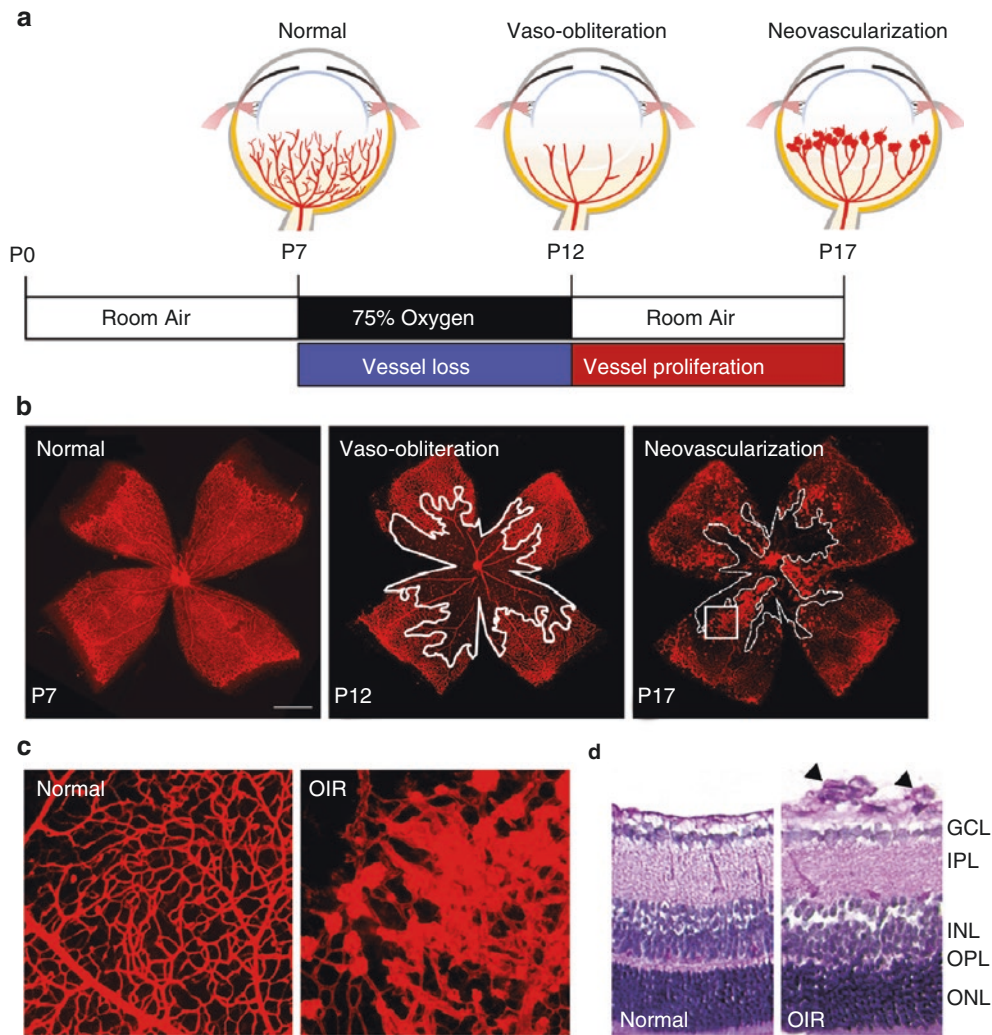


Fig. 2.2 The mouse model of oxygen-induced retinopathy (OIR). **(a)** A schematic diagram illustrates the procedure of mouse OIR. Neonatal litters are exposed to 75% ± 2% oxygen from P7-P12. Hyperoxia suppresses retinal vascular development and leads to the regression of existing immature retinal vessels, resulting in a central zone of vaso-obliteration. At P12, mice are returned to room air, and the relative hypoxia triggers vessel regrowth toward the vaso-obliteration zone as well as pathological neovascularization at the border between the vascular and avascular areas. The levels of neovascularization reach maximum severity at P17. **(b)** Representative images of retinal flat mounts with isolectin staining (red) in the normoxic and OIR retinas show the normal retinal vasculature at P7 (left panel), vaso-obliteration at P12 (middle panel; the area of vessel loss is outlined in white), and patho-

logic neovascularization at P17 (right panel; the vessel loss area is outlined with a white dashed line). Scale Bar: 1 mm. **(c)** Magnified images of isolectin-stained retinal flat mounts show the normal vasculature (left panel) from normoxia mice and pathological neovessels in the OIR mouse at P17. Right panel image was enlarged from the rectangular region in **(b)**. **(d)** Cross-sections of mouse retinas with normoxia (left panel) and OIR (right panel) at P17. The sprouting neovascular tufts (arrows) in the OIR mouse arise from the superficial retinal vascular layer and protrude into the vitreous. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Figure was adapted and reprinted with permission from Liu et al. [36]

VEGF's role in retinopathy. By utilizing the mouse OIR model, previous pioneering studies demonstrated that VEGF induces pathological retinal neovascularization [49] and that inhibition of VEGF suppresses retinal neovascularization in the OIR mouse eyes [48]. This work, together with studies in other ocular angiogenesis animal models [63–66], laid the experimental foundation for developing current anti-VEGF therapies to treat neovascular age-related macular degenera-

tion and ROP. Besides VEGF, other angiogenic factors, such as EPO [47, 50, 67] and additional signaling pathways, are extensively studied in the OIR model. In addition, factors critical in the third trimester and deficient after preterm birth, such as insulin-like growth factors and omega-3 polyunsaturated fatty acids [18, 19], have also been evaluated utilizing the mouse OIR model (which demonstrated that supplementing these factors is beneficial).

2.2.3 The Rat Model of OIR

The rat model of ROP was first demonstrated by Patz [16] in 1954. In that study, newborn rats were exposed to a constant level of high oxygen concentration (60–80%), resulting in preretinal neovascularization [16]. Subsequent studies using rats exposed to hyperoxia conditions also demonstrated abnormal vaso-proliferation with varying inconsistencies [68, 69]. Over the years, investigators explored and revised protocols for modeling human ROP in more efficient experiment settings [70–72]. Clinical studies revealed that, besides continuous oxygen supplement, oxygen fluctuation also contributes to the incidence of severe ROP [73, 74]. The partial pressure of dissolved arterial oxygen may fluctuate very quickly in premature infants, leading to the alternating occurrence of severe and extended hyperoxemia and hypoxemia [73], which in turn may lead to a higher risk of developing ROP. Based on these observations, Penn and colleagues developed the current rat model, which utilizes alternating hyperoxia–hypoxia cycles, in which the oxygen levels cycle between 50% and 10% every 24 hours for the first 14 days after birth followed by room air exposure through P20 [8, 11, 75] (Fig. 2.3). This rat model recreates oxygen tension fluctuation, which mirrors varying oxygen levels measured in preterm infants developing severe ROP [11], whereas in the mouse model the oxygen level remains constant during continuous oxygen exposure. Exposure to cycling levels of oxygen retards the retinal vascular development, leading to a peripheral avascular zone mimicking human ROP. After returning to room air, neovascular tufts grow at the boundary between vascular and avascular areas in the mid-peripheral retina. One main advantage of the rat OIR model lies in its clinically relevant feature of ROP—delayed development of retinal vasculature followed by pathologic neovascularization. The vaso-obliteration phenotype in the rat OIR model is highly reminiscent of the peripheral avascular zone observed in human ROP, whereas in the mouse model it is the central retinal vessels that are obliterated [76, 77]. Disadvantages of the rat model include lack of genetically modified animals, stain-dependent variation in response, and relatively inconsistent and low levels of neovascularization, which together limit its use in evaluating antiangiogenic therapies.

2.2.4 The Zebrafish Model

The zebrafish, albeit not directly relevant for clinical ROP, is an alternative model for studying angiogenesis-dependent ocular diseases and drug screening [78]. An adult zebrafish model of hypoxia-induced retinopathy was generated [30,

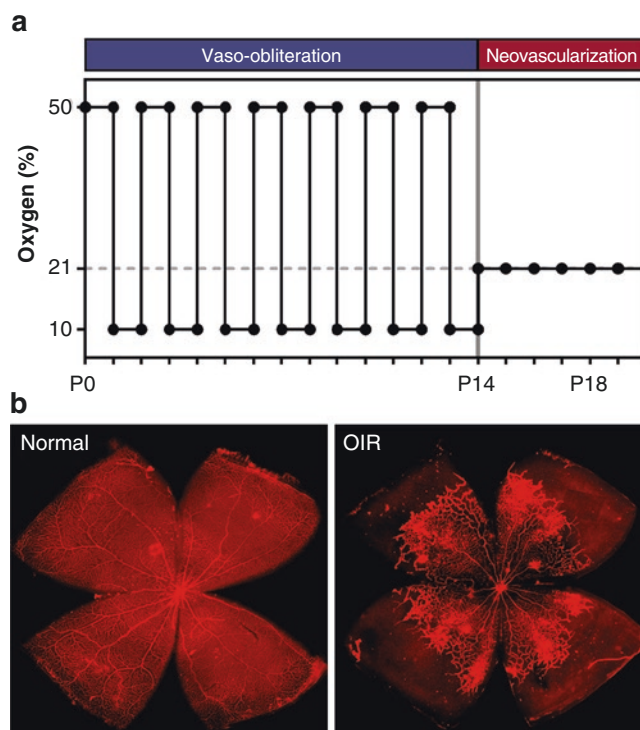


Fig. 2.3 The rat model of oxygen-induced retinopathy (OIR). (a) A schematic illustrates the rat OIR regimen, the 50/10 model. This model exposes neonatal rat litters to alternating 24-hour periods of 50% and 10% oxygen from P0 to P14. After 14 days of variable oxygen, rats are returned to room air (21% oxygen), inducing the development of neovascularization. (b) Representative retinal flat mount images of normoxic and the 50/10 OIR rat retinas. Retinas were collected from P18 normal (left panel) and OIR (right panel) rats followed by isolectin-staining to visualize the vasculature. Rat retinas with OIR show delayed vascular development in the peripheral area with a vaso-obiterated zone and neovascularization at the boundary of vascular and avascular areas. Panel b: Image courtesy of Dr. James D. Akula, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

79], utilizing blood vessel-specific stable fluorescent reporter transgenic zebrafish [80, 81]. When placed in a hypoxic aquarium with 10% air saturation (820 parts per billion) for 3–12 days, new retinal vessel sprouts reached a plateau of maximal angiogenic responses at day 12 [30, 79]. Subsequently, an embryonic model of ROP was also developed using hypoxia-inducing chemical agents to stimulate abnormal retinal angiogenesis in zebrafish embryos [82].

2.2.5 Hyperglycemia-Associated ROP Models

Neonatal hyperglycemia, common in extremely preterm infants, has been recognized as an ROP risk factor [20–23]. Neonatal hyperglycemia often occurs in preterm infants receiving glucose infusion and is related to incomplete sup-

pression of hepatic glucose production after infusion, inadequate pancreatic beta-cell response, and insulin insensitivity [83–86]. A hyperglycemia-associated retinopathy model was generated in neonatal rats by using a single injection of streptozotocin (STZ) at P1 [24]. Sustained hyperglycemia was induced rapidly from P2/3 to P6, and retinal angiogenesis is inhibited, with associated inflammatory response and inner retinal neuron degeneration within 2 weeks postnatally [24]. Recently, a similar mouse model of hyperglycemia-associated ROP was developed with daily injections of STZ from P1 to P9. Hyperglycemia was induced at P8, followed by delayed deep layer retinal vessel growth and photoreceptor dysfunction [25]. Together these models recapitulate delayed vascular development of ROP and may help elucidate the mechanisms of hyperglycemia-induced retinal damage in ROP.

2.3 Summary

ROP remains a leading cause of visual impairment and a challenge in neonatology worldwide. Animal models of ROP have evolved over the past several decades with vast amounts of research efforts directed toward better understanding of the pathogenesis of ROP, especially in the mechanisms governing retinal angiogenesis. The establishment of different experimental models allows investigators to explore the roles that many influencing factors (e.g., oxygen, light conditions, and hyperglycemia) play in ROP development. For instance, studies from the early feline, canine, and murine models of ROP provided compelling evidence that high levels of oxygen promoted obliteration of blood vessels in the developing retina [29, 69], suggesting the importance of titrating and close monitoring of exogenous oxygen administration to the preterm infants. Insights on an essential role of VEGF in ROP development were also derived from studies in OIR models, which facilitated the development of current anti-VEGF therapies.

Among several different species, the mouse model of OIR is the most representative model for studying ROP and other ocular diseases involving hypoxia-induced angiogenesis. As of December 2018, there are more than 700 PubMed publications using the mouse OIR model of ROP. These studies reflect the broad value of the mouse OIR model for studying mechanisms underlying pathological angiogenesis in retinal and tissue ischemia, including the roles of growth factors, fatty acids, inflammation, signaling cascades, metabolic and transcriptional regulation, and regulatory non-coding RNAs on ROP. In addition, the mouse OIR model, with its fast induction of consistent neovascularization, is also extensively used in evaluating antiangiogenic drugs, to facilitate the development of new therapies for ROP and other vascular eye disorders.

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Abstract

Retinopathy of prematurity (ROP) is a vasoproliferative disorder occurring in premature infants' retinas. With the advances in neonatal care in recent years, ophthalmologists are increasingly encountering premature babies, who have higher risks of developing ROP. The occurrence and severity of ROP depend on several factors, which have been reported by various clinical trials. This chapter demonstrates the evolution of several aspects of major clinical trials in ROP, including its treatment, the role of oxygen in ROP development, and the roles of light and nutrients in ROP.

Keyword

Retinopathy of prematurity · Clinical trials · Treatment
Oxygen · Light

3.1 Major Clinical Trials: ROP Treatment (Summary in Table 3.1)

3.1.1 The Multicenter Trial of Cryotherapy for ROP (CRYO-ROP) [1–5]

CRYO-ROP was one of the major trials in ROP, and most of the current clinical understanding of ROP derives from that study. This multicenter, randomized control trial of cryother-

apy for the treatment of ROP aimed to evaluate the efficacy and safety of the treatment in preventing serious sequelae of ROP in very-low-birth-weight infants. The study registered 9751 infants with a birth weight of less than 1251 g. The patients were then screened for the development of threshold disease; this was defined as five or more contiguous, or eight cumulative, clock hours of stage 3 ROP in zone 1 or 2, in the presence of plus disease. In all, 291 patients were included in the final cohort. Two hundred and forty eyes were classified as symmetric cases, in which both eyes developed threshold ROP. In this group, one eye was randomized to receive cryotherapy while the other eye was observed as a control. The remaining 51 eyes were in the asymmetric group, and only the eyes that developed threshold ROP were included in the study and randomized to either cryotherapy or observation.

If cryotherapy was assigned, it was performed within 72 h and prior to the development of stage 4A ROP. Usually, a conjunctival incision was not required. Cryotherapy was then started at the ora serrata, followed by more-posterior applications. The cryotherapy applications were contiguous and single, with an endpoint of the sudden whitening of the retina; this equated to approximately 2–3 s of freeze time. All avascular retina anterior to the ROP ridge was treated. When there were skipped or untreated areas with a persistence of plus disease, retreatment was performed. The outcomes were measured by the development of unfavorable outcomes (posterior retinal detachment, retinal fold involving macula, or retrolental tissue).

Overall, approximately 82% of the patients had a symmetric disease, and 11 cases needed retreatment with cryotherapy. In the third month after treatment, 31.1% of the eyes that received treatment developed unfavorable outcomes, versus 51.4% of the control eyes; this represented a risk reduction of approximately 40%. No major complications were found. As this result supported the efficacy of cryotherapy in reducing the risk of unfavorable outcomes from threshold ROP, cryotherapy was subsequently recommended for both eyes with stage 3+ ROP in zone 1. The report of CRYO-ROP at 5.5 years was the first study from CRYO-ROP to demonstrate the functional outcomes of

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Table 3.1 Summary of the major clinical trials in retinopathy of prematurity

Clinical trials	Objective	Criteria	Number enrolled	Intervention	Endpoint	Outcome measurement	Outcomes
CRYO-ROP (1986–2003)	To evaluate efficacy and safety of cryotherapy compared to observation	BW < 1251 g; survived to 28 days; and no major ocular or systemic congenital anomalies	291; 240 symmetric cases, and 51 asymmetric cases; one eye treated with cryotherapy, and the fellow eye as control; 247 patients followed and analyzed at year 10	Trans-scleral cryotherapy	Reports: Preliminary; 3 months; and 1, 3, 5.5, and 10 years	Structural unfavorable outcomes: Posterior retinal detachment, retinal folds involving macula, retrolental mass; visual function	At 3 mos: 31.3% cryo vs. 51.4% control had unfavorable outcomes. At 5.5 y: 47.1% cryo vs. 61.7% control had VA < 20/200; 26.9% cryo vs. 45.4% control had unfavorable structural outcomes. At 15.5 y: 44.7% cryo vs. 64.3% control had VA < 20/200; 30% vs. 51.9% had unfavorable structural outcomes.
ETROP (1999–2002)	To evaluate visual and structural outcomes of high-risk prethreshold ROP compared to threshold ROP	BW < 1251 g, and prethreshold ROP	828 prethreshold. 401 high risk (type 1) were randomized; 317 bilateral, and 84 asymmetric	Retinal ablation: Laser photocoagulation (cryotherapy allowed)	9 mos postmenstrual age	Visual function at 6 and 9 mos; structural outcomes at 9 mos.	Unfavorable VA; 19.5% threshold vs. 14.5% prethreshold (P = 0.01) and unfavorable structural outcomes; 15.6% threshold vs. 9.1% prethreshold (P < 0.001). Type 2: Wait-and-watch.
Laser compared to cryotherapy	Compare laser to cryo at 10 years for threshold ROP	Threshold ROP	118 eyes from 66 patients for treatment; 44 eyes from 25 patients were examined at 10 years	(1) laser vs. cryotherapy (2) refraction	10 years	(1) BCVA vs. retinal dragging (2) spherical equivalent	(1) mean BCVA: 20/66 laser vs. 20/182 cryo (P = 0.015); 7.2 times more retinal dragging in cryo eyes (95% CI, 1.54–3.36). (2) SE: –4.88 laser vs. –7.65 cryo (P = 0.019); lens power contributes to myopia.
BEAT-ROP (2008–2010)	To evaluate efficacy of bevacizumab for stage 3+ in zone I and posterior zone II	BW < 1500 g; GA < 30 weeks; and stage 3+ ROP in zone I or posterior zone II	150 infants (67 with zone I, and 83 with posterior zone II); 7 died; 75 laser, and 75 intravitreal injection	Intravitreal bevacizumab vs. laser	54 weeks postmenstrual age	Recurrence of ROP requiring treatment in either eye	4% bevacizumab vs. 22% laser (P = 0.002).

STOP-ROP (1994–1999)	Supplemental oxygen to reduce progression of prethreshold to threshold and need for retinal ablation	Prethreshold, and < 94% oxygen saturation in room air	325 conventional vs 324 supplemental	Conventional, SaO ₂ 89%–94% vs. supplemental, SaO ₂ 96%–99%	3 mos	Threshold ROP requiring treatment	Progression to threshold: 48% conventional vs. 41% supplemental. OR for progression after ROP baseline adjustment, 0.72 (95% CI, 0.52–1.01). Use of supplemental oxygen did not reduce ROP requiring treatment.
HOPE-ROP (1996–1999)	Compare to infants from STOP-ROP (SaO ₂ < 94%)	Prethreshold in one eye, and median SaO ₂ > 94%	136 infants vs. 229 STOP-ROP	None	Progressed to threshold ROP or resolved	Number of progressions	Progression to threshold: 25% HOPE-ROP vs. 46% STOP-ROP (OR, 0.607; 95% CI, 0.359–1.026); not statistically significant.
SUPPORT (2005–2009)	Compare target of oxygen saturation	GA 24 wks to 27.6 wks; decision for full resuscitation; and no major anomalies.	1316 infants; 654 (lower group) vs. 662 (higher group)	Target oxygen saturation: 85%–89% vs 91%–95%	36 wks postmenstrual age or did not need ventilator for 72 h, whichever first	Composite outcomes of severe ROP (threshold; need intervention or intravitreal injection), death, or both	No significant difference between the two groups in terms of composite outcomes (P = 0.21). Death excluded, the lower group: Less cases of severe ROP (P < 0.001). More deaths before discharge in the lower group (P = 0.04).
LIGHT-ROP (1995–1997)	Assess the effects of lighting conditions on the incidence in high-risk infants	BW < 1251 g, and GA < 31 wks	188 with goggles, and 173 in control	Goggles	Postconceptional age of 31 weeks or 4 weeks after birth	Any ROP	ROP development: 54% with goggles vs. 58% control (P = 0.50).
Vitamin E (1978–1981)	Increased serum vitamin E reduced stage 3+ ROP	BW ≤ 1500 g.	1414 VLBW infants; 704 vitamin E vs. 714 controls	Vitamin E prophylaxis	Stage 3+ ROP	Incidence of ROP stage 3+	Stage 3+ incidence: 2.4% vitamin E vs. 5.3% control (P < 0.02). 52% reduction in the incidence of stage 3+ ROP. Unable to assess adverse effects.

Abbreviations: *cryo* cryotherapy, *BCVA* best-corrected visual acuity, *BW* birth weight, *CI* confidence interval, *GA* gestational age, *OR* odds ratio, *ROP* retinopathy of prematurity, *SaO₂* oxygen saturation, *SE* spherical equivalent, *VA* visual acuity, *VLBW* very low birth weight