REVIEW ARTICLE

Retinal degenerative and hypoxic ischemic disease

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Abstract A broad spectrum of retinal diseases affects both the retinal vasculature and the neural retina, including photoreceptor and postreceptor layers. The accepted clinical hallmarks of acute retinopathy of prematurity (ROP) are dilation and tortuosity of the retinal vasculature. Additionally, significant early and persistent effects on photoreceptor and postreceptor neural structures and function are demonstrated in ROP. In this paper, we focus on the results of longitudinal studies of electroretinographic (ERG) and vascular features in rats with induced retinopathies that model the gamut of human ROP, mild to severe. Two potential targets for pharmaceutical interventions emerge from the observations. The first target is immature photoreceptors because the status of the photoreceptors at an early age predicts later vascular outcome; this approach is appealing as it holds promise to prevent ROP. The second target is the interplay of the neural and vascular retinal networks, which develop cooperatively. Beneficial pharmaceutical interventions may be measured in improved visual outcome as well as lessening of the vascular abnormalities.

Keywords Retina · Photoreceptors · Retinal blood vessels · Electroretinogram · Image analysis · Retinopathy of prematurity · Development

While categorized as a review article, this paper presents the authors' perspective on potential pharmaceutical interventions for retinal degenerative and hypoxic ischemic retinal diseases; it is not offered as a review of research into these conditions.

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As a system, the mammalian retina is vulnerable to diseases that affect the exquisitely balanced interplay of the neural retina and the vasculature that nourishes it. Visual loss occurs when this balance is disturbed. On the one hand, diseases such as photoreceptor degenerations that primarily affect the neural retina also affect the retinal vasculature. On the other hand, diseases that are clinically characterized by abnormality in the choroidal or retinal vasculature, such as age related macular degeneration, diabetic retinopathy, and retinopathy of prematurity (ROP), also affect the retinal neurons. Thus, all such diseases fall within the broad group of hypoxic ischemic disorders of neural tissue. Photoreceptors, specialized cells that have the highest oxygen requirements of any cell in the body [1], are likely important in all hypoxic ischemic diseases of the retina.
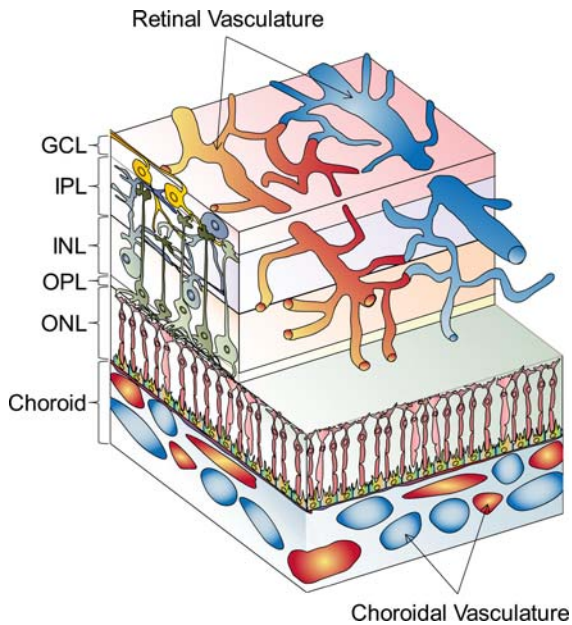
Fig. 1 Diagram of the neural retina and its vascular supplies (not to scale). The layers of the neural retina (ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer nuclear) are indicated. Blood flow through the choroidal vessels is swift. The retinal vasculature, visible by ophthalmoscopy, lies among the ganglion cells on the vitreal surface of the retina and extends capillary networks deep into the postreceptor layers. The caliber of the retinal arterioles adjusts to perturbations in blood oxygen levels ("autoregulation")

Translation from animal models to patients

The photoreceptors are nestled closely to the choroidal vasculature (Fig. 1). Highly organized postreceptor retinal neurons form layers that are supplied by the retinal vessels. Although the choroid is the principal supply to the photoreceptors, degeneration of the photoreceptors is, nonetheless, associated with attenuation of the retinal arterioles [2]. Because the photoreceptor layer is such an extraordinary oxygen sink, it is presumed that, as photoreceptors degenerate, their metabolic demands wane and the retinal vasculature becomes attenuated consequent to the neural retina's chronic lower requirement for oxygen [2].

A tight link between the photoreceptors and the retinal vascular network is evident in the developing retina. Postreceptor cells differentiate before the photoreceptors, which are the last retinal cells to mature. As the formation of rod outer segments advances in a posterior to peripheral gradient, so too does vascular coverage. Thus, concurrent and
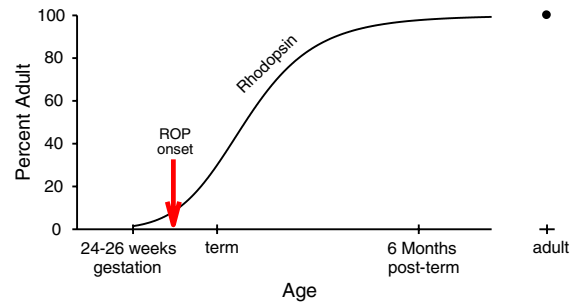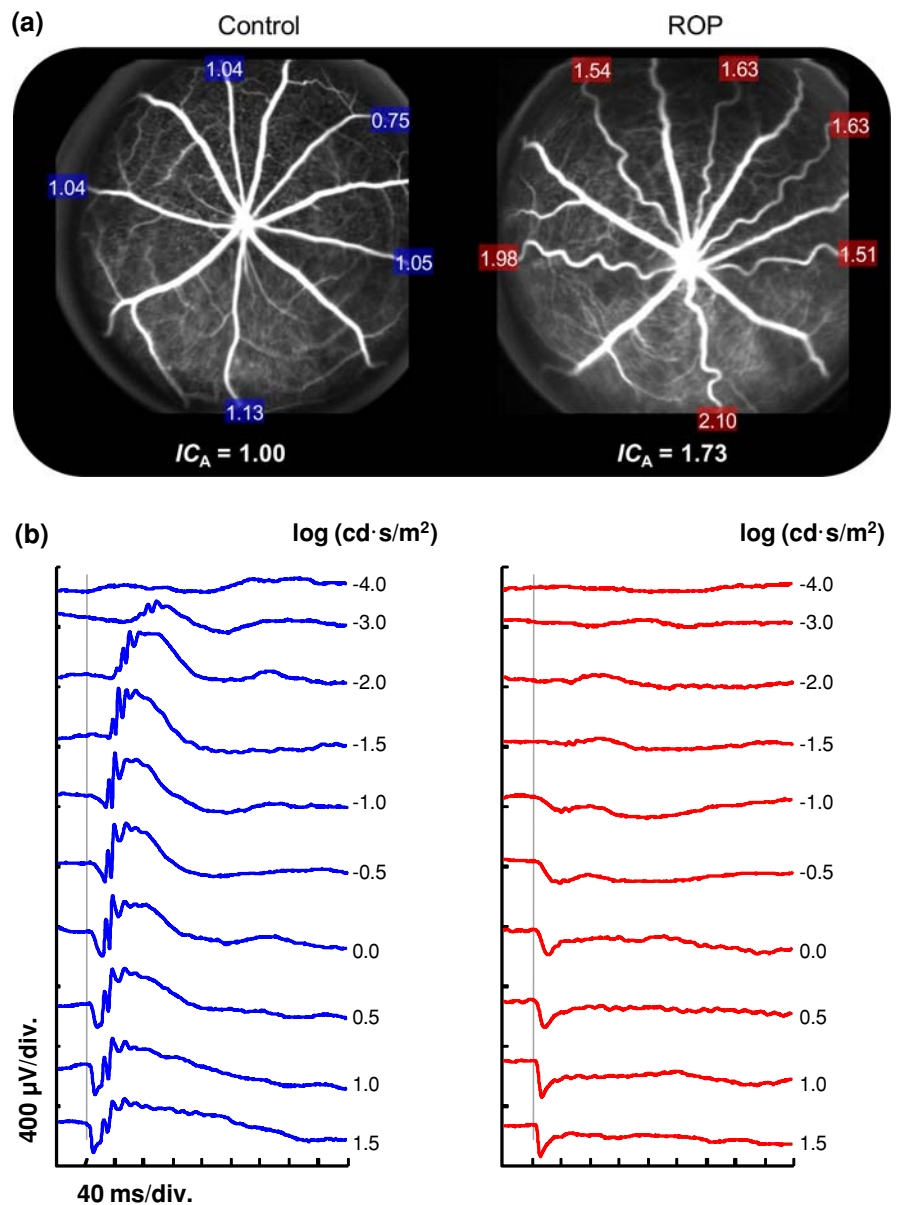

Fig. 2 Logistic growth curve showing human rhodopsin content [40] as a function of age. The red arrow indicates the age of ROP onset in preterm infants [41]

cooperative development of the neural and vascular components characterize normal retinal maturation.

In the preterm infant, the age of onset of ROP is around the age of rapid developmental increase in rod outer segment length and consequent increase in rhodopsin content (Fig. 2). This observation is circumstantial evidence of a role for the rod photoreceptors in the ROP disease process. In addition to immature photoreceptors and retinal vasculature, the preterm infant has immature lungs that create a precarious respiratory status with attendant risk of hypoxic injury to immature cells. This is countered by administration of supplemental oxygen. Both high and low oxygen levels are known to injure the immature photoreceptors [3, 4]. Indeed, rat models of ROP are induced by rearing pups in habitats with alternating periods of relatively high and low oxygen during the critical period of rod outer segment elongation [5–10]. Following induction, abnormalities of the retinal vasculature ensue, as do abnormalities of the structure and function of the neural retina (Fig. 3) [3, 5–9, 11]. The abnormalities in the morphology of the retinal vasculature and in the function of the neural retina in ROP rats are similar to those found in pediatric ROP patients (Fig. 4) [7–9, 11–16]. Thus, translation from the rat models to the human condition may be justified.

We use albino rat models of ROP to study the neural and vascular characteristics of the retina during development [5, 6, 8, 9]. Different schedules of oxygen exposure induce a range of effects on the retinal vasculature and the neural retina that model the gamut of retinopathy, mild to severe, observed in human ROP cases. As shown in Fig. 5, the oxygen exposures are timed to impact the retina during the ages when the rod outer segments are elongating and the rhodopsin content of the retina is increasing. Longitudinal measures of

electroretinographic (ERG) responses and retinal vascular features are obtained in infant (~20 days old), adolescent (~30 days old), and adult (~60 days old) rats. Our results from longitudinal investigations in ROP rats suggest two retinal targets for drug therapies.

Assessment of neural function

We use the ERG to characterize neural function. ERG responses to full-field stimuli over a range of intensities are recorded from the dark-adapted animal as previously described in detail [5]. To summarize rod photoreceptor activity, a model of the activation of phototransduction is fit to the *a*-waves [17–20] and the resulting sensitivity (S_{ROD}) and saturated amplitude (R_{ROD}) parameters are calculated. Postreceptor activity is represented by the *b*-wave. The stimulus/response functions are summarized by the saturated amplitude (V_{max}) and the stimulus producing a half-maximum response ($\log \sigma$); these parameters are derived from the Michaelis–Menten function fit to the
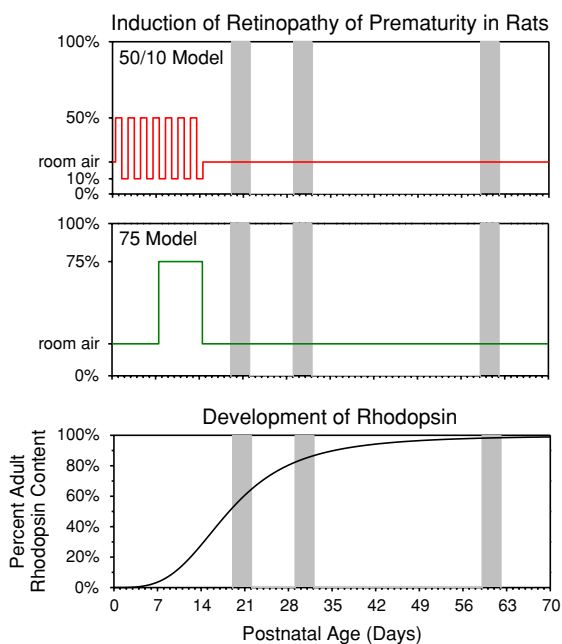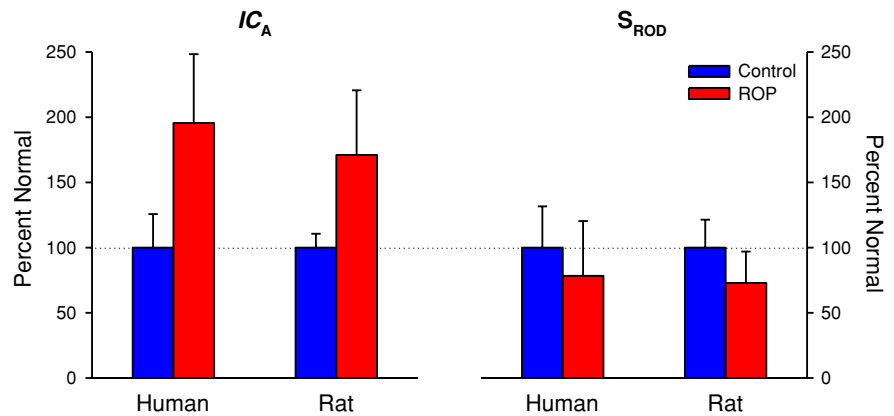
Fig. 5 Rat models of ROP. Two models, the "50/10 model" and the "75 model," are induced by exposing infant rats to alternating periods of relatively high and low ambient oxygen; room air is 21%. For both models the exposures are delivered at ages during which the rod photoreceptors are immature, as indicated by the low rhodopsin content. ERGs and fundus images are obtained longitudinally in infant (20 ± 1 days old), adolescent (30 ± 1 days old), and adult (60 ± 1 days old) rats (*gray bars*)

b-wave amplitudes [5]. Although we find significant deficits in the amplitude parameters (R_{ROD} and V_{max}), it is the sensitivity parameters (S_{ROD} and log σ) that, as described in the following sections, direct us to the postulated intervention sites.

Assessment of vascular characteristics

We derive retinal vascular parameters using image analysis software applied to digital fundus photographs [5, 21, 22]. Integrated curvature (*IC*), which agrees well with subjective assessment of vascular tortuosity reported by experienced clinicians [14], is used to specify the vascular status of each fundus. Both arterioles and venules are significantly affected by ROP. We find, however, that the arterioles are markedly affected, while the venules are less so [5, 9, 14], and therefore use the arteriolar parameter IC_A in our analyses.

Relation of retinal sensitivity and vasculature

Rod photoreceptor sensitivity (S_{ROD}) at a young age (20 days) predicts retinal vascular outcome as specified by IC_A. Better sensitivity at an early age is associated with better (less tortuous) vascular outcome (Fig. 6) [5]. After cessation of the inducing oxygen exposure, recovery of postreceptor neural retinal sensitivity (*b*-wave log σ) and decrease of vascular tortuosity occur hand in hand (Fig. 7). However, at a given age, sensitivity and vascular status are not correlated. Thus, simple compromise of vascular supply to the postreceptor neurons is, at most, an incomplete explanation for the low sensitivity. We note that the regulation of developing retinal neurons and blood vessels is complex, being under the cooperative control of several growth factors, such as vascular endothelial growth factor (VEGF), semaphorin, and their neuropilin receptors
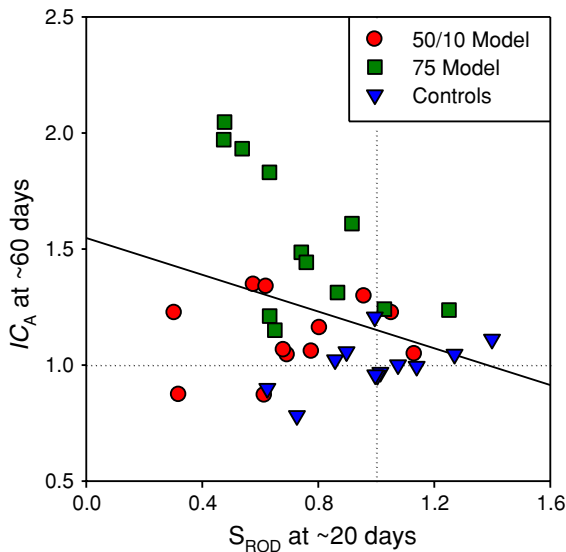
Fig. 6 Arteriolar curvature (IC_A) and photoreceptor sensitivity (S_{ROD}) in 50/10 model, 75 model, and control rats expressed as a proportion of the mean in controls (*dotted lines*). For each subject, IC_A measured at $\sim$60 days is plotted as a function of that subject's S_{ROD} measured earlier, at $\sim$20 days. The *solid line* is a linear regression through all the data. Early rod sensitivity predicted vascular outcome ($r = -0.332$; $P = 0.024$). Adapted from Fig. 8, Akula et al. [5]; copyright of Association for Research in Vision and Ophthalmology
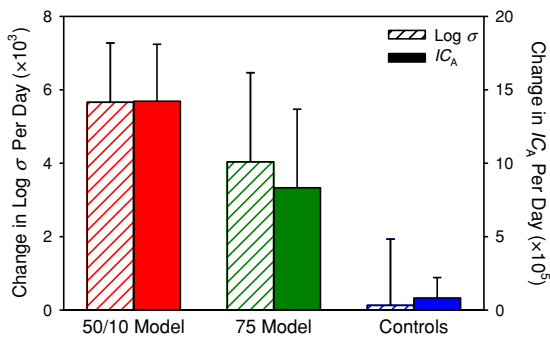

Fig. 7 Mean (SEM) rate of change in postreceptor sensitivity (log σ) and arteriolar curvature (IC_A) for 50/10 model, 75 model, and control rats. Adapted from Fig. 7, Akula et al. [5]; copyright of Association for Research in Vision and Ophthalmology

[23]. In rat models of ROP, expression of these growth factors is altered [24].

Identification of targets for pharmaceutical interventions

The longitudinal data from rat models of ROP identify two targets for pharmaceutical intervention: (1) the immature photoreceptors and (2) the molecular cross talk between neurons and retinal vasculature. We outline the rationale for each.

VEGF promotes development of retinal vasculature. Hypoxia promotes expression of VEGF. The rods are the most demanding of aerobic energy of any cells in the body [1]. Thus, rod-instigated hypoxia must lead to up-regulation of growth factors that promote retinal vascular development. Low rod sensitivity at an early age predicts the poorer vascular outcome at older ages (Fig. 6). Because low rod sensitivity appears to be indicative of early injury to the rod [3, 11], therapy designed to relieve the immature rod of its burgeoning oxygen-based energy requirements should be beneficial. It is at the ages during which the developing rod outer segments elongate with consequent escalation of energy demands that ROP has its onset (Fig. 2). Arden et al. [25] proposed treatment with light to reduce the ROP rods' energy requirements by suppressing the circulating current. Light has a mild beneficial effect on a rat model of ROP [26]. Careful regulation of supplemental oxygen so that arterial oxygen levels are neither too high nor too low may also have some beneficial effect on ROP [27–30]. One can argue that optimal timing for light and oxygen interventions has yet to be specified, but, to date, adjustments in light and oxygen have not been a panacea for ROP. Thus, pharmaceutical approaches are warranted. We theorize that improved management, or, better yet, prevention of ROP may be achieved through timely pharmaceutical suppression of rods' energy demanding processes such as turnover of outer segment material [31] and generation of the circulating current [19].

The second target for pharmaceutical intervention, the molecular cross talk between the retinal vasculature and postreceptor neurons, deserves attention first because abnormal retinal vasculature provides the usual clinical definition of ROP, and also because the postreceptor neurons have a great capacity to remodel [32] in the face of irreversible damage to the photoreceptors [33]. Damage to photoreceptors in ROP may be unavoidable as there is evidence of persistent damage to the rods even in mild cases [13, 34]. Conceivably, treatment of the retinal vasculature could result in improved visual function (e.g., contrast sensitivity) by an associated beneficial effect on the postreceptor neurons. However,

treatment of ROP by manipulating growth factors must be approached with considerable caution because the same growth factors regulate the developing photoreceptors and postreceptor retinal neurons [35, 36]. Indeed, particular caution in use of anti-VEGF treatment in immature retina has been advised specifically because of the potential for adverse effects on the developing neurons [37]. Further knowledge about neurovascular interactions in the immature retina, coupled with analysis of the function of those networks, is essential to delineate the potential therapeutic role of growth factors in ROP.

Conclusions

A systems biology approach is applicable not only to animal models but also to the human retina. Such an approach is nicely supported by the combination of full-field ERG analysis of the neural retina and image analysis of the retinal vasculature. Using the multifocal ERG and high resolution optical coherence tomography (OCT), we are now also applying the systems approach to study of disease of the macula in ROP [38, 39]. In summary, the perspective that we illustrate by study of ROP may also yield novel perspectives on other hypoxic ischemic retinal disorders, including diabetic retinopathy and age related macular degeneration, diseases which are characterized by both vascular and neural abnormalities of the retina.

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