Pathogenesis of retinopathy of prematurity

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Abstract

Retinopathy of prematurity (ROP) is a major cause of blindness in children in developed countries. ROP is a two-phase disease, beginning with delayed retinal vascular growth after premature birth (Phase I). Phase II follows when Phase I-induced hypoxia releases factors to stimulate new blood vessel growth. Both oxygen-regulated and non-oxygen-regulated factors contribute to normal vascular development and retinal neovascularization. Vascular endothelial growth factor (VEGF) is an important oxygen-regulated factor. A critical non-oxygen-regulated growth factor is insulin-like growth factor-I (IGF-I). In knockout mice, lack of IGF-I prevents normal retinal vascular growth, despite the presence of VEGF, important to vessel development. In vitro, low IGF-I levels prevent VEGF-induced activation of Akt, a kinase critical for vascular endothelial cell survival. We found that premature infants who develop ROP have low levels of serum IGF-I compared to age-matched infants without disease. IGF-I is critical to normal vascular development. Low IGF-I predicts ROP in premature infants, and restoration of IGF-I to normal levels might prevent ROP.

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1. Introduction

Retinopathy of prematurity (ROP) is still a major cause of blindness in children in the developed world [1] despite current treatment of late-stage ROP. Although laser photoocoagulation or cryotherapy of the retina reduces the incidence of blindness by 25%, the visual outcomes after treatment often are poor. Preventive therapy for ROP is sorely needed.

ROP was first described in the late 1940s and was soon associated with excessive oxygen use [2–4]. As a result, supplemental oxygen is delivered to premature infants to maintain adequate blood levels, but it is monitored carefully [5]. Even with controlled oxygen use, the number of infants with ROP has increased further [6], due most likely to the increased survival rate of very low birth weight infants [7].

2. Pathogenesis: two phases of ROP

In the human fetus, retinal blood vessel development begins during the fourth month of gestation (Fig. 1) [8,9]. Therefore, the retinas of infants born prematurely are incompletely vascularized, with a peripheral avascular zone, the area of which depends on the gestational age. In the first phase of ROP, the normal retinal vascular growth that would occur in utero ceases, and loss of some of the developed vessels occurs. With maturation of the infant, the resulting non-vascularized retina becomes increasingly metabolically active and increasingly hypoxic.

Retinal neovascularization, the second phase of ROP, is hypoxia induced [10,11] and occurs at about 34 weeks post-menstrual age. The hypoxia-induced retinal neovascularization phase of ROP is similar to other proliferative retinopathies.

To study the molecular pathways in ROP, we developed a mouse model of the disease to take advantage of the genetic manipulations possible in the murine system. The eyes of animals such as mice, rats and cats – though born full term – are incompletely vascularized at birth and resemble the retinal vascular development of premature infants. Exposure of these animals to hyperoxia causes vascular obliteration and cessation of normal retinal blood vessel development, which mimics Phase I of ROP [11–15]. When mice return to room air, the nonperfused portions of the retina become hypoxic, which in turn causes retinal neovascularization, similar to Phase II of ROP and of other retinopathies.
2.1. Vascular endothelial growth factor and Phase II of ROP

Hypoxia is a driving force for proliferative retinopathy, or Phase II of ROP. Vascular endothelial growth factor (VEGF) is a hypoxia-inducible cytokine [16–18] and is a vascular endothelial cell mitogen [18].

Retinal hypoxia stimulates an increase in the expression of VEGF before the development of neovascularization in the mouse [13,19]. Further, inhibition of VEGF decreases the neovascular response [20,21], indicating that VEGF is a critical factor in retinal neovascularization. VEGF also has been associated with ocular neovascularization by other investigators in other animal models, confirming the central role of VEGF in neovascular eye disease [22–26]. These results correspond to what is seen clinically. VEGF is elevated in the vitreous of patients with retinal neovascularization [27,28]. VEGF was found in the retina of a patient with ROP in a pattern consistent with mouse results [24].

2.2. VEGF and Phase I of ROP

VEGF is required for normal blood vessel growth. Thus, Phase I of ROP is also VEGF dependent in animal models. As the retina develops anterior to the vasculature, there is increased oxygen demand, which creates localized hypoxia. Induced by a wave of physiologic hypoxia that precedes vessel growth [29,30], VEGF is expressed in response to the hypoxia, and blood vessels grow toward the VEGF stimulus. As the hypoxia is relieved by oxygen from the newly formed vessels, VEGF mRNA expression is suppressed, moving the wave forward.

Supplemental oxygen interferes with that normal development in the mouse and rat models of ROP. Hyperoxia in animal models causes cessation of normal vessel growth through suppression of VEGF mRNA, causing loss of the physiological wave of VEGF anterior to the growing vascular front. Furthermore, hyperoxia-induced vaso-obliteration is caused by apoptosis of vascular endothelial cells and vaso-obliteration can be at least partially prevented by administration of exogenous VEGF [30,31]. This indicates that VEGF is required for maintenance of the immature retinal vasculature and explains, at least in part, the effect of hyperoxia on normal vessel development in ROP.

Although VEGF and oxygen play an important role in the development of retinal blood vessels, it is clear that other biochemical mediators also are involved in the pathogenesis. Inhibition of VEGF does not completely inhibit hypoxia-induced retinal neovascularization in Phase II of ROP. In Phase I of ROP, although hyperoxia is clearly the cause of both cessation of vascular growth and vaso-obliteration in animal models, it is clear that clinical ROP is multifactorial. Despite controlled use of supplemental oxygen, the disease persists as ever-lower gestational aged infants are saved, suggesting that other factors related to prematurity itself also are at work.

2.3. Growth hormone and insulin-like growth factor I in Phase II of ROP

Prematurity is the most significant risk factor for ROP, which suggests that factors relating to growth and development are critical to the disease process. Because growth hormone (GH) has been implicated in proliferative diabetic retinopathy [32–34], we considered GH and insulin-like growth factor I (IGF-I), which mediates many of the mitogenic aspects of GH, as potential candidates for this factor.

Proliferative retinopathy, Phase II of ROP [35], is substantially reduced in transgenic mice expressing a GH-receptor antagonist or in normal mice treated with a somatostatin analogue that decreases GH release. GH inhibition of neovascularization is mediated through an inhibition of IGF-I, because systemic administration of IGF-I completely restores the neovascularization seen in control mice. During GH and IGF-I inhibition, hypoxia-induced VEGF production is unchanged, indicating that IGF-I does not directly act through VEGF under these physiological conditions. Direct proof of the role of IGF-I in the proliferative phase of ROP in mice was established with an IGF-I receptor antagonist, which suppresses retinal neovascularization without...
altering the vigorous VEGF response induced in the mouse ROP model [36]. These findings suggest a permissive role of IGF-I in retinal neovascularization [35].

IGF-I regulates retinal neovascularization at least in part and is mediated through control of VEGF activation of p44/42 MAPK, establishing a hierarchical relationship between IGF-I and VEGF receptors. These studies establish a critical role for IGF-I in angiogenesis in an ROP model. IGF-I acts permissively to allow maximum VEGF stimulation of new vessel growth. Low levels of IGF-I inhibit vessel growth despite the presence of VEGF. This work suggests that IGF-I serves a permissive function and VEGF alone may not be sufficient for promoting vigorous retinal angiogenesis. Therefore, IGF-I is likely to be one of the non-hypoxia regulated factors critical to the development of ROP.

2.4. IGF-I and Phase I of ROP

IGF-I is critical both in the first phase of ROP [37], and in the normal development of the retinal vessels. The degree of Phase I determines the degree of Phase II, the later destructive phase of ROP, since normal vessel development in the retina precludes the development of ROP. After birth, IGF-I levels decrease from in utero levels due to the loss of IGF-I provided by the placenta and the amniotic fluid. We hypothesized that IGF-I is critical to normal retinal vascular development and that a lack of IGF-I in the early neonatal period is associated with poor vascular growth and with subsequent proliferative ROP. We examined normal retinal vascular development in IGF-I knockout mice to determine if IGF-I is critical to normal blood vessel growth. Retinal blood vessels grow more slowly in these mice than in normal mice, a pattern very similar to that seen in premature babies with ROP. It was determined that IGF-I controls maximum VEGF activation of the Akt endothelial cell survival pathway. This finding explains how loss of IGF-I could cause the disease by preventing the normal survival of vascular endothelial cells.

These observations were confirmed in patients with ROP [38]. In 84 premature infants, the mean IGF-I was significantly and proportionately lower in post-menstrual age-matched babies with each stage of ROP than without ROP (Fig. 2). These findings suggest the intriguing possibility that replacement of IGF-I to uterine levels might prevent the disease by allowing normal retinal vascular development. If Phase I is aborted, the destructive second phase of vaso-proliferation will not occur.

3. A rationale for the evolution of ROP

A rationale for the evolution of ROP has emerged based on this new understanding of the roles of VEGF and IGF-I in both phases of ROP. Blood vessel growth is dependent on both IGF-I and VEGF. In premature infants, the absence of IGF-I (normally provided by the placenta and the amniotic fluid) stops blood vessel growth. As parts of the eye mature, they become oxygen starved, sending signals to increase VEGF. As the infant’s organs and systems then continue to mature, IGF-I levels rise again, suddenly allowing the VEGF signal to produce blood vessels. This neovascular proliferation then becomes ROP and can cause blindness.

4. Clinical implications

The discovery of the importance of VEGF and IGF-I in the development of ROP is a step forward in our understanding of the pathogenesis of the disease. These studies suggest a number of ways to intervene medically in the disease process, but also make clear that timing is critical to any intervention. Inhibition of either VEGF or IGF-I early after birth can prevent normal blood vessel growth and precipitate the disease; whereas, inhibition at the second neovascular phase might prevent destructive neovascularization. Similarly, replacement of IGF-I early on might promote normal blood vessel growth; whereas, late supplementation with IGF-I in the neovascular phase of ROP could exacerbate the disease. In the fragile neonate, the choice of any intervention must be made very carefully to promote normal physiological development of both blood vessels and other tissue. In particular, the finding
that later development of ROP is associated with low levels of IGF-I after premature birth suggests that physiologic replacement of IGF-I to levels found in utero might prevent the disease by allowing normal vascular development.

References


