Angiogenesis (2007) 10:133–140
DOI 10.1007/s10456-007-9066-0

ORIGINAL PAPER

Retinopathy of prematurity

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Received: 2 November 2006 / Accepted: 24 January 2007 / Published online: 27 February 2007

Abstract Retinopathy of prematurity (ROP) is a common blinding disease in children in the developed world despite current treatment, and is becoming increasingly prevalent in the developing world. ROP progresses in two phases. The first phase begins with delayed retinal vascular growth after birth and partial regression of existing vessels, followed by a second phase of hypoxia-induced pathological vessel growth. Two major risk factors of ROP are the use of oxygen and a decreased gestation period. Excessive oxygen contributes to ROP through regulation of vascular endothelial growth factor (VEGF). Suppression of VEGF by oxygen in phase I of ROP inhibits normal vessel growth, whereas elevated levels of VEGF induced by hypoxia in phase II of ROP precipitate pathological vessel proliferation. Insulin-like growth factor 1 (IGF-1) is a critical non-oxygen-regulated factor in ROP. We have found that serum levels of IGF-1 in premature babies directly correlate with the severity of clinical ROP. IGF-1 acts indirectly as a permissive factor by allowing maximal VEGF stimulation of vessel growth. Lack of IGF-1 in preterm infants prevents normal retinal vascular growth in phase I of ROP, despite the presence of VEGF. As infants mature, rising levels of IGF-1 in phase II of ROP allows VEGF stimulated pathological neovascularization. These findings suggest that restoration of IGF-1 to normal levels might be useful in preventing ROP in preterm infants.

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Keywords Hypoxia · IGF-1 · Neovascularization · Prematurity · Retinopathy · VEGF

Introduction

Retinopathy of prematurity (ROP) is a major cause of blindness in children in the developing and developed world despite current surgical treatment in the late-stage of the disease [1]. Formerly known as retrolental fibroplasia, ROP was originally described in the 1940s by Terry who first connected the condition with premature birth [2]. At that time, no treatment for ROP was available. Major advances in ROP treatment came in the 1980s and 1990s, when cryotherapy and laser photocoagulation of the avascular retina were shown to be partially effective in preventing blindness in ROP infants. However, although these ablation treatments can reduce the incidence of blindness by 25% in infants with late-stage disease, the patients often still have poor visual acuity after treatment. Preventive and less destructive therapies for ROP would be much more desirable, and understanding the molecular basis of the disease is important to the development of such medical interventions.

Risk factors of ROP

First observed in the nursery [3, 4] and then supported by animal studies [5, 6], ROP was associated with excessive oxygen use shortly after the initial description of the disease. Controlled supplemental oxygen is now delivered to premature infants to maintain

adequate blood levels of oxygen [7]. However, even with monitored oxygen use, the number of infants with ROP has increased, most likely due to the increased survival of very low birth weight infants [8].

Besides oxygen, other factors that might contribute to ROP have been investigated over the years, including vitamin E and light exposure. Since premature infants have lower vitamin E levels in serum, the role of vitamin E in ROP was considered. However, conflicting results were reported in clinical studies of vitamin E and the efficacy of vitamin E prophylaxis remain inconclusive [9, 10]. Studies on light reduction have concluded that reduction of light exposure does not reduce the progression of ROP in either a mouse model of ROP [11] or in the clinic [12].

At present, oxygen use and gestational age/birth weight of the baby appear to be the major risk factors for ROP [13–15], although recent reports suggest that elevated glucose may also play a role [16–18].

Pathogenesis: two phases of ROP

ROP is a biphasic disease consisting of an initial phase of vessel loss followed by a second phase of vessel proliferation. For a better understanding of the process, one needs to examine the development of retinal vasculature. Retinal vascularization originates from the center of optic disc and progresses radially outwards towards the ora seratta. In the human, retinal blood vessel development is initiated during the fourth month of gestation and reaches the retinal periphery just before birth [19]. Therefore, infants born prematurely have incompletely vascularized retinas with a peripheral avascular zone. The gestational age at birth of the premature infant determines the area of the avascular zone.

In premature infants vascular growth that would normally occur in utero slows or ceases and is accompanied by regression of developed retinal vessels. The relative hyperoxia of the extrauterine environment as well as supplemental oxygen given to premature infants are thought to be responsible for this process. Normally in utero, the blood is only ~70% saturated compared to 100% in full term infants in room air. The normal PaO_2 in utero is 30 mm Hg, while a normal infant breathing room air will have a PaO_2 of 60–100 mm Hg [20]. As the infant matures, the non-vascularized retina becomes increasingly metabolically active and, in the absence of an adequate vascular system, leads to tissue hypoxia. The first phase of ROP occurs from birth to postmenstrual age approximately 30–32 weeks.

The second phase of ROP is characterized by hypoxia-induced retinal neovascularization and begins around 32–34 weeks postmenstrual age. This phase of ROP is similar to the neovascularizations in other proliferative retinopathies such as diabetic retinopathy. New vessels form at the junction between the vascularized retina and the avascular zone of the retina. Over time, this pathological growth of vessels produces a fibrous scar extending from the retina to the vitreous gel and lens. Retraction of this scar tissue can separate the retina from the retinal pigment epithelium, resulting in a retinal detachment and likely blindness.

Animal models of ROP: the murine model of oxygen-induced ROP

Much of our understanding regarding the underlying mechanism of the disease process comes from the use of animal models. Many animals such as mice, rats, kittens, and beagle pups have incompletely vascularized retinas at birth and resemble the immature retinal vascular development of premature infants.

We have developed a mouse model of ROP to study the molecular pathways in the disease as well as to take advantage of the genetic manipulation in mice [21]. Neonatal mice are exposed to 75% oxygen from postnatal day 7 until day 12. When mice are exposed to hyperoxia, vessel regression and the cessation of normal radial vessel growth occurs mimicking the first phase of ROP. The association between oxygen exposure and vaso-obliteration has also been observed in other animal models such as rats, kittens, and beagle pups [5, 6, 22, 23]. The extent of vaso-obliteration can be determined by measuring the non-perfused area in retinal whole mount.

Upon return to room air, the non-perfused portions of retina become hypoxic, thereby inducing the expression of angiogenic factors and resulting in retinal neovascularization. The neovascular phase in the oxygen-induced animal model is similar to the second phase of ROP in humans and, in addition, mimics certain aspects of proliferative diabetic retinopathy. The formation of vascular tufts are measured by quantifying neovascular nuclei extending into the vitreous in cross sections of retina, or by quantifying the area of vascular tufts in retinal whole mounts. The mouse model has proven useful in delineating the molecular changes in both phases of the neovascular eye diseases.

The roles of vascular endothelial growth factor (VEGF) and oxygen in ROP

Excessive oxygen use has been associated with development of ROP since the 1950s. It has become widely accepted that hyperoxia-induced vessel loss results in retinal hypoxia, which stimulates the release of factors that influence blood vessel growth [24]. It became clear that a growth factor or factors regulated by hypoxia and hyperoxia is important in the development of ROP. VEGF was discovered initially as a permeability factor [25] and later shown to be an endothelial cell mitogen [26]. VEGF mRNA increases with hypoxia [27] and plays a key role in tumor [28, 29] and retinal associated angiogenesis [30–32].

The role of VEGF in phase I of ROP

VEGF is essential in the development of the retinal vasculature. During normal retinal development, blood vessels grow from the optic nerve to the periphery. As the neural retina develops anterior to the vasculature, the increased oxygen demand of the developing neural tissue generates a wave of ''physiological hypoxia'' that precedes vessel growth [33]. In response to the hypoxia, astrocyte expression of VEGF stimulates blood vessel growth that follows the astrocyte template [34]. As new vessels form and retinal hypoxia decreases, VEGF expression and further vascular growth is reduced via a local feedback mechanism [35].

Supplemental oxygen to premature infants, however, interferes with normal VEGF driven vascular development. In phase I of the murine model of ROP, hyperoxia suppresses VEGF expression, resulting in the loss of the physiological wave of VEGF anterior to the growing vascular front [36]. Cessation of normal vessel growth and regression of existing vessels subsequently occurs. The hyperoxia-induced vaso-obliteration is caused by apoptosis of vascular endothelial cells and can be partially prevented by administration of exogenous VEGF or PIGF-1, a VEGFR-1 specific ligand [37–39]. This finding indicates that VEGF signaling through VEGFR-1 is required for survival of the immature retinal vasculature and explains, at least in part, the effect of hyperoxia on normal vessel development in ROP [39].

The role of VEGF in phase II of ROP

In the second phase of ROP, driven by hypoxia, there is a temporal and spatial relationship between VEGF and proliferative retinopathy. Following oxygen-induced vessel loss and subsequent hypoxia, VEGF expression is increased in retina, resulting in pathological neovascularization [21, 36]. Inhibition of VEGF following the intravitreal injection of either an anti-VEGF antisense oligonucleotide or with a VEGF binding molecule (VEGF receptor/IgG chimera) in this phase significantly decreases the neovascular response [40, 41], indicating that VEGF is a critical factor contributing to retinal neovascularization. Studies with other animal models also support the central role of VEGF in ocular neovascularization [42–46]. These results correspond with the observations from clinics. Elevated VEGF levels are found in the vitreous of patients with retinal neovascularization [31, 47]. In retinal specimens from an ROP patient, VEGF expression was found to occur in a pattern consistent with that found in the mouse model of ROP [46]. Based on these and other studies, an anti-VEGF aptamer as well as an anti-VEGF antibody fragment injected intravitreally is now available to treat neovascularization associated with age-related macular degeneration and is in clinical trials for diabetic eye disease. Clinical trials are planned for evaluation of treatment of the proliferative phase of ROP with anti-VEGF intravitreal treatment.

The roles of growth hormone and insulin-like growth factor (GH/IGF-1) in ROP

Although oxygen, acting in part through VEGF, plays a central role in retinal vessel development and in ROP, it is important to note that other biochemical mediators are also involved in the pathogenesis of retinopathy. Inhibition of VEGF does not completely inhibit hypoxia-induced retinal neovascularization in the second phase of ROP. In the first phase of the disease, although oxygen alone can cause cessation of vessel growth and vaso-obliteration, the results from animal models clearly indicate that clinical ROP is multi-factorial. Despite controlled use of supplemental oxygen, the disease persists, suggesting that other factors related to prematurity itself are also at work.

The fact that prematurity is the most significant risk factor for ROP suggests that factors involved in growth and development are critical. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1), which mediates many of the mitogenic aspects of GH, have been investigated as non-oxygen-regulated factors in this process. GH and IGF-1 have been suspected to play a role in retinal neovascularization since the discovery that pituitary ablation has an ameliorative effect on

proliferative diabetic retinopathy [48–50]. Using the ROP mouse model, we have determined that IGF-1 is critical to both phases of ROP, a fact supported by clinical results.

The role of IGF-1 in phase I of ROP

IGF-1 is critical to phase I of ROP and to the normal development of retinal vessels. The IGFs (IGF-1 and IGF-2) are important in fetal growth and development during all stages of pregnancy [51]. The serum concentration of IGF-1, but not IGF-2, increases with gestational age and correlates with fetal size [52, 53]. IGF-1 levels rises significantly in the third trimester of pregnancy, but after birth decreases due to the loss of IGF-1 provided by the placenta and the amniotic fluid [51].

We hypothesized that IGF-1 is critical to normal retinal vascular development, and that lack of IGF-1 is associated with lack of vascular growth and subsequent proliferative ROP. Results from animal studies show that normal retinal blood vessels grow more slowly in IGF-1 knockout mouse than in wild type controls, a pattern very similar to that seen in premature babies with ROP. In addition, IGF-1 controls maximum VEGF activation of the Akt endothelial cell survival pathway [54]. These findings help to explain how the loss of IGF-1 expression could cause ROP by preventing the normal survival of vascular endothelial cells.

The critical role of IGF in phase I of ROP is also supported by clinical observation from infants with ROP. The mean serum levels of IGF-1 in age-matched premature babies are directly correlated with the severity of clinical ROP [54–57]. IGF-1 appears to be as strong a determinant of risk for ROP as postmenstrual age at birth and birth weight [54, 55]. Low postnatal IGF-1 levels in preterm infants also correlates with brain development and might account for abnormal neural retinal function in ROP [56]. In patients with genetic defects of the GH/IGH-1 axis, very low level of IGF-1 directly causes decreased vascular density as evidenced by fewer vascular branching points [58]. This accumulated evidence suggests that low IGF-1 levels during development are associated with vessel loss and may be contributing to early vessel degeneration in phase I that sets the stage for hypoxia-induced proliferative retinopathy.

Taken together, these findings raise the possibility that early restoration of IGF-1 to uterine levels might prevent the disease by allowing normal retinal vascular development. Since normal vessel development of the retina precludes the development of ROP, the extent of the later destructive phase of ROP is determined by phase I. If we can prevent the vessel loss in the phase I of ROP, the destructive second phase of neovascularization should not occur. Clinical trials are being planned to supplement IGF-1 and IGFBP-3 to in utero levels in premature infants in an effort to evaluate if restoration of IGF-1 to normal levels can prevent or reduce the severity of ROP.

The role of IGF-1 in phase II of ROP

The first study showing that IGF-1 is important in retinopathy came from work in the proliferative phase of ROP (phase II). In the second phase of ROP, retinal neovascularization is substantially reduced in transgenic mice expressing a GH receptor antagonist or normal mice with a somatostatin analogue that decreases GH release [59]. This inhibition of neovascularization by GH is mediated through inhibition of IGF-1, since systemic administration of IGF-1 completely restores neovascularization in normal mice with decreased GH release. Direct proof of the role of IGF-1 in the proliferative phase of ROP in mice was established using an IGF-1 receptor antagonist, which was found to suppress retinal neovascularization without altering VEGF levels induced in mouse ROP [60].

Other studies have investigated the role of both IGF-1 and insulin in the ROP mouse model with a vascular endothelial cell specific knockout of the IGF-1 receptor or insulin receptor [61]. Both types of transgenic mice showed substantial reduction in retinal neovascularization compared with control. In the mice with the insulin receptor knockout, the reduction of neovascularization was associated with a decrease in VEGF expression. These finding suggest that both insulin and IGF-1 signaling in the vascular endothelium may be involved in the regulation of retinal neovascularization.

The interaction of VEGF and IGF-1 in the evolution of ROP

With GH and IGF-1 inhibition in the proliferative phase of ROP, hypoxia-induced VEGF production is unchanged, indicating that IGF-1 does not directly act through VEGF under these physiological conditions. It was suggested that IGF-1 regulates retinal neovascularization at least in part through control of VEGF activation of p44/42 MAPK, establishing a hierarchical relationship between IGF-1 and VEGF receptors

[54, 60]. IGF allows maximal VEGF stimulation of new vessel growth. Reducing IGF-1 levels inhibit vessel growth despite the presence of VEGF. These studies suggest that IGF-1 serves a permissive function, and VEGF alone might not be sufficient for promoting vigorous retinal angiogenesis.

Based on the understanding of the roles of VEGF and IGF-1 in both phases of the disease, a rationale for the evolution of ROP has emerged (Fig. 1). Retinal vessel growth requires both IGF-1 and VEGF. In premature infants, IGF-1, which is normally supplied by placenta and the amniotic fluid, is at very low levels after birth, since the premature liver cannot replace the loss. Blood vessel growth therefore slows or stops since IGF-1 is needed to allow VEGF signaling for vascular endothelial growth and survival. When supplement oxygen is given to the preterm infants, VEGF itself is suppressed. Thus, both prematurity and oxygen are contributing to the suppression of normal vessel growth and vessel loss in phase I of ROP. As the infant grows and the retina matures without an adequate supply of blood oxygen, it becomes hypoxic and induces increased expression of VEGF. In the mean time, as the infant's organs and systems continue to mature, IGF-1 levels rise, suddenly allowing the now-high-levels of VEGF to stimulate blood vessels growth. The result is the destructive neovascular proliferation in the phase II of ROP, leading to blindness.

Future therapeutic targets

The discovery of the importance of VEGF and IGF-1 in the development of ROP is a step forward in our understanding of the pathogenesis of this disease. These studies suggest a number of ways to intervene medically in the disease process. The use of anti-VEGF therapy is the first medical treatment for neovascularization in age-related macular degeneration and is likely to be useful for proliferative retinopathy. However, prevention of vessel loss will be even more important in the treatment of ROP since the extent of the second destructive phase of ROP is determined by the amount of vessel loss in the first phase. The finding that late development of ROP is associated with low levels of IGF-1 after premature birth suggests that physiological replacement of IGF-1 to levels found in utero might prevent the disease by allowing normal vascular development. In addition, the use of a specific agonist to VEGFR-1, PIGF-1, might be used early in the disease process to prevent vessel loss without promoting proliferative disease.

The current understanding of ROP pathogenesis also makes clear that timing is critical in any medical intervention, since the two phases of ROP require very different approaches. Inhibition of either VEGF or IGF-1 early after birth can prevent normal blood vessel growth while, at the second phase, might prevent pathological neovascularization. Similarly, providing VEGF or IGF-1 early on might promote vessel growth whereas late supplementation in the neovascular phase could exacerbate the disease. In the fragile neonate, any intervention must be made very carefully to promote normal physiological development of both blood vessels and other tissues.

Investigations of rare diseases with similar retinal vessel defects as ROP might provide insight to the treatment of ROP. Other conditions with incomplete retinal vascularization are Norrie's disease, familial exudative vitroertinopathy and incontinentia pigmenti. One form of familial exudative vitroertinopathy is caused by Frizzled-4 gene defect [62]. Both norrin, the protein product of norrie's disease gene, and Frizzled-4, a Wnt receptor gene, cause similar defects in humans and mice with incomplete peripheral retina vascularization and the lack of deep retinal vessels [62–64]. It has been shown recently that the norrin ligand binds to frizzled-4, a Wnt receptor [64, 65] and ectopic norrin restores retinal vessels in Norrie disease mice [66]. These findings raise the possibility of a new pharmacological therapy for eye disease with incomplete vascularization of peripheral retina such as ROP.

There are currently many active areas of research that suggest manipulation of currently approved pharmacological interventions or even dietary interventions may help to prevent the ischemia that results in the destructive aspects of neovascularization in proliferative retinopathy including ROP. Anti-inflam-

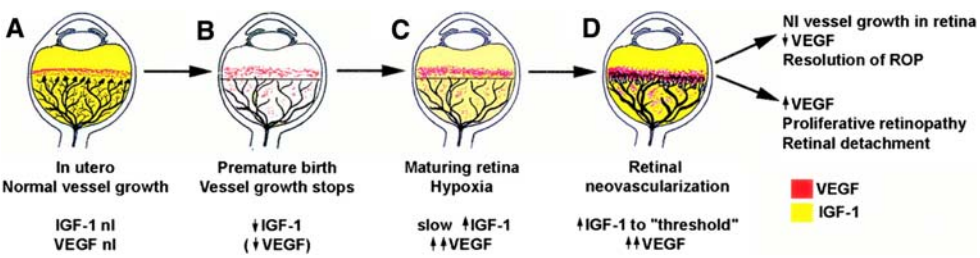

Fig. 1 Schematic representation of IGF-I/VEGF control of blood vessel development in ROP. (**A**) In utero, (**B**) Premature birth, (**C**) Retinal maturation, (**D**) Retinal neovascularization. Nl: normal

matory agents may also be important in future preventative treatment of retinal neovascularization. Further studies on disease mechanism and development of strategies to allow normal retinal and brain development may lead to a significant reduction in the incidence of ROP.

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