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Role of macular xanthophylls in prevention of common neovascular retinopathies: Retinopathy of prematurity and diabetic retinopathy

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ABSTRACT

Retinopathy of prematurity (ROP) and diabetic retinopathy (DR) are important causes of blindness among children and working-age adults, respectively. The development of both diseases involves retinal microvascular degeneration, vessel loss and consequent hypoxic and inflammatory pathologic retinal neovascularization. Mechanistic studies have shown that oxidative stress and subsequent derangement of cell signaling are important factors in disease progression. In eye and vision research, role of the dietary xanthophyll carotenoids, lutein and zeaxanthin, has been more extensively studied in adult onset macular degeneration than these other retinopathies. These carotenoids also may decrease severity of ROP in preterm infants and of DR in working-age adults. A randomized controlled clinical trial of carotenoid supplementation in preterm infants indicated that lutein has functional effects in the neonatal eye and is anti-inflammatory. Three multicenter clinical trials all showed a trend of decreased ROP severity in the lutein supplemented group. Prospective studies on patients with non-proliferative DR indicate serum levels of lutein and zeaxanthin are significantly lower in these patients compared to normal subjects. The present review describes recent advances in lutein and zeaxanthin modulation of oxidative stress and inflammation related to ROP and DR and discusses potential roles of lutein/zeaxanthin in preventing or lessening the risks of disease initiation or progression.

Introduction

Retinal neovascularization (NV)[1] from ischemia-induced retinopathy are common causes of blindness in children (retinopathy of prematurity, ROP) and working-age adults (diabetic retinopathy, DR) in the developed world. Ischemic retinopathies are characterized by microvascular degeneration and retinal ischemia, which can lead to secondary aberrant neovascularization, hemorrhages and blindness [1–3]. This pathologic angiogenesis could be prevented either by direct inhibition of the pathologic NV or by reducing retinal vessel loss, thus, decreasing the hypoxic stimulus that drives the NV. Current therapeutic strategies for ischemic retinopathies have focused on the former approach of inhibiting later stages of pathologic NV. There is great potential in the alternative strategy of reducing vessel loss or promoting normal vascular repair, thereby reducing the hypoxic stimulus that drives NV [4–6]. Such a strategy could result in reducing retinal vascular degeneration, increasing retinal revascularization, or the combination of both. It is, therefore, of great benefit to learn more about the factors and mechanisms that govern the extent of vessel loss and normal vascular regrowth in ischemic retinopathies.

In human retina, two vascular systems (retinal and choroidal) provide blood supply to the highly oxygen-consuming retinal layers. The retinal vascular system provides oxygen and nutrients to the inner retina, whereas the choroidal vasculature supplies the outer retina [3]. In ROP, a delay in physiologic retinal vascular development in preterm infants, suppression of growth factors due to hyperoxia and increase in metabolic demand is associated with hypoxic retinal injury [7]. The hypoxic retina stimulates expression of oxygen-regulated proangiogenic factors, which stimulates retinal NV with sprouting of abnormal vessels from the retina into the vitreous [8,9]. In diabetes, elevated blood glucose and decrease in blood flow result in hyperglycemia and hypoxia in the retina [10,11].

The retina is highly susceptible to oxidative damage by reactive oxygen species (ROS). During pathological conditions, such as retinal ischemia, the imbalance between the production of ROS

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[1] *Abbreviations used:* NV, retinal neovascularization; DR, diabetic retinopathy; ROP, retinopathy of prematurity; AMD, age-related macular disease; MPOD, macular pigment optical density; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor-1; BCO2, β-carotene 9′,10′-dioxygenase; MAPK, mitogen-activated protein kinase; PARP, poly(ADP-ribose) polymerase-1

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and the ability to scavenge these ROS by endogenous antioxidant systems is exaggerated. ROS triggers several signaling pathways, affects DNA and lipids inside the cell, and subsequently leads to cell death. Antioxidants that can inhibit or prevent the oxidative processes can protect retinal cells from oxidative damage. Antioxidants inhibit microvascular degeneration in animal models of DR [12,13] and oxygen-induced retinopathy (OIR) [14,15]. Recent clinical trials of lutein supplementation in term [16] and preterm infants point to lutein functional effects in the neonatal eye [17].

The carotenoids, lipophilic pigments, are important antioxidants, anti-inflammatory agents and regulators of development, reproduction, cellular differentiation and vision protection. They are characterized by an extended conjugated π-electron system that can only be synthesized by plants and microorganisms. Of the >700 described natural carotenoids, approximately 50–60 are typically consumed in the human diet, but only 15–20 are usually detected in human serum and tissues [18], including α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin. Of these, the xanthophylls (lutein and zeaxanthin) account for 20–30% of total carotenoids in human serum but 80–90% of the total carotenoids in the human retina [19]. In early primate development, lutein is the predominant retinal carotenoid. Over time, zeaxanthin levels rise, partly due to conversion of lutein to zeaxanthin. *Meso*-zeaxanthin, a specific lutein metabolite localized in the retina, has not been detected elsewhere in the body [20]. These xanthophylls are most dense at the center of the fovea in the yellowish pigmented area called the macula lutea and are referred to as macular pigment [21,22]. The macular pigment is tissue protective, acting via antioxidant, anti-inflammatory and light-screening properties [23,24].

Low systemic and retinal levels of lutein and zeaxanthin are adversely associated with the risk of age-related macular disease (AMD), ROP and DR [25–29]. Nevertheless, the molecular mechanisms underlying xanthophyll actions in the retina still remain elusive and whether dietary lutein/zeaxanthin can prevent or lessen severity of ROP and DR in practice remains somewhat inconclusive. To date, the majority of the evidence on protective effects of lutein and zeaxanthin in visual health has addressed AMD and cataract. In this review, we describe various aspects of ROP and DR pathogenesis and discuss the potential role of lutein and zeaxanthin in these common neovascular retinopathies of younger individuals.

Retinal vascular development and pathogenesis of retinopathy of prematurity

Retinal vascular development comprises two phases: vasculogenesis and angiogenesis. Vasculogenesis is characterized by *de novo* formation of blood vessels from endothelial precursor cells within the central retina. Angiogenesis is characterized by the development of new vessels that sprout from preexisting vessels [30] and is responsible for increasing the vascular density and peripheral vascularization in the inner retina. During fetal development, the relative hypoxic intrauterine environment promotes production of vascular endothelial growth factor (VEGF), stimulating retinal vascular development. The development of the human retinal vasculature starts at approximately the 16th week of gestation in a central-to-peripheral wave at a rate of about 0.1 mm/day and continues throughout gestation; it reaches an adult pattern at term (*i.e.*, the 40th week of gestation) [31]. Hence, when the infant is born prematurely, her/his retinal blood supply is incomplete. This immaturity in vascular development predisposes the retina to complications. In addition, the developing retina is highly susceptible to oxidative stress [32]. Prematurity further complicates the infant's ability to deal with oxidative stress via imbalanced antioxidant to oxidant ratio, resulting in increased ROS.

ROP is a retinal ischemic and vasoproliferative disease associated with premature birth [33]. It is a major cause of blindness in children in the developed and developing worlds, despite current disease treatment. ROP is characterized initially by a delay in physiologic retinal vascular development, and subsequently by aberrant angiogenesis in the form of intravitreal neovascularization [34]. The more profound the immaturity at birth and persistence of developmental arrest due to exposure of the retina to harmful factors, coupled with deficiencies of factors normally provided *in utero*, the more aggressive the later pathological response. The development of ROP progresses through two postnatal phases [35], possibly preceded by a prophase of antenatal sensitization via inflammation [36,37]. The first phase begins with the interruption of normal retinal vascular development at the time of preterm birth, accompanied by a sudden reduction in insulin-like growth factor-1 (IGF-1) and VEGF [38]. Premature infants are exposed to higher oxygen tension after birth compared to that *in utero*, which leads to downregulation of the major hypoxia-triggered VEGF and subsequent regression of developed retinal vessels. This relatively avascular preterm retina responds to increasing hypoxia and metabolic demand by triggering an abnormal proliferation of vessels leading to neovascularization, the second phase of ROP progression (Fig. 1) [39]. In this phase, overproduction of hormones and growth factors to ensure adequate perfusion to the now hypoxic retina occurs; in particular, VEGF, but also IGF-1 are produced. These factors influence production of extracellular matrix proteins (vitronectin, fibronectin and fibrinogen), deposit adhesive fibrins and induce growth, differentiation and migration of endothelial cells [40].

Preterm birth also is associated with reduction of enzymatic and nonenzymatic antioxidants which are produced or accumulated later in gestation, such as superoxide dismutase (SOD), catalase, vitamin C, vitamin E and lutein/zeaxanthin [41]. Moreover, preterm newborns poorly auto-regulate oxygen delivery to tissues during oxygen administration in intensive care [39]. One example of the presence of oxidative stress is levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), an indicator of oxidative stress, are elevated in leukocytes and urine from preterm infants with ROP [42].

During the development of ROP in the rat OIR model, the retina is subjected to fluctuating oxygen tensions, resulting in retinal hypoxia that triggers overproduction of ROS, which activates nitric oxide synthase (NOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [4]. Intravitreal NV is promoted by resulting activation of signaling pathways such as Janus kinase and signaling transducer and activator of transcription 3 (JAK/STAT3) [43]. Inhibiting ROS with the NADPH oxidase inhibitor apocynin reduces the avascular retina by interfering with apoptosis [4]. Increased levels of NOS, which contribute to the nitric oxide (NO) production, is observed in neonatal retina exposed to hypoxia [44]. In the animal model of ROP, increased formation of retinal peroxynitrite and apoptosis of endothelial cells were seen in association with increased tyrosine nitration of PI3K, cleaved caspase-3, activation of p38 MAPK signaling pathways, and decreased protein kinase B (Akt) phosphorylation. Blocking tyrosine nitration of PI3K with epicatechin or N-acetylcysteine (NAC) reversed the nitro-oxidation-induced pathogenesis [45]. In addition, application of a NOS inhibitor or genetic ablation of endothelial NOS effectively attenuates the severity of ROP in mice, demonstrating the importance of nitro-oxidative stress in ROP [46].

Repeated oxygen fluctuations in the rat OIR model also increase VEGF production, a major signaling molecule involved in hypoxia-induced NV and in ROP. In the retina, VEGF is primarily secreted by Müller cells and astrocytes. In the second phase of ROP, relative hypoxia induces VEGF production and, hence, promotes pathological vessel proliferation [47]. Levels of IGF-1, another key factor in retinal development, are inversely related to ROP. IGF-1