Early Human Development 87 (2011) 683–690

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Mediators involved in retinopathy of prematurity and emerging therapeutic targets

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ARTICLE INFO

Article history:
Received 7 February 2011
Received in revised form 27 May 2011
Accepted 28 May 2011

Keywords:
Retinopathy of prematurity
Mediators
Molecules
Pathogenesis

ABSTRACT

Retinopathy of prematurity (ROP) is a potentially blinding disease of premature infants and despite timely treatment some infants develop retinal detachment and sight loss. Current treatment utilises laser therapy which causes destruction of treated retinal tissue resulting in field loss. There is considerable research work ongoing on neovascular eye disease which is likely to result in antiangiogenic approaches that will arrest the development of ROP by specifically targeting the involved molecular mediators. Some of these new therapeutic interventions have entered clinical trials. This article reviews new information available on the molecular pathogenesis of ROP which may result in novel treatments for ROP; it does not discuss the well-known role of oxygen in the development of ROP.

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1. Introduction

Retinopathy of prematurity (ROP) is a serious complication of prematurity. The rate of blindness varies between countries: it accounts for 3% of childhood blindness in the UK and 13% in the United States. In middle income countries the incidence is considerably higher and it is now becoming a problem in the rapidly developing economies of India and China [1]. Most babies who develop ROP have only mild to moderate disease that spontaneously regresses without treatment; however, severe disease leads to retinal detachment, resulting in sight loss.

ROP screening programmes are well established in most developed countries, and treatment undertaken when required (cryotherapy initiated in 1980s and laser photocoagulation in 1990s). Whilst laser therapy has a reported >90% success rate there are still treatment

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doi:10.1016/j.earlhumdev.2011.05.009

failures and laser treatment involves equipment and training which may not be readily available in less economically developed countries which are currently experiencing an increasing incidence of ROP. Laser treatment, whilst highly effective, is destructive of treated retinal tissue resulting in long-term field loss. There is therefore a need to find other therapeutic methods that are simple, safe, effective and minimally invasive.

The clinical phases of ROP have been well identified and a mouse model of OIR (Oxygen-Induced Retinopathy) has given helpful information. Research on the pathogenesis of ROP, and a better understanding of the mechanisms regulating angiogenesis should enable the development of better, targeted, therapeutic agents to treat sight threatening ROP.

This review focuses on the newer information regarding mediators that are involved in the pathogenesis of ROP, and may be potential therapeutic agents to treat ROP.

2. Pathogenesis and phases of ROP

ROP is a two-phase disease characterised by delayed vessel formation in the first phase, followed by uncontrolled vessel growth in the second phase [2,3]. Vascularisation of the retina begins at the optic disc at 16 weeks gestational age, and is completed by approximately 40 weeks, at term. Premature delivery interrupts this normal vascular growth and the retina is left with a peripheral avascular zone. The extra-uterine environment is significantly more hyperoxic than that in utero, and in addition supplemental oxygen is often required in treatment of premature babies. Normal retinal vascularisation is delayed by this exposure to a relatively hyperoxic retinal environment. This is thought to occur in part as a consequence of increased free oxygen radicals caused by hyperoxia inducing cytotoxicity and resulting in vessel obliteration through apoptosis of endothelial cells. The “vaso-obliterative” Phase I of ROP occurs from birth up to approximately 30–32 weeks.

Although there may be a delay in retinal vascularisation the neuroretina continues to develop with increasing metabolic demands and the retina eventually becomes hypoxic with overproduction of growth factors, orchestrated by vascular endothelial growth factor (VEGF) and other factors that induce neovascularisation. New vessel formation occurs at the junction between vascularised and avascular retina and can lead to bleeding followed by traction and retinal detachment. This "vaso-proliferative" Phase II of ROP occurs most frequently at a postmenstrual age of 32–46 weeks.

A number of complex interactions between multiple mediators come into play during the above processes. Timing is crucial, as the same mediator may have pro- or anti-angiogenic effect during the first and second phase of the disease.

2.1. Vascular endothelial growth factor (VEGF)

The promising role of anti-VEGF agents for the treatment of retinal neovascular disease has given rise to intense research about this factor, and the involved signalling pathways.

VEGF is a term encompassing a number of proteins belonging to a family of heparin binding growth factors. In humans this family has five members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and Placental Growth Factor (PlGF) (Table 1). Of these, VEGF-A was discovered first, is the best characterised so far, and is often simply referred to as VEGF.

VEGF-A is secreted in response to hypoxic and inflammatory stimuli by a number of different cells (macrophages, T cells, astrocytes, pericytes, smooth muscle cells, and in the retina, glial cells, vascular endothelial cells, retinal pigment epithelial cells and ganglion cells) [10]. VEGF-A acts on several cells including vascular endothelial cells (EC). It can have a variety of effects (Table 1), but its main role is to orchestrate the development and growth of blood vessels, by promoting EC proliferation (mitogenesis), migration and tube formation, under physiological and pathological conditions.

Table 1
VEGF family members and isoforms.

Molecule	Isoforms	Action — Effect
VEGF-A		Vasculogenesis, angiogenesis, vasodilation, increases vascular permeability [4]
	VEGF-A_{165}	Angiogenesis, neuroprotection [4]
	VEGF-A_{165}b	Anti-angiogenesis and cytoprotection [4–6]
	VEGF-A_{189}	Tumour angiogenesis, tumour xenotransplantability [7,8]
	VEGF-A_{121}, VEGF-A_{145}, VEGF-A_{206}	Not as extensively studied, so undetermined role so far
VEGF-B		Survival effect on vascular endothelial cells, pericytes, smooth muscle cells, and vascular stem/progenitor cells [4], lipid metabolism in the heart [9]
VEGF-C		Lymphangiogenesis [9]
VEGF-D		Lymphangiogenesis [9]
PlGF		Recruits angiogenic macrophages to tumours [9]

Alternative splicing of the VEGF-A gene generates different variants called VEGF isoforms which have different, sometimes opposite, properties. Proximal splice site selection in the last exon (exon 8) of the gene produces pro-angiogenic isoforms, denoted as VEGF-A_{xxx}, whilst distal splice site selection yields anti-angiogenic isoforms, denoted as VEGF-A_{xxx}b (whereby xxx is the number of aminoacids in the mature protein). The pro- or anti-angiogenic tissue effect is determined by the isoform balance which is controlled by mRNA splicing [11]. This, in turn, is influenced by a number of factors, including hypoxia inducible factor-1 (HIF-1), insulin growth factor-1 (IGF-1) [12], cytokines, hormones and tumour suppressor genes [13]. The exact mechanisms of the regulation of VEGF-A gene expression and the production of specific splice variants remains unclear.

VEGF isoforms differ in structure, with different receptor interactions and consequent functions. There are three known VEGF receptors (VEGFR), of which VEGF-A binds to VEGFR1 and VEGFR2. Molecules such as neuropilin-1 and -2 (NRP-1, NRP-2) and heparin sulphate proteoglycans (HSPGs) lying near VEGFRs on the cell surface act as VEGF co-receptors, enhancing binding and leading to prolonged signal transduction. VEGF-A binding to the extracellular part of the receptor produces activation of intracellular signalling proteins, such as protein kinase C (PKC), VEGF receptor-associated protein (VRAP), and mitogen-activated protein kinase (MAPK). These molecules initiate activation of genes mediating cell migration, mitogenesis, or survival signalling.

2.1.1. VEGF-A_{xxx} isoforms

Amongst the variants of VEGF-A currently identified (Table 1), VEGF-A_{165} which is the major form found in the human eye, appears to be the one responsible for pathological ocular neovascularisation and represents the major therapeutic target. Inhibition of VEGF-A_{165} by anti-VEGF-A full and partial-length monoclonal antibodies, bevacizumab (Avastin; Genentech, San Francisco, CA) and ranibizumab (Lucentis; Genentech, San Francisco, CA), respectively, is already in common use for treatment of age-related macular degeneration and has dramatically changed its treatment and prognosis [14,15].

Anti-VEGF-A_{165} therapy may potentially play a similar role in ROP. In the first publications in 2007 on this topic, a study of intravitreal bevacizumab injection complementary to laser therapy in three patients [16], and two case reports [17,18] showed regression of aggressive ROP. By 2010, they were followed by six small case series or case reports [19–24] (Table 2). In a review of these case reports, it was summarised that in 74 out of a total of 89 treated eyes in varying stages of ROP the disease remained stable or regressed, and in 11 it progressed [24]. The