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Immunohistochemical analysis and neovascular AMD

Towards a rational approach to combination therapy for neovascular age-related macular degeneration

Jorge G Arroyo

Multifactorial Immunohistochemical Analysis of Choroidal Neovascularisation

Neovascularisation is controlled by the temporal and spatial distribution of agonising and antagonising membrane-bound and diffusible substances.¹ In 1948, Michaelson² hypothesised that a diffusible, hypoxia-induced, angiogenic “factor X” was responsible for iris and retinal neovascularisation associated with ischaemic retinopathies.

Decades later, in 1983, a candidate glycoprotein was partially characterised by Dvorak *et al*³ and initially termed vascular permeability factor. Further work by Ferrara and Henzel⁴ expanded our understanding of this endothelial cell-specific glycoprotein, leading to its current name, vascular endothelial growth factor (VEGF). Since then,

researchers have carried out studies on both animals and humans that strongly suggest that the diffusible, hypoxia-induced, endothelial cell-specific factor VEGF^{5–7} most probably represents Michelson’s retinal tissue “factor X”.

The fields of oncology and ophthalmology have profited from the discovery of VEGF and its critical role in physiological and pathological neovascularisation. The development of various antagonists, such as antibodies, antibody fragments, aptamers, traps, small interfering RNA fragments, has resulted in a leap forward in the treatment of neovascularisation associated with age-related macular degeneration (AMD),^{8–9} an oxidative/ischaemic/inflammatory retinopathy, and promises to make important inroads in the treatment of neovascularisation associated with ischaemic retinopathies, such as diabetic retinopathy, retinal vein occlusions and retinopathy of prematurity.

As with most biological systems, physiological neovascularisation is controlled by a dynamic balance between positive and

negative control mechanisms. Factors that stimulate angiogenesis include the VEGF-A isoforms and the α and β fibroblast growth factors. As a counterbalance, factors that inhibit neovascularisation include pigment epithelial-derived factor, angiotatin (29 kDa fibronectin), platelet factor-4, E-selectin, α -interferon and thrombospondin-1. These agonising and antagonising mediators work in a see-saw fashion to orchestrate the biochemical and cellular cascades that eventually lead to neovascularisation.¹

How some of these angiogenic factors respond, both temporally and spatially, to photo-occlusion by verteporfin (Visudyne, QLT, Vancouver, British Columbia, Canada) photodynamic therapy (PDT) is the focus of the paper by Tatar *et al*¹⁰ (see page 166) in this issue of *BJO*. These investigators studied retrospectively the choroidal neovascular membranes (CNVs) obtained during the course of 360° macular translocation in patients with AMD, who had and had not been previously treated with PDT. Similar to other studies,¹¹ they found that E-selectin (an important cofactor for endostatin) seems to be unaffected by PDT treatment between 3 and 655 days before surgery compared with controls. However, this group is the first to look at the relationship between VEGF and endostatin in CNV from patients with AMD treated with PDT. Within 3 days of PDT treatment, VEGF is up regulated, whereas endostatin is barely present, thereby creating an angiogenic microenvironment. At time points >3 days,

VEGF levels seem to moderate and endostatin levels recover, leading to vascular remodelling and maturation.

Such temporal, spatial, multifactorial immunohistochemical studies of pathological tissue are needed to clarify our understanding of the pathophysiology, and put forward novel treatment strategies for neovascular AMD. These studies not only provide a rationale for the incorporation of anti-VEGF agents with vaso-occlusive treatments such as PDT and potentially vaso-ablative treatments such as thermal laser photocoagulation, but also open the door to possible combined pharmacological treatments with agents that augment the presence of inhibitory factors, such as endostatin or pigment epithelial-derived factor,¹² helping to shut off the switch that controls angiogenesis.

Obviously, vasodestructive, vaso-occlusive and pharmacological treatments of CNV are not the ideal long-term solution for AMD. Instead, treatments need to be developed that target the underlying oxidative/ischaemic/inflammatory retinopathopathy associated with AMD, which creates the permissive microenvironment for pathological neovascularisation in the first place.

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Intravitreal triamcinolone and photodynamic therapy

Combined photodynamic therapy and intravitreal triamcinolone injection for the treatment of choroidal neovascularisation secondary to pathological myopia: a pilot study

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Intravitreal triamcinolone associated with PDT should preferably be used among elderly patients who usually show lower visual acuity and a poorer response to PDT, or among patients with recurrent or greater CNVs

High myopia is a major cause of legal blindness in many developed countries, affecting 2% of the general

population. Myopic maculopathy, mainly in the form of subfoveal choroidal neovascularisation (CNV), is the most common

cause of vision loss in patients with high myopia. The appearance of lacquer cracks and the presence of high levels of vascular endothelial growth factor and pigment epithelium-derived factor¹ are probably involved in the development of myopic CNV. These findings have given way to new theories and approaches to the treatment of myopic CNV.

Different therapeutic approaches have been tried for this condition. Photocoagulation² and surgical removal of subfoveal or juxtafoveal myopic CNV³ are no longer performed because of poor final visual acuity. During the past few years, the two main approaches to subfoveal myopic CNV have been macular translocation⁴ and photodynamic therapy (PDT). PDT has been a major advance in the management of this condition after the good results achieved by the Verteporfin in Photodynamic 1 Study.^{5,6} The response of myopic CNV to PDT is considerably better than that of age-related macular degeneration (AMD), requiring less PDT sessions and resulting in better final visual acuity.⁷ This is