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Role of vascular permeability factor/vascular endothelial growth factor in eye disease

Reinier O Schlingemann, Victor W M van Hinsbergh

The eye contains highly vascularised and completely avascular tissues in close apposition. This specialised anatomy requires tight regulation of the balance between vascular quiescence and vascular growth. Growth normally occurs in ocular embryonic development, but is virtually absent from the eye in adult life. In eye diseases associated with angiogenesis, this delicate balance is disturbed. Angiogenesis plays a crucial role in disorders responsible for most blind registration in the Western world—that is, diabetic retinopathy (DR), retinopathy of prematurity (ROP), age-related macular degeneration (AMD), and a large number of other eye conditions. Because of its importance in wound healing, tumour growth, and other pathological situations, angiogenesis has been extensively studied in the fields of oncology, rheumatology, cardiology, and ophthalmology. The main interest in these efforts has been the notion that inhibiting angiogenesis may influence the course of tumour growth or other disease. In ophthalmology, inhibition of angiogenesis may also prove to be of great value.

Angiogenesis is a tightly controlled process which involves both endothelial cells and pericytes, and is influenced by numerous agonist growth factors, inhibiting factors, and extracellular matrix proteins. Its importance in embryonic retinal vascularisation and eye disease (Fig 1) has long been recognised, and it was as early as 1948 that Michaelson suggested that it is the avascular fetal retina itself that produces a diffusible ‘biochemical factor’ capable of inducing vascular ingrowth, the production of which is associated with retinal metabolism. Later, when the association of neovascularisation with retinal non-perfusion and ischaemia in pathological conditions such as diabetic retinopathy was recognised, Ashton and colleagues suggested that hypoxia may be the impetus for the production of this presumed factor.

In recent years, an important candidate for Michaelson’s ‘factor’ has emerged: vascular permeability factor or vascular endothelial growth factor (VPF/VEGF). In 1982 the name vascular endothelial growth factor was first used to denote endothelium specific mitogenic activity isolated from calf retina. Although this growth factor had similarities with the molecule presently known as VPF/VEGF, it was incompletely characterised. VPF/VEGF was isolated in 1983 by Senger et al as the result of a search for a major angiogenesis and vascular permeability factor.

**DISCOVERY OF VPF/VEGF**

In 1982 the name vascular endothelial growth factor was first used to denote endothelium specific mitogenic activity isolated from calf retina. Although this growth factor had similarities with the molecule presently known as VPF/VEGF, it was incompletely characterised. VPF/VEGF was isolated in 1983 by Senger et al as the result of a search for a major angiogenesis and vascular permeability factor.
for tumour derived factors causing increased microvascular permeability.24 These authors hypothesised that angiogenesis in tumours and healing wounds depends on a common pathway that involves the deposition of fibrin and other extravasated macromolecules in the stroma, rather than being initiated directly by angiogenic factors.24–26 The isolation of a tumour derived diffusible molecule enhancing this extravasation supported their hypothesis, hence the early name vascular permeability factor (VPF).24 It took 6 years to recognise that this molecule, apart from its effect on microvascular permeability, effectively had all the properties of a true angiogenesis factor.27–29 This coincided with the isolation and subsequent cloning of two ‘new’ endothelial mitogens named vascular endothelial growth factor (VEGF) and vasculotropin30–33 which in the following years were recognised to be identical to VPF.29,34,35 In this review we will adhere to the most commonly used designation for this factor: VEGF.

**BIOCHEMICAL PROPERTIES**

VEGF is a glycoprotein dimer of 34–45 kDa with some structural homology to platelet derived growth factor (PDGF), and close homology with placenta growth factor36–40 and two other gene products, VEGF-B41 and VEGF-C.42–43 VEGF is a secreted factor in contrast with certain other highly angiogenic cytokines, such as acidic and basic fibroblast growth factor (aFGF, bFGF).44–46 Many different cell types, including endothelial cells, produce VEGF in vitro.47–49 Four different isoforms of VEGF (with 206, 189, 165, and 121 amino acids) arise from the same gene by alternative splicing.27,50 The isoforms appear to have identical biological activity but differ in molecular weight and affinity for heparin which may affect their bioavailability in tissues.51–53 Thus, the 121 and the predominant 165 species are soluble factors while the larger forms with higher heparin affinity are cell or extracellular matrix associated.54 Enzymatic cleavage of these larger forms by plasmin can lead to the release of active diffusible VEGF and this may provide one mechanism by which VEGF activity is regulated in vivo.55–54

**IN VIVO AND IN VITRO ACTIVITIES RELATED TO ANGIOGENESIS**

In vivo, VEGF potently increases microvascular permeability of post capillary venules and capillaries,54,55,56 and can rapidly induce fenestrations in continuous endothelia when injected in skin or muscle.56 This effect on endothelial permeability is probably involved in the pathogenesis of malignant ascites,57 ovarian hyperstimulation syndrome,58 oedema around brain tumours,59–61 bullous skin diseases, and psoriasis.62–65

The extravasation of plasma proteins caused by VEGF may be a crucial step in the neovascular response.55 However, VEGF has a number of other biological activities consistent with a role as a direct angiogenesis factor: it induces neovascularisation in several in vivo angiogenesis assays.18,27,28,64–66 It is mitogenic for cultured vascular and lymphatic endothelial cells27,76–80 and stimulates their migration and tube formation, the so called ‘angiogenesis in vitro’ (Fig 2).77–79 It also enhances endothelial glucose transport,80 and induces expression of interstitial collagens, the urokinase-type and tissue-type plasminogen activators and the urokinase receptor in these cells.70–72 These proteases and plasminogen activators are proteases that enable migration of endothelial cells through the interstitium or a fibrinous exudate during angiogenesis.70–72 In addition, VEGF induces the expression of a, b, integrin and osteopontin,44 proteins involved in cell-matrix interaction and angiogenesis.73–75 In conclusion, VEGF is able to stimulate all major functions of endothelial cells needed in angiogenesis—increased permeability, migration, proliferation, and tube formation.

**THE VEGF RECEPTORS**

Two high affinity receptors for VEGF, named VEGFR-1 or Flt-1 and VEGFR-2 or KDR (Flk-1 in mouse)75,76–80 have been characterised. They are both transmembrane proteins with cytoplasmic tyrosine kinase domains,76 and are expressed on endothelial cells, including retinal endothelial cells.70 The two receptors appear to mediate different biological effects of VEGF.80 VEGF receptors are upregulated in vivo in the microvasculature in conditions with overexpression of VEGF.13,14,35,36–37 In vitro, hypoxia can induce endothelial VEGF receptor expression, possibly by a paracrine mechanism.81 In cultured retinal endothelial cells, hypoxia initially downregulates VEGFR-2/KDR via adenosine and the adenosine A2 receptor, while chronic hypoxia leads to increased VEGFR-2 expression.83 On the other hand, transforming growth factor β downregulates the expression of the VEGFR-2 in cultured endothelial cells.84 The VEGF receptors and specifically VEGFR-1/Flt-1 have been demonstrated in many other cell types, including retinal glia, retinal pigment epithelial cells, monocytes, mast cells, corneal endothelial cells, and cultured pericytes.14,90–93 In most of these cell types, the growth factor stimulates migration through binding to VEGFR-1/Flt-1, but not proliferation.90,92,97,99,100 Thus, VEGF may help to recruit pericytes, mast cells, monocytes, and other cells during angiogenesis or inflammation.
The mitogenic effect of VEGF, which is mediated in cultured endothelial cells through binding to the VEGFR-2/KDR receptor, is restricted to endothelial cells in vivo and to endothelial cells, bovine retinal pericytes, and some epithelial cell types in vitro, including retinal pigment epithelial cells.\textsuperscript{101-103}

ROLE OF VEGF AND ITS RECEPTORS IN EMBRYONAL DEVELOPMENT

Recent evidence shows that VEGF plays a major role in the development of the vascular system in embryogenesis.\textsuperscript{104-107} In early embryos, VEGFR-2 is expressed in mesodermal cells giving rise to angioblasts, and in later stages both VEGF and its two receptors are highly expressed in a distribution suggesting a paracrine mechanism of vascular development induction.\textsuperscript{108-110} For example, in the development of brain and retina, expression of VEGF correlates temporally and spatially with the ingrowth of capillaries, consistent with Michaelson's hypothesis of a retina derived factor.\textsuperscript{111}\textsuperscript{106} In the developing retina, VEGF may also act as a survival factor for endothelium.\textsuperscript{112} 115 The two VEGF receptors appear to mediate distinct functions in this process as mutations in the two receptor genes show differential lethal developmental abnormalities in transgenic mice.\textsuperscript{113} 116 Mice lacking functional VEGFR-2/KDR fail to develop blood islands and show no blood vessel development, while embryos deficient in VEGFR-1/Flt-1 form endothelial cells with normal differentiation but abnormal assembly of the cells in disorganised blood vessels.\textsuperscript{117} This may be consistent with a role of VEGFR-2 in mediating a mitogenic/differentiation response of VEGF on embryonic endothelial cells and an effect on migration of vascular cells via VEGFR-1.

Mice embryos lacking one allele encoding for VEGF also have abnormal vascular development and die in utero, suggesting a tight dose dependent regulation of embryonic vascular development by VEGF.\textsuperscript{118} 119

INTERACTIONS WITH OTHER GROWTH FACTORS

Other growth factors and cytokines can also potently induce or inhibit angiogenesis.\textsuperscript{12} 19 63 82–86 107 145 146 Angiogenesis in the cycle of the female reproductive organs correlates with VEGF expression,\textsuperscript{147-150} and is possibly under hormonal control.\textsuperscript{147} 149 Angiogenesis is also associated with upregulation of VEGF messenger RNA in tissue responses to injury, such as wound healing\textsuperscript{46} and formation of collateral vascularisation in cardiac ischaemia,\textsuperscript{139-141} and in pathologically angiogenesis in solid tumour growth,\textsuperscript{14} 50 52 95 133 145 rheumatoid arthritis,\textsuperscript{151} psoriasis,\textsuperscript{152} capillary haemangiomas,\textsuperscript{153} 153 and atherosclerosis.\textsuperscript{154}

In many human and experimental tumours VEGF expression correlates with tumour vascularity, tumour progression, and metastatic potential.\textsuperscript{12} 63 82–86 133 134 The marked effect on the growth of certain angiogenesis in the cycle of the female reproductive organs correlates with VEGF expression,\textsuperscript{147-150} and is possibly under hormonal control.\textsuperscript{147} 149 Angiogenesis is also associated with upregulation of VEGF messenger RNA in tissue responses to injury, such as wound healing\textsuperscript{46} and formation of collateral vascularisation in cardiac ischaemia,\textsuperscript{139-141} and in pathologically angiogenesis in solid tumour growth,\textsuperscript{14} 50 52 95 133 145 rheumatoid arthritis,\textsuperscript{151} psoriasis,\textsuperscript{152} capillary haemangiomas,\textsuperscript{153} 153 and atherosclerosis.\textsuperscript{154}

In 1992, Shweiki et al showed that VEGF messenger RNA was mainly expressed in the vicinity of necrotic and presumably hypoxic foci in glioblastoma multiforme and they demonstrated that hypoxia upregulated VEGF expression in glioma cells in vitro.\textsuperscript{145} Since then other tumour cells\textsuperscript{155} 165 vascular smooth muscle cells,\textsuperscript{122} 123 126 pericytes,\textsuperscript{167} 168 retinal pigment epithelial cells,\textsuperscript{167} and endothelial cells\textsuperscript{167} 167-170 were found to have de novo (endothelial cells), or upregulated (other cell types) expression of VEGF when grown under hypoxic conditions.\textsuperscript{155} In vascular smooth muscle cells this effect is synergistically enhanced by bFGF or PDGF-BB.\textsuperscript{122} 123 126 In multicell spheroids of glioma cultured in vitro, VEGF expression is found in the inner hypoxic cells.\textsuperscript{122} 124 It is not entirely clear how hypoxia upregulates VEGF at the molecular level.\textsuperscript{173} 174 This appears to be partly due to transcriptional activation of the VEGF gene, in a manner similar to hypoxia induced erythropoietin gene expression,\textsuperscript{159} 177 178-181 but mostly by post-transcriptional

ROLE OF VEGF in hypoxia induced angiogenesis

ROLE OF VEGF IN PHYSIOLOGICAL AND PATHOLOGICAL ANGIOTGENESIS

VEGF and its receptors are overexpressed in many tissues with blood vessel growth, often together with other angiogenesis factors.\textsuperscript{12} 19 63 82–86 107 145 146 Angiogenesis is also associated with upregulation of VEGF messenger RNA in tissue responses to injury, such as wound healing\textsuperscript{46} and formation of collateral vascularisation in cardiac ischaemia,\textsuperscript{139-141} and in pathologically angiogenesis in solid tumour growth,\textsuperscript{14} 50 52 95 133 145 rheumatoid arthritis,\textsuperscript{151} psoriasis,\textsuperscript{152} capillary haemangiomas,\textsuperscript{153} 153 and atherosclerosis.\textsuperscript{154}
stabilisation of VEGF messenger RNA. Furthermore, adenosine, which is released from hypoxic tissues, can upregulate VEGF expression through the adenosine A(2a) receptor and the cAMP dependent protein kinase A pathway, as has been demonstrated in retinal vascular cells.

In addition to hypoxia and low glucose, mutations in the p53 cancer suppressor gene and the von Hippel Lindau tumour suppressor gene, or activation of the ras oncogene may also induce upregulation of VEGF expression in human cancer cells, providing a possible mechanism of the neoplastic progression associated with these genes.

Does VEGF play a role in eye disease?

OCULAR ANGIogenesis

Angiogenesis is crucial in the development of the eye as well as in the pathogenesis of a variety of ocular diseases. Corneal neovascularisation, rubeosis of the iris, proliferative retinopathies, and subretinal neovascularisation may result from a multitude of unrelated disease states. It should be realised that in all these conditions vascular growth is part of a wound healing response which usually also involves inflammatory cells, (myo)fibroblasts, and in the posterior segment, glial cells or activated pigment epithelial cells. In the past three years, extensive evidence has become available that VEGF is involved in probably all forms of ocular angiogenesis. In fact, it corresponds well with the diffusible retinal vasomotor ‘factor’ as originally proposed by Michaelson in 1948. However, the available data imply that other factors also contribute to the initiation or maintenance of ocular neovascularisation.

VEGF AND THE CORNEA

Exogenous VEGF potently stimulates angiogenesis in the cornea of experimental animals with no or little inflammatory response. However, the role of the factor in pathological corneal neovascularisation, as occurs in contact lens wearers, after corneal trauma and transplantation or in inflammatory conditions, is unclear. Recently it has been demonstrated that VEGF is produced in whole rabbit corneas kept in culture, and is upregulated in the rat cornea in vivo after 2 weeks of atmospheric hypoxia. Epithelial cells from normal avascular cornea produce VEGF, but do so at a lower level than epithelial cells from vascularised cornea or conjunctiva. These preliminary data suggest some role for the factor in corneal angiogenesis. Another interesting finding is the expression of the Flt-1/VEGFR-1 and VEGF in dedifferentiated corneal endothelial cells in vitro and the migration, but not proliferation, of these cells in response to VEGF.

RETINAL ISCHAEMIA

In clinical ophthalmology it is evident that diseases of the retina which are associated with impaired capillary or venous circulation can lead to retinal neovascularisation (proliferative retinopathies) (Figs 1 and 3). Viable ischaemic retinal tissue seems to be a prerequisite for an angiogenic response in these conditions, probably through the release of an angiogenic factor by retinal cells triggered by hypoxia. Oxygen measurements in experimental animals and patients indeed have shown that retinal PO2 is very low in ischaemic retinal conditions. Surprisingly, panretinal photocoagulation, which is an effective treatment modality for proliferative retinopathies, can restore retinal PO2 levels, most probably through a decrease of outer retinal oxygen consumption and improved oxygen diffusion from the choroid.

Strong evidence now suggests that VEGF is a major cytokine produced by hypoxic retina. In a primate model of retinal ischaemia produced by branch vein occlusions it was shown that the resulting vascular dilatation, leakage, and endothelial proliferation in the iris temporarily correlate with upregulated expression of VEGF messenger RNA in the ischaemic retina, and with elevated levels of growth factor in aqueous. A neutralising antibody to VEGF injected into these eyes prevented the vascular changes in the iris, but had no effect on the retinal production of the growth factor, indicating that VEGF is indeed required for the observed effect on the iris. In this model, scatter photocoagulation led to recovery from retinal hypoxia, and a decrease of VEGF expression in the retinal areas treated with laser. Comparable results have been obtained in other experimental models of retinal ischaemia in rabbits and rats. These studies suggest that VEGF plays a key role in ischaemia induced permeability and angiogenesis.

Several cell types, including ganglion cells, appear to express VEGF in the ischaemic retina in vivo. These findings correlate with in vitro experiments that have shown that retinal pericytes, endothelial cells, and glial cells produce VEGF in response to hypoxia. Possible mechanisms for increased
VEGF production in ischaemic retina in vivo are by a paracrine effect of increased levels of adenosine and by hypoxia induced VEGF messenger RNA stabilisation.218 219 The conclusions from the animal studies cited above are supported by recent reports showing that recombinant VEGF injected into the vitreous of mice is sufficient to induce vascular changes in the iris and eventually neovascularisation and neovascular glaucoma.207–209 In the retina itself, intravitreal VEGF induces vascular leakage, hyperplasia, and the formation of microaneurysms in monkeys210 and formation of preretinal new vessels in rabbits.211 Remarkably, in these models, the presence of exogenous or upregulated endogenous VEGF is always associated with marked early effects on vascular permeability,212 but does not necessarily lead to angiogenesis. The typical preretinal neovascularisation as seen by clinicians in the posterior segment of patients appears only late in VEGF injected rabbits and not at all in monkeys.190 191 199 201 208 209 These findings suggest that other factors are important and necessary to lead to true retinal neovascularisation.

This is further emphasised by recent work indicating that VEGF and its receptors may also be involved in retinal conditions that are not associated with angiogenesis or ischaemia. Expression of VEGF and both its receptors was found in cultured human RPE and glial cells at the RNA level,194–197 and immunohistochemical VEGF staining was found to be increased in situations where breakdown of the blood-retinal barrier is prominent such as experimental autoimmune uveitis in rats, human uveitis, and aphakic/pseudophakic macular oedema.201

ROLE OF VEGF IN DIABETIC RETINOPATHY AND VENOUS OCCLUSIVE DISEASE

Diabetic retinopathy (DR) is characterised by the development of microvascular leakage and focal areas of retinal ischaemia in non-proliferative DR (NPDR), by neovascularisation originating from the disc and peripheral retina in response to widespread retinal ischaemia (proliferative DR).

Retinal vein occlusions may also show extensive retinal non-perfusion with leakage and ischaemia (Fig 3B) leading to retinal or iris neovascularisation.2, 3 212–214 Based on the previously described findings in animal models it is not surprising that upregulation of VEGF messenger RNA, and marked immunohistochemical microvascular staining for the factor have been demonstrated in the retinas of patients with proliferative DR and central retinal vein occlusions (CRVO).201 205 207 Furthermore, several studies have demonstrated significantly elevated levels of VEGF in ocular fluids from patients with active diabetic proliferative disease or ischaemic CRVO compared with patients with inactive proliferative disease, after extensive laser surgery, or those with NPDR.217–219 None of the other known angiogenesis factors, except insulin-like growth factor-1 (IGF-1),5 126 220 show such a strong correlation with ischaemia related ocular angiogenesis in humans.217–219 221 In addition to hypoxia, elevated glucose levels alone may also upregulate VEGF in diabetics.222 However, it is remarkable that a significant number of diabetic patients with established active neovascularisation have undetectable levels of VEGF in ocular fluids.217–219 Although this observation does not exclude the possibility that VEGF plays a primary role in the onset of retinal angiogenesis, possibly when widespread retinal ischaemia leads to a certain critical level of the growth factor in the retina or vitreous, it does indicate that other factors also can contribute to active neovascularisation in the eye.

More localised retinal expression of VEGF may only lead to increased microvascular permeability as recent work suggest that VEGF is also upregulated in NPDR and other causes of retinal oedema.201 212 216–218 Surprisingly, increased immunohistochemical staining for VEGF could even be demonstrated in glial cells in the retina of patients with diabetes without signs of DR.221 Still, in diabetic rats, increased microvascular permeability, as demonstrated by extravasation of endogenous albumin, correlates with elevated expression of VEGF messenger RNA and immunohistochemical demonstration of the growth factor in microvessels.222 223 Retinal microvascular leakage is a major cause of visual loss in NPDR, vein occlusions, and other conditions and the above results suggest that VEGF inhibition may be used to treat these disorders.

RUBEOSIS OF THE IRIS

Growth of leaky new blood vessels on the surface of the iris is a feared complication of uncontrolled proliferative DR, ischaemic CRVO, and tumours of the posterior segment of the eye.216 In particular, neovascularisation of the chamber angle with contraction of scar tissue may occur and lead to neovascular glaucoma and, potentially, loss of the eye. Rubeosis has long been attributed to diffusion of a vasomorphic factor from ischaemic posterior segment structures.2, 212. Recent reports indicate that VEGF corresponds well with this factor.215 223 In a series of 38 patients with iris neovascularisation 85% of the cases with active rubeosis had detectable levels of VEGF in the aqueous or vitreous fluid, which was only one out of five (20%) cases with inactive iris neovascularisation.213 This difference was statistically significant. Another study demonstrated increased microvascular immunohistochemical staining for VEGF in the iris and other ocular structures of enucleated eyes with choroidal melanoma, but did not mention a relation with rubeosis.224 Furthermore, the experimental animal models described above show a convincing relation between retinal VEGF expression induced by ischaemia and increased vascular permeability, dilatation, and endothelial proliferation in iris vessels. 190 191 203 However, these changes occur much earlier after onset of experimental retinal ischaemia than rubeosis occurs is ischaemic CRVO seen clinically.212 214 They have been interpreted to represent true iris neovascularisation.190 191 However, they could just represent changes in pre-existing iris vessels,220 and as such may be quite different from the sprouting new vessels on the surface of the iris seen in clinical rubeosis.

RETINOPATHY OF PREMATURITY

Morphological studies on the mechanism of the normal development of retinal vasculature, and on the pathogenesis of retinopathy of prematurity (ROP) helped to form the concept of a hypoxia induced retinal vasoformative factor as put forward by Michaelson and Ashton.15–17 196 In the mammalian embryo, sprouting of vessels occurs from the hyaloid vessels at the disc towards the retinal periphery, preceded by mesenchymal cells and astrocytes.15–17 196 Indeed, recent work has shown that in newborn rats and fetal kittens, early expression of VEGF by these astrocytes in the inner retina is closely followed by formation of the inner vascular network,104 226 227 and that expression of the factor by Müller cells in a later stage precedes the ingrowth of blood vessels from the superficial network to the level of the inner nuclear layer and subsequent formation of the outer capillary plexus.216 This pattern led these authors to propose that hypoxia caused by the onset of neuronal activity is the stimulus for strategically located astrocytes and Müller cells to produce VEGF at different stages of development, leading to horizontal or vertical vascular ingrowth, respectively.216 When hypoxia is relieved by the new vessels, VEGF expression decreases and angiogenesis
comes to a halt.\textsuperscript{136} This reconstruction is consistent with the concepts proposed by Michaelson and Ashton.\textsuperscript{135} Retinopathy of prematurity may be the result of a distortion of these processes.\textsuperscript{135} This disease is characterised by the obliteration of newly formed retinal vasculature, and cessation of peripheral vascular outgrowth in the premature infant as a result of hyperoxia from artificial respiration with high oxygen levels. When the child returns to breathing room air, a neovascular response follows consisting of abnormal, leaky, preretalinal vessels on the edge of the avascular tissue, which may invade the vitreous and can lead to tractional retinal detachment and blindness.\textsuperscript{134} The role of VEGF in ROP has been extensively studied in experimental animal models of the disease. In these models, newborn rodents, pigs, or kittens are first kept in hyperoxic (70–80% oxygen) conditions for 4–5 days, and are then returned to room air. In such a model in the rat, 2 days of hyperoxia led to downregulated expression of VEGF, and this preceded endothelial apoptosis and vascular closure.\textsuperscript{11} These vascular effects could be prevented by a single intravitreal injection of VEGF before exposure to hyperoxia.\textsuperscript{111} Similar results were obtained in an identical mouse model.\textsuperscript{12} In these models, after return of the animals to room air, VEGF was markedly upregulated in the affected retinal areas, presumably in Müller cells, before preretalinal and vitreal neovascularisation occurred.\textsuperscript{124} Inhibition of VEGF in these mice significantly diminished the number of (presumed) endothelial cells in the vitreous, interpreted by these authors as a measure of retinal neovascularisation.\textsuperscript{232–233} Surprisingly, in these rodent models vascular closure caused by hyperoxia occurs in the posterior retina, while the already fully formed peripheral vasculature remains unaffected,\textsuperscript{121,124} the reverse pattern from human ROP. In the feline model of ROP developed by Ashton, which resembles human ROP more closely,\textsuperscript{126} similar results were obtained.\textsuperscript{125} In these kittens, overexpression of VEGF in peripheral avascular retina after return to room air was apparently situated in the ganglion cell layer and associated with degeneration of the pigment epithelium (RPE)-chorioid interface, most notably age-related macular degeneration (AMD). It is characterised by the growth of a fibrovascular tissue derived from the choroid. Macrophages, myofibroblasts, and transdifferentiated pigment epithelial cells accompany the growing blood vessels, leading to the formation of a fibrotic scar. Several angiogenic and other growth factors have been found in these membranes,\textsuperscript{11} and a role for VEGF in this common pathway of subretinal angiogenesis has been suggested by recent experimental data. The growth factor has been shown by immunohistochemistry in macrophages,\textsuperscript{136} transdifferentiated RPE,\textsuperscript{136} and by in situ hybridisation in fibroblasts and inflammatory cells\textsuperscript{15} in surgically removed specimens of these membranes, and in the RPE and retina of postmortem eyes of patients with age-related maculopathy with or without CNV.\textsuperscript{137} These observations are supported by the expression of VEGF by cultured RPE cells and choroidal fibroblasts.\textsuperscript{138,139} Furthermore, significantly elevated levels of VEGF were found in the vitreous of patients with CNV.\textsuperscript{140} How all these different observations fit together to explain the pathogenesis of CNV remains unclear.

POSSIBLE HOUSEKEEPING FUNCTIONS OF VEGF IN THE NORMAL EYE

As in other adult tissues, low constitutive expression of VEGF messenger RNA has been demonstrated in almost all tissues of the normal eye, most notably in the ciliary body, conjunctiva, RPE/choroid, and lens.\textsuperscript{137} In vitro, RPE cells produce VEGF,\textsuperscript{137,141,142} and in a culture system this factor is partly responsible for enhanced choroidal endothelial tube formation induced by overlying RPE cells.\textsuperscript{143} Therefore, it has been hypothesised that RPE derived VEGF may act as a trophic factor for the choriocapillaris.\textsuperscript{11} In general, however, the function of VEGF expression in the normal eye is unknown.\textsuperscript{144}

VEGF and its receptors as targets in ocular therapy

CLINICAL CONSIDERATIONS IN VEGF INHIBITION

Despite considerable therapeutic advances consequent upon the introduction of laser treatments, pathological angiogenesis and increased vascular permeability are still major causes of visual loss.\textsuperscript{5} Laser treatment is often unpleasant for the patient and its therapeutic effect depends on ablation of viable normal tissue, therefore leading to a loss of function.\textsuperscript{227} VEGF has been implicated in the pathogenesis of all the common conditions where laser treatment is currently used: NPDR and proliferative DR, RVO, ROP, and CNV. Inhibition of VEGF activity in these patients may prove a valuable alternative or additional therapeutic option and this could mean a major advance in ophthalmology.

From the previous paragraphs it is reasonable to conclude that VEGF plays an important and probably a pivotal role in ocular angiogenesis and increased microvascular permeability. Whether this role is mainly in initiation (directly or indirectly) or in maintenance of a neovascular response and/or vascular leakage is not known. This would have important implications for the use of VEGF inhibitory agents, as a significant number of patients still present with more or less established neovascularisation or macular oedema. Preventive neutralisation of VEGF in the experimental rodent models of ROP\textsuperscript{222,223} or in the cynomolgus monkey venous occlusion model\textsuperscript{199} has a significant inhibitory effect on preretinal neovascularisation or iris vascular changes, respectively. However, it remains to be seen how ocular VEGF inhibition will affect active or advanced neovascularisation or established oedema.
In the past 25 years several indications for laser therapy in DR and vein occlusions have been established through carefully designed randomised clinical trials. It seems reasonable to use these indications as starting points for planning clinical trials with VEGF inhibitory agents. Initial trials should use such agents only in situations that are known not to benefit from laser or in cases that have not responded to laser therapy, as it would be unethical to deny patients the established beneficial effect of the present laser treatments. Subsequent trials may evaluate their use as adjuvants or replacements for laser therapy.

**THERAPEUTIC USE OF VEGF**

Therapeutic use of VEGF could also be useful in certain situations, such as in stimulating collateral formation in limb ischaemia resulting from arterial occlusion.\(^{264, 245}\) In the developing retina VEGF appears to act as a survival factor for the microvasculature, so that exogenous VEGF may be able to prevent vascular obliteration in ROP.\(^{246, 247}\)

**STRATEGIES IN ANTI-VEGF THERAPY**

The actions of VEGF may be inhibited in several ways. Which of these approaches is clinically useful remains to be determined, as all are still in an experimental stage of development. Some proposed strategies interfere with the basal or hypoxia induced production of VEGF by effector cells. Others are aimed at neutralising soluble VEGF or to prevent its actions by blocking receptors or the transduction of receptor mediated intracellular pathways in target cells. Finally, VEGF induced angiogenesis may be inhibited by interference with the microvascular cellular response to the growth factor.

**Inhibition of VEGF production**

Antisense oligodeoxynucleotides can impair production of VEGF in effector cells at the RNA level.\(^{248}\) These compounds have the pharmacological advantage that they are small and highly specific. They may have the theoretical disadvantage of blocking both basal and induced VEGF expression. In the mouse model of retinopathy of prematurity, injection of these agents before the development of preretal neovascularisation led to partial inhibition of VEGF production and preretal angiogenesis.\(^{249}\)

Recent work has shown that hypoxic induction of VEGF can be mediated by adenosine\(^{250}\) and the adenosine A(2a) receptor,\(^{251}\) and this may lead to therapeutic strategies aimed to specifically inhibit hypoxia induced VEGF production. However, this approach may be complex, because the action of adenosine proceeds via cyclic AMP, and cAMP is also a physiological mediator countering increased endothelial permeability.\(^{252}\)

**Neutralisation of free VEGF**

Free VEGF can be neutralised by specific antibodies.\(^{253, 254, 249}\) Intravitreal injections with such antibodies were able to prevent iris vascular changes in the monkey model of retinal ischaemia.\(^{255}\) Alternatively, soluble VEGF receptor chimeric proteins may inhibit VEGF, as was shown to be effective in the mouse model of ROP.\(^{256}\)

**VEGF receptors and intracellular signal transduction pathways**

Another approach in VEGF inhibition is aimed at blockade of the VEGF tyrosine kinase receptors or their intracellular transduction pathways.\(^{257, 258, 259, 260, 261}\) In several experimental tumour models, transfection with a dominant negative VEGF receptor Flk-1 mutant was able to suppress tumour growth.\(^{262, 263}\)

The mitogenic effect of VEGF on endothelial cells appears to be mediated by certain isoforms of protein kinase C (PKC),\(^{264, 265}\) and specific PKC inhibitors are able to inhibit VEGF induced endothelial proliferation and angiogenesis.\(^{266, 267}\) However, such inhibitors are not specific for the eye, and much has to be learnt about how they may affect wound healing and collateral formation.

**Endothelial cellular response to VEGF**

As outlined before, VEGF induces several functional changes in endothelial cells related to microvascular permeability or angiogenesis. These cellular response to the cytokine (and other angiogenesis factors) may be specifically inhibited—for example, by interfering with endothelial migration through integrin antagonists\(^{241, 268, 269}\) or inhibitors of cell associated plasminogen activation.\(^{270}\)

**POTENTIAL SIDE EFFECTS OF VEGF INHIBITION**

A matter of concern in the clinical use of VEGF inhibition is the possible role of the growth factor in physiological angiogenesis and other vital functions. Wound healing, the female reproductive cycle, collateral formation in cardiac ischaemia and other tissue responses may be influenced by interfering with VEGF activity (see earlier). Furthermore, the constitutive expression of the molecule in normal quiescent ocular\(^{271, 272, 273}\) and other tissues\(^{274, 275, 276}\) suggests unknown but potentially important functions. For example, basal production of VEGF by epithelia may maintain certain capillary beds, as has been suggested for the choroid plexus and the kidney.\(^{277, 278}\) Retinal pigment epithelium (RPE) in culture has been shown invariably to produce VEGF.\(^{279, 280, 281}\) If a similar mechanism is operative between the RPE and the choroidal capillaries,\(^{282, 283}\) VEGF inhibition could lead to choroidal atrophy. Therefore, both systemic and local ocular inhibition of VEGF may have serious side effects.

**PHARMACOLOGICAL ASPECTS AND DRUG DELIVERY OF VEGF INHIBITORY AGENTS**

Application of medical inhibition of ocular angiogenesis or macular oedema will present ophthalmologists with many problems in practical clinical management. Certain conditions may require short term or long term application, possibly through new drug delivery devices. Recently, several new drug delivery systems have been developed.\(^{284, 285}\) This technology could help to target stable VEGF inhibitors to the posterior segment. For less stable protein agents, gene therapy aimed at temporary expression of these compounds by the retinal pigment epithelium may prove useful in the future.

**Conclusion**

VEGF appears to play an important role as a vascular permeability and angiogenesis factor in the developing eye and in pathological eye conditions. It is amazing that the predictions of investigators like Michaelson and Ashton have been confirmed after almost 50 years. In the past few years our understanding of the molecular mechanisms of ocular angiogenesis has dramatically increased. More important, inhibitors of this growth factor may provide clinicians with powerful tools to treat the common ocular diseases in which angiogenesis or loss of the blood-retinal barrier cause visual impairment.

However, it should be realised that there is still much to be learnt about the exact role of VEGF and other angiogenesis factors, and their interactions, in pathological conditions as well as in the normal eye.\(^{286}\) As with other growth factors, the actions of VEGF seem to be contextual, depending upon expression of receptors, the extracellular matrix, extravasated plasma proteins, other
cytokines and other, maybe unknown, influences. Furthermore, the experimental animal models of ocular angiogenesis, from which we have learned most of the role of VEGF in the eye, are quite different from the human disease states, most notably in their time course and pathogenesis.

However, when all the experimental findings are taken together, VEGF has emerged as a major angiogenesis factor in the developing and adult human eye. Modulation of VEGF expression and its effect on angiogenesis and vascular permeability will give us new insights in pathophysiological mechanisms at play in the eye. It is through these insights and by learning the interactions of VEGF with other mediators of angiogenesis that we can hope to develop new means of preventing or treating conditions which all too often lead to significant visual loss or blindness.

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