Reversal of Retinal Vascular Changes Associated with Ocular Neovascularization by Small Molecules: Progress toward Identifying Molecular Targets for Therapeutic Intervention

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Abstract: The elucidation of the molecular pathogenesis of ocular disease provides candidate targets for treatment. Animal models allow for identification and quantitation of ocular diseases. By gaining insight regarding the molecular signals involved in various types of ocular angiogenesis, general concepts can emerge that may apply to other settings, including tumor angiogenesis. The hypoxia inducible factor-1 (HIF-1) pathway is relatively well understood and serves as a good example of how knowledge of the biological responses to hypoxia can translate into new therapies. Furthermore, HIF pathway can be used as a therapeutic target and that the manipulation of the HIF pathway at several points has potential use for the treatment of oxygen-dependent diseases in retina. However, there are numerous other molecular and cellular responses to hypoxia that are independent of HIF-1, perhaps each with unique oxygen sensors. Despite participation of multiple stimulatory factors for ocular neovascularization (NV), vascular endothelial growth factor (VEGF) emerges as a pivotal player, thus manipulation of VEGF signaling represents an important therapeutic strategy. While most studies have focused on prevention of ocular NV, regression of new vessels is desirable and is achievable with various small molecules. Screens are underway to identify and test the efficacy of these small-molecules to target various mechanisms involved in ocular NV. These small molecules might represent an important component of novel combination therapies to target various molecular signaling mechanisms in neovascular tissues.

Keywords: Antiangiogenic drugs, neovascularization, reversal, small molecules, HIF-1, YC-1.

INTRODUCTION

Diabetic retinopathy (DR) is a leading cause of visual disturbance in adults. In the early stage of the disease, retinal vascular permeability can increase even before the appearance of clinical retinopathy [1]. Retinal vascular leakage and thickening of the retina lead to diabetic macular edema (DME). In the late stage of DR, abnormal increases in vascular permeability result from retinal ischemia due to nonperfusion of the retina or a decrease in oxygen tension [2]. During this stage, over-proliferation of capillary endothelial cells (ECs) results in retinal NV, abnormal formation of new vessels in the retina and in the vitreous, leading to proliferative diabetic retinopathy (PDR) [3]. Additionally, during the late stages of DR, the ischemia-induced pathological angiogenesis ultimately causes severe vitreous cavity bleeding and/or retinal detachment, resulting in severe loss of vision.

Retinal NV is the major cause of severe vision loss and irreversible blindness in developed countries, affecting people of all ages. These clinical and pathologic manifestations occur in DR, retinopathy of prematurity (ROP), age-related macular degeneration (AMD) and retinal vein occlusion (RVO). Angiogenic factors, such as VEGF, play a prominent role in promoting retinal NV. Retinal ischemia is the major driving force behind the induction of VEGF, which plays a crucial role in ocular pathogenesis [4]. VEGF has a profound impact on multiple functions in ECs, such as proliferation, migration, survival, tube formation, and vascular permeability [5]. Previous studies have indicated that VEGF is an important mediator of NV induced by hypoxic retinopathies [6]. It has been reported that there is increased VEGF production in both vitreous [7], and ocular fluids [8] of patients with DR. Suppression of VEGF receptor interaction, VEGF expression and VEGF-induced signaling has been shown to inhibit NV in animal models of retinal ischemia [9] (Fig. 4).

Screens are underway to identify and test the efficacy of various small molecules to target a plethora of signaling pathways that contribute to ocular NV. YC-1; (3-(5'-hydroxymethyl-2'furyl)-1-benzyl indazole), is a small molecule that inhibits cGMP breakdown and potentiates nitric oxide (NO)-induced soluble guanylyl cyclase (sGC) stimulation [10]. Data from our lab have revealed that YC-1 suppressed retinal new vessel growth and formation in human retinal microvascular ECs, and retinal explants. Furthermore, we have demonstrated that YC-1 down-regulates HIF-1α, HIF-2α, VEGF, erythropoietin (EPO), endothelin-1 (ET-1), and matrix metalloproteinase-9 (MMP-9) protein levels in the human retinal microvascular ECs [11, 12]. In addition, oxygen induced retinopathy (OIR) mouse
model [13] and (Fig. 1) has produced several clinical manifestations that resemble the ones that are exhibited during the diabetes-induced retinopathies. Our data have revealed for the first time that YC-1 selectively inhibits retinal NV (Fig. 2B), while concomitantly promotes physiological revascularization in a mouse model of OIR [14]. Retinal treatment with YC-1 induced the reversal of the vasculature growth to a state that was comparable to the normal retinas that were grown under normoxic conditions (Figs. 1, 2A). There are various small molecules that are currently in use to treat patients with ocular pathologies (Table 1). In this review, we will attempt to shed some light on these molecules and the role that they play in targeting a wide range of signaling pathways that are relevant to ocular NV in different tissues and in animal models.

INHIBITORS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

The concept that growth factors mediate retinal angiogenesis was postulated by Michaelson in 1948 [15]. More recently, attention has focused on VEGF as the primary mediator of intraocular angiogenesis and permeability in PDR [as well as in other conditions, including; DME, exudative AMD, RVO, and ROP]. Several features of VEGF make it a plausible mediator of retinal NV and vascular permeability in ischemic ocular conditions. It is produced by numerous types of ocular cells, including retinal ECs, which in addition express abundant VEGF receptors. Furthermore, VEGF expression is markedly increased in response to hypoxia, and is a potent inducer of vasopermeability [16]. Consequently, the retina may become
Fig. (2). (A) Retina from P17 “control” room air-reared mouse shows a homogeneous normal delicate vessel pattern throughout the retina. The retina is completely vascularized without avascularized areas. No blood vessel tufts are present. The main vessels show no tortuosity and no dilation. (B) Retina from P17 non-treated oxygen-injured group shows an engorged tortuous appearance of the blood vessels, large central avascular area (yellow arrows), and high presence of blood vessel tufts (red arrows).
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“ischemic”, resulting in the growth of new abnormal blood vessels that can cause further vision loss and more serious complications. VEGF levels correlate closely with active intraocular NV and very high VEGF intraocular levels in patients with PDR decline after successful laser photocoagulation. These observations have led to the hypothesis that anti-angiogenic agents could be used to inhibit the development of proliferative retinopathy, much like their use in cancer, which was originally proposed by Folkman more than 30 years ago [17]. It is believed that anti-VEGF treatment may help decrease vascular permeability and edema and prevent the growth of abnormal new blood vessels in the retina in patients with central retinal vein occlusion (CRVO). To date, most clinical trials of anti-angiogenic therapy for ocular disease have focused on agents that specifically sequester VEGF, thus preventing VEGF-receptor engagement (Figs. 3, 4). Such agents include Avastin (bevacizumab), a recombinant humanized murine monoclonal antibody (MoAB) that binds to and inhibits the biologic activity of all human VEGF-A isoforms [18] (Fig. 3). Avastin inhibits all isoforms of VEGF by blocking its interaction with membrane-bound tyrosine kinase (TK) receptors VEGFR-1 and VEGFR-2 [19] (Figs. 3, 4). Thus inhibiting VEGF-induced cell proliferation, survival, permeability, nitric oxide production, migration, and tissue factor production [16]. Early preclinical work demonstrated activity against tumors from a human colorectal carcinoma (CRC) cell in mice [20], and phase I evaluations of Avastin revealed good tolerability when used alone and in combination with 5-fluorouracil (5-FU) and leucovorin (LV) [21]. Currently, Avastin is an FDA approved drug for intravenous use in the treatment of metastatic colorectal carcinoma. Furthermore, in the quest to help patients with neovascular AMD who do not respond to current therapies, injections of Avastin, as an off label intravitreal agent into the eye has been employed. Avastin has produced promising results in inhibiting NV in DR [22] and neovascular AMD [23, 24]. When administered into the vitreous cavity, Avastin penetrates the retina and disperse through the retinal pigment epithelium (RPE) and into the choroid, scavenging extracellular VEGF molecules. The bound VEGF molecules cannot activate VEGF receptors, so blood vessels can’t grow or leak. There are no long-term results on safety and effectiveness of the use of intravitreal Avastin for neovascular AMD, but short-term data indicate that it may be of value. Intravitreal Avastin may lead to complete regression of neovascular formation. Clinically, data have indicated that there was a dramatic and rapid response to the injection, with complete resolution of the vascular leakage in most cases. Animal studies have suggested that Avastin was too large to cross the retina into the subretinal space [25]; however, other results [23] have confirmed the effects of intravitreal Avastin on choroidal neovascularization (CNV). One possibility is that this large molecule is able to cross the diseased retina more readily. Another possibility is that Avastin inhibits VEGF in the vitreous, the surface of the retina, or inside the retina, which may be enough to prevent further growth and leakage from the CNV. In a study of 32 patients, intravitreal injections of Avastin were associated with a rapid regression of retinal and iris NV secondary to PDR [24]. Another small uncontrolled study showed improvement of visual acuity and decreased retinal thickness after intravitreal injection of Avastin [26]. Yet another study showed that intravitreal Avastin injections were followed by reduction of fluorescein leakage from persistent active NV without loss of vision in patients with DR [27]. Finally, it is possible that the results of the animal studies may not apply to the human eye, and Avastin is able to cross the retina. There are several potential adverse effects associated with the systemic use of VEGF inhibitors, such as increased risk for thromboembolic events, hypertension, epistaxis, hemoptysis, proteinuria, delayed wound healing after surgery, and impaired reproductive function [25, 28]. Visual loss in the fellow eye may occur by inhibiting VEGF, which would cause regression of the choiropapillaris [29]. In one primate study, an intravitreal injection of Avastin was noted to cause occlusion of the choiropapillaris from thrombocytes and leucocytes and a reduction in choroidal capillary fenestration [30]. Apart from a low incidence of endophthalmitis associated with repeated intravitreal injections of anti-VEGF agents, no significant adverse effects have been noted clinically [31]. Fears that therapies blocking the action of all VEGF isoforms (Fig. 3) might have adverse effects have not been realized in practice [31].

The second “small molecule” of this category that target VEGF is Macugen (pegaptanib); an aptamer that binds specifically to the VEGF165 isomer and neutralizes the isoform’s ability to bind to its cognate receptors VEGFR-1 and VEGFR-2 (Fig. 4). Aptamers are a new class of therapeutics macromolecules composed of chemically synthesized single-stranded nucleic acids, which maintain a highly specific three-dimensional conformation that allows binding with selective affinity to a molecular target in a manner similar to the binding of antibody to antigen. Previous investigations have demonstrated that blocking VEGF165 with Macugen was as effective as blocking all the isoforms in preventing pathologival NV (Figs. 3, 4) [32]. Therefore, Macugen was the first aptamer to receive FDA approval for use in humans. In a phase II clinical trial, patients treated with intraocular injections of Macugen had better visual acuity outcomes, were more likely to show reduction in central retinal thickness and were deemed less likely to need additional therapy with photocoagulation at follow-up as compared with the sham-injected control subjects [33].

The third member of this group that specifically target VEGF is Lucentis (ranibizumab) (Fig. 4), which is another modified humanized MoAb fragment against all human VEGF [18] that is currently used clinically for ocular NV. Studies with patients showed that intraocular injections of Lucentis significantly reduced foveal thickness and improved visual acuity [34]. Although the previous drugs have shown very promising results, one setback is that they often need repeated delivery. All three agents; Avastin, Macugen and Lucentis are given by intravitreal injection and appear to be well tolerated. The lower molecular weight Lucentis is cleared more rapidly (3 days) [35] from the vitreous than the higher molecular weight Avastin (9.8 days) [36] and10 days for pegaptanib. Although both Macugen and Lucentis have been shown in randomized controlled clinical trials (e.g., ANCHOR, MARINA, V.I.S.I.O.N.) to be effective in the control of CNV in AMD with a consequent improvement in vision [31], neither preparation has been used extensively in
VEGF receptors and related ligands include VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4), neuropilin-1, and neuropilin-2. The interaction of VEGFR with either neuropilin-1 (NRP-1) or heparin sulfate proteoglycan may facilitate the presentation and binding of VEGF to its receptor. These receptors have numerous immunoglobulin G-like extracellular domains and intracellular TK activity. There are several splice variants of VEGF, including VEGF121, 145, 165, 189, and 206, with VEGF165 being the predominant form. Other members of the VEGF family include; VEGF-B, -C, and -D and PlGF. VEGF binds to VEGFR-1 and -2 and are involved in triggering angiogenesis. PlGF is localized to the placenta and binds only to VEGFR-1. VEGF-B also binds only to VEGFR-1 and appears to be involved in coronary vascularization and growth. VEGF-C and VEGF-D activate VEGFR-2 and -3 but not VEGF-1. VEGF-C is implicated in lymphangiogenesis.

Small molecules targets VEGF-signaling pathway by; (1) inhibiting the angiogenic factor VEGF; (2) Inhibition of VEGF-secretion; (3) Inactivation of VEGF; (4) Blockade of VEGF receptors on ocular ECs; (4) Inhibition of postsynaptic VEGF induced cell activation.
the management of macular edema from inner blood-retinal barrier (iBRB) breakdown. FDA has approved formulations for AMD include Macugen and Lucentis, both intravitreous injections requiring repeated injections. However, Avastin is approved for systemic use in certain metastatic cancers and has been effectively used as an intravenous or intravitreous agent for human neovascular AMD [23, 28].

A systemically delivered, modified VEGF receptor; Aflibercept (VEGF-TRAP) is another small molecule “fusion protein”, which is specifically designed to bind all forms of VEGF-A and placental growth factor (PLGF); which are both involved in the abnormal growth of new blood vessels. Counteracting the effects of VEGF-A and PLGF may provide a significant therapeutic effect in patients suffering from these disorders (Fig. 4). In addition to AMD; Regeneron and Bayer HealthCare extended the development program for VEGF Trap-Eye to include CRVO. Recent data from the Phase I study of intravenous VEGF Trap (aflibercept) in patient with advanced solid studies have indicated that IV VEGF Trap was well tolerated at the dose levels tested. In addition, pharmacodynamic and pharmacokinetic markers were indicative of VEGF blockade [37].

Delivery of a small interfering RNA (Ribonucleic Acid Interference) (RNAi) is a gene-silencing mechanism utilized by cells to specifically target the expression of genes corresponding to a double-stranded RNA or siRNA that has been introduced into the cell and to reduce the levels of the specific protein product in the targeted cells. This method could be used in vitro and in vivo [38, 39]. Therefore, RNA interference has potential for application to studies of retinal biology and for the treatment of a variety of retinal diseases, including that involving abnormal blood vessel growth [40]. In the case of neovascular AMD, investigators have focused on downregulating VEGF and its receptor [40, 41]. In addition, Adenovirus vector has been used as the delivery vehicle for the siRNA targeting VEGF. This model overcomes the main hindrance to the therapeutic potential of siRNA, its limited stability, and the need to specifically deliver it to the pathologic cells. Furthermore, previous data have shown that VEGF messenger RNA (mRNA) silencing of >90% in a human RPE cell line and over 80% reduction in the size of CNV in a murine model of neovascular AMD by adenovirus-mediated subretinal delivery of siRNA targeting VEGF [41]. Interestingly, previous investigations have shown that siRNAs 21-nucleotide or longer dsRNAs were antiangiogenic and were able to suppress CNV in mice regardless of the sequence they are targeting via their interaction with a cell surface receptor called toll-like receptor 3 [42]. Their study has shown that two investigational siRNAs in clinical trials owe their antiangiogenic effect in mice not to target knockdown but to TLR3 activation. These findings have supported investigations of non-targeted dsRNAs as generic anti-CNV agents as effective as anti-VEGFA antibodies, while avoiding potential neurotoxicity resulting from chronic administration of the latter. In addition, previous studies have used a vector-based siRNA expression system, which overcomes the limitations of transience and high cost in synthetic siRNAs, to specifically inhibit VEGF165 expression in the murine model of proliferative retinopathy [43]. Their data confirmed the potential VEGF165 inhibitors for the treatment of ocular angiogenesis. Previously, Acuity Pharmaceuticals reported that in a primate disease model, its lead product, Cand5 (bevasiranib), significantly inhibits both NV and vascular leakage that lead to vision loss in wet AMD. Cand5 is a siRNA that targets VEGF. The study data demonstrated that a single dose of Cand5 safely and significantly reduced both NV and vessel leakage, in a dose-dependent manner for more than five weeks. At the highest dose used in the study Cand5 reduced the incidence of clinically significant vascular leakage to zero by week three and for the duration of the study, and at day 35 NV was inhibited by greater than 65% in the high dose group. No adverse effects were observed [44]. Cand5 has entered a Phase III trial for the treatment of wet AMD. The study assess whether Cand5 administered every 8 or 12 weeks is safe and has equivalent efficacy in preventing vision loss as Lucentis administered every 4 weeks. In a Phase II trial, Cand5 was demonstrated to be safe and well tolerated and revealed benefits against several end points, including near vision and lesion size. Furthermore, AGN211745 (formerly Sirna-027 is in development for wet AMD by Allergan, has reached Phase II; in earlier trial, it was reported to improve or stabilize visual acuity in a subset of patients. A third siRNA in wet AMD, RTP801i (Quark Pharmaceuticals), entered into Phase I clinical trials.

INHIBITORS OF THE TYROSINE KINASE SIGNALING PATHWAYS

Recent investigations in tumor biology have focused on multi-kinase inhibitors, such as Sutent, (PTK787) (Fig. 4) [45], and Axitinib (AG013736) to block angiogenesis [46]. Recently, Pfizer announced that the Phase III AXIS 1032 trial, studying axitinib, (AG013736) [47], in previously treated patients with metastatic renal cell carcinoma, has met its primary endpoint, demonstrating that axitinib significantly extended progression-free survival when compared to sorafenib (Nexavar, Bayer Healthcare/Onyx Pharmaceuticals), in the study population. Consistent with previous analyses, axitinib demonstrated a generally manageable safety profile in this study. Several studies have utilized two similar compounds, AG013711 and AG013764, which are small molecule inhibitors of VEGFR-1, VEGFR-2 and related receptor tyrosine kinases (RTK) such as platelet-derived growth factor receptor (PDGFR) [48]. Combined activity against VEGF and PDGF signaling transduction is beneficial since both growth factors are involved in angiogenesis (Fig. 5). VEGF induces endothelial cell proliferation, migration, tube formation, increases vascular permeability, leukocyte trafficking, and is critical in ensuring the survival of proliferating ECs. PDGF induces pericyte recruitment and supports vascular maturation. Inhibitors of PDGFRs have been shown to produce pericyte loosening or detachment from ECs of tumor vessels [49]. In addition, blood vessels lacking pericytes are more susceptible to VEGF deprivation. Therefore, inhibitors targeting both VEGFR and PDGFR can contribute to the regression of actively proliferating ECs in tumors [46] and in multiple models of ocular NV [50]. Both compounds; AG013736 and AG013958 target both VEGF and PDGF pathways and have the potential to induce regression of CNV beyond what Macugen or Lucentis can accomplish individually. Previous data have shown that Sub-Tenon administration of AG-
The Open Diabetes Journal, 2011, Volume 4 DeNiro and Al-Mohanna

013958 (Pfizer, Inc.) in 21 patients with subfoveal CNV associated with AMD resulted in minimal levels of systemic exposure, which was similar to levels found in 13 cynomolgus monkeys dosed with the same compound [51]. Several studies have indicated that intravitreal injections of AG013764 or AG013711 reduced the level of CNV by approximately 60% compared to control [52]. The discovery of a multiple VEGFR inhibitors representing a variety of pharmacophores offers the opportunity to generate new molecules with increased potency or improved pharmaceutical properties by rational design based on cocrystals with VEGFR-2. Several investigations have revealed that 3-[5-methyl-2-(2-oxo-1, 2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid (SU10944) is an inhibitor of VEGFR-2. SU10944 can be administered in vivo by the oral route and achieves sufficient exposure to inhibit nearly all VEGF-stimulated NV and vascular permeability. In addition, a compound of this nature will be valuable in delineating the role of VEGF in various forms of pathological angiogenesis where multiple kinases may play contributing roles. Previous studies have demonstrated that SU10944 inhibited VEGF-induced receptor autophosphorylation as well as downstream signaling [53]. Furthermore, SU10944 exhibited potent inhibitory activity against VEGFR-1; weak activity against other related subgroup members, including stem cell factor receptor (SCFR), PDGFR, and FGFR-1; and no detectable activity against other protein tyrosine kinases such as epidermal growth factor receptor (EGFR), Src, and hepatocyte growth factor receptor (HGFR). In cellular assays, the selectivity for SU10944 to inhibit VEGFR is maintained compared with other tyrosine kinases. Oral administration, SU10944 gave a clear dose response in the corneal micropocket model. Moreover, SU10944 potently inhibited VEGF-induced vascular permeability.

Specific multi-targeted RTK inhibitors have demonstrated broad and potent anti-angiogenic activities by targeting several angiogenic-signaling pathways. Sutent...
Reversal of Retinal Vascular Changes

SU11248 is a small, orally bioavailable molecule that has been identified as a low-nanomolar inhibitor of the angiogenic receptor tyrosine kinases; PDGFR-α, and PDGFR-β, as well as VEGFR-1 and -2, KIT (stem cell factor receptor), and FLT3 (Fms-like tyrosine kinase-3 receptor) (Fig. 4). This agent has demonstrated anti-angiogenic and/or antitumor activity in preclinical studies, including tumor regression in murine models of human epidermal (A431), colon (Coto205 and HT-29), lung (NCI-H226 and H460), breast (MDA-MB-435), prostate (PC-3-M-luc), and renal (786-O) cancers, and suppression or delay in growth of many other tumor models, including subcutaneous C6, GL261, and SF763T glioma xenografts [54]. More recently, SU11248 has demonstrated efficacy with acceptable tolerability in a phase III clinical trial involving patients with gastrointestinal stromal tumor and two phase II trials involving patients with metastatic renal cell carcinoma [45], and it is under-going earlier stage clinical trials for various other cancers, including breast and non-small-cell lung tumors. Promising SU11248 clinical results to date support the hypothesis that targeting VEGF- and PDGFR-mediated signaling is an effective approach in the treatment of human cancers. Furthermore, SU11248 effectively blocked hypoxia-induced retinal NV in zebrafish, while the formation of new vascular branches was virtually totally inhibited [55].

To date, most clinical trials of anti-angiogenic therapy for ocular disease have evaluated agents that specifically bind VEGF, thus preventing VEGF-receptor activation. ZM323881 (5-[7-(benzoxo) quinazolin-4-yl] amino]-4-fluoro-2-methylphenol) is a selective inhibitor of human VEGFR-2/KDR activity [56]. Data have indicated that ZM323881 selectively inhibits VEGFR-2 over VEGFR-1 and a range of other receptor tyrosine kinases such as PDGFRβ, FGFR1, EGFR and erythroblastic leukemia viral oncogene homolog 2 (erbB2) [57]. Angiopoietins and Tie2 inhibitors were recently identified as an endothelial cell-specific ligand-receptor system that is critical for vascular development and postnatal pathologic angiogenesis by mediating vascular integrity. Tie2 and angiopoietins are upregulated in the retina from patients with ischemic retinal disorders [58]. Recent studies demonstrated that Tie2 plays a key role in mediating both retinopathy and CNV. Immunohistochemistry with a monoclonal antibody to human Tie2 showed a prominent expression of Tie2 around and within the base of newly formed blood vessels of retinal and choroidal neovascular lesions. Several small-molecule Tie2 inhibitors have been identified. These molecules have blocked Ang1-induced Tie2 autophosphorylation and downstream signaling. Further optimization of these molecules has yielded improved selectivity, aqueous solubility, micromolar stability and cytochrome P450 profile for one of the compounds. Some of these compounds have inhibited endothelial cell tube formation. Intravitreal injection of sTie2-Fc, soluble Flt-1 fusion protein (sFlt-1-Fc), and both chimeric proteins suppressed retinal angiogenesis in a murine model of retinal ischemia in the order of sTie2-Fc < sFlt-1-Fc < sTie2-Fc+sFlt-1-Fc [58]. In addition, systemic soluble Tie2 expression inhibited corneal NV [59].

Phosphatidylinositol 3-kinase (PI3 kinase) inhibition is also considered as an attractive pathway to modulate the development of angiogenesis in the eye. One example is LY294002, which shows dose-dependent anti-angiogenic effects, without perturbing existing vessels. The anti-angiogenic effect of LY294002 can be phenocopied using an inhibitor to AKT, which itself is known to act downstream of PI3K. In addition, intraocular administration of LY294002 selectively inhibits angiogenesis in the eye but does not diminish visual function [60]. Moreover, the inhibition of the splicing factor kinase 1/2 (SRPK1/2) via a small molecule SRPIN340 has previously been shown to block the RNA-binding splice factor ASF/SF2 phosphorylation, while the inhibition of distal splice site selection is brought about by the Cdc2-like kinase (Cdk) inhibitor TGF003. TGF003, an inhibitor of the Cdk family of kinases implicated in splicing control by phosphorylating splicing factors, inhibited TGF-β1 induced VEGF mRNA expression. Data have indicated that TGF-β1 stimulates the synthesis of VEGF mRNA isoforms by p38 MAPK and Cdk kinases [61]. Previous studies have indicated that intravitreous injection of SRPIN340 significantly reduced leakage of lesions in fluorescein angiography and significantly inhibited CNV in a mouse model [62]. In addition, several scientific investigations have utilized the concepts of targeting VEGF-receptor family and the inhibition of the fibroblast growth factor receptor (FGFR) and PDGFR tyrosine kinases as an alternative therapeutic modality to target vascular formation. Several groups have previously shown that JNJ-17029259 interferes with VEGF-stimulated signal transduction in human umbilical vein endothelial cells (HUVECs) cells including proliferation, VEGF-R2 receptor phosphorylation and MAP kinase activation [63]. These studies have shown that JNJ-17029259 significantly inhibited normal development of the eye in the chick embryo in a dose-responsive manner, presumably by interfering with formation of the blood supply to the eye. In the corneal micropocket assay of retinopathy in both rabbits and mice, oral administration of JNJ-17029259 blocked the FGF-induced growth of new vessels in the eye. In these models, both vessel length and vessel area were significantly reduced in a dose responsive manner. These results demonstrate the utility of VEGF receptor kinase inhibitors in treatment of ophthalmic neovascular disorders [64]. Another small molecule inhibitor of PI3 kinase is Wortmannin, which blocks the catalytic activity of PI3-kinase without affecting the upstream signaling events. Previous data have shown that Wortmannin can inhibit proliferation and migration of ECs induced by Müller cell conditioned medium (HGMCM) [65].

Downstream signals from VEGF; like the small GTPase, Ras homolog gene family, member A (RhoA) have been targeted (Fig. 5). H-1152 is a cell permeable, highly specific, potent and ATP-competitive inhibitor of Rho kinase (ROCK). In addition, H-1152 inhibits the phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS) in cells stimulated by lysophosphatidic acid, as well as it inhibits the prostaglandin E receptor EP3 subtype-stimulated NO formation. Several groups have utilized the mouse model of OIR, ROCK I/II inhibition by H-1152, which resulted in increased angiogenesis [66]. This enhanced angiogenesis, however, was completely blocked by the VEGF-receptor antagonist Vatalanib (PTK787)/ZK222584. Loss-of-function experiments in ECs revealed that inhibition of ROCK I/II using the pharmacological inhibitor H-1152 and ROCK I/II-specific small-interfering RNAs resulted in a
rise of VEGF-driven sprouting angiogenesis. Other investigations have recently indicated that H-1152 plays a major role in promoting cell survival and reducing reactive gliosis in the rodent retina via the attenuation of glial cell reactivity [67].

Several inhibitors of the kinase activity of VEGF receptors, PDGF receptors, and c-kit have been shown to completely inhibit retinal NV. Studies from Campochiaro’s lab have indicated that PTK787 completely inhibited retinal NV in murine OIR and partially inhibited retinal vascularization during development [68]. Previous studies have indicated that tyrosine kinase inhibitors are promising substances not only in cancer therapy, but also in the treatment of ischemic retinopathies that are mediated by VEGF [69]. Furthermore, TG100572; a topically applied small molecule VEGFR2/Src kinase inhibitor permeates the cornea and is present and active in the retina [70]. This small molecule evolved through a structure-based drug design approach to potent Src inhibitors of the benzo triazine class [71]. TG100572 potently inhibits a number of tyrosine kinases responsible for mediating vascular leak and/or angiogenesis. TG100572 was shown to stop NV and to decrease inflammation, both of which are characteristics of wet macular degeneration. TargeGen, Inc. have demonstrated that topical (eye drop) administration of the prodrug, TG100801, may be effective for the treatment of retinal disease and may also be used in combination with approved products. TG100801 converts to the active drug TG100572 as it penetrates the eye. TG100801 demonstrated the ability to reduce VEGF-mediated retinal leakage, angiogenesis and inflammation after topical instillation. Data have suggested that TG100801 is well tolerated in humans at the low and high doses tested when applied topically twice daily for 14 days.

Certain small molecules have exhibited the ability to target the signaling pathways of several kinases. PTK787 is a great example, which targets the kinase activity of several isoforms of PKC (Fig. 5). VEGF receptors, PDGF receptors, and c-kit, but not of receptors of several other growth factors that have been tested [72] (Fig. 4). In either rho/PDGF-B or rho/PDGF-A mice, oral administration of PKC412 or PTK787, but not SU1498 or imatinib mesylate, significantly reduced ERM formation. PKC412 reduced the incidence of severe retinal detachments in both models and PTK787 did so in homozygous rho/PDGF-A mice [73]. Furthermore, PKC412 (protein kinase C [PKC] inhibitor) decreased leakage caused by prostaglandins, which implicate PKC in retinal vascular leakage caused by prostaglandins.

Sorafenib is a multikinase inhibitor, which blocks the action of VEGF, PDGF and the MAP kinase pathway. Sorafenib blocks the action of receptor tyrosine kinases, a class of molecules that transmit the signal that a receptor has been activated from the receptor to the nucleus of the cell. Sorafenib is a drug that is traditionally used to treat cancer, has shown promise as a neovascular AMD treatment. This study investigated the effects of sorafenib on light-induced overexpression of growth factors in human RPE cells. Human RPE cells were exposed to white light and incubated with sorafenib. Light exposure decreased cell viability and increased expression and secretion of VEGF, PDGF and PIGF. These light-induced effects were significantly reduced when cells were treated with sorafenib. The investigators conclude that sorafenib has promising properties as a potential antiangiogenic treatment for AMD [74]. Another small-molecule kinase inhibitor is pazopanib, which blocks VEGFR1, VEGFR2, and VEGFR3. Furthermore, pazopanib also has substantial activity directed against PDGFRα, PDGFRβ, c-Kit, FGFR1, FGFR3, and c-fms. Therefore, pazopanib has an interesting inhibitory profile with regard to potential effects in angiogenic diseases. Previous data investigated the effects of pazopanib in mouse models of subretinal NV [75]. Data indicated that orally administered pazopanib has good bioavailability to the retina/choroid and strongly suppresses CNV in mice. Treatment with pazopanib after CNV is established causes dose-dependent regression of CNV.

Mammalian target of rapamycin (mTOR) is a protein kinase that controls cell growth, proliferation, and survival (Fig. 6). mTOR signaling is often upregulated in cancer and there is great interest in developing drugs that target this enzyme. Rapamycin and its analogs bind to a domain separate from the catalytic site to block a subset of mTOR functions. These drugs are selective for mTOR and are already in clinical use for treating cancers, but they could potentially activate an mTOR-dependent survival pathway that could lead to treatment failure. By contrast, small molecules that compete with ATP in the catalytic site would inhibit all of the kinase-dependent functions of mTOR without activating the survival pathway. Rapamycin inhibits the translation and activity of HIF-1α, a stress-activated protein that regulates numerous survival proteins involved in angiogenesis and hyperpermeability [76]. In fact, HIF-1α is a potent stimulator of VEGF, and its inhibition affects VEGF at the production level and at the receptor level [77]. Moreover, several non-selective mTOR kinase inhibitors have been described and here we review their chemical and cellular properties. Further development of selective mTOR kinase inhibitors holds the promise of yielding potent anticancer drugs with a novel mechanism of action. Furthermore, previous investigations have indicated that Rapamycin significantly reduced the extent of NV in both the CNV and the ROP model [78]. In addition, Rapamycin suppressed corneal NV, possibly by inhibiting proinflammatory cytokines [79]. In a recent randomized pilot study of systemic immunosuppression in the treatment of AMD with CNV; patients were randomized to 1 of 3 systemic arms immunosuppressive agents (dacilizumab, rapamycin, or infliximab) for 6 months plus intraocular anti-VEGF therapy if indicated, compared with a group who received only anti-VEGF therapy if indicated. Thirteen patients were randomized; comparing anti-VEGF injections before and during the study. Results indicated that there was a decrease in the number of injections from 0.73 injections per month to 0.42 for daclizumab and from 0.67 to 0.34 for sirolimus, while no apparent decrease was seen for either infliximab or observation. Visual acuities were maintained in all groups. Preliminary data have suggested that some immunosuppressive agents given systemically can alter the clinical course of the wet form of the disease and support the notion that more definitive clinical trials of immune mediation of AMD are indicated [80].
MacuSight, Inc. in collaboration with the Retinal Consultants of Arizona/Spectra Eye Institute in Sun City, AZ, have completed two phase 1 studies; one for DME, and one for wet AMD. The randomized, open-label, dose-escalation study ran in parallel with a study of the same drug for treatment of DR. Perceiva (a proprietary ocular formulation of sirolimus) is injected either subconjunctivally or intravitreally for up to three months. Developers have indicated that sirolimus is different from other anti-angiogenic drugs in that it is “a highly-potent, broad-acting compound that has demonstrated the ability to combat disease through multiple mechanisms of action including immunosuppressive, anti-angiogenic, anti-migratory, anti-proliferative, anti-fibrotic and anti-permeability activity,” and that it may, therefore, serve as a potentially highly-efficacious therapeutic for a wide range of ocular diseases and conditions. Investigators found that the drug was well-tolerated at all doses for both administration methods tested, with no reported IOP elevations, inflammatory effects or indications of cataract progression. There were no dose-limiting toxicities, ocular inflammation, or increase in intraocular pressure. In addition, investigators from both studies noted improvements in visual acuity and reductions in retinal thickness with observed beneficial anatomical changes following a single administration of. These findings were consistent across both routes of administration. These promising results for Perceiva delivered with subconjunctival injection provide the first evidence for the potential of treating retinal disease without direct injection into the back of the eye. The majority of ocular adverse events were related to the injection procedure were mostly mild and transient in nature. Systemic exposure was negligible and insufficient for systemic immunosuppression. As an mTOR inhibitor, sirolimus possesses a broad spectrum of therapeutic action which MacuSight recognized as potentially relevant to the treatment of ocular diseases, including the inhibition of inflammation, angiogenesis, vascular permeability, proliferation and fibrosis. Designed

Fig. (6). Schematic Illustration of Insulin/IGF-I and Other Factors that Regulate mTOR Signaling Pathway. Insulin/IGF-I stimulates activation of the PI3K-PDK1 pathway, leading to phosphorylation of p70S6K in the activation loop (Thr229). Nutrients, such as amino acids and glucose, regulate the mTOR signaling pathway through an uncharacterized mechanism, and protein X represents an unknown molecule that senses the amount of amino acids in the cell and inhibits the TSC complex or activates the mTOR pathway somewhere between the TSC complex and mTOR complex, but most likely involve activating the small G protein Rheb. Activation of the PI3K-PDK1-Akt pathway may indirectly enhance p70S6K phosphorylation at the HM site via inhibition of the tuberous sclerosis protein complexes TSC1 and/or TSC2, which are inhibitors of the mTOR signaling pathway.
for minimally invasive, sustained, local administration, Perceiva may offer more comfort to patients and more convenience to physicians, while minimizing the risk of complications and adverse events. In 2010, Perceiva is being advanced in a broad Phase 2 clinical program across multiple large ocular indications including diabetic DME, wet AMD, uveitis and dry AMD. Additionally, as part of this Phase 2 program, the company is using Perceiva to evaluate the potential role for sirolimus in the treatment of dry eye syndrome (DES).

Retinal NV has been targeted at the molecular chaperone level. Investigators have used antagonists to the heat shock protein 90 (Hsp90) [81] (Fig. 5). The Geldanamycin analog, 17-allylamino-17-desmethoxygeldanamycin (17-AAG), is now in Phase I clinical trial at the NCI and several other sites. This drug represents a class of natural products known as benzoquinone ansamycins, which also contains herbinycin A and macbecin. Benzoquinone ansamycins are characterized by linkage of a quinone moiety to a planar macrocyclic ansa-bridge structure. Although these drugs were previously thought to be tyrosine kinase inhibitors, the mechanism of action of some benzoquinone ansamycins, including Geldanamycin and 17-AAG, must now be reevaluated following their demonstrated ability to specifically bind to and antagonize the function of the chaperone protein heat shock protein 90 (Hsp90). The ansamycin antibiotic GA and its modified derivative 17-AAG bind to a conserved pocket in the Hsp90 protein and inhibit its function [82]. 17-AAG inhibits the Ras/Raf/MEK and PI3-Kinase signaling pathways and down-regulates VEGF expression (Fig. 5). Previously, data have demonstrated that an IP injection of 17-AAG was able to reduce angioproliferative retinopathy in a mouse model for OIR [83]. Furthermore, they have indicated that the mechanism does not involve a direct or indirect reduction of the VEGF mRNA level, but acts downstream of the VEGF pathway. Thus, 17-AAG probably does not work by PI-3 kinase inhibition but via the Ras/Raf/MEK pathway (Fig. 5).

INHIBITORS OF THE CELL CYCLE

Blocking the progression of the cell cycle has been investigated by several research groups as an ideal venue to target retinal NV. TNP-470, a synthetic analog of fumagillin, has been undergoing clinical trials for treating a variety of cancers. TNP-470 has been shown to block endothelial cell cycle progression in the late G1 phase. Although the direct molecular target for TNP-470 has been identified as the type 2 methionine aminopeptidase (MetAP2). Studies have reported that treatment of endothelial and other drug-sensitive cell types leads to the activation of the p53 pathway, causing an accumulation of the G1 cyclin-dependent kinase inhibitor p21WAF1/CIP1 [84]. These results shed light on the mechanism of cell cycle inhibition by TNP-470 and suggest an alternative method of activating p53 in ECs to halt angiogenesis and tumor progression. Several studies have demonstrated that TNP-470 inhibits the growth and the capillary-like tube formation of ECs more sensitively than other types of cells [85]. Furthermore, Studies evaluated the efficacy of the conjugate TNP-470-PVA in vivo, where it was shown to inhibit CNV in rabbits [86]. Their studies have also demonstrated that TNP-470-PVA inhibited the growth of HUVECs in a biphasic manner similar to that of TNP-470. On the other hand, the bovine retinal pigment epithelial cells (BRPECs) exhibited less sensitivity to TNP-470-PVA than did the HUVECs, which suggested that TNP-470-PVA preserves the original bioactivity of TNP-470 and that, if this relationship between the two types of cells corresponds to that between choroidal ECs and RPE cells, this conjugate may inhibit the growth of ECs and produce less interference in the proliferation of RPE cells. These studies have suggested that targeted delivery of TNP-470-PVA may have potential as a treatment modality for CNV.

Other small molecules that induce cell cycle arrest have been investigated in various models of ocular pathologies. IMS2186 is an anti-CNV drug in which its mechanism of action is proposed to be the arrest of the cell cycle at G2 phase, and the inhibition of prostaglandin E2 (PGE2) and TNF-α production; the latter contributing to anti-inflammatory and anti-angiogenic effects [87]. Therefore, IMS2186 acts more upstream of the angiogenesis, rather than simply blocking the VEGF. Furthermore, IMS2186 has inhibitory effects in vitro on not only angiogenesis, but also on the mobilization of macrophages and the proliferation of fibroblasts and other cell types.

INHIBITORS OF THE BINDING SITES / RECEPTORS

The ability to target the binding sites/receptors both in vitro and in vivo by applying blocking antibodies, antisense oligonucleotides, or soluble receptor has heralded modulation of growth factors as the way forward in treating PDR. Studies have shown that the heparin analog 5-amino-2-naphthalenesulfonate (5-amino-2-NMS) reduced the mitogenic activity of fibroblasts. In addition, 5-Amino-2-NMS blocked the heparin-binding site of FGF1 or FGF2 and thus hampered the interaction of FGF with its receptor at the cell membrane level. Furthermore, the induction of NV in mice by a subcutaneous FGF1-containing sponge was inhibited by the intraperitoneal injection of 5-amino-2-NMS [88]. The effect of (5-amino-2-NMS) on retinal NV was investigated in the OIR mouse model [89]. Data have indicated that a single intravitreal injection of 5-amino-2-NMS reduces significantly angioproliferative changes compared to the contralateral control.

INHIBITORS OF THE CALCIUM-ENTRY

A number of anti-angiogenic strategies work through mechanisms distinct from those described above. CAI (beta-hydroxypropyl cyclodextrin ([βHPCD]) is an inhibitor of calcium influx, which is currently in phase I studies in combination with Paclitaxel against solid tumors (Fig. 5). In vivo studies have demonstrated that CAI treatment reduced choroidal neovascular lesion volume. These studies have shown that intravitreal suspension CAI was highly effective in the CNV model, and possesses an ocular pharmacokinetic profile that may be superior to the standard 4-6 week administration interval and was well tolerated in the rat based on both anatomic and physiologic testing [90].

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) PATHWAY TARGETING

Peroxisome proliferator-activated receptors (PPARs) are members of the steroid receptor superfamily and, as such, are ligand-activated transcription factors. PPARγ is a nuclear
receptor that functions as a transcription factor to mediate
ligand-dependent transcriptional regulation. PPARs
signaling pathway regulates cell proliferation, survival,
synthesis of cell growth and division machinery, G1-S cell
cycle progression, and tumor cell VEGF production (Fig. 6).
Several studies have reported on the potent and novel
inhibitory activity of PPARγ ligands on HUVEC
differentiation into tube-like structures and proliferation in vitro,
and the inhibition of EGF elicited angiogenesis in vivo
(Fig. 6). These studies demonstrated that PPARγ is an
important molecular target for the development of small
molecule inhibitors of angiogenesis, which may be useful
therapeutic agents in the treatment of cancer and other
vasculoproliferative disorders. Activation of PPARγ by the
naturally occurring ligand, 15-deoxy-D12,14-prostaglandin
J2 (15d-PGJ2), or members of a new class of oral anti-
diabetic agents, e.g. BRL49653 and ciglitizone, has been
linked to adipocyte differentiation, regulation of glucose
homeostasis, inhibition of macrophage and monocyte
activation, and inhibition of tumor cell proliferation. Data
have indicated that activation of PPARγ by the specific
ligands 15d-PGJ2, BRL49653, or ciglitizone, dose-
dependently suppresses HUVEC differentiation into tube-
like structures in 3D collagen gels. Moreover, treatment of
HUVEC with 15dPGJ2 also reduced mRNA levels of VEGF
receptors 1 (Flt-1) and 2 (Flk/KDR) and urokinase
plasminogen activator and increased plasminogen activator
inhibitor-1 (PAI-1) mRNA [91]. Administration of 15d-
PGJ2 inhibited VEGF-induced angiogenesis in the rat cornea
[91]. In addition, previous data have indicated that
Thiazolidinediones (TZDs) inhibits experimental retinal NV
with an effect that is primarily downstream of VEGF
expression [92].

TUBULIN-BINDING AGENTS

Combretastatin A-4 (CA-4) is an anti-tumor agent, which
binds specifically to the colchicine-binding site on tubulin, a
key protein in the process of cell division. Both molecules
(colchicine and combretastatin A-4) cause a decrease in
elasticity, therefore preventing cell division. Studies from
Campochiaro’s lab have indicated that in rho/VEGF mice,
daily intraperitoneal injections of CA-4-P starting at
postnatal day (P) 7, the time of onset of transgene
expression, resulted in a significant reduction in the number
of neovascular lesions and total area of NV per retina at P21,
compared with vehicle-injected mice. In mice with laser-
induced rupture of Bruch’s membrane, daily intraperitoneal
injections of CA-4-P resulted in a significant reduction in the
area of CNV at rupture sites compared with vehicle-injected
mice. In mice with established CNV, daily intraperitoneal
injections of CA-4-P for 1 week resulted in a significant
reduction in CNV area at rupture sites compared with the
baseline area before treatment or the area of CNV in vehicle-
treated mice. These studies have revealed that CA-4-P
suppresses the development of VEGF-induced NV in the
retina and both blocks development and promotes regression
of CNV. Therefore, CA-4-P shows potential for both
prevention and treatment of ocular NV [93].

INHIBITORS OF INTEGRINS

Integrin α5β1 is the only integrin expressed on the apical
surface of RPE cells in the mammalian eye, where it controls
the phagocytosis of photoreceptor outer segments [94, 95]
and probably the adhesion of RPE cells to the retina [94]
(Fig. 5). In contrast, integrins αvβ3, α6β1, and α3β1 are
expressed on the basal lateral membrane of RPE cells where
they enable their attachment to Bruch’s membrane [96].
Moreover, α5β1 was highly expressed on surgically excised
proliferative membranes, and proliferating and migrating
RPE cells in vitro, yet normal retinal tissues showed little
α5β1 expression. Together, these findings suggest that no
impairment of physiological RPE function is to be expected
during pharmacological therapy that specifically targets
α5β1. The integrin α5β1-fibronectin (α5β1-FN) interaction
is a promising target for the treatment of proliferative
vitreoretinopathy (PVR) with the potential to inhibit multiple
pathogenic pathways, including the intraretinal proliferation
and migration of RPE cells, the proliferation of Müller cells,
the inflammatory response and resulting fibrosis, and, most
likely, the transdifferentiation of RPE cells, the activation of
Müller cells, and the response after the contraction of epiretinal membranes. Among these pathogenic processes,
the activated Müller cells [97] and transdifferentiated RPE
cells [98] are proposed to be the most important triggers of
PVR.

JSMD6427: 3-2-[1-alkyl-5-[(pyridin-2-ylamino)-methyl]-pyrrolidin-3-yl oxy]-(acetylamino)-2-(alkylamino)-propionic acid, is a small molecule inhibitor of the α5β1-FN interaction
[99], with good tissue penetration and stability in
physiological buffers. Furthermore, JSMD6427 is a
competitive integrin inhibitor mimicking the natural integrin
recognition motif Arg-Gly-Asp. It inhibits the integrin α5β1-
fibronectin interaction [99]. JSMD6427 has a selectivity at
least 1,700-fold greater for α5β1 than for other integrins
expressed in normal RPE [100] and has been shown to result in
a dose-dependent reduction in FN-mediated ERK-1/2
phosphorylation in the spontaneously arising retinal pigment
epithelium cell line (ARPE-19) [101]. Furthermore, JSMD6427
resulted in the inhibition of multiple α5β1-mediated effects
in vitro and in vivo, suggesting that it may provide some
therapeutic benefit in the treatment of PVR [53]. It has been
shown that JSMD6427 is a promising treatment for PVR, with
data suggesting that inhibition of α5β1-fibronectin
interactions addresses multiple pathways involving retinal pigment epithelial, glial, and inflammatory cells. Previous
data have demonstrated that single intravitreal injections
of JSMD6427 are well tolerated in patients with neovascular
AMD and may show early indications of biological activity
[102]. The repeat dose cohorts have completed enrollment
and are in early follow-up. In addition a sustained-release
formulation is in development for the prevention as well as
the treatment of neovascular AMD.

INHIBITORS OF THE RENIN-ANGIOTENSIN SYS-
TEM (RAS)

The hypothesis that an ocular RAS is involved in
the development of PDR is supported by evidence that all
components of the RAS are present in the retina [103, 104]
and that Ang II, the effector molecule of this system, is
angiotensic [105]. The renin-angiotensin system is well
known as a major controller of systemic blood pressure.
Angiotensin II (Ang II), a final product of the system, has
two cognate receptors: the angiotensin II type 1 receptor
(AT1-R) and the angiotensin II type 2 receptor (AT2-R)
Because major Ang II-related systemic functions are mediated by AT1-R signaling, its antagonist is widely used for the treatment of hypertension and cardiac diseases. In contrast, several reports have recently suggested that Ang II plays crucial roles in promoting tumor angiogenesis and cardiovascular remodeling through the proliferation of smooth muscle cells and the induction of various growth factors [107-110]. Blockade of Ang II signaling seems to be a useful strategy for the improvement of inflammation-related diseases. AT1 receptor blockers as well as ACE inhibitors attenuated preretinal pathological angiogenesis when administered during retinal ischemia [108, 109, 111]; in addition, it reduced inflammation [111]. Furthermore, ACE inhibitors reduce OIR in mice when administered during the hyperoxic period and this is associated with downregulation of ET-1 [112].

Studies have shown the localization of renin to the macroglial Müller cell [103] making this the likely site for pathophysiological processes involving the retinal RAS. Data have suggested that retinal RAS plays an important role in the pathogenesis of NV in the ROP model [108]. This information provides a rationale for the use of agents that interrupt the RAS in the prevention of proliferative retinopathy related to retinal hypoxia and possibly ischemia. Furthermore, several reports have suggested that Ang II plays a key role in various inflammatory processes, including not only the expression of chemokines and adhesion molecules for the recruitment of inflammatory cells, but also the differentiation and proliferation of inflammatory cells, per se [113]. Hypertension is well known to be a worsening factor in PDR [114]. A recent report [115] has shown the increased levels of Ang II in the vitreous fluid of patients with proliferative diabetic retinopathy, although its role in pathogenesis remains undetermined. More work is needed to establish the validity of this therapeutic approach in vision-threatening ischemic retinopathies. It has been demonstrated that the AT1, but not AT2, antagonist partially, but significantly, reversed the increase in retinal vascular hyperpermeability. In addition, data have demonstrated that olmesartan medoxomil partially, but significantly, inhibited the retinal vascular hyperpermeability induced by hypoxia. In contrast, PD123319 did not show a significant effect. The VEGF and HIF-1alpha protein levels were significantly elevated in the OIR retina; however, there was no significant effect of olmesartan medoxomil on the expression of either protein [116]. Moreover, losartan (enalapril maleate), an angiotensin converting enzyme (ACE) inhibitor, prevents the synthesis of angiotensin II [117]. This compound is used to treat high blood pressure, heart and renal abnormalities [118]. In zebrafish, this compound is toxic at higher concentrations and causes embryonic lethality. At lower concentrations, it causes a widening of intraocular blood vessels, while vessel thickness in the trunk does not display this effect. To test whether losartan activity in zebrafish is mediated via the inhibition of ACE, certain groups have demonstrated that morpholino knockdown of the ACE gene had no apparent effect on the blood vessel morphology in the eye [119]. Recent data have revealed early blockade of the renin-angiotensin system with losartan (100 mg daily), losartan (20 mg daily), in patients with type 1 diabetes did not slow nephropathy progression but slowed the progression of retinopathy [120]. ACE inhibitors have been shown to suppress the progression of human DR to its proliferative (angiogenic) stage [121].

Olmesartan Medoxomil is another small molecule, which belong to the class of medicines called angiotensin II receptor antagonists to treat high blood pressure. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis. Olmesartan has more than a 12,500-fold greater affinity for the AT1 receptor than for the AT2 receptor. A previous study has demonstrated that both the AT1 and AT2 receptor mRNA are upregulated in OIR rat retinas [122], suggesting that these receptors may be involved in the pathogenesis of the OIR. Unlike the angiotensin receptor antagonist losartan, olmesartan does not have an active metabolite or possess uricosuric effects. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and circulating angiotensin II levels do not overcome the effect of olmesartan on blood pressure. Since olmesartan medoxomil is now available clinically as an antihypertensive drug, olmesartan medoxomil might have a beneficial effect in treating ischemic retinal diseases, such as DME. Additionally, research investigations on neonatal transgenic (mRen-2)27 rats, which overexpress renin in tissues, have indicated that ACE inhibitor lisinopril (Prinivil) and the angiotensin type 1 receptor antagonist losartan both increased retinal renin levels and prevented inner retinal blood vessel growth [108]. Furthermore, lisinopril reduced both retinal VEGF and its type 2 receptor mRNA in ROP rats, whereas losartan had no effect. It is predicted that agents that interrupt the renin-angiotensin system may play an important role as retinoprotective agents in various forms of proliferative retinopathy. Moreover, the hypothesis that an ocular RAS is involved in the development of PDR is supported by evidence that all components of the RAS are present in the retina [103, 104] and that Ang II, the effector molecule of this system, is angiogenic [105]. Previous studies have localized renin to the macroglial Müller cells, making this the likely site for pathophysiological processes involving the retinal RAS [103]. The Müller cell is also the site of synthesis of the potent angiogenic factor VEGF and its tyrosine kinase receptors [123]. There is evidence of an association between VEGF, the RAS, and retinal NV, because both VEGF and prorenin increase in the vitreous of patients with PDR [8] and Ang II increases VEGFR-2 receptor mRNA in retinal ECs [110].

Candesartan cilexetil (candesartan) is in a class of drugs called angiotensin receptor blockers which includes losartan. Candesartan blocks the ability of the chemical angiotensin II to raise the blood pressure by constricting or squeezing arteries and veins. This leads to a reduction in blood pressure. In addition, by reducing the pressure against which the heart must pump blood, candesartan reduces the work of the heart and is useful in patients with heart failure. A 5-year study of candesartan treatment in type 1 diabetes reduced the incidence of retinopathy by two or more steps in severity by 18% and, in a post hoc analysis, reduced the incidence of retinopathy by three-step progression by 35%. In type 1 diabetes patients there was no effect on progression of established retinopathy. In contrast, in type II diabetes, 5
years of candesartan treatment resulted in 34% regression of retinopathy. Most significantly, an overall significant change towards less-severe retinopathy was noted in both type I and II diabetes [124, 125].

GROWTH HORMONE INHIBITORS

Growth hormone (GH) may be involved in the development of ROP and DR (Fig. 5). The initial association between GH and DR came from studies in which pituitary ablation was linked to the remission of DR [126, 127]. In subsequent studies, DR was found to be approximately three times more prevalent in Type I diabetic patients who are GH sufficient than those who were GH deficient [126]. GH deficient dwarfs with diabetes were free of microvascular complications [128], and GH replacement therapy for patients with GH deficiency induced a diabetic-like retinopathy, which is attenuated after discontinuation of GH treatment [129]. In terms of ROP, Smith and colleagues reported that retinal NV is reduced in transgenic mice expressing a GH antagonist gene that were subjected to experimental ROP [130].

GH has indirect effects, which are mediated primarily by insulin-like growth factor-1 (IGF-1), a hormone that is secreted from the liver and other tissues in response to GH. A majority of the growth promoting effects of GH is actually due to IGF-1 acting on its target cells. GH secretion is part of a negative feedback loop involving IGF-1; where high blood levels of IGF-1 lead to decreased secretion of GH not only by directly suppressing the somatotroph, but by stimulating release of somatostatin from the hypothalamus. The type 1 IGF receptor (IGF1R) is a transmembrane tyrosine kinase that is frequently overexpressed by tumors, and mediates proliferation and apoptosis protection. IGF signaling also influences hypoxia signaling, protease secretion, tumor cell motility and adhesion, and thus can affect the propensity for invasion and metastasis. Therefore, the IGF-1R is now an attractive anti-cancer treatment target. Design of specific IGF-1R inhibitors has been problematic due to close homology with the insulin receptor, but recently it has proved possible to design selective IGF-1R inhibitors. These compounds and IGF-1R antibodies are showing promise in preclinical models of human cancer, and several agents are now in early phase clinical trials. Both classes of agents affect insulin receptor signaling, either by direct kinase inhibition or antibody-induced insulin receptor downregulation. This effect may lead to clinical toxicity, but could be therapeutically beneficial in blocking signaling via variant insulin receptors capable of a mitogenic response to IGF-IL. Specificity for IGF-1R targeting can be achieved by antisense and siRNA-mediated IGF-1R downregulation; these approaches have undoubted utility as research tools, and may in future generate nucleic-acid-based therapeutics. It will be important to use data from preclinical and early clinical trials to establish the molecular correlates of sensitivity to IGF-1R blockade, and the optimum means of combining this new approach with standard treatment modalities. Therapeutic strategies for DR included early approaches to block the actions of GH such as hypophysectomy and pituitary radiation [127], and more recently, the use of the GH receptor (GHR) antagonist, pegvisomant, or inhibiting the secretion of GH from the pituitary using somatostatin or its analogues such as octreotide [130-132]. Previous investigations have tested whether systemically delivered antisense oligonucleotide could inhibit NV in mice with oxygen induced retinopathy (OIR) [133]. They reported the design and optimization of a “5-10-5” 2’-O-(2-methoxy)ethyl (2’-MOE) modified antisense oligonucleotide (ASO) directed to the mouse GHR, which suppresses GHR mRNA levels in vitro and in vivo and reduces binding of GH to liver cells in normal mice [133]. Their approach described the effect of this ASO; ATL 227446 on retinal NV in a mouse model of ROP. It was concluded that treatment with the GHR ASO, ATL 227446, reduced pathological retinal NV in OIR to a greater extent than octreotide or control oligonucleotides [133]. Systemically delivered GHR ASO may be a potential treatment for ocular NV related disorders such as ROP and DR [133].

INHIBITORS OF INFLAMMATION AND VASCULAR LEAKAGE

Corticosteroids have a specific effect on reducing vascular permeability [134]. Steroids have anti-angiogenic, anti-inflammatory, anti-apoptotic, anti-proliferative and anti-edematous activity [135]. Hypoxia induces an inflammatory response in the retina with increased levels of inflammatory cytokines and macrophage attractants. As corticosteroids are anti-inflammatory, the actions of corticosteroids in conditions of hypoxia induced inner blood-retinal barrier breakdown (iBRB) include both anti-inflammatory activity and vascular permeability. In many clinically important conditions including diabetic retinopathy, central or branch retinal vein occlusion (BRVO), and some respiratory diseases retinal hypoxia results in a breakdown in the blood retinal barrier (BRB) [136]. Disruption of the iBRB with increased vascular permeability causes vasogenic retinal edema and tissue damage, with consequent adverse effects upon vision. Factors such as enhanced production of VEGF, NO, oxidative stress and inflammation underlie the increased permeability of the iBRB and inhibition of these factors may be therapeutically beneficial. In the eye the effect of most steroids is short-lived, requiring repeated or continuous administration to maintain therapeutic effect. Recent studies have demonstrated the usefulness of intravitreal injection of triamcinolone acetonide (TCA) in the reduction of inflammation [137, 138], vascular permeability [138] fibrovascular proliferation [139], and macular thickening due to diffuse DME, at least in the short-term [139]. In rabbit animal models TCA inhibited VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown [140]. The relatively insoluble steroid TCA has much more prolonged activity in the eye [141]. Although some doubt must be raised about the acceptance of visible remnants of vitreal TCA as an indicator of the activity of this steroid within the vitreous, it does seem to be the case that the retention time of TCA is much longer than is the case with other anti-VEGF intravitreal preparations used. Adverse effects of intravitreal TCA include elevated intraocular pressure and cataract formation [142]. The general conclusion from various studies is that TCA alone or in combination with other treatments improves best-corrected visual acuity (BCVA) in DME and reduces central retinal thickness during the early few months after commencement of treatment and that the results may be
better than with laser treatment at this time. In a report of long-term results, however [143], laser treatment was more effective and had fewer side effects than intravitreal TCA.

Another anti-inflammatory agent under this category is dexamethasone (DEX), which is a potent and effective glucocorticoid (GC) [9-fluoro-glucocorticoid] ocular agent that is topically applied in ocular conditions, such as keratitis, uveitis, and iritis [144]. However, the adverse effects of prolonged use of DEX include decreased aqueous humor outflow and increased intraocular pressure (IOP), which may cause the onset of secondary glaucoma. The exact molecular mechanism of glucocorticoid-induced glaucoma (GIG) is still elusive, but evidence points to excessive extracellular matrix (ECM) material aggregation within the outflow channels in trabecular meshwork (TM) tissues as a result of ECM degradation inhibition, which subsequently leads to increased outflow resistance [145]. GC-induced ocular hypertension shares some clinical features with primary open-angle glaucoma (POAG). Besides IOP elevation, both secondary and primary glaucoma have selective retinal ganglion cell death that causes visual field changes, nerve fiber layer defects, and eventual irreversible blindness [145]. Several studies have noted that the GC-induced changes in TM can partially reflect the pathological mechanisms of POAG [146]. Investigations into the molecular mechanisms of GIG may provide new insights into the pathology of POAG.

INHIBITORS OF ANGIOGENESIS

Tryptophanyl-tRNA synthetase (TrpRS) is an aminoacyl-tRNA synthetase and a smaller proteolytic product in which the entire NH2-terminal domain has been deleted. It is involved in protein synthesis and regulation of RNA transcription and translation and is an inhibitor of angiogenesis. Previous studies have investigated the angiostatic activity of T2-TrpRS [147]. Their data have demonstrated that the 20-40% inhibition of NV demonstrated by other anti-angiogenic compounds, preclinical studies of TrpRS have revealed that in 70% of cases there was 100% inhibition. Another potential advantage of TrpRS fragments is that they represent naturally occurring and, therefore, they could avoid the problems of toxicity and potential immunogenicity that are associated with other potential antiangiogenic drugs. Thus, these molecules can be delivered via targeted cell- or viral vector-based therapy.

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2-naphthalenesulfonate reduces retinal neovascularization in mice.


