Chapter 9
Antiangiogenic Agents and Photodynamic Therapy

Atul Kumar, S.N. Mohanraj, Kavitha Duraipandi, and Anuradha V. Pai

Abstract Diabetic retinopathy, Age Related Macular Degeneration (ARMD) and Retinopathy of Prematurity(ROP), are few of the many pathological conditions which are vision threatening. Their classical feature is neovascularization. Angiogenic factors such as VEGF, PEDF, Tyrosine Kinase etc., have a significant role in the causation of angiogenesis in the hypoxic areas of the ocular tissues. This chapter covers the causative factors of ocular angiogenesis and the possible pharmacological intervention to inhibit these factors in order to improve the vision of the patients. This chapter also deals with the possible benefits of the photodynamic therapy in the ocular neovascularisation.

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9.1 Antiangiogenic Agents

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9.1.1 Introduction

Angiogenesis is the formation of new blood vessels from preexisting vessels. It is a normal process in growth and development as well as in wound healing. However, this process can occur in pathological conditions such as in transition of tumors from dormant to malignant state. The process of angiogenesis is tightly regulated by balance of two sets of counteracting cellular signaling molecules: angiogenic activators and inhibitors. Vascular Endothelial Growth Factor (VEGF) is one of the principal molecules among a large number of proangiogenic growth factors that induce migration, proliferation, and formation of tubes by endothelial cells of the basal lamina.

Ocular angiogenesis is the hallmark of many ocular diseases like diabetic retinopathy (DR), vascular occlusions, age-related macular degeneration (ARMD), and retinopathy of prematurity (Campochiaro and Hackett 2003; Gariano 2003; Gariano and Gardner 2005; Witmer et al. 2003). The major trigger for angiogenesis in these conditions is tissue hypoxia which arises due to compromised vascular supply that cannot cope up to high metabolic demand of associated neuronal tissue. This condition is further aggravated by oxidative stress and inflammatory mediators.

As in generalized angiogenesis, vascular endothelial growth factor (VEGF) plays a major role in the process of retinal neovascularization (Blaauwgeers et al. 1999; Gariano and Gardner 2005; Witmer et al. 2003). VEGF is mainly released by Muller cells, the retinal pigment epithelium (RPE), astrocytes, ganglion cells, and endothelial cells. Muller cells and astrocytes are the largest sources of VEGF in hypoxic conditions (Gerhardt et al. 2003; Gogat et al. 2004; Patan 2000).

Angiogenesis is known to occur in a series of stages with immediate early events caused due to growth factors and signaling, followed by early events that comprise of change in genetic programming of vascular cells, followed by invasive angiogenesis culminating in a resolution stage wherein cells revert to their quiescent stage. The process of angiogenesis is an orderly set of events as follows (Cheresh and Stupack 2008):

1. The blood vessels providing nutrients and oxygen throughout the body are composed of an inner lining of endothelial cells that completely encircle the lumen. The basal lamina surrounds the endothelial cells and pericytes (Hynes 1992). Pericytes are responsible for regulating endothelial cell proliferation, survival, migration, differentiation, and vascular branching via selective inhibition of endothelial cell growth (Hellstrom et al. 2001). The basal lamina provides mechanical support for cell attachment, serves as a substrate for cell migration, separates adjacent tissue, and acts as a barrier for the passage of macromolecules (Hynes 1992).

2. In healthy adults, a balance of growth factor signaling keeps endothelial cells in a quiescent or resting state to monitor and supply sufficient amounts of oxygen to surrounding tissues. To achieve this, blood vessels have receptors for sensing oxygen and hypoxia that allow minute-to-minute readjustment of vessel diameter (to adjust the blood flow) to keep pace with oxygen demand.
3. Hypoxia or endogenous signals induce the release of signaling factors (such as VEGF, bFGF, Ang-2, and chemokines) to promote endothelial cell migration (Rousseau et al. 2000).
4. As a result, there is disruption of endothelial cell junctions leading to vascular permeability. As a result, various plasma-borne components leak into local interstitial tissues (Lamalice et al. 2007).
5. Consequently, pericytes detach from the vessel (Ang-2 signaling), and as the vessel dilates, endothelial cells lose their contact with the basal lamina.
6. This is followed by coordinated cytoskeletal remodeling and disruption of cell-cell junctions: this is assisted by signaling factors such as neuropilin, VGEF/VGEFR, NOTCH/DLL4, and JAGGED1 which release matrix metalloproteases (MT1-MMP) to degrade the basement membrane and remodel the extracellular matrix.
7. New focal contacts (tip cells) are formed which extend numerous filopodia (via semaphorin, ephrin, and integrin guidance signals) toward angiogenic stimuli (VEGF gradient).
8. Stalk cells follow the tip cell and proliferate and extend the sprout. Proliferating stalk cells establish junctions with neighboring endothelial cells and release molecules such as EGFL7 (an endothelial cell chemoattractant expressed by proliferating endothelial cells) that bind to extracellular membrane components and regulate vascular lumen formation.
9. Fusion of neighboring branches occurs when two tip cells encounter each other, establish EC-EC junctions (VE-cadherin, Ang-1), and form a continuous lumen. Extracellular matrix is deposited to establish a new basement membrane (TIMPs), endothelial cell proliferation ceases, and pericytes are recruited to stabilize the new vessel (PDGFR/PDGF-B, Ang-1).
10. Once blood flow is established, the perfusion of oxygen and nutrient reduces angiogenic stimuli (VEGF expression) and inactivates endothelial cell oxygen sensors, reestablishing the quiescent state of the blood vessel (Cheresh and Stupack 2008).

9.1.2 Factors that Modulate Angiogenesis

Angiogenesis is controlled by a number of growth factors and inhibitors. Some of the well-known angiogenic (stimulatory) growth factors are angiogenin, angiopoietin-1, Del-1, and fibroblast growth factors: acidic (aFGF) and basic (bFGF), follistatin, granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor

(HGF)/scatter factor (SF), interleukin-8 (IL-8), leptin, midkine, placental growth factor, platelet-derived endothelial cell growth factor (PD-ECGF), platelet-derived growth factor-BB (PDGF-BB), pleiotrophin (PTN), progranulin, proliferin, transforming growth factor-alpha (TGF-alpha), transforming growth factor-beta (TGF-beta), tumor necrosis factor-alpha (TNF-alpha), and vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF).

Angiogenic inhibitors are angioarrestin, angiostatin (plasminogen fragment), antiangiogenic antithrombin III, arrestin, chondromodulin, canstatin, cartilage-derived inhibitor (CDI), CD59 complement fragment, endostatin (collagen XVIII fragment), endorepellin, fibronectin fragment, fibronectin fragment (anastellin), Gro-beta, heparinases, heparin hexasaccharide fragment, human chorionic gonadotropin (hCG), interferon alpha/gamma, interferon-inducible protein (IP-10), interleukin-12, kringle 5 (plasminogen fragment), metalloproteinase inhibitors (TIMPs), 2-Methoxyestradiol, PEX, pigment epithelium-derived factor (PEDF), placental ribonuclease inhibitor, plasminogen activator inhibitor, platelet factor-4 (PF4), prolactin 16kD fragment, proliferin-related protein (PRP), prothrombin kringle 2, retinoids, soluble Fms-like tyrosine kinase-1 (S-Flt-1), targeting fibronectin-binding integrins, tetrahydrocortisol-S, thrombospondin-1 (TSP-1) and −2, transforming growth factor-beta (TGF-b), troponin I, tumstatin, vasculostatin, and vasostatin (calreticulin fragment)

9.1.3 Angiogenesis in Disease

In various disease conditions, dysregulated angiogenesis has been seen. Angiogenesis-dependent diseases result when new blood vessels either grow excessively or insufficiently. Excessive angiogenesis occurs in diseases such as cancer, ischemic heart or limbs, rheumatoid arthritis, psoriasis, peptic ulcers, bowel atresias, vascular malformations, hemangiomas, diabetic blindness, age-related macular degeneration, and more than 70 other conditions. As a result, diseased cells and tissue produce abnormal amounts of angiogenic growth factors, overwhelming the effects of natural angiogenesis inhibitors. New blood vessels grow and feed diseased tissues; new vessels are abnormal and leaky and can destroy normal tissues. In the case of cancer, the abnormal vessels allow tumor cells to escape into the circulation and lodge in other organs (tumor metastases). Antiangiogenic therapies, aimed at halting new blood vessel growth, are used to treat these conditions. Insufficient angiogenesis occurs in diseases such as coronary artery disease, stroke, and chronic wounds. In these conditions, blood vessel growth is inadequate, and circulation is not properly restored, leading to the risk of tissue death. Insufficient angiogenesis occurs when tissues do not produce adequate amounts of angiogenic growth factors. Therapeutic angiogenesis therapies, aimed at stimulating new blood vessel growth with growth factors, are being developed to treat these conditions.

9.1.4 Types of Pathological Angiogenesis

Pathological ocular angiogenesis is classified into two major forms: preretinal angiogenesis, arising from the retinal vessels, and subretinal (or choroidal neovascularization) angiogenesis, arising from the choriocapillaris.

Preretinal angiogenesis generally develops during vascular occlusion (where there is capillary non-perfusion along with ischemia) in conditions like diabetic retinopathy wherein VEGF is secreted by the RPE, Muller cells, and astrocytes. During this condition, abnormal vessels breech the internal limiting membrane of the retina and breakthrough into the vitreous cavity. These vessels in the vitreous cavity acquire fibrous component and form fibrovascular membrane that bleed leading to severe vision loss. Fibrovascular membranes also cause tractional retinal detachment when not treated adequately by retinal photocoagulation. When the retinal ischemia is global, angiogenesis and scarring occur in the anterior chamber angle of the iris and cause neovascular glaucoma which responds poorly to antiglaucoma medications.

Subretinal neovascularization is associated with pathological changes in the retinal pigment epithelium, Bruch's membrane, and the choriocapillaris. Any breech in the Bruch's membrane triggers the healing response in the form of neovascularization. Oxidative stress, local inflammatory mediators, and hypoxia also play an important role in the sequence of events. The exact role of each of these contributory factors is yet to be ascertained. Subretinal neovascularization can develop either between the retinal pigment epithelium and Bruch's membrane (occult choroidal neovascularization), or between the retinal pigment epithelium and the neurosensory retina (classic choroidal neovascularization).

Subretinal neovascularization can regress, leaving an area of retinal atrophy, or can progress to be ultimately replaced by fibrotic scar over a period of time. Neovascular membranes can also bleed producing massive subretinal hemorrhage which can break through the internal limiting membrane into the vitreous cavity. Ultimately, the overlying neuroretina will degenerate, leading to loss of central vision, contrast sensitivity, and color vision.

In this chapter, we discuss various antiangiogenic agents which inhibit the vasculogenesis cascade at different steps. These include the ones which have been approved for clinical use, the ones which are used off label, and the ones still undergoing trials.

9.1.5 Tyrosine Kinase Inhibitors

The PI3K/AKT/MTOR (phosphoinositide 3 kinase/Akt/mammalian target of rapamycin) pathway is an intracellular signaling pathway important in regulating cell cycle. Second-generation inhibitors of PI3K/Akt/mTOR pathway have been found to be efficacious in managing various stages of disease progression in DR thus presenting a unique opportunity for the management of neovascularization.

During early stages of neovascularization, mTOR inhibitors suppress HIF-1α and VEGF thus preventing leakage and breakdown of the blood-retinal barrier. These inhibitors impart a pronounced inhibitory effect on inflammation (which is an early component with diverse ramifications) influencing the progression of DR. mTOR inhibitors suppress IKK and NF-κB along with downstream inflammatory cytokines, chemokines, and adhesion molecules. These inhibitors also suppress several growth factors that play pivotal roles in the induction of pathological angiogenesis (Jorge and David 2011).

Inhibition of the PI3K/Akt/mTOR pathway that disrupts the Akt-RhoB interaction is postulated to promote endothelial cell death based on experimental evidence (Adini et al. 2003). Prevention of endothelial cell proliferation and enhancement of endothelial cell apoptosis could serve as a treatment modality to delay or prevent progression of vasculopathies as observed in diabetic retinopathy since enhanced migration of endothelial cells is a requirement for neovascularization to occur.

Sirolimus (MacuSight, Union City, Calif, USA) and Palomid 529 (Paloma Pharmaceuticals, Inc. Jamaica Plain, Mass, USA) and everolimus (Novartis) are currently being evaluated in NIH-sponsored trials for ocular indications. Sirolimus is in phase II trial to treat diabetic macular edema and advanced ARMD. Palomid 529 is being evaluated for ARMD (Clinical Trial 2008; Sherris 2007). Everolimus in combination with ranibizumab is in phase II trial for patients with wet ARMD. However, several adverse events have been reported with the tyrosine kinase inhibitors particularly in diabetics such as gastrointestinal effects (Soefje et al. 2011), hematological (Sofroniadou and Goldsmith 2011), decreased glucose tolerance, hyperglycemia, and hypertriglyceridemia (Majewski et al. 2004). Cutaneous and oral ulceration with delayed wound healing (Mills et al. 2008), renal toxicity (Letavernier and Legendre 2008), and infertility (Boobes et al. 2010) have also been reported. However, these adverse events have been found to be reversible and medically manageable.

9.1.6 Small Interfering RNA (siRNA)

Small interfering RNA (siRNA) is another approach that is used to inhibit VEGF expression. PF-655 is a synthetic siRNA known to inhibit the expression of DNA damage-inducible transcript 4 protein (DDIT4; also known as RTP801) associated with pathological retinal neovascularization in animal models (Brafman et al. 2004). Expression of RTP801 is rapidly upregulated in response to ischemia, hypoxia, and/or oxidative stress. The mechanism of action of PF-655 is different from that of different types of VEGF inhibitors (Shoshani et al. 2002). Intravitreal injection of PF-655 in preclinical animal models of laser-induced choroidal neovascularization (CNV) has been found to inhibit RTP801 expression and induce expression of anti-angiogenic and neurotrophic factors leading to subsequent reduction of CNV volume, vessel leakage, and infiltration of inflammatory cells into the choroid. It is currently in phase II trials for diabetic macular edema and wet ARMD (clinical trial).

Bevasiranib is a naked, 21-nucleotide-long siRNA that specifically targets VEGF. Upon introduction into the cell, this siRNA binds to and activates the RNA-induced silencing complex (RISC). The RISC in turn targets and degrades mRNA molecules that are complementary to the introduced siRNA. A single activated RISC complex can bind to and destroy hundreds of mRNAs, thus preventing translation and protein synthesis (Liu et al. 2010).

Bevasiranib is known to downregulate VEGF-A mRNA. However, this drug has failed to achieve the expected endpoint of "stabilization of vision" in treated individuals. This could be because siRNAs need to be formulated to achieve cell permeation in order to cause bona fide RNA interference. They also need to be modified to avoid off-target effects and recognition of their nucleotide structure by the innate immune system (Hornung et al. 2005; Kleinman et al. 2008; Sledz et al. 2003).

9.1.7 Pigment Epithelium-Derived Factor (PEDF)

PEDF is one of the most potent known antiangiogenic proteins found in humans. Ad(GV)PEDF.11D, is an E1-, partial E3-, E4-deleted replication-deficient, adenovirus serotype 5, gene transfer vector. The transgene in this vector is cDNA for human pigment epithelium-derived factor (PEDF). While Ad(GV)PEDF.11D is able to transduce many somatic cell types, the natural barrier to other tissues created by the retina limits the ability of Ad(GV)PEDF.11D to affect tissues other than in the eye.

Intravitreal administration of Ad(GV)PEDF.11D provides a convenient means of delivering PEDF to the relevant cells within the eye that is likely to result in a more prolonged duration of effect than administration of the PEDF protein alone (Rasmussen et al. 2001). Pigment epithelium-derived factor (PEDF) has an antagonistic effect on endothelial cell proliferation and migration, and its levels are decreased in ARMD (Bhutto et al. 2008; Dawson et al. 1999; Holekamp et al. 2002). A phase I clinical trial has been initiated using an adenoviral vector (AdGVPEDF.11D) to deliver PEDF via intravitreal injections.

9.1.8 Anti-VEGF Agents

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a signal protein produced by cells under normal as well as pathological conditions like hypoxia, ischemia, and neoplasia. VEGF stimulates vasculogenesis and angiogenesis. It is a part of the system that attempts to restore the oxygen supply to tissues under hypoxic and ischemic conditions. Serum concentration of VEGF has been found to be high in bronchial asthma and diabetes mellitus (Cooper et al. 1999).

The VEGF family in mammals comprises of five different molecules, namely, VEGF-A, placenta growth factor 1 and 2 (PGF- 1 and 2), VEGF-B, VEGF-C, and

VEGF-D. VEGF-A acts mostly on cells of the vascular endothelium. It also has effects on a number of other cell types like monocytes and macrophages stimulating their migration in neurons, cancer cells, and kidney epithelial cells. In vitro, VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor. There are several isoforms of VEGF-A, including VEGF-A121, VEGF-A145, VEGF-A148, VEGF-A183, VEGF-A189, and VEGF-A206; the best characterized proangiogenic isoform of VEGF is VEGF-A165 (Harper and Bates 2008; Nowak et al. 2010).

Monoclonal antibodies against VEGF were first developed as an intravenous treatment for metastatic colorectal cancer (Homsi and Daud 2007; Los et al. 2007). The three available anti-VEGF agents for intravitreal use are bevacizumab (Avastin) and ranibizumab (lucentis) and pegaptanib. Ranibizumab is a shorter 48-kDa antibody fragment (κ isotype) that binds to the receptors of biologically active VEGF-A, including VEGF-110. It blocks the binding of VEGF-A to VEGR receptors, VEGFR1 and VEGFR2 receptors on endothelial cells (Patan 2000). Bevacizumab, however, is a larger whole antibody of 149 kDa and possesses two antigen-binding domains for its receptors Flt-1 and KDR. It binds to all isoforms of VEGF7. Pegaptanib sodium is a 50-kDa aptamer, a pegylated modified oligonucleotide that adopts a specific 3D configuration and has a high affinity for extracellular VEGF-165, the isoform of VEGF-A implicated in ocular angiogenesis (Eyetech Study Group 2002).

The difference in molecular weights of these molecules may determine their potential difference in efficacy and their duration of action. Detailed safety profiles and risks of adverse effects are now available for these agents as they have been used extensively in patients for the treatment of ARMD, diabetic macular edema, and retinal vascular occlusions (Tolentino 2011). Furthermore, the incidence of raised IOP and development of lens opacity with anti-VEGF agents is negligible when compared with intravitreal steroid injections (Jager et al. 2004; Sampat and Garg 2010).

9.1.9 VEGF Trap

It's a fusion protein comprised of segments of the extracellular domains of human vascular endothelial growth factor receptors 1 (VEGFR1) and 2 (VEGFR2) fused to the constant region (Fc) of human immunoglobulin G1 (IgG1) with potential anti-angiogenic activity. Aflibercept acts as a soluble decoy receptor, binds to proangiogenic vascular endothelial growth factors (VEGFs), thereby preventing VEGFs from binding to their cell receptors.

Aflibercept binds to circulating VEGFs and acts like a "VEGF trap" (Stewart 2012). It thereby inhibits the activity of the vascular endothelial growth factor subtypes VEGF-A and VEGF-B, as well as to placental growth factor (PGF), inhibiting the growth of new blood vessels in the choriocapillaris (Saishin et al. 2003). VEGF trap has been found to be beneficial in ARMD (Cho et al. 2013) and diabetic macular edema (Korobelnik et al. 2014). Aflibercept has been approved by the FDA for the manage-

ment of ARMD since Nov 2011 and DME from July 2014 (FDA-approved aflibercept for diabetic retinopathy in patients with diabetic macular edema in March 2015).

9.1.10 VEGF Inhibitors: AAV2-sFLT01

AAV2-sFLT01 is an adeno-associated virus (AAV) (Maclachlan et al. 2011) that expresses a modified soluble Flt1 receptor designed to neutralize the proangiogenic activities of vascular endothelial growth factor (Maclachlan et al. 2011; Takahashi et al. 2009) (VEGF) AAV2-sFLT01 which produces a soluble VEGFR1 that can bind to free VEGF (Ambati et al. 2006; Kendall and Thomas 1993) to interrupt VEGF signaling. VEGF-A binds primarily to VEGFR1 and VEGFR2, both of which are tyrosine kinases (Ferrara et al. 2003). It is under phase I trial in the management of wet ARMD via an intravitreal injection (clinical trial).

9.1.11 Chemokine CC Motif Receptor 3 Blockers

Another approach that is currently undergoing testing involves the blockade of chemokine CC motif receptor 3, which mediates angiogenic signaling initiated by eotaxins (Takeda et al. 2009). Takeda et al. (2009) demonstrated that the eosinophil/mast cell chemokine receptor CCR3 is specifically expressed in choroidal neovascular endothelial cells in humans with ARMD and that despite the expression of its ligands eotaxin-1, eotaxin-2, and eotaxin-3, neither eosinophils nor mast cells are present in human choroidal neovascularization (CNV). Genetic or pharmacologic targeting of CCR3 or eotaxins has been found to inhibit injury-induced CNV in mice. CNV suppression by CCR3 blockade has been found to be due to direct inhibition of endothelial cell proliferation (and was uncoupled from inflammation, since it occurred in mice lacking eosinophils or mast cells) and to be independent of macrophage and neutrophil recruitment. CCR3 blockade has been more effective at reducing CNV than VEGF-A neutralization when used in the treatment of ARMD and, unlike VEGF-A blockade, is not toxic to the mouse retina. In vivo imaging with CCR3-targeting quantum dots located spontaneous CNV in mice where diagnosis with fluorscein angiography was not possible. Takeda et al. (2009) concluded that CCR3 targeting might reduce vision loss due to ARMD through early detection and therapeutic angioinhibition (Takeda et al. 2009).

9.1.12 Other Tyrosine Kinase Inhibitors

Pazopanib is a tyrosine kinase inhibitor that inhibits VEGFR1, VEGFR2, VEGFR3, PDGFR, c-KIT, and FGFR1 (Kumar et al. 2007). Pazopanib is effective in preclinical models of CNV (Takahashi et al. 2009). Pazopanib, formulated as eye drops, is

under investigation for the treatment of neovascular ARMD as a self-administered, potentially lower burden therapy (clinical trial). Pazopanib inhibits the same pathway that has been clinically validated in ARMD with anti-VEGF therapies, but with inhibition at the tyrosine kinase receptor level. Additional benefit may come from inhibition of the proangiogenic platelet-derived growth factor pathway. Inhibition of PDGFR-α and PDGFR-β, in combination with inhibition of the VEGF pathway, may lead to regression of aberrant blood vessels (Jo et al. 2006; Takahashi et al. 2009).

Vatalanib (PTK787 or PTK/ZK) is a small molecule protein kinase inhibitor that is orally administrated and inhibits angiogenesis. Oral administration of vatalanib blocks phosphorylation of VEGF and PDGF receptors and provides inhibition of retinal neovascularization. There is no effect on mature retinal vessels. Vatalanib has been tested previously as a treatment for CNV in phase I and phase II clinical studies (clinical trial).

AL39324 is an intravitreally administered tyrosine kinase inhibitor that is in a phase II clinical trial in combination with ranibizumab (clinical trial).

TG100801 is a tyrosine kinase inhibitor that binds and inhibits VEGFR and PDGFR. It is administered as an eye drop and has been found to suppress CNV and retinal edema (Palanki et al. 2008). A phase I trial was completed successfully, but a phase II trial was discontinued because of corneal toxicity.

9.1.13 Nicotinic Acetylcholine Receptor (nAChR) Pathway Inhibitors: Mecamylamine

VEGF inhibition can also be achieved by preventing the activation of the nicotinic acetylcholine receptor (nAChR) pathway in the vasculature, which inhibits VEGF-induced angiogenesis in endothelial cells (Heeschen et al. 2002). ATG-3 is an eye drop formulation of mecamylamine, an antagonist of the nAChR pathway (Kiuchi et al. 2008). It is the first antiangiogenic eye drop that has been studied in humans. ATG-3 has successfully completed two phase II clinical trials, one in patients with diabetic macular edema (Campochiaro et al. 2010) and the other in patients with wet ARMD who were receiving anti-VEGF treatments.

9.1.13.1 Anti-Sphingosine-1-Phosphate (S1P) Monoclonal Antibody

Sphingosine-1-phosphate (S1P) is the extracellular ligand for S1P receptor 1 (also known as EDG1), a G protein-coupled lysophospholipid receptor, and it is a proangiogenic and profibrotic mediator (Ozaki et al. 2003; Watterson et al. 2007). Intravitreous injection of an anti-S1P monoclonal antibody inhibited CNV formation and subretinal collagen deposition in a preclinical model of laser-induced CNV and in a preclinical model of diabetic retinopathy (Caballero et al. 2009; Xie et al. 2009). A humanized S1P monoclonal antibody (iSONEP) is under clinical trial in patients with wet ARMD (clinical trial). iSONEP was well tolerated, and no drug-related serious adverse events were observed in any of the patients. iSONEP also

exhibited positive biological effects (including lesion regression, reduction of retinal thickness, and resolution of pigment epithelium detachment). The mechanisms responsible for the positive effects exerted by S1P appear to be independent to those of anti-VEGF agents, indicating the potential of S1P antibodies to serve as a monotherapy (or an adjunct therapy to anti-VEGF agents) for the treatment of neovascular ARMD and polypoidal choroidal vasculopathy.

9.1.13.2 Anti-α5β1 Integrin Monoclonal Antibody

Endothelial cell migration involves interactions between integrins (transmembrane heterodimeric proteins) and extracellular matrix ligands. α5β1 integrin is expressed on the surface of vascular endothelial cells and mediates cell migration (Orecchia et al. 2003). Intravitreal JSM6427 (Jerini Inc) is a potent and selective inhibitor of integrin α5β1. Its potency as an inhibitor of angiogenesis has been demonstrated in animal models of CNVM blocking angiogenesis through inhibition of integrin-mediated signaling and has the potential to inhibit the cellular responses to growth factors, cytokines, and other inflammatory mediators.

Intravitreal administration of volociximab has resulted in strong inhibition of rabbit and primate retinal neovascularization. In monkeys with laser-induced choroidal neovascularization (CNV), volociximab significantly inhibited CNV proliferation and reduced the degree of lesion formation. In a rabbit model, volociximab administered either intravenously or intravitreally prior to the onset of neovascularization significantly reduced angiogenesis as compared to control.

Thus, JSM6427 and volociximab both bind to α5β1 integrin and block the migration of endothelial cells. Both agents have been shown to inhibit CNV formation in preclinical models (Zahn et al. 2009). They are in phase I trial for the treatment of ARMD (clinical trial).

9.1.13.3 Fosbretabulin (Combretastatin A-4 Phosphate)

Fosbretabulin is a vascular disrupting agent, and its active metabolite is combretastatin A4 (CA4) (Pettit et al. 1995). The active metabolite CA4 binds to tubulin and prevents microtubule polymerization in proliferating endothelial cells, thus inducing vessel regression (Dark et al. 1997). In animal models, CA4 has been demonstrated to inhibit retinal neovascularization and suppress the development of CNV (Griggs et al. 2002; Nambu et al. 2003). Currently phase II trials are underway to evaluate the safety and efficacy of fosbretabulin in patients with myopic CNVM (clinical trial).

9.1.13.4 E10030: A Pegylated Aptamer

E10030 (Ophthotech), an antiplatelet-derived growth factor (anti-PDGF-B) aptamer, strongly binds to PDGF-β. Platelet-derived growth factor-B plays a key role in recruiting the pericytes that envelop the new vessels and make them more resistant

to the anti-VEGF attack. Pericyte recruitment is a crucial step in vascular maturation and stabilization. PDGF has a key role in this process (Benjamin et al. 1998; Jo et al. 2006).

With this in mind, E10030—a pegylated aptamer that inhibits PDGF-β—was developed. Inhibition of PDGF results in the stripping of pericytes from endothelial cells, which increases the sensitivity of mature CNV to VEGF inhibition. PDGF inhibition may therefore synergize with VEGF inhibition in CNV treatment. In a phase I dose-escalating study, some CNV regression was observed in over 90 % of patients receiving the combination therapy of E10030 and ranibizumab, compared to about 10 % of patients receiving ranibizumab alone (Boyer and Ophthotech Anti-PDGF in AMD Study Group 2009). A phase II study is currently underway.

9.2 Photodynamic Therapy

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9.2.1 Introduction/Definition

Also known as photochemotherapy, it is a form of phototherapy using a photosensitizer (a nontoxic light-sensitive chemical which when selectively exposed to light becomes toxic) and causes damage to target malignant and other diseased cells. This method is used for both disease diagnosis and treatment (Jia and Jia 2012).

The spectrum of possible applications of PDT encompasses the entire range of infectious (viral, bacterial, fungal, protozoal) disorders, epidermal and dermal inflammatory diseases, tumors of lymphocytes, adnexal diseases, and premature skin aging due to sun exposure. With PDT, it is possible to eradicate pathogens in simple locations such as oral cavities as well as in deep-seated bone marrow. Nowadays' PDT is being explored as a new line of antimicrobial therapy in order to eradicate drug-resistant pathogens.

Photodynamic therapy (PDT) has also recently grown as a proven method of treatment for various types of cancers with a proven potential to target even HIV and MRSA (methicillin-resistant *Staphylococcus aureus*). Another key indication is for small areas of cancer that are unsuitable for or have persisted or recurred after conventional management. It can be applied in areas already exposed to the maximum safe dose of radiotherapy. Photodynamic therapy (PDT) is of particular value for precancer and early cancers of the skin and mouth because of good cosmetic and functional results.

PDT also has considerable potential in arterial diseases for preventing restenosis after balloon angioplasty and in the treatment of infectious diseases, where the responsible organisms are accessible to both the photosensitizer and light. New developments on the horizon include techniques for increasing the selectivity for cancers, such as coupling photosensitizers to antibodies, and for stimulating immunological responses, but many further preclinical and clinical studies are needed to establish PDT's role in routine clinical practice (Bown 2013).

9.2.2 Photodynamic Therapy Process

Most PDT applications involve three key components: a photosensitizer, a light source, and tissue oxygen. A photosensitizer is a chemical compound that gets converted to an excited state upon absorption of light and forms free radicals which react with oxygen to produce a highly reactive state of oxygen (reactive oxygen species (ROS)) known as singlet oxygen. Exposure of cell to light energy wavelengths is typically in the visible region. ROS produces cell inactivation and death through modification of intracellular components thus becoming highly cytotoxic within few microseconds of activation. Generally, the photosensitizer is applied locally to target area or the photosensitive targets are locally excited with light. In the localized treatment of internal tissues and cancers, photosensitizers are administered intravenously and light is delivered through endoscopes or fiber-optic catheters. Once the photosensitizer is localized to the intended area (tissue/organ), an appropriate wavelength of light is chosen in order to excite the photosensitizer.

9.2.2.1 Mechanism of Photodynamic Therapy

Photodynamic process begins when photosensitizer absorbs a photon and gets converted into an excited (singlet) state. This singlet state of photosensitizer undergoes simultaneous or sequential decay resulting in intramolecular energy transfer reactions which are of three types. Type I reaction involves photooxidation by radicals, type II reaction involves photooxidation by singlet oxygen, and in type III reaction, photoreaction does not involve oxygen. Photosensitizer in excited singlet state readily decays back to the ground state with the emission of light (fluorescence) or heat. Sometimes it goes into a "triplet state." In addition, excited molecules also undergo type III reactions. Photosensitizer in triplet state reacts with ground state oxygen to produce singlet state oxygen, then decay to ground state by phosphorescence, or undergo type I and III reactions. Singlet oxygen generated during the process is a highly reactive form of oxygen and is highly damaging species with the potential to cause blood flow stasis, vascular collapse, and/or vascular leakage ultimately leading to tumor ablation. PDT has also been demonstrated to induce apoptosis causing orderly elimination of unwanted cells (Sibata et al. 2000).

9.2.2.2 Light Used

The light source for PDT can range from an ordinary light bulb to a diode array (emitting a broad band incoherent spectrum) or a laser. Broad-spectrum light sources such as xenon arc lamps or slide projectors equipped with red filters (to eliminate short wavelengths) are used for in vitro and preclinical in vivo studies of tumors. Lasers are standard light sources for PDT as they are monochromatic, have high power output, and can be easily coupled to fiber optics for endoscopic light delivery to localized areas in body cavity. The most common lasers are tunable dye lasers

(Sibata et al. 2000). Argon lasers have been employed for the treatment of human corneal neovascularization (Sheppard et al. 2006) and neovascular maculopathy (Barbara et al. 1991). Copper-pumped dye laser, a double laser consisting of KTP (potassium titanyl phosphate/YAG (yttrium aluminum garnet)) medium, has been used in dermatology and in facial telangiectasias (Cassuto et al. 2000), while LED (light-emitting diode) has been used in treatment of viral warts (Ohtsuki et al. 2009).

9.2.2.3 Dyes Used

Photofrin was the first US FDA-approved photosensitizer for treatment of cancer. Thereafter, a variety of dye sensitizers have been developed and approved for PDT treatment of skin and organ diseases ranging from simple bacterial infections (acne vulgaris) to more serious cancers. Some of the very common dyes used in the treatment of photodynamic therapy are listed as follows:

(i) Psoralen compounds in conjunction with UVA (300–400 nm) radiation are used in the treatment of psoriasis, atopic dermatitis, seborrheic dermatoses, histiocytosis, lichen planus, mycosis fungoides, polymorphous light eruption, pityriasis lichenoides, lymphomatoid papulosis, prurigo, palmar and plantar pustulosis, and vitiligo. Examples of psoralen photosensitizers include 5-methoxypsoralen, 8-methoxypsoralen, and trioxsalen.
(ii) Porphyrinoid photosensitizers porphyrin, chlorin, bacteriochlorin, pheophorbide, bacteriopheophorbide, texaphyrin, porphycene, and phthalocyanine.
(iii) Non-porphyrin dyes are anthraquinones, phenothiazines, xanthenes, cyanines, and curcuminoids.

9.2.2.4 Molecules Used

Photofrin is the only photosensitizer approved by US FDA for palliation of various types of cancer such as cancer of the esophagus and Barrett esophagus, endobronchial cancer, certain skin cancers such as basal cell carcinoma and squamous cell carcinoma, and some tumors of the vagina, vulva, and cervix that can be reached by activating light. Aminolevulinic acid has been approved for the treatment of actinic keratosis of face or scalp. Methyl ester of ALA has been approved by US FDA in July 2004 for treatment of some types of actinic keratoses of face and scalp. Verteporfin (Visudyne) had been approved for treatment of pathologic myopia, ocular histoplasmosis, and for age-related macular degeneration.

9.2.2.5 Side Effects

Photofrin comes with the side effects of accumulation. Also skin and eyes of the patient become photosensitive with Photofrin treatment. Application of aminolevulinic acid causes redness and tingling or burning sensation to skin. Photosensitivity

reactions can also be observed with methyl ester of ALA, and therefore, this drug is not recommended for people whose skin is sensitive to light, in immunosuppressed individuals, and those with peanut or almond allergy.

Some of the other general adverse effects include burns, swelling, pain, and scarring of surrounding tissues (Dolmans et al. 2003). Skin and eyes become sensitive to light. Stenosis and perforation of hollow organs have also been observed in some cases (Wittmann et al. 2014). PDT has also been reported to cause damage to DNA such as strand breaks, degradation, DNA-protein cross-links, and chromosomal aberrations and mutations. Other shortcomings include limitation of treatment depth due to ineffective penetration of light. Revascularization of treated areas is one of the biggest adverse effects that pose threat to benefits of PDT. In some cases, damage to vascular endothelium leading to increased vascular permeability, platelet aggregation, blood flow stasis, vasoconstriction, and ultimately vascular occlusion has been observed. Vascular damage further leads to hypoxia-related re-angiogenesis necessitating re-treatment (Verteporfin in Photodynamic Therapy Study Group 2001).

9.2.2.6 Advantages of PDT

(i) It has no long-term side effects when used properly.
(ii) It is less invasive than surgery.
(iii) It usually takes only a short time and is most often done as an outpatient.
(iv) It can be targeted very precisely.
(v) It can be used to treat one lesion at a time.
(vi) Unlike radiation, PDT can be repeated many times at the same site if needed.
(vii) Since this therapy uses nonionizing radiation, there is relatively rapid recovery.
(viii) There is little or no scarring after the site heals.
(ix) It often costs less than other cancer treatments.
(x) It can also be used in combination with other therapies so as to achieve synergistic effects.

Calculations for doses applied/based on body surface area: Successful treatment with PDT is highly dependent upon the light dose delivered to the target tissue. The unit of total energy of light is joule (J) and is determined by watt (W) multiplied by time (s). The number of photons (N) in a joule depends on the wavelength (λ) of light. If two different wavelengths of light are used, the number of photons per joule varies as the inverse ratio of the wavelength (hc/λ, where $h = 6.623 \times 10–34$ J is Planck's constant and $c = 2.998 \times 108$ m/s is the speed of light). Another factor that needs to be considered is fluence rate (i.e., rate of light delivered; fluence rate = W/area). Fluence rate, and thus treatment time, depends on the light source used. It the light is delivered at a high rate, significant heating of the tissue and its surrounding may take place. Generally, fluence rates less than 200 mW/cm^2 (for microlens) or 400 mW/cm (for cylindrical diffusers) should be used in order to avoid thermal damage to the normal tissues (Sibata et al. 2000).

9.2.2.7 Photodynamic Therapy in Ophthalmic Disorders

PDT finds widespread use in treatment of ocular diseases. In ophthalmology, PDT is used to treat ARMD and malignant cancers. This is because maintaining the mechanical integrity of hollow organs is easy with PDT therapy since its biological effect is different from surgery, radiotherapy, and chemotherapy as connective tissues like collagen are largely unaffected. In ophthalmology, it is established for all types of ARMD such as nonexudative, exudative, dry, and wet ARMD. PDT with verteporfin along with laser photocoagulation and administration of pegaptanib sodium is known to reduce the risk of vision loss in selected cases of neovascular ARMD as well as wet ARMD (55–56). PDT with verteporfin causes stabilization or improvement of visual acuity in patients suffering from chorioretinal anastomosis (Silva et al. 2004).

Some of the other applications of PDT are in treatment of ocular herpes using antimetabolites ara A and F3T (Pavan-Langston and Langston 1975), to reduce subretinal fluid in choroidal nevus with serous macular detachment (Garcia-Arumi et al. 2012), to induce closure of superficial vasculature of pigmented choroidal melanoma with verteporfin (Tuncer et al. 2012) and with bevacizumab (Canal-Fontcuberta et al. 2012), for the treatment of progressive keratoconus using collagen cross-linking by the photosensitizer riboflavin and UVA light (Wollensak 2006), for the treatment of chronic cases of central serous chorioretinopathy with verteporfin (64–66), for the treatment of polypoidal choroidal vasculopathy [using ICGA-guided photodynamic therapy (PDT) with verteporfin, combined PDT, and antivascular endothelial growth factor (VEGF) therapy (anti-VEGF therapy)] (Wong and Lai 2013). PDT with intravitreal bevacizumab has also shown good visual improvements in cases of polypoidal choroidal vasculopathy (Fan et al. 2014). Preoperative PDT has also been useful in reducing the potential of bleeding at the time of tissue biopsy (Canal-Fontcuberta et al. 2012).

9.2.3 Photodynamic Therapy in Angiogenesis

PDT is clinically approved for the treatment of angiogenic disorders, including certain forms of cancer and neovascular eye diseases. PDT for ocular angiogenesis is generally a two-step process, consisting initially of an intravenous (within the vein) injection of photosensitizer followed by laser treatment to the targeted sites of neovascularization in the retina after 15 min. The laser treatment is intended to selectively damage the vascular endothelium. In case choroidal neovascularization persists, patients are re-treated. In some cases, PDT is combined with angiostatic agents intended to target various parts of angiogenic pathway: mRNA, VGEFs, endothelial cell proliferation, migration, and proteolysis. Some of the angiostatic agents under study are verteporfin, pegaptanib, ranibizumab, bevacizumab,

anecortave acetate, squalamine, vatalanib, and triamcinolone acetonide. Some of the typical cases of angiogenesis treated with PDT are:

(i) *Subfoveal and juxtafoveal choroidal neovascularization*: Choroidal neovascularization is the most common vision-threatening complication of high myopia. In this disease, new vessels grow under the retina distorting vision and leading to scarring. Photodynamic therapy (PDT) is a new experiment treatment for CNV that combines the application of low-intensity light with a photosensitizing agent in the presence of oxygen to produce tissue effects. It uses the noninvasive potential of the laser light to cause a nonthermal localized chemotoxic reaction and obtain highly selective occlusion of the neovascular channels while sparing the overlying photoreceptors (Donati et al. 1999). Verteporfin photodynamic therapy is used in these cases due to its angio-occlusive mechanism of action that reduces visual acuity loss and underlying leakage associated with lesions. Verteporfin PDT has also been associated with encouraging treatment outcomes in case studies involving patients with choroidal vascular disorders such as polypoidal choroidal vasculopathy, central serous chorioretinopathy, choroidal hemangioma, angioid streaks, and inflammatory CNV (Chan et al. 2010). Verteporfin photodynamic therapy has also been found to be beneficial as a part of triple therapy for neovascular ARMD (Ehmann and Garcia 2010). PDT therapy has been found to effectively induce tumor regression and resolution of exudative subretinal fluid, improving or stabilizing vision in circumscribed choroidal hemangioma (Elizalde et al. 2012). Visudyne has already been approved by US FDA for the treatment of subfoveal choroidal neovascularization (Rivellese and Baumal 2000) and juxtafoveal neovascularization (Blair et al. 2004).

(ii) *Corneal neovascularization* (*CNV*): It is excessive ingrowth of blood vessels from the limbal vascular plexus into the cornea as a result of oxygen deprivation. CNV causes significant visual loss because of the scarring and lipid deposition that frequently accompany it and is generally induced by nonspecific inflammatory stimuli mediated primarily by polymorphonuclear neutrophils. CNV can also be caused due to specific corneal immune reactions such as herpes simplex keratitis. Photodynamic therapy with argon laser following intravenous injection of hematoporphyrin derivative or purified dihematoporphyrin ether (DHE) has been used to suppress tumor growth and blood vessel growth in the eye (Epstein et al. 1987; Sheppard et al. 2006). Currently, verteporfin in conjunction with photodynamic therapy has been reported to be effective against CNV (Al-Torbak 2012).

9.2.3.1 Mechanism of Action of Molecules Used for PDT in Angiogenesis

(i) Verteporfin: it is composed of two semisynthetic porphyrin isomers and is four times more powerful as compared to porphyrin sensitizers when used alone. It is a chlorin-type molecule and an efficient generator of singlet

oxygen. It has a maximum absorption in the UVA range with an additional absorption peak between 680 and 695 nm. Therefore, it can be activated with a low-power light that can penetrate blood, melanin, and fibrotic tissue. It binds with LDL to form a complex within abnormally proliferating cells. Thereafter, it becomes bound to intracellular or membrane components and, when activated through light, causes cellular damage (Schmidt-Erfurth and Hasan 2000).

(ii) Pegaptanib: it is a pegylated anti-VGEF aptamer (single strand of nucleic acid that binds with a particular target). It is a selective antagonist of 165 isoform of VGEF. As a result, growth of blood vessels is curtailed to control leakage and swelling in angiogenesis (Ng et al. 2006).

(iii) Bevacizumab: it is the first monoclonal antibody synthesized for treatment of angiogenesis in cancer. It is a recombinant monoclonal antibody that blocks VGEF-A, which is an angiogenesis stimulator. It finds application in treatment of certain metastatic cancers such as colon cancer, ovarian cancer, and renal cancer. In eye diseases, it is used in the treatment of diabetic retinopathy (Agarwal et al. 2014), neovascular glaucoma (Jiang et al. 2014), diabetic macular edema (Stewart 2014), and retinopathy of prematurity.

(iv) Ranibizumab: it is a monoclonal antibody fragment similar to bevacizumab with similar effectiveness and clinical applications (Agarwal et al. 2014; Jiang et al. 2014; Stewart 2014).

(v) Anecortave acetate: it is a unique angiostatic agent derived from glucocorticoid cortisol acetate and is a nonselective inhibitor of different angiogenic factors. It is useful in the treatment of iatrogenic glaucoma (Razeghinejad and Katz 2012) and wet ARMD (Augustin 2006) and shows promise in the treatment of choroidal neovascularization (Slakter 2006).

(vi) Squalamine: it is an aminosterol compound obtained from dogfish shark. It has potent antimicrobial activity. It is a cationic peptide that binds to phospholipid membranes and interacts with plasma membranes of infective bacteria to prevent their function. It shows promise in the treatment of neovascular ARMD (Emerson and Lauer 2007) and exudative ARMD (Connolly et al. 2006).

(vii) Vatalanib: it is a VEGF substrate that inhibits tyrosine kinase receptor that is an important enzyme contributing to formation of new blood vessels. It has been found to be useful in treatment of neovascular ARMD (Emerson and Lauer 2007) and wet ARMD (Ni and Hui 2009).

(viii) Triamcinolone acetonide: it is a synthetic fluorinated corticosteroid and is about eight times more potent as compared to prednisone. It possesses good anti-inflammatory action and therefore used in the management of ocular inflammatory disease. Its use is being explored in the treatment of diabetic macular edema (Ciulla et al. 2014).

Recently, the concept of angiostatic targeted therapy is under rapid development. It involves use of clinically effective angiogenesis inhibitors in combination with PDT (Weiss et al. 2012). However, this therapy has not got a full-fledged entrance

into the clinical management of cancer mainly because of secondary complications such as inflammation and neoangiogenesis.

The recent development of anti-VEGF substances for use in clinical routine has markedly improved the prognosis of patients with neovascular ARMD. Intravitreal treatment with substances targeting all isotypes of vascular endothelial growth factor (VEGF), for the first time in the history of ARMD treatments, results in a significant increase in visual acuity in patients with neovascular ARMD. Overall, antiangiogenic approaches provide vision maintenance in over 90 % and substantial improvement in 25–40 % of patients. The combination with occlusive therapies like photodynamic therapy (PDT) potentially offers a reduction of re-treatment frequency and long-term maintenance of the treatment benefit.

9.3 Summary

Photodynamic therapy is a unique treatment modality wherein a photosensitizer localized to specific tissue/organ is activated by use of light of specific wavelength. This method has revolutionized therapeutic strategies for the treatment of several infectious as well as angiogenic diseases. It also finds promising applications in several malignant as well as nonmalignant conditions of the ocular system.

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