REVIEW

Clinical biomarkers and molecular basis for optimized treatment of diabetic retinopathy: current status and future prospects

Rohit Saxena[1]
Digvijay Singh[2]
Ravi Saklani[3]
Suresh Kumar Gupta[3]

[1]Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, [2]Division of Ophthalmology, Medanta-The Medicity, Gurgaon, [3]Ocular Pharmacology Laboratory, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India

Abstract: Diabetic retinopathy is a highly specific microvascular complication of diabetes and a leading cause of blindness worldwide. It is triggered by hyperglycemia which causes increased oxidative stress leading to an adaptive inflammatory assault to the neuroretinal tissue and microvasculature. Prolonged hyperglycemia causes increased polyol pathway flux, increased formation of advanced glycation end-products, abnormal activation of signaling cascades such as activation of protein kinase C (PKC) pathway, increased hexosamine pathway flux, and peripheral nerve damage. All these changes lead to increased oxidative stress and inflammatory assault to the retina resulting in structural and functional changes. In addition, neuroretinal alterations affect diabetes progression. The most effective way to manage diabetic retinopathy is by primary prevention such as hyperglycemia control. While the current mainstay for the management of severe and proliferative diabetic retinopathy is laser photocoagulation, its role is diminishing with the development of newer drugs including corticosteroids, antioxidants, and antiangiogenic and anti-VEGF agents which work as an adjunct to laser therapy or independently. The current pharmacotherapy of diabetic retinopathy is incomplete as a sole treatment option in view of limited efficacy and short-term effect. There is a definite clinical need to develop new pharmacological therapies for diabetic retinopathy, particularly ones which would be effective through the oral route and help recover lost vision. The increasing understanding of the mechanisms of diabetic retinopathy and its biomarkers is likely to help generate better and more effective medications.

Keywords: mechanism, pharmacotherapy microvascular changes, neurodegeneration, laser, progression

Introduction

Diabetic retinopathy (DR) is a highly specific neuroretinal and microvascular complication of diabetes caused by various abnormal metabolic pathways triggered by uncontrolled hyperglycemia. Initially, the disease is asymptomatic, but prolonged poor control of diabetes leads to permanent pathological changes in the retina resulting in blurred vision, floaters, distortion, and complete loss of vision.[1–6] These pathological changes include microaneurysms, hemorrhages and exudates (nonproliferative DR [NPDR]), formation of new abnormal blood vessels or retinal neovascularization (proliferative DR [PDR]), and diabetic macular edema (DME).

DR is emerging as one of the leading causes of blindness in both developing and developed countries. It is estimated that nearly 400 million people are affected with diabetes worldwide and the number is likely to reach close to 600 million by 2035.[7,8] Over 90 million people are estimated to be suffering from DR, of which 17 million have PDR, 21 million have DME, and 28 million have severe vision-threatening DR.[9,10]

Correspondence: Rohit Saxena
Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, Room No 377, New Delhi 110029, India
Tel +91 11 2569 3182
Email rohitsaxena80@yahoo.com

A thorough and enhanced understanding of the various pathways and clinical biomarkers involved in the pathogenesis of DR is a prerequisite to developing therapeutic strategies. At the molecular level, the etiology of DR is highly complex, with numerous interlinked mechanisms causing adaptive, functional, and structural changes in both the microvasculature and neuroglia. These in turn lead to cellular damage in the retina which is often permanent. However, despite extensive research, the understanding of these pathways remains limited and the initiator of the DR cascade is unclear.[3,5,9]

It is currently understood that prolonged hyperglycemia causes increased polyol pathway flux,[11,12] increased advanced glycation end-product (AGE) formation,[13,14] abnormal activation of signaling cascades such as activation of protein kinase C (PKC) pathway,[15–17] increased hexosamine pathway flux,[10,18–22] and peripheral nerve damage.[23] All these changes lead to increased oxidative stress[19,24–27] and inflammatory assault[28–31] to the retina resulting in adaptive structural and functional changes.[9,32,33]

Progression of DR is characterized by loss of pericytes, basement membrane thickening, formation of microaneurysms, neovascularization, and blood–retinal barrier breakdown.[9] The disease progresses through increased vascular permeability and retinal ischemia resulting in retinal neovascularization and retinal thickening.[34]

Conventionally, the clinical classification, etiology, and management strategy of DR were solely based on microvascular changes in the retina. The role of neuroretinal alterations in the etiology of DR were not recognized until the 1960s, when Wolter[35] and Bloodworth[36] documented the pathological degeneration of neurons in the retina of diabetic patients. Although the exact relation between neuroretinal changes and DR is still unclear, research over the past decade has enhanced our understanding about various neuroretinal pathways and clinical biomarkers involved in the pathogenesis of DR. The role of neuroretinal alterations and neuroretinal inflammation in the formation and progression of DR is apparent, and therapies targeting the prevention of neuroretinal damage from diabetes are also underway in different stages of clinical and preclinical trials.[37,38]

Biomarkers and mechanisms for microvascular dysfunction
Glycemic level
Evidence of hyperglycemia and its duration as a major risk factor in the progression of DR is plentiful.[10,39] In diabetes, due to prolonged exposure to high blood glucose concentration, endothelial cells lining the microvasculature experience oxidative stress and cause increased adhesive interactions between circulating inflammatory cells and additionally activate them. This leads to increased synthesis of inflammatory mediators by blood and endothelial cells promoted by cytokines.[26]

Lipid level
Dyslipidemia is found to increase the risk of DR, especially DME.[40–42] The exact role of an increased lipid level in the progression of DR remains unclear, but it is hypothesized that high lipid levels cause endothelial dysfunction due to a decreased bioavailability of nitric oxide and peroxidation of lipid by increased oxidative stress associated with hyperglycemia which is responsible for retinal exudate formation in DR.[40]

Aldose reductase (polyol pathway)
In a hyperglycemic state, excess intracellular glucose enters the polyol pathway and is reduced by aldose reductase to sorbitol accompanied by expenditure of NADPH and reduction of glutathione level. Increased sorbitol levels cause osmotic stress, and reduced levels of NADPH and glutathione cause impairment of antioxidant defense mechanisms resulting in oxidative damage to retina and capillary cells. Increased expression of aldose reductase has been implicated in the pathogenesis of DR and inhibition of the same may control progression of DR.[11,12,43]

TGF-β1 and plasminogen activation inhibitor-1 (hexosamine pathway)
In a hyperglycemic condition, excess glucose is diverted from glycolysis to the hexosamine pathway, which produces substrates such as proteoglycan and *O*-linked glycoproteins. These substances are responsible for many manifestations of DR. Substrates produced in this pathway cause intracellular glycation of transcription factor SP1 at serine and threonine location in the transcribed protein, which is consequently responsible for increased expression of TGF-β1 and PAI-1. TGF-β1 is involved in the production of extracellular matrix protein and PAI-1 inhibits the fibrinolysis. Overexpression of these two biomarkers together causes retinal vein occlusion.[44]

Vascular endothelial growth factor (angiogenic biomarkers)
Vascular endothelial growth factor (VEGF) is an important endothelial cell-specific angiogenic and permeability-inducing inflammatory agent. It is an important biomarker and plays a causative role in the development of DR. If the level of

VEGF is maintained, then the progression of DR is controlled substantially.[41]

PKC-β

PKC-β is an important biomarker in DR and mediates a variety of undesirable functional and structural changes in vascular tissues. In diabetes, prolonged hyperglycemia results in increased diacylgycerol level, which triggers the activation of PKC-β. The PKC-β level is raised in the retina of patients with DR. Activation of PKC-β causes phosphorylation of many important intracellular proteins leading to initiation of a cascade of reactions resulting in activation or inhibition of many enzymes responsible for abnormal changes in blood flow, loss of capillary pericytes, and increased endothelial permeability. It also causes retinal leakage, ischemia, and neovascularization in conjunction with angiogenic growth factors, all of which lead to progression of DR.[16,45]

Tumor necrosis factor alpha (inflammatory biomarker)

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine responsible for inflammation and apoptotic cell death of retinal endothelial cells. The introduction of TNF-α antagonists for the management of DR in an early stage can reduce the diabetes-induced retinal cell death and progression of DR.[28,46,47]

IL-1β (inflammatory biomarker)

IL-1β is a proinflammatory cytokine, levels of which are reported to be upregulated in the vitreous humor of patients suffering from PDR.[9,10] Elevated vitreous levels of IL-1β are implicated in modulating the inflammatory process by stimulating various cells to release inflammatory signals which cause disruption of the blood–retinal barrier and retinal capillary cell apoptosis.[46–48] Another inflammatory biomarker is IL-6, which is associated with increased apoptosis and cell death.

Basement membrane thickening

Thickening of the capillary basement membrane is the hallmark of DR. Prolonged hyperglycemia stimulates the messenger RNA expression of extracellular matrix proteins in retinal endothelial cells, which leads to increased generation and decreased degradation of the extracellular matrix proteins and thickened capillary basement membrane.[49,50]

Growth hormone

The role of growth hormone in the progression of diabetes is not clear, but its role in the development of DR is confirmed by improvement in retinopathy following hypophysectomy in diabetic patients.[51,52]

AGEs and receptor of AGEs

Hyperglycemia causes nonenzymatic glycation of proteins, lipids, or nucleic acids producing irreversible cross-linked complex compounds known as AGEs. Important examples of AGEs associated with progression of DR in humans are carboxyethyllysine, carboxymethyllysine, and pentosidine.[9] Increased levels of AGEs are found accumulated in retinal pericytes of patients suffering from DR. Interaction of AGEs with the receptor of AGEs (RAGEs) form AGE–RAGE complexes which provoke the activation of critical cell signaling pathways, such as p21 ras, mitogen-activated protein kinases (MAPKs), and nuclear factor-κB (NF-κB). All this leads to the activation of proinflammatory responses, causing injury to endothelial cells and disruption of the blood–retinal barrier.[53,54]

Oxidative stress

Prolonged hyperglycemia causes alteration in regular biochemical pathways and reduction in antioxidant enzyme (SOD, CAT, or GSH) and coenzyme (NADH) levels in the retina. Impairment of the body's antioxidant defense mechanism causes oxidation of cellular macromolecules, causing further oxidative damage to the retina.[55–57]

Biomarkers and mechanisms for neurodegeneration

Neurodegeneration is a very early event in the progression of DR, preceding the clinically apparent signs associated with DR. Changes in physiological structure and function in the neuroretinal compartment of diabetic patients manifest as alterations in normal physiological constriction and dilation of blood vessels (which occur in response to stimuli such as oxygen and flickering light, respectively).[58–62] Other preclinical pathological changes observed include decreased multifocal electroretinogram potentials,[63,64] decreased contrast sensitivity,[65] delayed dark adaptation,[66,67] abnormalities on frequency doubling perimetry,[66,68] and reduction in retinal ganglion cell layer thickness on optical coherence tomography (OCT).[37,69,70]

Insulin receptors

Insulin receptors in the retina are important for anabolic synthesis, survival, growth, and development of neurons.[71–73] In diabetes, a deficiency of insulin and/or its action is associated with apoptosis of neurons and ganglion cells leading to

DR. This is shown by the prevention of neurodegeneration and DR in diabetic rats treated with insulin.[74,75]

Molecular pathways

Activation of a PKC pathway, increased flux by the polyol pathway, formation of AGEs, and increased oxidative stress provoked by a prolonged hyperglycemic condition is also found to damage the neuroretinal compartment along with retinal microvasculature.[37,38]

Neuroinflammation

In diabetes, the level of inflammatory markers such as TNF-α is raised and plays an important role in the leukostasis and apoptosis of neural cells which are responsible for the progression of DR.[37,38]

Glutamate excitotoxicity

Glutamate plays an important role in communication between neurons. Prolonged hyperglycemia causes a reduction in the activity of the enzyme glutamine synthetase and oxidation of glutamate, which leads to a glutamate and glutamine disequilibrium in neurons and glial cells. The increased level of glutamate in diabetes is associated with glutamate excitotoxicity which increases calcium influx and initiates proapoptotic signaling cascades.[37,38] Such events are likely to be involved in the apoptosis of neurons within the retina in diabetes.[76–78]

Deficiency of neuroprotective factors

The neuronal compartment possesses certain neuroprotective local factors including pigment epithelium-derived factor (PEDF),[79–82] brain-derived neurotrophic factor (BDNF),[83,84] and nerve growth factor (NGF).[85,86] It is postulated that they protect against oxidative stress and glutamate excitotoxicity, thereby having a critical role in the prevention of neuronal damage associated with DR. In diabetes, the level or activity of these factors is reduced and neurodegeneration occurs.[37]

Apoptosis

In diabetes, the exact mechanism of apoptosis of retinal neurons and vascular cells is not known. It is believed that apoptotic cascades are triggered by the combined action of hyperglycemia, glutamate excitotoxicity, and neurotrophins, ultimately leading to neuronal cell death and DR.[75,85,87]

Interlinked molecular pathways

DR has a multifactorial etiology with various interconnecting biochemical pathways acting in conjunction and leading to oxidative and inflammatory damage to the retina.[88] In prolonged hyperglycemia, surplus glucose enters the polyol pathway and is reduced to sorbitol, which gets further converted to fructose. An increased sorbitol level in the retina causes osmotic stress and predisposes the retina to oxidative damage due to expenditure of NADPH and a reduced glutathione level. In addition, the by-products of the polyol pathway, fructose-3-phosphate and 3-deoxyglucosone form irreversible cross-linked complex compounds with proteins, lipids, and nucleic acids (AGEs). AGE interaction with its receptor forms AGE–RAGE complexes which further provoke the critical cell signaling pathways such as the PKC pathway, p21ras, MAPKs, NF-κB, and poly (ADP-ribose) polymerase. This leads to endothelial cell injury and disruption of the blood–retinal barrier. An increased flux through the hexosamine pathway causes increased TGF-β expression, PKC activation, and extracellular matrix production, which escalate the blood–retina barrier breakdown. All these pathways also cause activation of a proinflammatory response, oxidative stress, and growth factor imbalances leading to progression of DR.[9]

Clinical biomarkers
Microaneurysm turnover

The rate of formation of new microaneurysms is shown to be linked to increased risk of development of clinically significant macular edema. A higher rate of microaneurysm formation indicates a higher likelihood of disease progression.[89,90] Microaneurysm turnover is also associated with disease severity as measured by the ETDRS (Early Treatment Diabetic Retinopathy Study) severity level.[91]

OCT changes

OCT has become a mainstay in the diagnosis and management of DR, particularly macular involvement. Detection of subclinical macular edema has added an enhanced sensitivity to detection of the disease and its progression.[69,70,92–94]

Visual acuity

Patients with DR are shown to have lower visual acuity than those without a retinopathy. Visual acuity has shown a negative correlation with disease duration and severity of ocular damage in patients with retinopathy.[95]

Perimetry

Visual field deterioration does not correlate with a change in retinopathy.[66] By using perimetry with an analysis tailored for monitoring diabetic subjects, it has been possible to demonstrate progression of retinal dysfunction over time, which may

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represent early signs of retinal neurodegeneration. Special forms of perimetry including frequency doubling perimetry have also been studied as potential biomarkers for DR.[66,68,96,97]

Fluorescein angiography

Studies have found that a greater macular ischemia grade as noted by the increasing foveal avascular zone is independently predictive for progression, and the progression of diabetic macular ischemia itself is predictive of the loss of visual function. The role of peripheral ischemia as a biomarker for progression is uncertain.[98,99]

Electrophysiology

In DR, the oscillatory potentials of electrooculography can monitor the progression of disease and indicate neuronal alterations rather than diabetic angiopathy. Multifocal electroretinogram has also been shown to predict onset and development of localized DR.[63,64,82,94,100,101]

Management of DR

DR is a disorder with a multifactorial etiology and hence its management is also complex. It requires a multidisciplinary approach and needs to be individualized according to severity of the disease and patient response. Table 1 describes the various stages of DR and preferred clinical treatment modality.

Primary/preventive interventions
Glycemic control

Good glycemic control early in the course of the disease is beneficial in reducing the incidence and progression of DR. According to a Diabetes Control and Complications Trial (DCCT) report, the risk of progression of retinopathy in diabetes can be reduced by 25% in type 1 and 39% in type 2 diabetes with each 1% decrease in glycated hemoglobin (HbA_{1c}) level.[104] Maintaining a glycemic level below 6.5 mmol/L or 117 mg/dL (fasting plasma glucose) and HbA_{1c} level below 7% is the target in the ideal management of DR. If these targets are met, then the progression of DR can be stopped or slowed down significantly.[102,103] Though the risk of early worsening of DR following the initiation of intensive insulin therapy was documented in a DCCT report, it is reversed by 18 months, and, in the long term, intensive glycemic control is beneficial in reducing the incidence and progression of DR.[104]

Blood pressure control

Evidence that blood pressure is a major risk factor in the progression of DR is not apparent, but it is a modifiable risk factor in the progression of DR.[105,106] Controlling systolic (<130 mmHg) and diastolic blood pressure is found to be beneficial in reducing loss of vision due to DR. According

Table 1 International DR disease severity scale and recommended management strategies[146,147]

Disease severity level	Signs	Management options
No apparent retinopathy	No abnormalities.	Optimize medical therapy of glucose, blood pressure, and lipids.
Minimal NPDR	Microaneurysms only.	Optimize medical therapy of glucose, blood pressure, and lipids.
Mild-to-moderate NPDR	More than just microaneurysms but less than severe NPDR.	Optimize medical therapy of glucose, blood pressure, and lipids and ophthalmology referral.
Severe NPDR	Any of the following: more than 20 intraretinal hemorrhages in each of four quadrants; definite venous beading in two or more quadrants; and prominent intraretinal microvascular abnormalities in one or more quadrants and no signs of proliferative retinopathy.	Consider scatter (panretinal) laser treatment for patients with type 2 diabetes. Optimize medical therapy of glucose, blood pressure, and lipids.
PDR	One of the following: neovascularization or vitreous/preretinal hemorrhage.	Strongly consider scatter (panretinal) laser treatment, without delay for patients with vitreous hemorrhage or neovascularization within one disc diameter of the optic nerve head. Optimize medical therapy of glucose, blood pressure, and lipids.
Macular edema		
Absent	No retinal thickening or hard exudates in posterior pole.	Laser treatment for PDR and center not involving DME.
Present	Mild – some retinal thickening or hard exudates in posterior pole but distant from the macula.	Intravitreal drug administration for center involving DME and eventual additional later laser treatment.
	Moderate – retinal thickening or hard exudates approaching the center of the macula but not involving the center.	Vitrectomy as a microsurgical approach for traction-induced DME, later changes in PDR such as vitreous hemorrhage, and tractional retinal detachment, sometimes in conjunction with prior intravitreal anti-VEGF treatment.
	Severe – retinal thickening or hard exudates involving the center of the macula.	

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; NPDR, nonproliferative DR; PDR, proliferative DR.

to the UKPDS, lowering the systolic blood pressure by 10 mmHG decreases the risk of DR progression by 13% irrespective of blood glucose level.[107] Certain antihypertensive agents may actually be responsible for the beneficial effect of lowering blood pressure on the progression of diabetes rather than reduced blood pressure itself. This is supported by the benefit documented in using certain agents in normotensive diabetic patients. The angiotensin-converting enzyme (ACE) and renin–angiotensin system[108] are expressed in retina where these agents are hypothesized to be affecting VEGF expression. Lisinopril,[109] an ACE inhibitor, is proved to be beneficial in reducing the progression of DR. β-Blockers are also found to be efficient in reducing the progression of DR.[107]

Lipid control

Studies suggest fenofibrate and simvastatin play a role in reducing the incidence of DME and need for laser treatment by controlling the macular hard exudate deposition.[110,111] However, another study found no significant role of lipid level in progression of DR or improvement in vision loss in patients treated with hypolipidemic agents.[40] Due to the contradictions of previous studies, the roles of lipid control and hypolipidemic agents remain inconclusive in DR, but treatment with hypolipidemic agents in diabetic patients with a high lipid level is considered beneficial and adjunctive to conventional treatment.

Secondary/therapeutic interventions
Retinal laser photocoagulation
Retinal laser photocoagulation refers to the process of firing laser spots at the retina in order to burn a part of the diseased retina. It can either be panretinal photocoagulation (PRP) or focal laser photocoagulation. PRP is the gold-standard technique in the treatment of very severe NPDR, PDR, and DME.[112–114] In this technique, a laser is used to deliver spots on the retina to burn and destroy ischemic retina which is releasing proinflammatory and proangiogenic compounds that increase the severity of retinopathy. PRP reduces the risk of vision loss by 50% in PDR. In mild-to-moderate NPDR, however, it is discouraged over primary interventions due to its adverse effects, which include risk of severe vision loss due to traction retinal detachment or accidental laser burn of the fovea, vitreous hemorrhage, field constriction, color blindness, night blindness, glaucoma, and macular edema exacerbation.[115] Focal laser photocoagulation refers to using a laser beam on a small area of the retina or a localized lesion in order to seal faulty, leaky blood vessels, thereby maintaining the function and thickness of the retina. It aims to alleviate

loss of vision to a great extent, particularly in the less severe stages of DR. Adverse effects include accidental central retinal burns causing permanent central vision loss. The role of laser treatment for DR is now dwindling with the advent of newer drugs which help control macular edema and retinal neovascularization effectively. However, due to the unavailability of an effective oral drug and limited period of action of these newer antiangiogenic agents, laser still forms the mainstay of managing severe NPDR, PDR, and DME.

Vitrectomy
Vitreous surgery is indicated in cases of DR causing vitreous hemorrhage or retinal traction or a retinal detachment. It is also beneficial in case of widespread or diffuse DME not resolved by laser treatment. While generally it is advocated after 6 months of waiting for a vitreous hemorrhage to clear, early vitrectomy is recommended in type 1 diabetic patients (within 3 months of severe hemorrhage).[116–119]

Vitreolysis
The vitreoretinal interface plays an important role in the progression of DR. Vitreolysis involves the use of enzymes to liquefy the vitreous gel and induce posterior vitreous detachment. Vitrase, ie, hyaluronidase (bovine), a US Food and Drug Administration (FDA)-approved drug, is often used off-label in clearing vitreous hemorrhage. Research is helping establish the safety and efficacy of intravitreal injection of autologous plasmin enzyme as an adjunct to vitreous surgery in the management of PDR and DME. Some studies have demonstrated the efficacy of plasmin enzyme in reduction of macular thickening in DME and posterior vitreous detachment.[120,121]

Pharmacotherapy of DR
Pharmacotherapy of DR is still unsatisfactory, as treatment options are limited and display poor efficacy in outcomes. None of the currently available drugs have been able to prevent DR progression or treat the DR. Intravitreal anti-inflammatory and antiangiogenic drugs restrict or slow down the further progression of DR and work as an adjunct to other, more definitive therapeutic interventions. Table 2 describes the available and under-research drugs for the treatment of DR.[4,122,123]

Anti-VEGF/angiogenesis inhibitors
Antiangiogenic agents are emerging as the new gold standard in the management of DR, especially PDR and DME.[124,125] The most common target of these is VEGF,

Table 2 Drugs currently available or under investigation for management of DR and their status

Drug or formulation	Mechanism or category	Clinical status/ regulatory approval	Remarks
Anti-inflammatory			
Intravitreal injection of triamcinolone acetonide	Corticosteroid	Off-label use	Off-label use in combination with anti-VEGF agents and as adjunct with surgical intervention for PRP.
Dexamethasone implant	Corticosteroid	FDA approved	
Fluocinolone acetonide	Corticosteroid	FDA approved	
Ketorolac	NSAID	Phase II study	Under different clinical and preclinical development phases.
Nepafenac	NSAID	Phase II study	development phases.
Etanercept	TNF-α inhibitor	Preclinical studies	Under different clinical and preclinical development phases.
Infliximab	TNF-α inhibitor	Phase III study	development phases.
Mab2F1	Monoclonal antibody: Wnt coreceptor blocker	Animal study	
VEGF inhibitors			
Bevacizumab	VEGF inhibitor: monoclonal antibody	Off-label use	FDA-approved anticancer, off-label use for DR in conjunction with anti-VEGF agents and surgical intervention.
Ranibizumab	VEGF inhibitor: monoclonal antibody	FDA approved	FDA approved for DME in 2014 (AMD 2006; RVO edema 2010).
Pegaptanib	VEGF inhibitor: RNA aptamer	Off-label use	FDA approved for AMD in 2004; Phase III trial completed for DME.
Aflibercept	VEGF inhibitor: decoy receptor fusion protein	FDA approved	FDA approved for DME in 2014; also FDA approved for macular edema following RVO and neovascular AMD.
KH902	VEGF inhibitor: VEGFR decoy	Phase II study	
Rapamycin	mTOR inhibitor	Phase II study	
Decursin	VEGFR2 phosphorylation	Animal study	
Bevasiranib	Short interfering RNA targeting VEGF	Phase II study	
Angiogenesis inhibitor			
TG100801	Tyrosine kinase inhibitor	Phase II study	
Pazopanib	Tyrosine kinase inhibitor	Animal study	
Aldose reductase inhibitor			
ARI-809	Aldose reductase inhibitor	Animal study	
Epalrestat	Aldose reductase inhibitor	Animal study	
Fidarestat	Aldose reductase inhibitor	Animal study	
Sorbinil	Aldose reductase inhibitor		
Tolrestat	Aldose reductase inhibitor		
PKC inhibitors			
Ruboxistaurin	PKC-β inhibitor	Phase III study	
PKC-412	PKC-β inhibitor	Phase III study	
Fasudil	Rho-associated protein kinase inhibitor	Phase III study	
Growth hormone/insulin-like growth factor inhibitors			
Octreotide	Somatostatin analog	Phase III study	
Pegvisomant	IGF-1R inhibitor	Preclinical studies	
AGE inhibitors			
Aminoguanidine	AGE inhibitor	Phase III study	
GLY-230	Selective inhibitor of glycation	Phase II study	
OPB-9195	AGE inhibitor	Animal study	
ALT-711/Alagebrium	AGE inhibitor	Animal study	
LR-90	AGE inhibitor	Animal study	
N-Phenacylthiazolium bromide	AGE inhibitor	Animal study	
Antiplatelet agents			
Ticlopidine	ADP receptor inhibitor		
Vitreous clearing agents			
Vitrase (hyaluronidase)	Protease, spreading agent	Phase III	FDA approved as spreading agent; under Phase III clinical trial to investigate its promotion of the clearance of vitreous hemorrhage from PDR.

(Continued)

Table 2 (*Continued*)

Drug or formulation	Mechanism or category	Clinical status/ regulatory approval	Remarks
Plasmin	Proteolytic enzyme		
Ocriplasmin	Recombinant protease with activity against fibronectin and laminin	FDA approved	
Agents for blood pressure control			
ACE inhibitors: lisinopril, captopril, enalapril	NA		
Candesartan, losartan, telmisartan	Angiotensin-2 receptor antagonist		
PD 123319	Angiotensin-2 receptor antagonist	Animal study	
Valsartan	AT-1 receptor antagonist	Animal study	
Mecamylamine	Nicotinic acetylcholine receptor	Phase II study	
Neuroprotective agents			
PEgylated PEDF	PEDF bioactive derivative	Animal study	
S-Nitrosoglutathione	Nitric oxide synthase inhibitor	Animal study	
Antioxidants			
Baicalein	Flavonoid: lipoxygenase inhibitor	Animal study	
Apocynin	Reactive oxygen species inhibitor	Animal study	
Curcumin	PPAR-gamma upregulator	Animal study	
PJ-34	PARP inhibitor	Animal study	

Abbreviations: AGE, advanced glycation end-product; AMD, age-related macular degeneration; DME, diabetic macular edema; DR, diabetic retinopathy; PKC, protein kinase C; VEGF, vascular endothelial growth factor; FDA, Food and Drug Administration; NSAID, nonsteroidal anti-inflammatory drug; PDR, proliferative DR; PEDF, pigment epithelium-derived factor; TNF-α, tumor necrosis factor-α; PRP, panretinal photocoagulation; RVO, retinal vascular occlusion; NA, not applicable.

based on its well-established prominent role in the progression of DR. Presently, the anti-VEGF molecules which are under investigation for their role in the management of DR are pegaptanib (Macugen), ranibizumab (Lucentis), bevacizumab (Avastin), and aflibercept intravitreal (Eylea). Of all the available anti-VEGF agents, bevacizumab has attracted the most interest because it is economical and has shown good results during its current use as an off-label drug.[126] It is a recombinant humanized monoclonal antibody that binds to all VEGF-A isoforms. Ranibizumab is another VEGF inhibitor which acts by inhibiting the interaction of VEGF-A with its receptors and thereby suppresses endothelial cell proliferation, neovascularization, and vascular leakage. It has been FDA approved for the management of exudative age-related macular degeneration through intravitreal injections. Pegaptanib is an aptamer that binds specifically only with VEGF-A 165 isoform. Theoretically, it is supposed to be devoid of some side effects of bevacizumab and ranibizumab due to its selectivity. Aflibercept (VEGF Trap-eye) is the newest recombinant VEGF inhibitor that binds at all isoforms of VEGF including VEGF-A and VEGF-B. Unlike bevacizumab and ranibizumab (antibody-based binding), it binds with endogenous VEGF 1 receptor at the second binding domain and VEGF 2 receptors at the third binding domain to inhibit angiogenesis. While the complete significance of this binding is not yet known, it has been used for the management of subfoveal choroidal neovascularization since FDA approval. Its indications for use in DR include DME.

Corticosteroids

Corticosteroids are potent anti-inflammatory and antiangiogenic agents, which supports the rationale behind their use in the management of DME and PDR. Intravitreal injection of triamcinolone acetonide is an emerging therapy in the management of DME and has shown high efficacy and good outcomes.[127] Lately, a longer-acting intravitreal or retinal implant of fluocinolone acetonide has been developed which releases the steroid over a prolonged period of time. The side effects include cataract formation, glaucoma, and infection. At present, there is not sufficient evidence supporting the efficacy and safety of the implant on routine use for longer term.

Combination therapy

Intravitreal steroids and anti-VEGF drugs are often combined in the treatment of DR and macular edema (particularly refractory) with an objective of achieving synergistic activity of the two. Combination therapy has been found to have good results in reducing macular thickness in patients with DME who are otherwise unresponsive to laser therapy.[128]

Novel targets

With the enhanced understanding of the mechanisms and pathways involved in the development of DR, newer drugs

are being developed to target specific points in the molecular cascades.

Aldose reductase inhibitors

Aldose reductase is a rate-limiting enzyme in the polyol pathway and its inhibition is likely to play an important role in modifying the course of progression in DR. Aldose reductase inhibitor ARI-809, epalrestat, sorbinil, and tolrestat are potential agents in the prevention and treatment of DR, but showed no substantial results in clinical studies.[129–132] In routine clinical practice today, there is no defined role of this group of agents.

PKC inhibitors

The efficacy of PKC inhibitors is not clear, and results from current studies are expected soon. Ruboxistaurin, a PKC inhibitor, has shown some potential in reducing vision loss, but there is not sufficient evidence to prove its efficacy in preventing or treating progression of DR.

Growth hormone/insulin-like growth factor inhibitors

The association of growth hormone with progression of DR prompted studies investigating its role in management of DR.[52,133,134] Two trials investigating the role of a synthetic analog of somatostatin octreotide in DR have shown promising results.[135,136] However, various other studies have shown no benefit of this agent.[137,138] There are potentially substantial adverse effects such as hypoglycemic episodes, cholelithiasis, and diarrhea as well as high costs which have curtailed clinical use of this drug for management of DR in clinical practice today.[139]

AGE inhibitors

A pathogenic role for AGEs in DR is likely. AGE inhibitors such as aminoguanidine are currently being evaluated in trials, but no definitive evidence for their use is available today.

Antiplatelet agents

A variety of hematologic abnormalities are associated with hyperglycemia, including increased erythrocyte aggregation, increased sensitivity to platelet aggregating agents, decreased red blood cell deformability, and increased platelet aggregation and adhesion. These predispose to sluggish circulation, endothelial damage, and focal capillary occlusion, eventually leading to retinal ischemia. Based on these and other considerations, antiplatelet agents are expected to show efficacy in the management of DR, though existing studies have shown

no clear benefit from these agents.[140–142] In certain studies, aspirin, dipyridamole, and ticlopidine have been reported to prevent some signs of DR such as microaneurysms, but there is not sufficient evidence to prove the efficacy of antiplatelet agents in preventing or treating DR.[140–142]

Topical therapies

Therapeutically active eyedrop formulations can play an important role in management of DR, but currently there is no topical formulation available for the management of DR. Several approaches with existing molecules having anti-inflammatory, antioxidant, and anti-VEGF activity have been tried, but, in practice, results are not favorable for their clinical use.[143,144]

Nutritional and herbal products

There are many nutritional supplements and herbal products which are claimed to be effective in the management of DR. Many herbal products and phytoconstituents including curcumin and bioflavonoids possessing antioxidant and anti-inflammatory activity are likely to be efficacious in the management of DR.[26,145] Despite their popularity and frequent use worldwide, there is no scientific proof regarding their efficacy in clinical use.

Conclusion

Our understanding of the etiopathogenesis of DR has greatly enhanced over the past few decades, but is still far from satisfactory, as we continue to strive toward finding the perfect therapy for managing DR. The knowledge of clinical and molecular biomarkers has enabled us to provide much better clinical care to diabetic patients and preserve their vision.

The current pharmacotherapy, particularly anti-VEGF agents and corticosteroids, is the outcome of understanding the molecular mechanism of neovascularization in PDR. These drugs now form the mainstay of treatment in the majority of cases where they serve as an important adjunct to laser. Since these need to be used via an intravitreal route, certain risks are involved and research is focusing on drugs which may be administered orally.

Various agents such as aspirin, vitamin E, vitamin C, aldose reductase inhibitors, and PKC inhibitors which were expected to be promising theoretically have not shown adequate outcomes.

One thing that is now certain is that in view of the multiple and complex pathways involved in the formation of DR, no single standard treatment strategy is sufficient and multiple modes of treatment are needed as a part of the algorithm for

DR management. Primary prevention of DR and its progression through control of risk factors such as hyperglycemia and dyslipidemia is probably the prime aim of all clinicians.

Disclosure

The authors report no conflicts of interest in this work.

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