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Review Article

BIOCHEMICAL BASIS AND EMERGING MOLECULAR TARGETS TO TREAT DIABETIC RETINOPATHY

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ABSTRACT

Diabetes being considered as an epidemic, long term untreated complicated diabetes resulting in retinopathy will be a leading cause of blindness worldwide. Many cross-sectional studies reported a strong relationship between chronic hyperglycaemia and development, progression of retinopathy, however the underlying mechanism that cause retinal microvascular damage following prolonged hyperglycaemia, yet to be revealed. Continued research worldwide focuses on understanding the molecular basis with the ultimate goal to prevent diabetic retinopathy by developing newer therapeutic targets. This article reviews multiple biochemical pathways that are implicated in diabetic retinopathy. Recent advancement in the molecular basis of the disease as well as clinical trials undertaken to target these molecules in order to block the signalling cascade prevailing in diabetic retinopathy is also discussed. This review highlights the recent therapeutic targets to prevent the onset as well as the progress of retinopathy in diabetes.

KEYWORDS

Hyperglycaemia, microvascular damage, molecular basis, therapeutic targets, signalling cascade.

INTRODUCTION

Increased prevalence of Type-2 diabetes mellitus and insulin resistance (a pre diabetic condition) is a major health concern in India. As per International Diabetic Federation (IFD) estimates , the number of patients with diabetes is expected to be more than double by 2030.Diabetes has many underlying interrelated pathways that lead to potentially blinding complications like diabetic retinopathy.[1,2] Diabetic retinopathy is the most frequent microvascular complication of diabetes and is one of the major causes of vision loss worldwide. Review of population based studies revealed approximately 34.6% of diabetics have retinopathy.[3] Diabetic retinopathy occur both in type 1 and type 2 diabetes and is strictly related to poor glycaemic control over a prolonged duration of disease, however there are substantial differences between type 1 and type 2 diabetes in terms of clinical presentations and prevalence. Prevalence of retinopathy is more in type 1 than in type 2 diabetes and more in males than in female.[4] Intensive glucose control early in the course of the disease produced significant benefits on microvascular complications i.e. retinopathy both in type1 and type2 Diabetes mellitus.[5] In addition to the extent and duration of chronic hyperglycaemia , other biochemical mechanisms like Polyol accumulation, Protein Kinase C pathway, Oxidative damage, non-enzymatic protein glycosylation, increased hexosamine pathway flux as well as recently documented endothelium related dysfunction of the coagulant and anticoagulant pathway, cytokines, interleukins, Endothelin 1 also play a role in the pathogenesis of diabetic retinopathy. All these pathways ultimately lead to increased oxidative stress, inflammation, and vascular occlusion that cause upregulation of factors such as vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), stromal derived factor-1 (SDF-1), angiopoietins (Ang-2), tumour necrosis factor (TNF), and basic fibroblast growth factor-2 (bFGF) leading to capillary damage, ischaemia with unregulated angiogenesis that is pathognomic of diabetic retinopathy.[6]

Genetic studies revealed involvement of a number of genes in diabetic retinopathy. Aldose reductase (ALR2),receptor for advanced glycation end products (RAGE) ,endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), paraoxonase1(PON1), plasminogen activator inhibitor1 (PAI) are some of the important genes found to be associated with diabetic retinopathy. Polymorphisms at the regulatory regions of these genes have been evaluated as risk alleles for the progression of diabetic retinopathy.[7] According to the Diabetes Control and Complications Trial (DCCT) intensive treatment and improved glucose control delayed the onset of retinopathy and slowed down its progression in comparison to conventional treatment.[8] Laser

photocoagulation and Focal/grid photocoagulation have been effective in reducing further vision loss in diabetic retinopathy[9] ; however, these procedures are associated with potential complications.[10] To avoid these complications, new drugs and therapeutic targets must be identified which can disrupt the cascade of events underlying the pathogenesis of Diabetic retinopathy. So there is a need for better understanding of molecular basis of diabetic retinopathy in greater details so that newer therapeutic interventions can be developed for effective management.

Biochemical mechanisms of diabetic retinopathy:

Diabetes control and complications trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) group reported strong association between chronic hyperglycaemia and development and progression of diabetic retinopathy.[11,12] According to DCCT mean risk for development of any retinopathy was reduced by 76% in the intensive therapy compared to the conventional therapy. With established retinopathy the intensive group had a higher incidence of progression during the first year whereas from 3 years onwards, the progression of retinopathy was reduced in the intensive group by 54%.United Kingdom Prospective Diabetes Study (UKPDS) showed that patients who were assigned to intensive glucose control had less need for retinal photocoagulation .Both studies showed that glycaemic control is protective .Hyperglycaemia is involved in the pathogenesis of diabetic retinopathy (Figure 1), nephropathy, neuropathy and macro vascular disease via the following mechanisms i.e. increased flow through the aldose-reductase pathway ; non enzymatic glycation and glycoxidation with formation of advanced glycation end products (AGEs); increased de-novo synthesis of diacylglycerol from glucose, causing protein-kinase C (PKC) activation; oxidative-nitrosative stress with overproduction of reactive oxygen species (ROS).[13,14] Therefore beside the optimal glycaemic control , pharmacologic inhibition of these pathways might prevent loss of retinal pericytes, micro aneurysm formation, changes in retinal hemodynamics that ultimately result in neovascularization a characteristic feature in diabetic retinopathy.

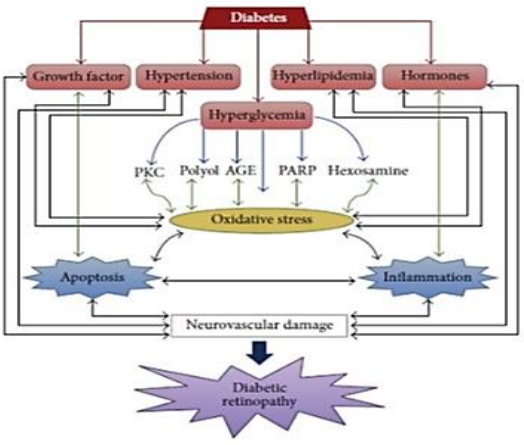

Figure 1. Hyperglycaemia induced biochemical alterations in diabetic retinopathy

A) Increased glucose flux through polyol pathway:

Aldose Reductase (AR) present in the retina reduces glucose into sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol dehydrogenase (SDH) with NAD+ as cofactor subsequently converts sorbitol into fructose. Since sorbitol is impermeable to cellular membranes, it accumulates within the cell and later metabolised to fructose. Built up of sorbitol (Figure 2) is an important cause of osmotic damage to retinal cells.[15] Fructose can be phosphorylated to fructose-3-phosphate that later can be degraded to 3-deoxyglucosone, both of which are strong glycating agents and result in the production of Advanced Glycated End Products i.e. AGEs.[16] Increased utilisation of NADPH as a cofactor in the polyol pathway results in less NADPH availability for use by glutathione reductase (enzyme involved in generation of reduced glutathione).Reduced glutathione being a free radical scavenger , its decreased level diminishes protective response against oxidative stress.[17] Exaggerated NADH oxidase activity due to increased cytosolic NADH/NAD+ ratio by SDH results in increased production of reactive oxygen species(ROS) within retinal cells.[18] Under euglycemic conditions, hexokinase enzyme has higher affinity for glucose hence the formation of sorbitol is very low . However, in hyperglycaemic state there is a substantial increase in intracellular sorbitol levels. Aldose reductase has a high capacity and a low affinity for glucose but sorbitol dehydrogenase (SDH) has a high affinity and a low capacity for sorbitol. So only during hyperglycaemic state Aldose reductase activity is increased and sorbitol oxidation is relatively independent of the sorbitol concentration within the physiological ranges.[19,20]

In diabetes, the sorbitol pathway activity is more in tissues like retina, kidney, peripheral nerves and blood vessels where insulin is not required for cellular glucose uptake. The polyol pathway seem to be an important mechanism for the ganglion cell apoptosis and Müller glial cell activation.[21,22] Aldose reductase is found in Ganglion and Müller cells of the retina. Since neuroglial changes may cause vascular changes the inhibition of the polyol pathway could also prevent the vascular abnormalities of diabetic retinopathy. Inhibition of aldose reductase was also able to prevent the early activation of complement in the retinal vessel wall as well as the apoptosis of vascular pericytes and endothelial cells and the development of acellularcapillaries.[23] Retinal endothelial cells showed aldose reductase immunoreactivity, and human retinas exposed to high glucose in organ culture increased the production of sorbitol.[24] Experimental evidences suggest that defects in the polyol pathway may produce thickening of the capillary basement membrane, loss of mural pericytes and micro aneurysm formation ,the vascular changes characteristics of diabetic microangiopathy. So polyol pathway has been an extremely attractive target for the treatment of diabetic retinopathy. Animal data showed that aldose reductase has an early role in the pathogenesis of diabetic retinopathy but studies of inhibition of polyol pathway in vivo have yielded inconsistent results .The Sorbinil trial also indicated that sorbinil did not prevent the worsening of the disease except for a slower progression rate.[25] Animal

models suggest that AR inhibitor fidarestat, is active in the treatment of diabetic retinopathy. Fidarestat (Figure 5) being an inhibitor of aldose reductase neutralizes diabetes-associated retinal oxidative stress and (ADP-ribose) polymerase formation.[26] This indicates the rationale for the development of aldose reductase inhibitors for counteraction of polyol pathway.[27] In the streptozotocin diabetic rats, fidarestat treatment decreased sorbitol and fructose concentrations in the retina. Similarly in the rat model with retinal ischemia reperfusion injury, fidarestat treatment caused increased AR expression and cell death with decreased sorbitol pathway intermediate accumulation.[28] Fidarestathas a role in ICAM-1 mRNA expression and leukocyte accumulation in the retina. Immunohistochemical study also revealed the suppressive effect of fidarestat on the expression of ICAM-1.[29] A double-blind study in patients with diabetic neuropathy documented the efficacy of sorbinil, an aldose reductase inhibitor, against morphological signs of degeneration associated with a decrease in the nerve sorbitol level.[30] Zenarestat , another aldose reductase inhibitor also reported same results.[31] Sorbinilretinopathy trial indicated that sorbinil had no clinically important effect on the course of human diabetic retinopathy.[25] However Zenerestat had a positive effect on diabetic neuropathy progression[32] thus creating a hope in the use of Aldose reductase inhibitors in diabetic retinopathy that needs further clinical trials.

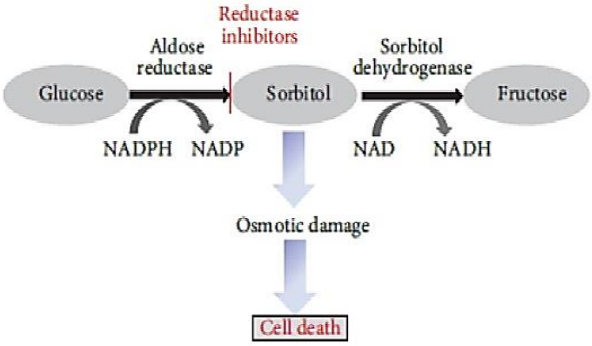

Figure 2. Polyol Pathway and role of Aldose reductase Inhibitor

A) Non enzymatic Protein Glycosylation:

Chronic hyperglycaemia leads to accumulation of Advanced Glycation End Products (AGES) that play an important role in pathogenesis of retinopathy in diabetes.[33] AGEs are heterogeneous molecules formed by nonenzymatic reaction of free amino groups of proteins, lipids and nucleic acids with the reducing sugars . Schiff base is formed that undergoes spontaneously rearrangement (Maillard reaction) on itself to produce an Amadori adduct (Figure 3). Most glycated proteins are eliminated in physiological conditions, they accumulate in the presence of diabetes and undergo further glycation and molecular rearrangement that lead to the formation of AGEs.[34]

Some of the AGEs in human are Carboxymethyllysine (CML), Carboxyethyllysine (CEL) and Pentosidine out of which CML have been localized to retinal blood vessels of diabetes patients and significant correlation has been found between CML and the degree of retinopathy pointing its role in Diabetic retinopathy.[35] AGEs are involved in microvascular and macrovascular complications through the formation of covalent crosslinks with molecules of the basement membrane of the extracellular matrix and the vessel wall components. Binding of AGEs with a variety of cell-surface AGE binding receptors(receptor for advanced glycation end products i.e. RAGEs) leads to cellular activation of prooxidant, proinfammatory events .Various signalling pathways (Figure 4) that are activated by AGE receptor binding include tyrosine phosphorylation of Janus kinase (JAK)/signal transducers and activators of transcription (STAT)[36], recruitment of phosphatidylinositol 3 kinase to Ras[37], activation of protein kinase C[38] and oxidative stress by NFk Band AP-1 transcription[39] .CML interact with vascular endothelium via RAGE activating nuclear factor kappa B (NF-κ B), that increase expression of adhesion molecules and secretion of tumour necrosis factor alpha (TNFα) and VEGF.

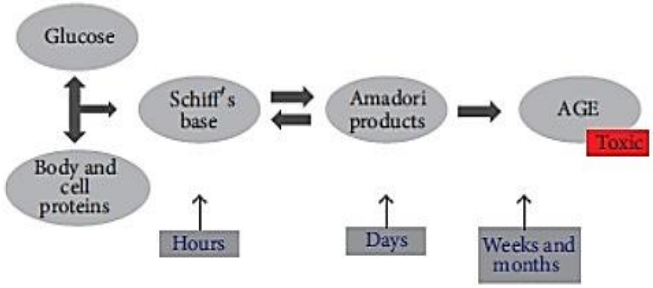

Figure 3. Formation of Advanced Glycation End products

Recent investigations suggest that the AGE-RAGE interaction might increase VEGF gene transcription by NADPH oxidase-mediated ROS generation and nuclear factor- κ.B (NF-κ.B) activation via Ras mitogen activated protein kinase (MAPK) pathway.[40,41] Knocking down of integrin-linked kinase (ILK) gene expression with siRNA inhibited the expression of VEGF and intercellular adhesion molecule 1 (ICAM-1) that indicates the ILK response to high glucose and its role in pathogenesis of diabetic retinopathy (DR).[42,43]

Evidences from animals studies suggest that exposure to high levels of AGEs cause vascular and renal complications.[44,45,46] Diabetic rats when treated with Aminoguanidine hydrochloride (AGE inhibitor), accumulation of AGE was significantly reduced that prevented formation of micro aneurysms, acellular capillaries and pericyte loss.[47] Treatment with vitamin B6 derivatives, an AGE inhibitor also found to be protective against capillary drop out in diabetic rats.[44] Such observations suggest that AGE accumulation and its interaction with RAGE are interconnected mechanisms in Diabetic retinopathy and inhibition of these pathways could be an important therapeutic avenue.[48] Current treatments focus on preventing the AGE formation, breaking established crosslinks and reducing receptor-ligand interactions .Recently Park et al. reported that the Wnt pathway inhibitor that i.e. a Pigment Epithelium-Derived Factor (PEDF), a serine proteinase inhibitor overexpression could attenuate Wnt signaling induced by retinal ischemia.[49] PEDF is also found to inhibit NADPH oxidase mediated ROS generation and VEGF

expression thus preventing AGE induced oxidative stress and apoptosis in retinal pericytes.[50] Yamagishi et al documented that injection of AGEs to normal rats increases RAGE and ICAM-1 expression that could be prevented by treatment with PEDF by inhibiting superoxide generation and NFkB activation in endothelial cells.[51] Aminoguanidine, a potent inhibitor of AGE mediated cross-linking, has been shown to reduce diabetic vascular complications including retinopathy in experimental animals.[52]

B) Oxidative stress:

Imbalance between the level of ROS and the antioxidant defence mechanisms in a biological system leads to oxidative stress. Hyperglycemia induces overproduction of ROS and oxidative stress (Figure 4) reflected by increased Malondialdehyde, isoprostane, nitrotyrosine or 8-hydroxy-2 deoxyguanosine levels and decreased antioxidant status.[53,54] Reactive oxygen species are produced by glucose auto-oxidation, protein glycation, increased flux through the polyol pathway, and prostanoid production. ROS and reactive nitrogen species (RNS) act on lipids, protein and DNA molecules leading to cross-link formation, lipid peroxidation and protein modification. Pericytes are highly sensitive to the oxidative stress and increased rate of apoptosis due to decreased level of antioxidant enzymes.[55] Progressive Pericyte loss leads to pore formation in the blood vessel wall thus proteins are leaked , a characteristic finding in non proliferative diabetic retinopathy i.e. hard exudates.

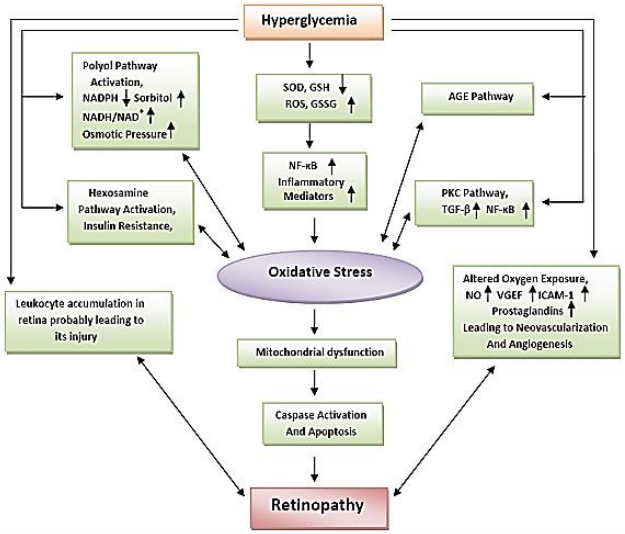

Figure 4. Interrelationship of Biochemical Mechanisms in diabetic retinopathy

Animal studies documented that oxidative stress cause development of retinopathy in diabetes and it prevent retinopathy to reverse even after euglycemic state is achieved.[56] In diabetes the antioxidant enzymes i.e. SOD, glutathione reductase, glutathione peroxidase, catalase are found decreased in the retina.[57] It is also reported that type 2 diabetes mellitus is associated with reduced plasma total antioxidant status and increased plasma oxidisability with reduced plasma ascorbic acid and vitamin E.[58] Lipoicacid scavenge ROS and reduces glutathione to maintain a healthy cellular redox state.[59] Lipoic acid supplementation prevents diabetes-induced increase in nitrotyrosine and activation of NF-κB , decrease VEGF and oxidatively modified proteins in the rat retina.[60] Vitamin E through non enzymatic mechanisms act as free radical scavenger in DR.[61] Trolox prevent the loss of pericytes in diabetic rats[62] Carotenoid, lutein and zeaxanthin prevented progression of DR and maintained normal retinal function, mitochondrial homeostasis and inflammatory mediators.[63]

C) Protein Kinase C pathway:

Protein kinase C (PKC) is a family of 10 enzymes, in which the β 1/2 isoforms are closely associated with the development of diabetic retinopathy.[64] So far 12 PKC isoforms have been isolated and can be divided into 3 groups i.e. classical, novel and typical. Classical isoform (PKC-α,β ½,δ) are enhanced by Diacylglycerol (DAG) and have been linked to vascular dysfunction and pathogenesis of DR.[65] Hyperglycaemia increase glucose flux through the glycolytic pathway, which increases DAG , the key activator of PKC.[66] activation of PKC has a cascade-like effect on several other pathways that influence endothelial permeability, retinal hemodynamic, and expression of vascular endothelial growth factor (VEGF) in the retinal along with leukocyte adhesion.[67,68] Upregulation of PKC contribute to the pathogenesis of diabetic retinopathy i.e. extracellular matrix (ECM) remodelling, differential synthesis of extra cellular matrix proteins, release of angiogenic factors, endothelial and leukocyte dysfunction leading to vascular changes pertaining to DR.[69]

PKC inhibitors are new potential therapeutics useful in DR. They can delay the onset or prevent the progression of vascular complications of diabetes. Isoquinoline sulphonamides and staurosporine the first and second generation PKC inhibitors are of therapeutic interest.[70,71,72,73] Ruboxistaurin mesylate was reported in science.[74] Vitamin E can inhibit PKC activity by decreasing DAG contents by activation of DAG kinase.[75,61] PKC β inhibitor Ruboxistaurin (Figure 5) reduces the mitogenic response to VEGF, in contrast with PKC-α inhibition.[76] Selective inhibition is very crucial to develop clinically safe therapeutic PKC inhibitors. Tuttle et al documented that PKC isoform selective inhibitors can be used for chronic clinical treatment with nominal side effects.[77] So, selective PKC inhibitors are likely new potential therapeutics in DR. Endothelium related dysfunction of the coagulant and anticoagulant pathway has been documented in diabetes.[78]

D) Miscellaneous Mechanisms:

- In case of proliferative diabetic retinopathy a hypercoagulable state is present due to conversion of the endothelium from a thermoresistant to a thermogenic surface with activation of extrinsic haemostatic pathway. Moreover finding of anti-pericyte and phospholipid binding autoantibodies (eg. Leupus anticoagulants) as well as presence of T lymphocytes, B lymphocytes , HLA DR/DQ expressing cells ,macrophages and Ig deposits in the vitreous and the pre retinal membrane suggest immunological basis of diabetic retinopathy.[79,80]

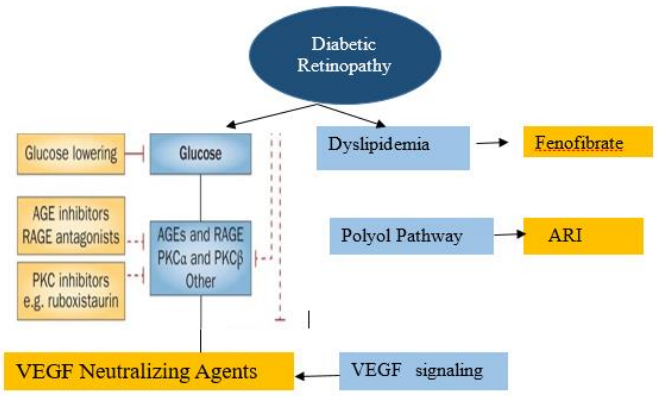

Figure 5. Biochemical basis and emerging molecular targets

- A number of growth factors have been associated with the development of Diabetic Retinopathy.[81,82] Basic fibroblast growth factor (b FGF).[83] Insulin like growth factor 1(IGF-1).[84] Angiopoitin 1 and 2[85,86], stromal derived factor 1 [87] ,Epidermal growth factor(EGF)[88], Transforming growth factor β 2(TGF- β 2)[89], Platelet derived growth factor[90] and Erythropoitin[91] have been found associated with DR.

- Anti VEGF agents are recently developed as a treatment modality in DR. Pegaptenib, Ranibizumab, Bevacizumab are some of the current anti VEGF agents.[92-95] Recent advances suggest cautious use of anti VEGF agents on long term basis to treat DR due to the fact that VEGF has a role as retinal neuron survival factor and its inhibition may lead to destruction of cells i.e. photoreceptors, muller glia that are involved in visual function.[96]

- Many studies highlighted the importance of subclinical inflammation in the development of DR.[97-99] Hyperglycaemia , hypertension, oxidative stress ,Advanced Glycation End products all contribute to inflammation and inflammatory response in tern regulates these pathways via cytokines, VEGF signalling, adhesion molecules, Enhanced RAGE expression, NFkB signalling. Leucostasis is another hallmark in the pathogenesis of DR as it causes capillary occlusion, ROS mediated cell death that enhance inflammatory response locally in retinal tissues.[100] Therefore subclinical inflammation in the retina leads to increased intra ocular pressure, formation of new, weak vessels and their increased permeability due to VEGF that cause retinal haemorrhage. Activation of microglia and immune cells is also revealed by many researchers.[101] This fact has been supported by the use of Minocyclin, an antibiotic and anti-inflammatory agent that block the activation of Microglia and prevent DR.[102] The use of intravitreal Triamcinolone acetonide and non-steroidal anti-inflammatory drugs i.e. Nepafenac has been reported to reduce VEGF expression, normalize vascular permeability and reduce apoptosis , leucostasis that improve visual acuity.[103,104] There is also great deal of interest in intraocular implants that deliver anti-inflammatory steroids.[105,106]

- A significant relationship between VEGF and IL-6 was reported by Funatsu et al. Aqueous levels of these two parameters were correlated with the severity of fundus findings.[107] Recently VEGF & IL-6 relationship & their levels in vitreous fluid has been documented[108,109] thus pointing towards the usefulness of their measurement as an analytical marker of pathogenesis of DR and to predict the progression of retinal diseases.

- Matrix Metalloproteinases (MMP) 2.9 and their tissue inhibitors (TIMP) were found elevated in vitreous of diabetic patients and were correlated with severity of retinopathy.[110, 111] MMP activity represent the " final common pathway" in retinal neovascularisation from whatever cause and therapeutic inhibition at this level may be more effective then targeting individual pathway.

- Endothelin-1 (ET-1) is a peptide produced by the endothelial cells that induces vasoconstriction. Studies reported that hypoxia induces ET-1 gene expression in endothelial cells.[112] A position association between ET-1 expression and PKC activation in early diabetes reflected the fact that PKC inhibitors could be able to reverse the upregulation of ET-1.[113] Therapeutic effect of long-term selective blockade of endothelin A (ETA) receptor has been recently evaluated in a genetic mouse model of non obese type-1 diabetes.[114] Such studies suggest a new strategy for preventing development of retinopathy in diabetes.

Fenofibrate (Peroxisome proliferator activated receptor PPAR-α agonist) is used to treat high triglycerides and low HDL or as adjunct to statin therapy. It regulates the expression of many genes that work against lipids, inflammation, angiogenesis, and cell apoptosis. The ACCORD Eye Study group involved a subset of 2,856 participants reported that the rates of progression of diabetic retinopathy were significantly reduced in the intensive glycemic control group and in the fenofibrate group.[115]

CONCLUSION

The pathogenesis of DR is very complex and many biochemical mechanisms have been proposed which are interactive and interdependent. This review provides better understanding of complex biochemical mechanisms and treatment modalities of recent Interest. At proliferative phase of retinopathy therapeutic interventions are effective but in advanced stage hypoxia induced VEGF production leads to disease progression. Overexpression of growth factors i.e. VEGF, IGF-1, stromal derived GF-1, angiopoetin- 1 & 2, fibroblast Growth Factor act in synergy in mediating process of angiogenesis and endothelial are proliferation. As there is a complex interplay between the biochemical pathways, understanding the molecular basis of these pathways in greater details will help in exploring newer pharmacological agents targeted to block different pathways that could provide a better insight in preventing the disease progression in Diabetic Retinopathy.

CONFLICT OF INTEREST

Nil.

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