Pathology and Mechanisms in Diabetic Retinopathy

Pathology of Diabetic Retinopathy
DR is a disorder of microvasculature of retina that occurs as a consequence of prolonged uncontrolled hyperglycemic state. It has been reported that more than 4 percent of world populations is diabetic and half of them are suffering from DR at some stage of disease progression. DR affects all patients with type-1 diabetes and more than 70 per cent of type 2 diabetic patients develop DR after 15 years of diabetes. There are large number of studies conducted in western world to set up database for the epidemiology DR (Klein et al., 1990; DCCT Group, 1997; Klein et al., 1998; Klein, 2007; Klein et al., 2009). However, there is a paucity of data on DR in India and other Asian countries despite the fact that DR has become the leading cause of vision loss in all Asian countries.

Classification of Diabetic Retinopathy
Based on the severity of the disorder, DR is classified into two types, (A). Non-proliferative diabetic retinopathy (NPDR) and (B). Proliferative diabetic retinopathy (PDR) (Frank, 2004).

(A). NPDR, commonly known as background retinopathy, is an early stage of diabetic retinopathy. In this stage, blood or fluid leaks from tiny blood vessels within the retina. Leaking fluid causes the retina to swell or to form deposits called exudates. NPDR is divided into mild, moderate and severe.

(i). Mild NPDR - It is the initial phase of NPDR and is characterized by the presence of at least one microaneurysm, and also by dot, blot or flame-shaped haemorrhages. Hard exudates and cotton wool spots are usually not a feature of mild NPDR (Fig.1).

(ii). Moderate NPDR – It is the next and more severe stage of NPDR. During this stage, some of the small blood vessels in the retina may become blocked. The blockage of these tiny blood vessels causes a decrease in the supply of nutrients and oxygen to certain areas of the retina (Fig.1).
Figure 1. A and B are representative fundus photographs from patient with moderate NPDR showing significant accumulation of hard exudates (arrow), microaneurysms and leakages at some areas (star); figure 1C is fundus photograph from patient with severe NPDR showing significantly high leakage at multiple areas (star) and appearance of hard exudates; figure D is fundus photograph from patient with severe NPDR showing tortuous blood vessels (arrow head), large deposits of hard exudates (arrow) and leakages in the fundus (star); figure E is a representative image from patient with PDR as evident from neovascularisation of the optic disc (arrow), tortuous vessel, appearance of hard exudates (arrow head) and retinal haemorrhage (star); figure F is a fluorescein fundus angiogram from a patient with PDR showing significant leakage of sodium fluorescein (star) and appearance of microaneurysms in the form of hyperfluorescent dots (arrow heads).
(iii). Severe NPDR - During severe NPDR significant number of small blood vessels in the retina become blocked. As a result more areas of the retina are deprived of nourishment and oxygen. A lack of sufficient oxygen supply to the retina results in a condition called “Retinal Ischemia”. To overcome ischemic insult a variety of compensatory growth factor cascade are activated eventually causing raised retinal Vascular Endothelial Growth Factor (VEGF) and Protein Kinase-C-β (PKC-β) levels. These angiogenic growth factors are highly responsible for increased retinal permeability and neovascularisation (Fig.1).

(B). PDR is a more advanced form of diabetic retinopathy and a major cause of vision loss in diabetic patients. It is characterized by neovascularization on optic disc and in other areas of fundus. Sometimes vessels grow out of inner limiting membrane and bleed in the vitreous leading to significant vision loss (Fig.1).

**Mechanisms in Diabetic Retinopathy**

**Hyperglycemia**

Complications of diabetes such as diabetic retinopathy are a result of multiple metabolic, vascular and inflammatory defects. Hyperglycemia is the key metabolic abnormality in diabetes mellitus. Hyperglycemia if prevented early in the disease course can help in preventing the onset or delaying the progression of microvascular disease (Engerman & Kern, 1987 Engerman, 1989). The Diabetes Control and Complications Trial (DCCT) demonstrated that the risk for the development and progression of diabetic retinopathy was significantly reduced in patients receiving intensive insulin therapy, however the significance was observed only after 6-7 years (DCCT research group, 1993). Nevertheless, the data clearly indicates that the early control of hyperglycemia may be instrumental in preventing the onset and progression of vascular changes associated with diabetic retinopathy.

**Oxidative Stress in Diabetic Retina**

In diabetes, the enzyme activities of antioxidant defense enzymes responsible for scavenging free radicals and maintaining redox homeostasis, such as SOD, CAT, and GSH are decreased in the retina (Wolf, 1987; Baynes, 1991; Kowluru et al., 2001; Haskins et al., 2003). However, the cell is equipped with intracellular antioxidant,
GSH; GSH is probably the most important defense the cell is equipped with. It can act as a reactive oxygen species scavenger and modulate intracellular redox state (Meister et al., 1988). The levels of this intracellular antioxidant are decreased in the retina in diabetes (Kern et al., 1994) and the enzymes responsible for its metabolism are compromised. Finally, a stage is reached when insufficient neutralization of free radicals occurs due to an imbalance between Pro-oxidants and Anti-oxidants, which is known as Oxidative Stress. Oxidative stress leads to oxidation of cellular lipids, proteins and nucleic acids causing further damage (Baynes et al., 1991).

**Angiogenic Mechanisms in Diabetic Retina**

**VEGF**

Vascular endothelial cell growth factor is an endothelial cell-specific angiogenic and permeability-inducing factor that has been implicated in the pathogenesis of diabetic retinopathy (Rajah and Grammas, 2002). Inhibition of VEGF function may control pathologic neovascularization such as in diabetic retinopathy and tumor growth, whereas enhancement of VEGF may stimulate new blood vessel growth in ischemic tissue (Robinson et al., 2001, jung et al., 2001). Oxidative stress mediates the hyperglycemia-induced pathological effects of VEGF on microvascular complications of diabetes (Caldwell et al., 2005). Retinal expression of VEGF is elevated by ROS.
(lu et al., 1998), and VEGF can also interact with other metabolic pathways important to the development of retinopathy such as PKC and the polyol pathway (Aiello et al., 1997; Frank et al., 1997). Both preclinical and clinical studies have shown that VEGF participated in the pathogenesis of proliferative diabetic retinopathy (Adamis et al., 1994, Aiello et al., 1994). Ocular VEGF levels are strongly correlated with neovascularisation in patients with diabetes (Aiello et al., 1994), and higher vitreous and retinal concentrations have been reported in diabetic patients. VEGF-induced vasopermeability in vivo and in vitro requires PKC activity. However, actual mechanism by which PKC regulates barrier properties is not fully understood (Aiello, et al., 1997; Geraldes and King, 2010; Ayo et al., 1991; Geraldes et al., 2009).

**PKC-β**

PKC-β is important mediator in VEGF signaling pathway. Various experimental studies have shown that angiogenic response, as a result of retinal ischemia, is increased in mice over-expressing the PKC-β isoform and also mitogenic action of VEGF has been found to be increased in retinal endothelial cells over expressing the PKC-β isoform (Suzuma et al., 2002). Further, others have also shown that raised VEGF and PKC-β levels in retinas of patients with DR (Aiello et al., 1994; Kim et al., 2010a, 2010b).

**Inflammation in Diabetic Retina**

DR shares many common features with chronic inflammatory disease. Earlier studies shows that levels of cytokines, includes IL-1β, IL-6 & TNF-α, are increased in the vitreous fluids of proliferative DR patients (Maimone et al., 1997; Yuuki et al., 2001; Demircan et al., 2006) and retinas of diabetic rats (Carmo et al., 1999). According to Demirican et al., 2006 both TNF-α and IL-1β are highly responsible for retinal angiogenesis activity and also responsible for retinal apoptosis.

**IL-1β**

IL-1β, a pro-inflammatory cytokine, is the key mediator in neuroinflammation & upregulated in various diseases like Alzheimer’s disease, Parkinson’s disease and also in DR (Carmo et al., 1999; Griffin et al., 1989; Kowluru and Odenbach, 2004). Role of IL-1β is very well studied in Blood Retinal Breakdown (Bamforth et al., 1997).
Further, IL-1β activation in diabetic retina helps in induction of retinal capillary cell apoptosis (Kowluru and Odenbach, 2004).

Figure 3. showing central role of Oxidative stress in development of diabetic retinopathy.

**TNF-α**

TNF-α is the prototypical member of a family of cytokines that also include Fas ligand, CD40 ligand and TNF-related apoptosis inducing ligand, and induce apoptosis, differentiation, cell activation, and inflammation (Joussen et al., 2003). The contribution of TNF-α to the pathogenesis of DR is clearly supported by a number of reports (Joussen et al., 2002; Joussen et al., 2003), and significantly higher levels of TNF-α are found in the plasma of diabetic patients versus age-matched healthy control subjects (Foss et al., 1992; Zorena et al., 2007). Joussen et al. (2002) have investigated the role of the inflammatory cytokine TNF-α in the apoptotic cell death of retinal endothelial cells during early and late stages of DR using a rat model of streptozotocin-induced diabetes and a mouse model of long-term galactosemia. Moreover, it has been studied that early introduction of the TNF-α antagonists to the treatment of young patients with type 1 diabetes mellitus, who show high serum activity of the cytokine may prevent development of DR (Joussen et al., 2002).
Apoptosis in Diabetic Retina

Bax/Bcl-2

Apoptosis plays an important role in cell death after retinal ischemia. Various studies have shown that an imbalance in the regulation between pro- and anti-apoptotic (Bax and Bcl-2) molecules triggers apoptosis (Luo et al., 2002; Xing et al., 2008). Further, studies performed on diabetic retina shows that Bax was upregulated following hyperglycemia induced ischemia, and conflicting reports are available Bcl-2 regulation (Kaneda et al., 1999; Tezel and Wax, 1999). Various studies have confirmed that Bax and Bcl-2 expression in retinal ganglion cells and inner retina in humans and rats (Podesta et al., 2000; Mizutani et al., 1998; Chen et al., 1994).

Caspase-3

Hyperglycaemia acts as a trigger that causes activation of various apoptotic proteins involved in apoptotic cell death, including members of the caspase family (Allen et al., 2005). Earlier studies showed that caspases play important role in the initiation and execution of apoptosis (Doonan and Cotter, 2004; Katai and Yoshimura, 1999). Moreover, caspase-3 has been studied to play an important role in diabetes and its complications (Kern et al., 2000; Kowluru and Koppolu, 2002b). Caspase-3 is activated at very late stage of apoptosis and play crucial role in activating proteolytic cascade. Therefore, detection of caspase-3 is a reliable marker for cells which are destined to undergo apoptosis (Kowluru and Koppolu, 2002b; El-Asrar., et al., 2004).

Expression of Tight Junction Proteins in Diabetic Retina

Tight junctions are composed of a combination of various proteins including the transmembrane proteins occludin, the claudin family, and members of the zonula occludens (ZO) family, and several regulatory proteins. Occludin and claudins are responsible for the direct cell-to-cell attachment in the tight junction barrier (Fanning et al., 1999; Matter et al., 1999) and are a crucial determinant of tight junction permeability properties in endothelial cells (Hirase et al., 1997; Turksen and Troy, 2004). Claudin-5 is necessary to preserve the vascular barrier to small (<0.8 kDa) molecules in the brain (Nitta et al., 2003), and it possibly also plays a similar role in the BRB. The zonula occluden proteins (ZO-1, -2, and -3) coordinate the assembly of the junctional complex and provide the interaction with components of the
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cytoskeleton (Wittchen et al., 1999), also important for BRB function (Leal et al., 2010).

Two mechanisms have been correlated with increased retinal vascular leakage in diabetes; first is increased paracellular leakage due to changes in the expression, content, phosphorylation, altered arrangement of tight junction proteins and second is increased transcellular uptake of fluids by pinocytosis mechanism (Klaassen., et al 2009; Antonetti et al., 1998, 1999). The principal tight junction (TJ) proteins which are universally present in vertebrate retina are Occludin, Claudin-5 and ZO-1. However, Increased Occludin expression has been more specifically found in vascular endothelium of brain and retina having greater barrier properties (Harhaj., et al 2006; Hirsae., et al 1997). Similarly, various proteins are involved in transcellular transport of pinocytotic vesicles contribute to retinal hyperpermeability (Klaassen., et al 2009).

Expression of Aquaporins in Diabetic Retina

The aquaporins (AQPs) are a family of small membrane-spanning proteins (monomer size ~30 kDa) that are expressed at plasma membranes in many cells types involved in fluid transport. AQP4 has more relevance in the pathogenesis of DR. AQP4, a water specific membrane-channel protein, is more specifically expressed in Müller cells and astrocytes, and its overexpression has been implicated in neuronal and glial swelling (Bringmann et al., 2005; Da and Verkman, 2004; Liu et al., 2007; Nagelhus et al., 2004). Earlier studies have shown that intense expression of AQP4 at Müller cell endfeets (Da and Verkman, 2004; Nagelhus et al., 1998). Further, various authors have suggested that AQP4 inhibition could be considered as novel target for therapeutic intervention (Bringmann et al., 2005; Da and Verkman, 2004; Nagelhus et al., 1998).

Basement Membrane Thickening in Diabetic Retina

A consistent feature of diabetic retinopathy is the thickening of the capillary BM (Yamashita and Becker, 1961; Siperstein et al., 1968; Lambert et al., 1996; Roy et al., 1994, 1996, 2000, 2003). Capillary BM thickening can result from increased production and decreased degradation of the extracellular matrix proteins (Roy et al., 1994, 1996, 2000, 2003). High levels of glucose can increase mRNA expression of ECM proteins, collagen and fibronectin, in the kidney mesangial cells and retinal
endothelial cells (Cagliero et al., 1991, 1998; Hua et al., 2001). These changes are brought upon as early as 8 weeks following onset of diabetes (Ljubimov et al., 1996). In the present study marked prevention in the progression of thickening of BM was observed in the curcumin-treated diabetic rats. Endothelium activated by hyperglycaemia and/or ischemia and may lead to the isolation of pericytes from endothelial cells and blood supply. This, in turn, may cause pericyte loss, release of endothelium, leading to neovascularisation (D’Amore, 1994; McAvoy and Chamberlain, 1990).

Polyol Pathway in Diabetic Retina
A key enzyme of the polyol pathway, aldose reductase (AR), is found to be increased in the diabetic retina and lens. AR inhibitors shown inhibition of thickening of the basement membrane of the retinal capillaries and formation of cataract in experimental studies (Yeh et al., 1986; Sun et al., 2006; Okuda et al., 1985; Obrosova et al., 2003, 2005). The polyol pathway was considered the most desirable target for adjunctive treatment to prevent DR. Based on favorable in vivo experiments using the aldose reductase inhibitor sorbinil (Robison et al., 1983; Frank et al., 1983), a clinical trial of sorbinil was conducted, but sorbinil did not affect the development of DR (Sorbinil Retinopathy Trial Research Group, 1990), and enthusiasm for the clinical application of aldose reductase inhibitor goes in vain.
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Introduction

Method- Literature search was done on online database, Pubmed, Google Scholar, clinitrials.gov and browsing through individual ophthalmology journals and leading pharmaceutical company websites.

Diabetic retinopathy (DR) is characterized by the progressive development of well-defined morphological abnormalities in the retinal microvasculature that can remain relatively stable, that is non-proliferative diabetic retinopathy (NPDR) or progress to diabetic macular edema (DME) and/or proliferative DR (PDR) (Shah, 2008; Zhang et al., 2011). One of the major hallmarks of DR is increased vascular permeability, which leads to the development of retinal hemorrhages and fluid accumulation in the macula, which is referred as DME (Scholl et al., 2010; Augustin et al., 2010).

Since the last two decades there have been significant developments in the emerging field of pharmacotherapy of DR. The advent of laser photocoagulation three decades back, was really useful in limiting vision loss in most of the cases and is still considered gold standard therapy for the treatment of DR. However, corticosteroids and anti-VEGF agents have shown promising results with regard to prevention of neovascularisation, but remained limited in use due to their short-duration effects. More importantly none of these agents have been able to substitute the remarkable durability and effectiveness of panretinal photocoagulation in preventing vision loss in the late stages of DR. Therefore, pharmacotherapy of DR is still an adjunct to panretinal photocoagulation [Table 1].

Anti-inflammatory Agents

Various inflammatory mediators are up-regulated during DR including Tumour Necrosis Factor - α (TNF-α), Interleukin-1β (IL-1β) and Vascular Endothelial Growth Factor (VEGF) are investigated to play significant role in the pathogenesis of DR. These inflammatory mediators are very well modulated with corticosteroids. In the past, corticosteroids have been incorporated as a treatment option for DME and DR, because of their anti-inflammatory and anti-angiogenic effects. However, they
remained limited in use as an adjunct to panretinal photocoagulation due to their short duration of effectiveness. The primary mode of delivery of corticosteroids is through the intravitreal route, so as to avoid the blood-retinal barrier limitations to reach the drug to the target site. However, these direct intravitreal injections are often associated with steroid-related adverse effects including cataract and elevation of IOP, and less common injection-related side-effects such as retinal detachment, vitreous hemorrhage, and endophthalmitis (Quiram et al., 2006; Sakamoto et al., 2011). Despite this fact, corticosteroid therapy has been effective for DME and DR as an adjunct to laser photocoagulation, and has also shown improvements in best corrected visual acuity (BCVA) (Bhagat et al., 2009).

Intravitreal triamcinolone acetonide (IVTA) has been studied for its potent anti-inflammatory effects and has shown improvement in DME and age-related macular degeneration (AMD) (Gillies et al., 2006; Becerra et al., 2011; Lam et al., 2007; Kim et al., 2008; Dehghan et al., 2008). With its anti-angiogenic effects, IVTA is a valuable option in the treatment of proliferative DR (Bandello et al., 2006). The US FDA has approved a couple of IVTA preservative-free injections, Triesence (40 mg/ml, Alcon) and Trivaris (80 mg/ml, Allergan), so as to lessen the incidence of non-infectious endophthalmitis and other complications.

The diabetic retinopathy clinical research (DRCR) network investigated the retinal thickness (by Optical Coherence Tomography) and visual acuity outcomes of two doses (1 mg and 4 mg) of travaris in comparison to macular photocoagulation for the treatment of macular edema in a large, multicenter randomized clinical trial. After three years of follow-up, treatment with macular photocoagulation was associated with improved BCVA and fewer complications. On the other hand, two major
complications of IVTA were cataract formation and ocular hypertension. However, the rate of endophthalmitis in patients enrolled in the DRDRnet and SCORE (Standard care versus Corticosteroid for Retinal vein occlusion study) trials was 0.05% (Bhavsar et al., 2007).

Moreover, various randomized clinical trials have shown that treatment with IVTA in the improvement of BCVA at three months but treatment was no longer effective at six months (Yilmaz et al., 2009). So this lack in the efficacy for chronic use and associated adverse effects at higher doses has resulted in the focus on the development of novel intravitreal steroid delivery devices that release a small quantity over a prolonged period of time.

### Sustained Drug Delivery Systems of Corticosteroids

For chronic inflammatory disorders like DR, there is urgent need for sustained-release corticosteroid therapy, which can help in reducing associated adverse effects. One

<table>
<thead>
<tr>
<th>Drug/Formulation</th>
<th>Company</th>
<th>Category/Mechanism</th>
<th>Clinical Phase</th>
<th>Regulatory Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triamcinolone (40 mg/ml), IVTA</td>
<td>Alcon Laboratories, Inc.</td>
<td>Anti-inflammatory</td>
<td>-</td>
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<td></td>
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<tr>
<td>Triamcinolone (80 mg/ml), IVTA</td>
<td>Allergan, Inc.</td>
<td>Anti-inflammatory</td>
<td>-</td>
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<tr>
<td>Preservative-free Injection</td>
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</tr>
<tr>
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<td>Suroptics, Inc.</td>
<td>Anti-inflammatory</td>
<td>Phase 1 completed</td>
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<tr>
<td>Fluocinonide</td>
<td>Allergan Sciences</td>
<td>Anti-inflammatory</td>
<td>Completed Phase III</td>
<td>Not FDA-approved</td>
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<td>Ozone, Dexamethasone IV implant</td>
<td>Allergan, Inc.</td>
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<td>Under Phase III</td>
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<td>Genentech, Inc.</td>
<td>Anti-VEGF</td>
<td>Under Phase III</td>
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<td>EyeTech Pharmaceuticals and Pfizer</td>
<td>Anti-VEGF</td>
<td>-</td>
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<td>VEGF Trap-Eye</td>
<td>Regeneron and Bayer Healthcare Collaboration</td>
<td>Anti-VEGF</td>
<td>Phase II (DME) Phase III (AMD and ORVO)</td>
<td>Not FDA-approved</td>
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<td>Vitrine, hyaluronidase</td>
<td>ISTA Pharmaceuticals</td>
<td>Viscous clearing agent</td>
<td>Undergoing Phase III</td>
<td>Not FDA-approved</td>
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such steroid drug delivery system in development for use in DME is the triamcinolone acetonide (TA) implant (I-vation®), which has already completed a Phase I trial in the long-term treatment of DME, and a Phase II clinical triamcinolone acetonide trial is being planned (Available at: http://www.clinicaltrials.gov/ct2/show/NCT00915837). It is composed of biodegradable polymers which slowly degrade over time, thereby bypassing the risk of secondary surgical complications upon removal as compared to non-biodegradable devices. Another potential new steroid delivery system is the sustained release fluocinolone acetonide non-biodegradable intravitreal insert (Iluvien®, Alimera Sciences). Iluvien is designed to release the drug fluocinolone acetonide up to three years. Importantly, the device is very small, which can be injected into the back of the eye with a 25-gauge needle creating a self-sealing hole. The insertion procedure is almost similar to an intravitreal injection. The two ongoing pivotal multicenter trials known collectively as FAME study have shown improvement in BCVA at low-dose insert (0.19 mg total, approx. 0.23 µg/day) out of the two doses studied (Kane et al., 2008; Kompella et al., 2010; Schwartz and Flynn, 2011). However, in response to new drug application (NDA) seeking approval to market ILUVIEN (fluocinolone acetonide intravitreal insert) for the treatment of DME, FDA has recently issued the complete response letter to communicate its decision that the NDA cannot be approved in its present form.

Another fluocinolone acetonide intravitreal implant, Retisert® (Bausch and Lomb) is FDA-approved for the treatment of chronic, non-infectious uveitis. A Phase III clinical trial conducted in patients with DME reported large cases of cataract and glaucoma (Kompella et al., 2010; Schwartz and Flynn, 2011).

Ozurdex® (allergen) is an extended-release biodegradable dexamethasone intravitreal implant that has been recently approved by the FDA for the treatment of macular edema secondary to retinal vein occlusions (RVO). A 2007 study found that dexamethasone (at 700 µg) was well tolerated and produced statistically significant improvements in BCVA and central retinal thickness at Day 90, but at Day 180 no significant difference in visual acuity was found and both treatments groups (350 µg and 700 µg) had an increased incidence of elevated IOP (Kuppermann et al., 2007; Haller et al., 2010).
The Cortiject implant (NOVA63035; Novagali Pharma) is a preservative- and solvent-free emulsion that contains a tissue-activated proprietary corticosteroid prodrug. Once released, the prodrug is converted into an active drug at the level of the retina. A single intravitreal injection of the emulsion provides sustained release of the corticosteroid over a six to nine-month period. An open-label, Phase I, dose-escalation clinical study to assess the safety and tolerability of NOVA63035 in patients with DME is currently under way (Available from: http://www.novagali.com/en/eye-therapy).

The Verisome delivery system (Icon Biosciences, Inc.) is also a sustained-release drug delivery system. When injected into the vitreous as a liquid via a standard 30-gauge needle, the liquid coalesces into a single spherule. The biodegradable vehicle provides controlled, extended drug release over a titratable period of up to one year. The drug delivery system degrades as the active agent is released over the intended duration. For its first clinical trial, the Verisome technology was formulated for the injectable intraocular sustained-release delivery of TA (IBI-20089) (Fung, 2010). The Phase I and II trial of IBI-20089 in patients with cystoids macular edema associated with RVO conducted using two dosing levels, (1) 25-μL dose designed to last six months, and (2) 50-μL dose designed to last one year. The patients were treated with a single intravitreal injection of Verisome containing TA through a 30-gauge needle in two sequential cohorts of five patients each. The first cohort received a lower dose containing 6.9 mg triamcinolone in 25 μL with sustained delivery calculated to last for six months. The second cohort received a higher dose containing 13.8 mg triamcinolone in 50 μL calculated to last for 12 months. Based on the results of clinical trials, it was concluded that Verisome was well tolerated by patients without any drug-related adverse events and demonstrated evidence of controlled release efficacy for a low dose of triamcinolone. The larger dose showed more evidence of efficacy than the smaller dose (Available from: http://www.iconbioscience.com/Overview.html).
Miscellaneous Anti-inflammatory Agents

Nepafenac (Nevanac®, Alcon) is an FDA-approved topical non-steroidal anti-inflammatory drug that has demonstrated efficacy against DME in one case report (Hariprasad et al., 2007). However, clinical trials are warranted.

Etanercept (Enbrel®, Amgen, Inc. and Wyeth) is a recombinant fusion protein having anti-TNF-α property and is FDA-approved for the treatment of psoriasis (Ducharme and Weinberg, 208). A small series of patients with refractory DME were treated with intravitreal etanercept, but no statistically significant improvement was found (Tsilimbaris et al., 2007).

Infliximab (Remicade®, Centocor) is another TNF-α antagonist that is FDA-approved to treat Crohn’s disease. An investigation of systemic treatment of DME with infliximab has led to a study of administration through intravitreal injection (Sfikais et al., 2005).

Angiogenesis Inhibitors

In addition to corticosteroids, anti-angiogenic agents are found to be effective for the treatment of proliferative DR and DME. Presently, novel and more specific anti-angiogenic agents are being explored in order to address the vascular leakage and, perhaps, neovascularisation associated with DME. The most popular target of these agents is the subfamily of proteins known as VEGF, whose over-expression is believed to play a role in numerous diseases including DR and AMD. After all, laser photocoagulation remains the gold standard therapy for the treatment of proliferative DR. Whereas, newly developed anti-VEGF agents are used as an adjunct to laser photocoagulation.

Bevacizumab (Avastin®, Genentech Inc.) is a complete full-length humanized antibody that binds to all subtypes of VEGF and is successfully used in tumor therapy as a systemic drug (Ferrara et al., 2004). Recent studies have demonstrated the usefulness of an intravitreal injection of bevacizumab in the reduction of vascular permeability and fibrovascular proliferation in macular edema secondary to central vein occlusion, retinal neovascularization secondary to PDR, and choroidal neovascularization secondary to AMD (Valiatti et al., 2011; Michels et al., 2005;
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Campa and Harding, 2011; Iturralde et al., 2006). Avery et al., 2006 reported regression of iris and retinal neovascularization after intravitreal avastin therapy. However, recurrence of neovascularization was noticed as early as two weeks following treatment, which is its major shortcoming when compared to laser therapy. Therefore, Avastin has gained popularity as a clinical adjunct to laser treatment in patients with PDR (Schmidinger et al., 2011; Mirshahi et al., 2008).

Ranibizumab (Lucentis®, Genentech, Inc.) is a recombinant humanized antibody fragment against VEGF-A, and was approved by the FDA for the treatment of exudative AMD in the year 2006 (Rosenfeld et al., 2006; Matsumiya et al., 2011). Recently, Roche announced (January, 2011) that one of the two Phase III (RISE Study) studies evaluating monthly Lucentis in patients with DME met its primary endpoint.

Moreover, in 2010, Lucentis has been approved by FDA for macular edema following RVO. The BRAVO study assessed the safety and efficacy profile of Lucentis for macular edema following branch-RVO. The CRUISE study assessed the safety and efficacy profile of Lucentis for macular edema following central-RVO. The primary endpoint of both studies was mean change from baseline in BCVA at six months compared with patients receiving sham injections.

In the BRAVO study, the percentage of patients in the Lucentis study group who gained 15 or more letters in BCVA from baseline at month six was 61% (compared with 29% in the sham injection study group). In the CRUISE study, the percentage of patients in the Lucentis study group who gained 15 or more letters in BCVA from baseline at month six was 48% (compared with 17% in the sham injection study group). At six months, patients in the BRAVO study who received Lucentis had a mean gain of 18.3 letters (compared to 7.3 letters in patients receiving sham injections). In the CRUISE study, at month six, patients who received Lucentis had a mean gain of 14.9 letters (compared to 0.8 letters for patients receiving sham injections) (Campochiaro et al., 2010; Brown et al., 2010).

VEGF Trap Eye (Aflibercept, EYLEA®, Regeneron Pharmaceuticals, Inc. and Bayer HealthCare Pharmaceuticals) is a recombinant fusion protein consisting of portions of
human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 that binds all forms of VEGF-A along with the related placental growth factor. Based on VIEW 1 and VIEW 2 clinical trials, it would be now possible to inject VEGF Trap Eye every two months against the use of contemporary therapy of ranibizumab every four weeks. Further, VEGF Trap Eye was found to be well tolerated following intravitreal injections.\textsuperscript{[43,44]} Recently, FDA has delayed its decision on VEGF Trap Eye for the treatment of wet macular degeneration. Bayer Healthcare has also submitted an application to European Medicines Agency for marketing approval of VEGF Trap Eye for the treatment of wet macular degeneration. Jerini AG (German biopharmaceutical company), has developed an anti-angiogenic compound, JSM6427, which has shown positive results and offers a more convincing treatment option. Intraocular implanted osmotic pump results in slow release of formulation for an extended period of six months. Phase I clinical trials have begun. TargeGen, Inc. announced that topical administration of the prodrug, TG100801, may be effective for the treatment of retinal disease and may also be used in combination with approved products. TG100801 demonstrated the ability to reduce VEGF-mediated retinal leakage, angiogenesis and inflammation after topical instillation. TG100801 converts to the active drug TG100572 as it penetrates the eye. The active drug, TG100572, was shown to overcome neovascularization and inflammation, both of which are characteristics of DR and wet macular degeneration.

CoMentis, Inc. (formerly Athenagen), announced the initiation of a Phase II clinical study of ATG3, a topical drop, for neovascular AMD. ATG3 acts as antagonist of the nicotinic acetylcholine receptor pathway which mediates angiogenesis. The drug was developed to effectively penetrate into the retina and choroid following topical eye drop administration as shown in animal experimental data. Further, ATG3 was evaluated in a randomized, double-masked, placebo-controlled Phase I study which enrolled 80 healthy volunteers in single and multiple dose ascending regimens for up to 14 days of therapy. ATG3 study demonstrated excellent ocular tolerability of ATG3 with no systemic side-effects.

GlaxoSmithKline is investigating Pazopanib, oral, once-daily angiogenesis inhibitor targeting vascular endothelial growth factor receptor (VEGFR), platelet-derived
growth factor receptor (PDGFR) and c-kit. Phase III studies are being planned (Takahashi et al., 2009).

**Vitreolytic Agents**

Vitrase (hyaluronidase ovine, ISTA Pharmaceuticals, Inc.) is the first and only pure, preservative-free, thimerosal-free, ovine hyaluronidase, which is FDA-approved as a spreading agent. Intravitreal vitrase has shown efficacy and safety in a Phase III clinical trial to investigate its promotion of the clearance of vitreous hemorrhage from PDR, although the agent is not FDA-approved for this purpose (Kupperman et al., 2005a, 2005b).

The induction of a posterior vitreous detachment also could be beneficial in the treatment of DME and PDR (Lopez-Lopez et al., 2009). A randomized, double-masked, sham-injection controlled, dose-ascending clinical trial primarily designed by Thrombogenics NV (Belgium) to compare multiple doses of intravitreal microplasmin versus sham injection for treatment of patients with DME (MIVI-II) has been completed. With this preliminary study the company believes that microplasmin may represent a major advance in this area, as detaching the vitreous from the retina has been associated with greatly reducing neovascularization of the retina, which plays a fundamental role in the loss of vision in many diabetic patients.

Two trials of MIVI-TRUST Phase III program for Focal Vitreomacular Adhesion showed that microplasmin: (1) was successful in resolving vitreomacular adhesion, (2) was able to cure full thickness macular hole without the need for surgery, (3) delivered an improvement in the vision of patients without the need for surgery, and (4) was safe and well-tolerated.

**Effects of Systemic Agents on Diabetic Retinopathy**

Various systemic agents which are actually not designed for the management of DR have shown evidence-based results in reducing the progression of DR. So, these agents should be considered as extra value, while they are primarily used for treating dyslipemia and hypertension in diabetic patients. Before these can be used clinically, further studies are warranted to elucidate their mechanisms in the prevention of DR.
Hypoglycemic Agents

**Insulin** – The main goal of insulin therapy is to limit the progression of long-term diabetic complications with either Type 1 or Type 2 diabetes mellitus. Further, various studies have justified that good glycemic control helps in delaying the progression of DR (UKPDS, 1998).

Multiple studies, for example Diabetes Control and Complications Trial (DCCT), have reported retinal pro-angiogenic effects of insulin following the initiation of intensive insulin therapy for a couple of years, therefore, DR can worsen for a short span of time. However, over the long term, intensive glycemic control is associated with improvement in DR (DCCT, 1998).

**Thiazolidinediones**- Thiazolidinediones are oral hypoglycemic agents used either as monotherapy or in combination with other hypoglycemic agents. According to the recommendations of the United Kingdom Prospective Diabetes Study (UKPDS), thiazolidinediones are extensively used for the improvement of glycemic control in Type 2 diabetic patients resulting in 1% reduction in % HbA1C values (UKPDS, 1998).

Thiazolidinediones result in the activation of peroxisome proliferator-activated receptor (PPAR) \( \gamma \) – a transcription factor responsible for regulating the expression of genes primarily located in the adipose tissue and also evident in the retina. The thiazolidinedione rosiglitazone, apart from its effect on glycemic control, possibly delays PDR by its anti-angiogenic effects through PPAR \( \gamma \) agonist activity (Shen et al., 2008).

**Biguanides**- Metformin is a good hypoglycemic agent and has known cardioprotective effects. Metformin is indicated in obese and overweight Type 2 diabetic patients prone for the development of cardiovascular complications. Various studies have shown the anti-inflammatory and anti-angiogenic activity of metformin by decreasing the concentration of plasminogen activator inhibitor 1 and increasing fibrinolytic activity (Nagi and Yudkin, 1993; Xavier et al., 2010).
Hypolipidemic Agents

**Fibrates** - Fenofibrate is most commonly used for the treatment of hyperlipidemia. Its main action is to lower triglyceride levels, but it also reduces total and low density lipoprotein (LDL) cholesterol, raises high density lipoprotein (HDL) cholesterol, and decreases concentration of small LDL cholesterol particles and apolipoprotein B. The FIELD study shows that subjects treated with fenofibrate required less photocoagulation in PDR by 30% and DME by 31% (Keech et al., 2007). In the ophthalmology sub-study, fenofibrate reduces the progression of retinopathy by 22% in all patients and 79% in patients with pre-existing retinopathy. This result was unrelated to serum lipid levels, which were statistically similar in both, the group treated with fenofibrate and the control group (Keech et al., 2005).

Apart from its beneficial hypolipidemic effects, fenofibrate also acts via various nonlipidemic mechanisms, being a PPAR-\(\alpha\) agonist, for prevention of DR are: (1) PPAR-\(\alpha\) is present in retinal endothelial cells, and its activation through PPAR-\(\alpha\) agonists (fenofibrate) inhibits expression of VEGF receptor 2 and neovascularization in human umbilical endothelial cells, (2) fenofibrate induces expression and activation of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidise, and activation of PPAR-\(\alpha\) induces apoptosis of human monocyte-derived macrophages, (3) PPAR-\(\alpha\) activation has neuroprotective effects (Inoue et al., 1998; Chen et al., 2007; Hukka et al., 2010).

**Statins** – Collaborative Atorvastatin Diabetes Study (CARDS), a randomized controlled trial in Type 2 diabetics, reported atorvastatin to be ineffective in reducing DR. However, there was a decrease in the need for laser treatment with atorvastatin (Colhoun et al., 2004). ACCORD Eye substudy evaluated the effect of fenofibrate in combination with simvastatin on the risk of three-stage progression of retinopathy using a modified ETDRS (Early Treatment Diabetic Retinopathy Study) severity scale or laser photocoagulation over a duration of four years. The result in comparison to placebo shows a 40% reduction in the relative risk of DR progression. The decrease in risk was related to a significant decrease in triglyceride levels and an increase in HDL levels (The ACCORD STUDY, 2010).
Anti-hypertensive agents

**Angiotensin-converting enzyme inhibitors** – It is very much evidenced from a large number of clinical trials that blood pressure is the major culprit for the development of diabetic retinopathy and efficient control of blood pressure may reduce the development of DR in both Type 1 and 2 patients. ACE inhibitors are the most preferred modalities for the treatment of hypertension in diabetic patients. Further, it has been proved that the rennin-angiotensin system is expressed in diabetic retina as well (Vaanjanen et al., 2010; Willis et al., 2010). The RAS study (RASS) was conducted to evaluate the effect of rennin-angiotensin system blockage with either ACE inhibitor (Enalpril) or ARB (losartan) as compared to placebo for a period of five years on both renal and retinal morphologic characteristics in normotensive Type 1 diabetics. The ratio of progression of DR by two or more steps was reduced by 65% with enalpril and by 70% with losartan, this effect was seen independent of changes in blood pressure or glycemic control (Mauer et al., 2009).

**Angiotensin-2 receptor blockers** - The Diabetic Retinopathy Candesartan Trial (DIRECT) program was conducted to check blockade of renin angiotensin system with AT1-receptor blocker (candesartan) could prevent the progression of DR in both Type 1 and 2 diabetes independent of their hypotensive effect. It is divided into three randomized double-blind placebo-controlled parallel-group studies: (1) A primary prevention study involving Type 1 diabetic patients without DR (DIRECT-Prevent 1), (2) A secondary prevention study involving Type 1 diabetic patients with DR (DIRECT-Protect 1), (3) A secondary prevention study involving Type 2 diabetic patients with diabetic retinopathy (DIRECT-Protect 2). In each trial patients were randomized to receive candesartan (16–32 mg/day) or placebo and the median follow-up was 4.7 years. Results of both studies, DIRECT-Prevent 1 and DIRECT-Protect 1, suggested that candesartan is not beneficial for the prevention of DR. DIRECT-Protect 2 showed a non-significant reduction in the progression of DR. However, a significant increase in diabetic retinopathy regression was observed—this effect was even more pronounced in patients with mild DR. Thus, data analysis
suggests an overall protective effect of candesartan in DR (Sjolie et al., 2008; Chautervedi et al., 2008).

**Miscellaneous Agents**

**Ruboxistaurin** - Ruboxistaurin (Arxxant®, Eli Lilly and Company) is an investigational agent which has shown results for the treatment of moderate to severe NPDR. It acts by limiting the over-activation of protein kinase C beta, which is directly involved in the pathogenesis of DR. It is a new class of compounds being tested for the management of moderate to severe NPDR (Amadio et al., 2010; Galvez et al., 2011).

Therapy with ruboxistaurin is associated with a reduction in the progression of DME and a reduction in the rate of vision loss in patients with DME, although ruboxistaurin has not received FDA approval (PKC-DMS Study, 2007; Davis et al., 2009).

**Somatostatin derivatives** - Octreotide (Sandostatin®, Novartis) is approved for acromegaly, carcinoid tumors, and vasoactive intestinal peptide tumors. Somatostatin is an endogenous growth hormone inhibitor with known anti-angiogenic properties. The somatostatin analogue octreotide has been associated with decreased rates of progression to high-risk PDR, vitreous hemorrhage and the need for vitrectomy in patients with at least severe NPDR (Grant et al., 2000; Shah et al., 2010).

**Antiplatelet agents** - It has been seen that in chronic hyperglycemia-induced retinal inflammation, platelet activation, aggregation and thromboxane A2 has been increased. Further, leucocytes start adhering to the endothelial surface resulting in increased propensity for microthrombus formation and finally capillary occlusion, leading to retinal ischemia and severe DR. The EDTRS study evaluated the effect of aspirin on the progression of diabetic retinopathy and showed no beneficial or deleterious effect either on disease progression or on the rates of vitreous hemorrhage. In another study, combinations of aspirin and dipyridamole have suggested possible benefits for slowing the progression of diabetic retinopathy. The Dipyridamole, Aspirin, Microangiopathy of Diabetes (DAMAD) study showed a small but
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statistically significant reduction in the formation of micro-aneurysms (The DAMAD Study, 1989).

Herbal Drugs for the Management of Diabetic Retinopathy

Various plant-based drugs have shown promising results in experimental studies for the prevention of diabetic retinopathy. The advantage of herbal drugs over current therapies is that, apart from their safety, they are producing hypoglycemic effects in addition to their retinoprotective effect. Recently, Gupta et al., 2011 have shown the effect of chronic oral administration of curcumin in rats for prevention of DR. Curcumin inhibited the over-expression of retinal VEGF levels in rats. Moreover, electron microscopy study has shown that curcumin prevented the thickening of the basement membrane by its anti-oxidant and anti-inflammatory mechanisms as measured in retinas of treated rats. Overall, they have found good preventive effect of curcumin in DR.

Pycnogenol® is a natural plant extract from the bark of the maritime pine tree which grows exclusively along the coast of southwest France in Les Landes de Gascogne. The extract possesses powerful anti-oxidant and anti-inflammatory properties. Various experimental and clinical studies have shown the efficacy of Pycnogenol® in the management of DR (Steigerwalt et al., 2009; Spadea et al., 2001).

In another study, Nakajima et al., 2001 have shown that chronic oral genistein can significantly reduce retinal vascular leakage in an animal model of DR. Further, it has been studied that genistein possesses good tyrosine kinase inhibitory activity. In a perspective study published earlier has proved that drinking green tea lessened the incidence of DR (Cao and Cao, 1999). Similarly, one experimental study showed that green tea can potentially prevent the onset of DR (Kumar et al., 2012). Further, hesperetin has shown a preventive effect on DR in an experimental rat model (Kumar et al., 2012). So there is lot of scope coming out of the herbal world.

Conclusion
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The scientific principles for the treatment of DR and prevention of blindness have been known for the past three decades. In spite of this, DR remains a major public health problem with large numbers of people with diabetes going blind worldwide (especially in India), which can be preventable. Patients are not going blind for lack of technology or treatment options. They are going blind because they are not receiving treatment that has been well established for more than a quarter of a century.

Certainly, in the last one decade there have been significant developments in the pharmacotherapy of retinal-related disorders and many such innovations are still in the pipeline which we can expect in the near future. But, still we cannot depend on the innovations of the future. So there is a need for improving the present system using existing techniques and options.

In conclusion, it can be stated that for the present scenario systematic use of available pharmacotherapy as an adjunct to laser photocoagulation, which is the gold standard therapy, can be a useful tool in the prevention of vision loss from DR and related disorders.